CORRELATION BETWEEN HEMATOLOGICAL PROFILE, ERYTHROCYTE SEDIMENTATION RATE, AND HISTOLOGICAL TYPE OF DIGESTIVE SPINAL METASTASIS

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CORRELATION BETWEEN HEMATOLOGICAL PROFILE, ERYTHROCYTE SEDIMENTATION RATE, AND HISTOLOGICAL TYPE OF DIGESTIVE SPINAL METASTASIS (Abstract). **The objective** is to analyze possible correlations between histological types, hematological tests and erythrocyte sedimentation rate that can be identified in vertebral metastases secondary to digestive cancers. **Materials and methods:** The present study is a retrospective observational research of 27 patients with spinal metastases identified in the Prof. Dr. N. Oblu Emergency Clinical Hospital in Iași over a period of 8 years (2014-2021). For each patient, were recorded: patient demographics, including age and gender; tumor histology; hematological analyses (erythrocyte count, hemoglobin, hematocrit) and erythrocyte sedimentation rate. **Results:** The mean age of the group was 64.3 years. The male: female ratio was 19: 8=2.375. The most frequent histological subtypes of vertebral metastases were colonic adenocarcinomas (CAC) (n=12 cases; 44.4%,) and hepatocellular carcinomas (HCC) (n=6 cases; 22.2%), followed by squamous cell carcinomas (SCC) of oral and rectal origin (n=3 cases; 11.1%), rectal adenocarcinomas (RAC) (n=2 cases; 7.4%), and cholangiocarcinomas (CC) (n=2 cases; 7.4%). RAC cases presented the lowest erythrocyte counts (2.62 $\times 10^6$/mL), while neuroendocrine carcinoma (NEC) of the small intestine showed the highest mean values (4.20 $\times 10^6$/mL). RAC cases presented the lowest hemoglobin levels (8.3 g/dL), while CC and NEC (small intestine) demonstrated relatively higher averages (>11 g/dL). RAC cases demonstrated the lowest mean hematocrit (~31%), while oral cavity SCC presented the highest mean values (~38%). Spinal metastases of CAC, HCC, or CC showed intermediate levels (36-37%). Spinal metastases of RAC cases presented with the highest mean ESR values (~60-70 mm/h), and NEC of small intestine showed the lowest averages (~25 mm/h). **Conclusions:** Hematological profile and erythrocytes sedimentation rate (ESR) in spinal metastases of digestive origin show reproducible, histology-specific patterns: rectal primaries cluster with profound iron-deficiency anemia and high ESR; colon adenocarcinoma and hepatocellular carcinoma show intermediate anemia with variable inflammation; cholangiocarcinoma and neuroendocrine tumors of small intestine often pre-

serve hematological parameters with lower ESR. Integrating RBC/Hb/Ht with ESR enhances clinical triage, informs supportive care, and may improve prognostication when embedded in multivariable models. **Keywords**: SPINAL METASTASES, DIGESTIVE SYSTEM CANCER, HEMATOLOGICAL PROFILE, ERYTHROCYTE SEDIMENTATION RATE.

INTRODUCTION

Bone metastases are one of the most common complications of advanced neoplastic disease, with a significant impact on the quality of life and prognosis of patients. Although the most well-known sources of spinal bone dissemination are breast, prostate, and lung cancers (1), tumors of digestive origin can also generate vertebral metastases (2), sometimes clinically underestimated.

The spine is the favorite site of bone metastases originating from digestive cancers (3), as the rich venous vascularization and lack of valves in the vertebral plexuses (Batson plexus) facilitate hematogenous dissemination of tumor cells (4).

There are studies that have shown that certain laboratory tests can be useful in the early detection of bone metastases. Some authors have reported that elevated serum calcium (>2.54 mmol/L) and alkaline phosphatase (>116 U/L), but lower values of hemoglobin (<37.5 g/L) are risk factors for developing bone metastases of bladder cancer (5). Some other researchers reported that serum levels of prostate specific antigen (PSA), alkaline phosphatase (ALP), bone sialoprotein (BSP), collagen type I pyridine crosslinking peptide (ICTP) are increased in patients with bone metastases, and their combined detection could improve their positive predictive value (6).

The objective of this study is to analyze possible correlations between histological types, hematological tests (number of erythrocytes/mL, Hb, Ht), and erythrocyte sedimentation rate that can be identified in vertebral metastases secondary to digestive cancers.

MATERIAL AND METHOD

Study design. The present study is a retrospective observational research of patients with spinal metastases identified in the Prof. Dr. N. Oblu Emergency Clinical Hospital in Iași over a period of 8 years (2014-2021). We retrieved and examined eligible cases through the hospital case management system, using the disease code, which was standardized according to the International Classification of Diseases, Tenth Revision (ICD-10), and patient information was obtained by analyzing the medical records of hospitalized patients.

We identified a sample of 27 hospitalized patients, diagnosed and surgically treated for vertebral metastases secondary to digestive system cancer. For each patient, the following indicators were recorded: patient demographics, including age and gender; tumor histology; hematological analyses (erythrocyte count, hemoglobin, hematocrit) and erythrocyte sedimentation rate.

The inclusion criteria were: (1) adult patients (≥18 years old); (2) Diagnosis of spinal metastasis of a histologically confirmed digestive cancer; (3) availability of a minimum set of biological data (erythrocytes, hemoglobin, hematocrit, and ESR) that could be analyzed retrospectively.

Exclusion criteria were: (1) patients younger than 18 years; (2) patients with other spinal diseases, tumoral or non-tumoral.

Statistical analysis. The data were entered into a digital file and processed using

Python statistical software. The following methods were applied: descriptive statistics (mean); Kruskal-Wallis test for comparing groups according to non-parametric variables; graphical representations: (pie chart and bar chart).

Ethics. The data was anonymized before analysis. The study was approved by the Ethics Committee of the Prof. Dr. N. Oblu Emergency Clinical Hospital, Iași, and by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy, Iași. The research complies with the ethical principles of the Declaration of Helsinki and national regulations on the processing of personal data for research purposes. Each patient/guardian gave informed consent to obtain samples and their use for study purposes.

RESULTS

Demographic data. The study group included 27 patients with spinal metastases originating from a primary tumor of the digestive system. The male: female ratio was 19: 8=2.375, in favor of the male gender. The mean age of the group was 64.3 years.

Histological types of spinal metastases in the present series. In the analyzed cohort (fig. 1), the most frequent histological subtypes of vertebral metastases were colonic adenocarcinomas (n=12 cases; 44.4%) (fig. 2) and hepatocellular carcinomas (n=6 cases; 22.2%). These were followed by squamous cell carcinomas of oral and rectal origin (n=3 cases; 11.1%), rectal adenocarcinomas (n=2 cases; 7.4%) (fig. 3), and cholangiocarcinomas (n=2 cases; 7.4%) (Fig. 4). Single cases were represented by a neuroendocrine tumor of the small intestine and by a gastric adenocarcinoma (n=1 case each; 3.7%) (fig. 5).

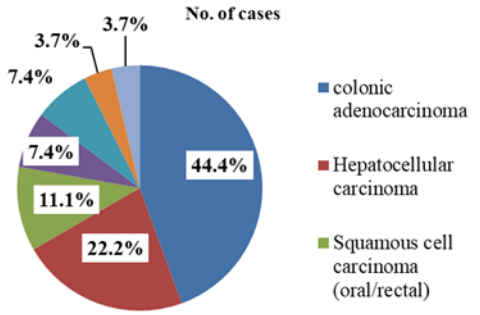

Fig. 1. Proportion of histological subtypes of vertebral metastases.

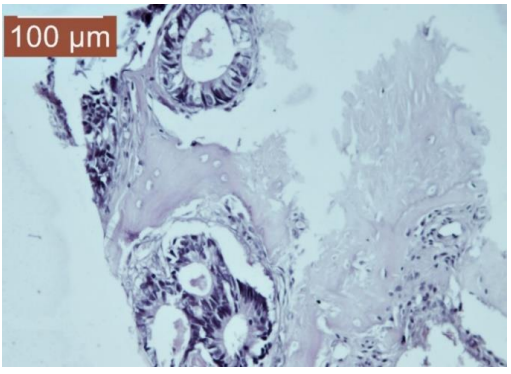

Fig. 2. Microscopic aspects: spinal metastasis originating from a moderately differentiated colonic adenocarcinoma (HE, x20).

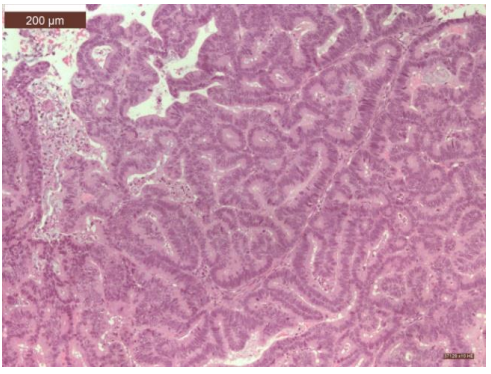

Fig. 3. Microscopic aspects: spinal metastasis originating from a moderately differentiated rectal adenocarcinoma (HE, x10).

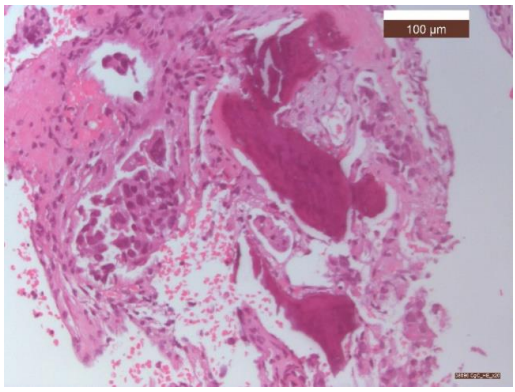

Fig. 4. Microscopic aspects: spinal metastasis of poorly differentiated cholangiocarcinoma (HE, x20).

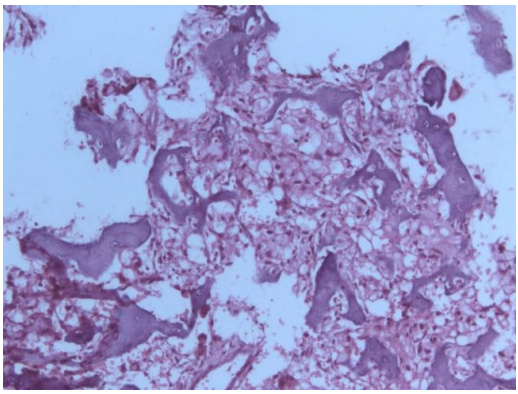

Fig. 5. Microscopic aspects: Spinal metastasis of gastric carcinoma with "signet ring cells" (HE, x10).

Correlations between the histological type of spinal metastasis and the number of erythrocytes

The Kruskal-Wallis test for the comparison of erythrocyte counts between the different histological types of metastases generated the following values: H = 6.21; p = 0.52, which means that there is no statistically significant difference between the values of erythrocyte counts and histological type ($p > 0.05$).

However, it can be observed that, at the moment of their spinal metastases, rectal adenocarcinoma cases presented the lowest erythrocyte counts (2.62×10^6/mL), while neuroendocrine carcinoma of the small intestine showed the highest mean values (4.20×10^6/mL). Intermediate levels were observed in rectal squamous carcinoma (3.3×10^6/mL), colon adenocarcinomas (3.74×10^6/mL), hepatocellular carcinoma (3.80×10^6/mL), and cholangiocarcinoma (4.06×10^6/mL) (fig. 6). These findings suggest potential variations in hematological impact depending on the histological subtype of the primary tumor.

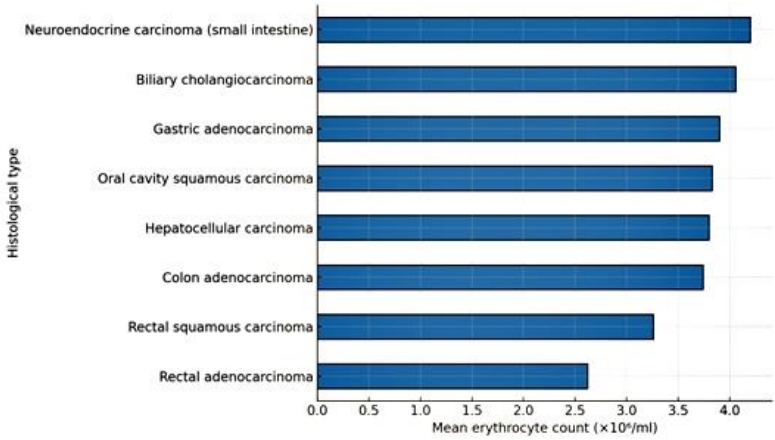

Fig. 6. Bar chart of average erythrocyte count values ($\times10^6$/mL) across different histological types of digestive cancers with spinal metastases in the present series.

Correlations between the histological type of spinal metastasis and hemoglobin value

The Kruskal-Wallis test for comparing hemoglobin values according to the histological type of metastasis generated the following values: H = 6.36; p = 0.50, which demonstrates that there is no statistically significant difference between hemoglobin levels and histological type ($p > 0.05$).

However, it is noted that vertebral metastases from rectal adenocarcinoma cases presented the lowest hemoglobin levels (8.3 g/dL), while cholangiocarcinoma and neuroendocrine carcinoma (small intestine) demonstrated relatively higher averages (>11 g/dL) (fig. 7).

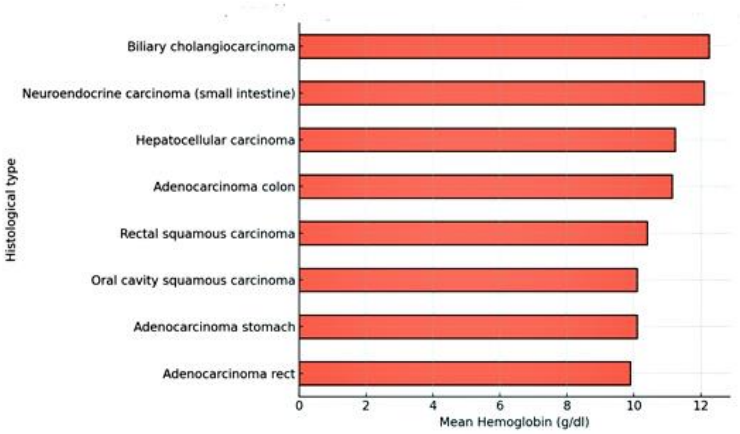

Fig. 7. Mean hemoglobin values (g/dL) by histological type in patients with digestive spinal metastases in the present series.

Correlations between the histological type of spinal metastasis and hematocrit value

The Kruskal-Wallis test for comparing hematocrit values between the different histological types of metastases generated the values: H = 6.21; p = 0.52, which means that there is no significant difference between hematocrit values and histological type ($p > 0.05$).

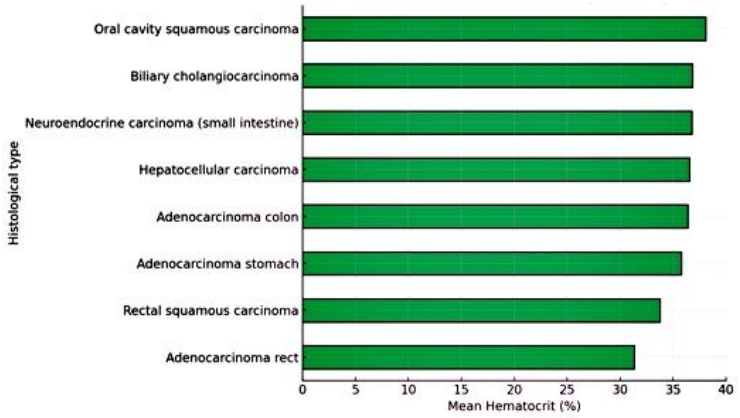

Fig. 8. Average hematocrit values (%) by histological type of digestive spinal metastases in the present series.

However, it is found that spinal metastases of rectal adenocarcinoma cases demonstrated the lowest mean hematocrit (~31%), while spinal metastases of oral cavity squamous carcinoma presented the highest mean values (~38%). Spinal metastases of colon adenocarcinoma, hepatocellular carcinoma, or cholangiocarcinoma showed intermediate levels (36-37%) (fig. 8).

Correlations between the histological type of spinal metastasis and the erythrocyte sedimentation rate value (ESR)

The Kruskal-Wallis test for the comparison of ESR values between different histological types of metastases generated the values: $H = 9.42$; $p = 0.22$, which means that there is no statistically significant difference between ESR values depending on the histological type ($p > 0.05$).

However, it is observed that spinal metastases of oral cavity squamous carcinoma and of rectal adenocarcinoma cases presented with the highest mean ESR values (~60-70 mm/h). Hepatocellular carcinoma (HCC) and colon adenocarcinoma demonstrated intermediate values (~45-55 mm/h), though one HCC patient exhibited an extreme value (130 mm/h). Neuroendocrine carcinoma of the small intestine showed the lowest averages (~25 mm/h). Colorectal adenocarcinomas exhibited median values (~50mm/h), but there were highly variable ESR values, reflecting the heterogeneity of systemic inflammation and disease burden in this subgroup (fig. 9).

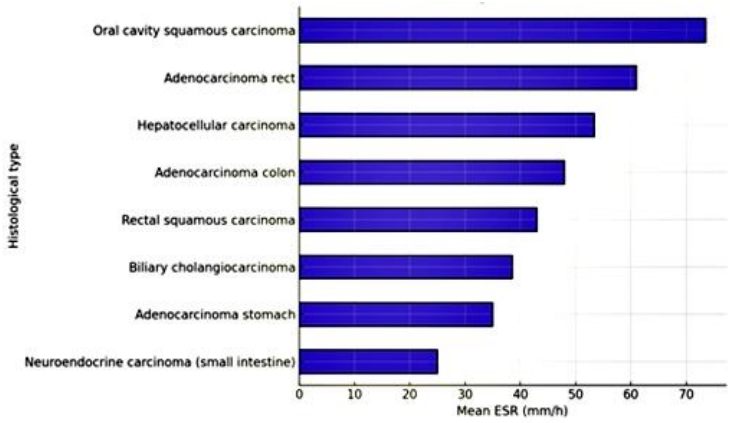

Fig. 9. Average erythrocyte sedimentation rate (ESR) values (mm/hr) by histological type of digestive spinal metastases in the present series

DISCUSSION

This study integrates erythrocyte count (RBC), hemoglobin (Hb), hematocrit (Ht), and erythrocyte sedimentation rate (ESR) from a 27-case cohort of spinal metastases of digestive origin, contextualizing patterns across histological subtypes and their clinical implications for diagnosis, prognostication, and supportive care.

Rectal adenocarcinoma showed the most profound anemia profile (lowest mean RBC, Hb, and Ht), together with higher ESR, suggesting combined iron-deficiency (chronic blood loss) and inflammation. Colon adenocarcinoma displayed intermediate anemia with moderately elevated ESR, indicating heterogeneous systemic inflammation. Our data are in line with literature reporting anemia as the most common extraintestinal symptom, appear-

ing in 30-75% of patients (7).

Hepatocellular carcinoma (HCC) presented intermediate anemia, but ESR values were highly variable (including an outlier at 130 mm/hr), consistent with chronic inflammation/hypersplenism rather than occult bleeding. Some authors have shown that ESR can be a marker of early HCC (8). These data may explain the identification of a high EST value in the present study in the case of spinal metastases of HCC.

Cholangiocarcinoma and neuroendocrine carcinoma of small intestine tended to maintain higher RBC/Hb/Ht and lower ESR, in keeping with relatively indolent bleeding profiles and lower marrow suppression.

Regarding squamous carcinomas, we found out some differences by site: oral cavity squamous carcinoma kept higher Ht/Hb and high ESR, but rectal squamous carcinoma showed lower Hb/Ht with mid-range ESR, probably due to anterior chemo- and radiotherapy. However, in case of rectal squamous carcinoma a higher pre-treatment value of Hb was an independent prognostic factor for overall survival of patients with anal canal cancer (9).

From a pathophysiological point of view, we can take into consideration the following mechanisms:

(1) Rectal primaries frequently drive iron deficiency via chronic bleeding, lowering RBC/Hb/Ht and raising ESR (7). Conversely, HCC-associated anemia is often normocytic and inflammation-driven, compounded by hypersplenism, but it can be significantly associated with mortality in HCC patients, independent of established liver- and tumor-related prognostic factors (10);

(2) Recent chemotherapy and radiotherapy of the primary tumor may transiently suppress erythropoiesis, worsening anemia independently of histology (11).

(3) ESR elevation often correlates with poor prognosis in several types of cancer. There are studies that have demonstrated this correlation in kidney cancer (12), prostate cancer (13), and also in colorectal cancer (14).

(4) Bone marrow involvement in metastatic disease and poor nutrition decrease Hb/Ht, especially in colorectal cancer (15), but NETs and cholangiocarcinoma typically show slower marrow impact and as such hematological indices will not decrease at lower values, but rare cases of cholangiocarcinoma can realize an extensive destruction of bone marrow and present anemia with lower values of Hemoglobin (16).

Our findings have some clinical implications. One of them is the possibility to realize a baseline risk stratification when combining erythrocyte number, Hb, Ht and ESR, because differences of these hematological indices can underline the anatomical site of the primary cancer, and comorbid conditions and may have prognostic and therapeutic relevance.

Some authors demonstrated that Hb is a prognostic factor for spinal metastases. The baseline Hb level is included as a prognostic variable in certain scoring systems for spinal metastasis (17-19).

ESR was also identified as an independent prognostic factor for patients with Spinal Metastasis from Esophageal Cancer (20). Across solid tumors, pre-treatment anemia and elevated ESR associate with poorer survival (21, 22).

In colorectal cancer, both low Hb and high ESR relate to advanced stage; in HCC, inflammatory markers reflect underlying hepatic dysfunction. The histology-linked hematologic patterns suggest that simple

blood tests can refine prognosis alongside imaging and clinical scores as Erythrocyte Sedimentation Rate revealed the inflammation that is associated with development and progression (23), and anemia, revealed by low values of erythrocytes, hemoglobin and hematocrit, often accompany the progression of the metastatic disease and its cause is a defect in iron handling rather than a lack of iron *per se* (24).

CONCLUSIONS

Hematological profile and erythrocytes sedimentation rate (ESR) in spinal metastases of digestive origin show reproducible, histology-specific patterns: rectal primaries cluster with profound iron-deficiency anemia and high ESR; colon adenocarcinoma and hepatocellular carcinoma show intermediate anemia with variable inflammation; cholangiocarcinoma and neuroendocrine tumors of small intestine often preserve hematological parameters with lower ESR. Integrating RBC/Hb/Ht with ESR enhances clinical triage, informs supportive care, and may improve prognostication when embedded in multivariable models.

Histology-specific anemia patterns therefore provide useful insights into prognosis and patient management as it may aid in risk stratification: rectal cancers with low hematocrit at the moment of the spinal metastases' diagnosis may indicate more aggressive systemic disease.

Monitoring hematological analysis dynamics could help guide supportive care, including transfusions and iron supplementation, especially in colorectal cancer patients at risk for profound anemia.

ACKNOWLEDGMENTS

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MANAGEMENT OF LEFT ILIAC FOSSA PAIN IN THE EMERGENCY DEPARTMENT

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MANAGEMENT OF LEFT ILIAC FOSSA PAIN IN THE EMERGENCY DEPARTMENT (Abstract): Left iliac fossa (LIF) pain is a common reason for emergency department (ED) visits and can be caused by a wide range of conditions, from benign to life-threatening, timely and accurately diagnosis being crucial for appropriate management. This study **aims** to highlight the management of LIF pain and to outline the etiology and imaging findings in a real-world ED setting. **Materials and methods:** We conducted a retrospective study of 144 patients who presented with LIF pain at the ED of Sf. Spiridon County Clinical Emergency Hospital Iași between August 2022 and December 2024. Imaging studies and final diagnoses were analyzed to assess diagnostic pathways and imaging concordance. **Results:** The patients had a mean age of 53.9 years, with a slight female predominance (56.25%), the most frequent diagnoses being acute diverticulitis (25%). Computed tomography (CT) was performed in 105 cases (72.92%) and provided clarification in nearly a third of the patients who had previously undergone ultrasound (US) and didn't have a final diagnosis. Overall, CT findings confirmed the ED's initial diagnostic hypothesis in 75.24% cases. **Conclusions:** This study offers a detailed analysis of CT utility in a real-world emergency setting, confirming that CT plays an important role in the differential diagnosis of LIF pain in the ED, influencing management decisions and improving clinical outcomes. Our data support the implementation of standardized diagnostic algorithms for LIF pain in the ED, incorporating early CT in selected cases in order to ensure a patient-centered approach. **Keywords:** LEFT ILIAC FOSSA PAIN, COMPUTED TOMOGRAPHY, DIVERTICULITIS.

INTRODUCTION

The most common condition for patients' presenting in ED is abdominal pain, the one in the LIF being a frequent symptom and affecting an older population compared to the right iliac fossa pain (1); it is important to find out the etiology (organic or functional, tumoral or infectious) as early as possible because it can be a symptom of an emergency in which case the patient requires an adequate treatment (2).

LIF includes intraabdominal structures (digestive and genito-urinary organs, vessels, lymph nodes) and abdominal wall (3), which can cause visceral, parietal and referred pain together and separately.

Plain abdominal X ray, US and CT, play an important role in the evaluation and management of LIF pain, being able to differentiate rapidly from conditions requiring no treatment to life-threatening emergencies. Plain abdominal X ray is

often the chosen imaging modality due to its availability, low cost, low radiation dose and high amount of information (evaluates bones, foreign bodies, air filed structures and offers limited detail on the soft tissues). US is non-invasive and fast but is operator dependent and doesn't pass through bone or gas (4). CT creates cross-sectional images, is standardized, uses a higher amount of ionizing radiation but provides a higher amount of diagnostic information than plain abdominal X ray (5,6); it is also fast (image acquisition takes 5 to 10 minutes from patient positioning to the end of the procedure and another 10 to 30 minutes for the written report), but sometimes patients can have adverse reactions to intravenous contrast medium. Radiologists and emergency doctors must adequately choose between plain abdominal X ray, abdominal and pelvic US and abdominal and pelvic contrast enhanced CT (CECT), depending on the suspected diagnosis and patient's specificity, in order to quickly assess injuries, identify life-threatening pathologies, and guide immediate patient management (7).

The most frequent causes of LIF pain are gastrointestinal diseases: constipation, irritable bowel syndrome (IBS), acute diverticulitis, colitis, bowel obstruction and tumors, sigmoid volvulus, hernias, epiploic appendicitis, digestive foreign bodies.

Colonic diverticulosis is the most frequent bowel condition in Europe (appearing in over 70% of the population by their eighties), most patients remaining asymptomatic (8). Diverticulitis can cause only localized inflammation but sometimes (12% of cases) is complicated with abscess, fistula, perforation, bowel obstruction or hemorrhage. Endoscopic procedures are generally contraindicated in cases of suspected acute diverticulitis, due to a high risk of perforation, so imaging methods play an important role in diagnosis (9).

Acute colitis reunites a wide range of bowel conditions, so discerning the specific cause of the disease is challenging, CECT frequently provides an initial evaluation, offering suggestive signs and aiding to clarify the underlying etiology (10).

CT is a valuable diagnostic tool for two self-limiting inflammations causing abdominal pain: acute epiploic appendicitis, mainly located frequently in LIF and omental infarction frequently on the right side of the abdomen, rarely causing pain in the LIF (11).

Colorectal cancer is an increasingly pressing global health concern, left side locations being more likely to be complicated with bowel obstruction, but also with progressive left iliac fossa pain, rectal bleeding and unexplained weight loss. Data indicates that men are more frequently affected (53% *vs.* 47%) and earlier in life (68 years *vs.* 72 years in women). The incidence of this condition in younger adults is also climbing. Many cases are sporadic, influenced by factors such as advanced age, racial background, diet, and a history of inflammatory bowel disease (12).

MATERIALS AND METHODS

We conducted a retrospective study on a cohort of 144 patients who presented to the Emergency Department (ED) of Sf. Spiridon County Clinical Emergency Hospital Iași between August 2022 and December 2024 with LIF pain, either as an isolated symptom or associated with other types of abdominal or lumbar pain.

The inclusion criteria were straightforward: the presence of LIF pain clearly documented in the ED observation sheet,

either in the patient's medical history or in the clinical examination. Cases were excluded if abdominal pain was mentioned without a clearly specified location or without identifying the point of maximum intensity.

A systematic review of ED observation sheets was carried out both manually and electronically, using keywords such as "LIF pain". Although not all parameters were available for every patient, the dataset included key clinical and administrative variables whenever possible. These included patient demographics such as age and sex; timing of ED events, including the moment of ED sheet initiation, time of first medical evaluation; signs and symptoms recorded during the visit, relevant medical history (previous surgeries, chronic conditions), imaging methods.

The statistical analysis aims to characterize the patient cohort, explore relevant clinical correlations and compare with findings reported in the literature.

RESULTS

The patients had an age range between 18 and 93 years old, with a mean age of 53,9 years. The most common age group was 61-70 years old, accounting for 33 cases, while the least represented were patients over 90 years old, with only one case. Gender distribution was nearly equal, with 47.36% males and 52.64% females, a ratio consistent with that of the general Romanian population. Minor variations may reflect the limited sample size, but they also raise relevant questions regarding differences in symptom perception and care-seeking behavior. Women are often more proactive in addressing health concerns, which might explain their slight predominance in this cohort.

Time spent in the ED plays a critical role in patient management and ensures prompt initiation of treatment. In this study, triage time (interval between the opening of the ED observation sheet and the first medical consultation) ranged from 0 to 250 minutes, with a mean value of 30 minutes. The cumulative time spent in the ED calculated from the time of completing the ED observation sheet until the time of closing the sheet due to admission, transfer to another hospital, discharge with recommendations, or leaving the ED without medical advice, ranged from one to 18 hours. During this time, patients underwent clinical evaluation (including consultations in gastroenterology and surgery departments), blood and urine tests, imaging investigations.

The longest recorded delay, 250 minutes (4 hours and 10 minutes), was noted in the case of a patient with uncomplicated acute diverticulitis. Such prolonged waiting times, although isolated, can increase patient anxiety and discomfort, and may lead to delays in diagnostic procedures and the start of appropriate treatment. At the other end were 9 patients (6.25%) triaged as Red Code (NOW: 0-1 minute), with the following diagnoses: LIF stab wound, ruptured ectopic pregnancy, septic shock (abscess and possible tumor recurrence), lipothymia (acute diverticulitis and pulmonary thromboembolism), syncope (small bowel intussusception on imaging and heart failure diagnosed by the cardiologist), 2 patients with generalized stercoral peritonitis due to sigmoid diverticulum perforation post-chemotherapy, 2 thoraco-abdominal contusions (incidental finding of colonic malignant tumors on CT scan). These findings prove that cardiovascular diseases and tumors associated with acute abdominal conditions must be careful-

ly evaluated by a multidisciplinary team (13, 14).

The cumulative distribution curve revealed that more than half of the patients (51%) were triaged within the first 15 minutes, and over 80% within one hour. In contrast, only 18% of patients completed their ED stay within 4 hours, while nearly two-thirds (65%) remained in the department for 4–8 hours. This discrepancy highlights the efficiency of the triage process but also indicates prolonged diagnostic and management phases within the ED workflow (fig. 1).

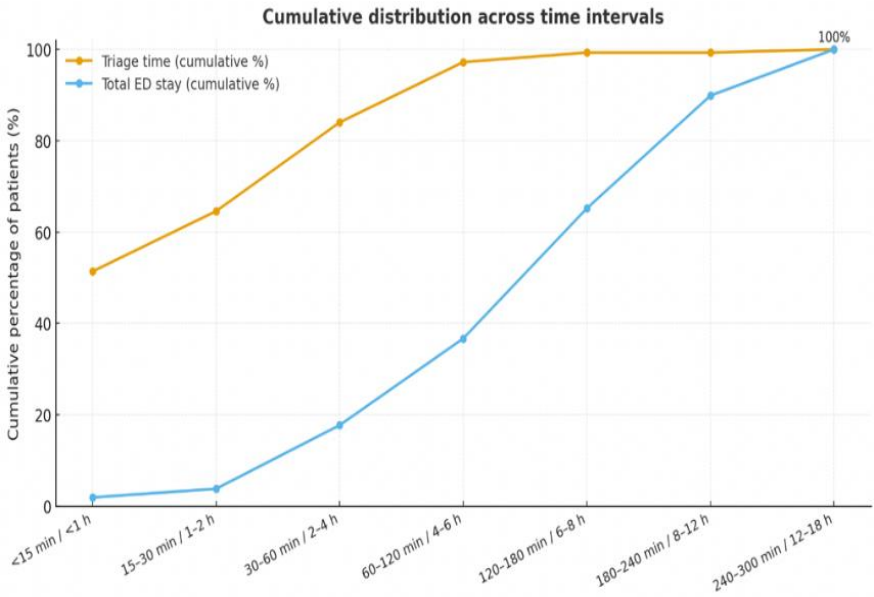

Fig. 1. Cumulative distribution across time intervals (triage time *vs*. total ED stay)

Patients presenting with LIF pain underwent the following imaging investigations: 128 patients (88.89%) underwent abdominal and pelvic US. Which was the most frequent imaging modality used, followed by abdominal and pelvic CECT (105 patients -72.92%), especially valuable when US was inconclusive. Plain abdominal X-ray was used in only 52 cases (36.11%), with limited diagnostic value compared to US and CT, diagnosing bowel obstruction or digestive tract perforation: 27 exhibited hydro-aeric levels (one of the patients also presented a metallic foreign body located in LIF), 23 showed no significant pathological findings, while one revealed a left ureteral calculus.

There was no evidence of pneumoperitoneum on plain abdominal X-ray, however, subsequent abdominal and pelvic CECT identified intraperitoneal air in two cases. This discrepancy underscores a higher CT sensitivity in detecting small volumes of extraluminal gas. The findings align with existing literature that highlights CT's enhanced diagnostic accuracy for pneumoperitoneum, particularly in cases where radiographic results are inconclusive. 7 (4.86%) patients underwent both US and plain abdominal X – ray, 47 (32.64%)

were examined by US and CECT, while in 45 (31.25%) cases, US, plain abdominal radiography and CECT were used together.

Among the 92 patients who underwent both abdominal US and abdominal and pelvic CECT, concordant findings were observed in 65 (29.35%) cases, and in 27 (70.65%) cases, US did not reveal any significant findings related to the patients' presenting symptoms, whereas CT imaging provided diagnostic insights. This discrepancy underscores the complementary roles of US and CT in the evaluation of LIF pain. While US is a non-invasive, radiation-free, and readily accessible imaging technique, its diagnostic accuracy can be limited by factors such as operator dependency and patient body habitus. Conversely, CT imaging offers higher sensitivity and specificity in detecting abdominal pathologies, particularly in complex or inconclusive cases.

CECT had a concordance rate of 75.24% (79 cases) with the initial ED diagnostic hypothesis; in 26 cases (24.76%) CT did not confirm the preliminary diagnosis. This finding demonstrates the significant role of CT in validating clinical assessments, while also highlighting its importance in identifying alternative diagnoses when initial clinical evaluation is inconclusive. The findings affirm the necessity of integrating imaging results with clinical judgment and laboratory testing, to enhance diagnostic accuracy and optimize patient management strategies.

The distribution of final diagnoses among patients presenting with LIF pain was diverse, reflecting a wide range of underlying pathologies (tab. I). The Gini coefficient (fig. 2) was applied to quantify the inequality in the distribution of final emergency department (ED) diagnoses. In this context, a Gini index of 0.45 indicates a moderate concentration of cases within a limited number of etiologic entities. This finding demonstrates that abdominal pain in the ED is not randomly distributed across a wide spectrum of conditions, but rather dominated by a few diagnostic categories-chiefly genitourinary disease (28.47%) and diverticular disease (27.08%)-which together accounted for more than half of all cases. Such a distribution pattern underscores the importance of focusing diagnostic resources and imaging protocols on the most prevalent and clinically significant causes, rather than applying uniform, broad-spectrum diagnostic pathways to all patients.

TABLE I.

ED final diagnoses of patients with LIF pain

ED final diagnosis	No. / %	Observations
Genito-urinary disease	41 (28.47%)	26 (18.05%) left renal colic
		10 (6.94%) left adnexal masses
		2 (1.38%) bladder tumors
		1 (0.69%) left ureter tumors
		1 (0.69%) ruptured ectopic pregnancy
		1 (0.69%) left-side testicular cancer
Diverticular disease (DS)	39 (27.08%)	36 (25%) diverticulitis
		3 (2.08%) uncomplicated DS
Intestinal tumors	15(10.41%)	14 (9.72%) colon cancer
		1 (0.69%) gastro-intestinal stromal tumor

ED final diagnosis	No. / %	Observations
Intestinal obstruction	13 (9.02%)	6 (4.16%) adhesions
		2 (1.38%) intestinal intussusception
		1 (0.69%) small bowel diverticulum
		1 (0.69%) sigmoid volvulus
		1 (0.69%) stenosis (Crohn's disease)
		1 (0.69%) fecal impaction
		1 (0.69%) stoma complication
No significant pathological findings	10 (6.94%)	-
Epiploic appendicitis	7 (4.86%)	6 (4.16%) acute
		1 (0.69%) chronic
IBS	6 (4.16%)	-
Abdominal wall pathology	5 (3.47%)	2 (1.38%) abscesses
		3 (2.08%) hematomas
Abscesses	4 (2.77%)	2 (1.38%) intra-abdominal
		2 (1.38%) iliopsoas region.
Other findings	4 (2.77%)	1 (0.69%) stab wound
		1 (0.69%) acute appendicitis
		1 (0.69%) left inguinal hernia
		1 (0.69%) ruptured aortic aneurysm

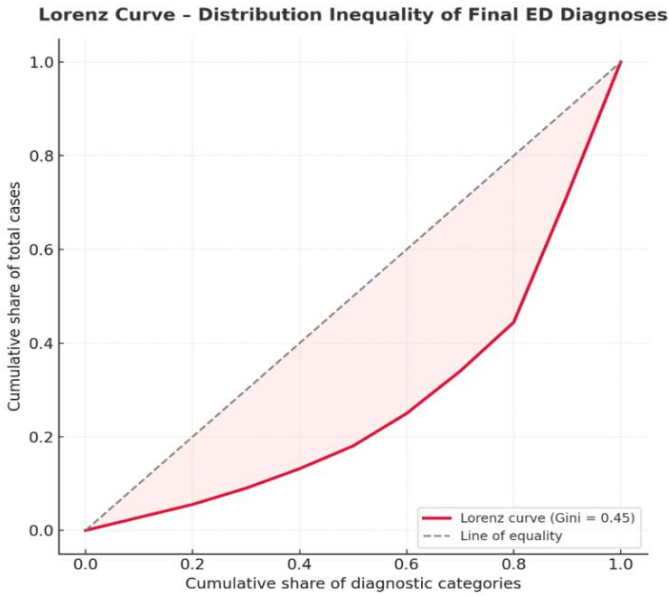

Fig. 2. Lorenz curve illustrating the unequal distribution of final ED diagnoses.

Patients included in the studied group had coexisting chronic health conditions, among them 52 patients (36.11%) had heart conditions, often with two or more diseases present simultaneously, 10 (6.94%) reported respiratory phenomena (dyspnea, cough) or declared non-specific symptoms: headache (37 patients, 25.69%), lipothymia (2 patients, 1.38%). Within the studied cohort, 50 patients (34.72%) had a history of prior surgical interventions, among them two patients (1.38%) had mastectomy and 48 (33.33%) abdominal and pelvic surgery, including 14 (9.72%) with surgery related to various malignant tumors (digestive tract, ovaries, breast, thyroid, and testes). The presence of previous abdominal and pelvic surgery is clinically significant, as it may predispose patients to postoperative adhesions, leading to bowel obstruction or chronic pelvic pain, and can influence both the differential diagnosis and the choice of imaging modalities. Furthermore, a history of cancer-related surgery requires careful consideration, as these patients may have an increased risk of disease recurrence or metastasis, which could manifest as abdominal pain. Therefore, their prior oncological history should be integrated into the diagnostic process to ensure comprehensive evaluation and appropriate management (fig. 3).

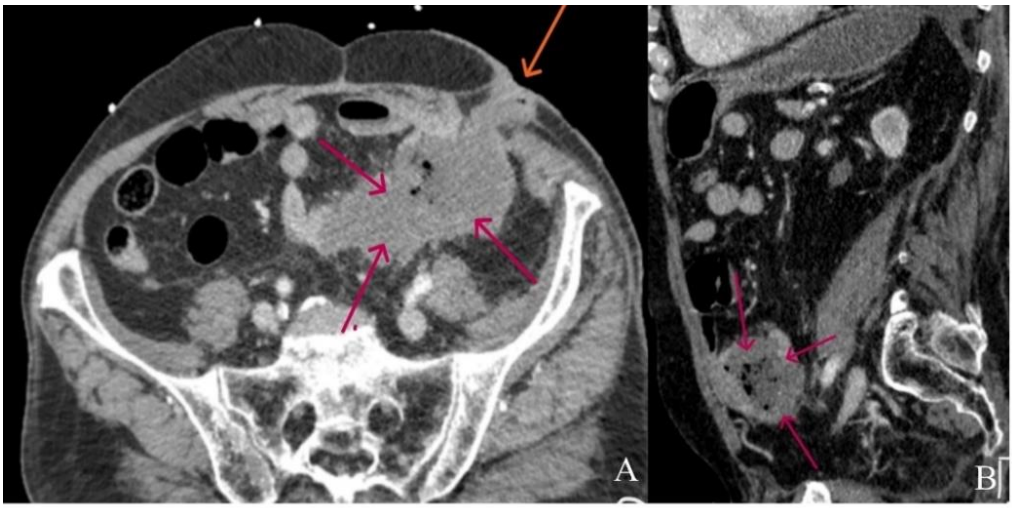

Fig. 3. CECT - colostomy for colonic cancer (orange arrow), collection with gas bubbles (red arrow): axial section (A), coronal multiplanar reconstruction (B)

The Gini coefficient (0.46) indicates that a small number of diagnostic entities account for a disproportionately large share of emergency abdominal presentations.

DISCUSSION

The results of this study have limitations. Being a retrospective, single-centered study, the results may be influenced by the specific practices of this unit and have limited generalizability. Additionally, this analysis is based on data available in patients' charts, which may be incomplete or have variations in the detail

of the information.

Due to its low cost, accessibility, rapid results and lack of ionizing radiation, abdominal US is the first imagistic method of choice for investigating left iliac fossa pain, especially in young female patients (15), but CT has an overall view of the entire patients' pathology.

The main purpose is to analyze the role and added value of CECT in the differential diagnosis of LIF pain. Our study demonstrated that CT is an effective imaging modality and identified a wide range of causes of LIF pain, highlighting the limitations of clinical examination and laboratory tests in establishing a precise etiological diagnosis in the ED and underscores the necessity of using advanced imaging methods (16).

In case of patients with malignancies or complex inflammatory conditions, early identification of complications such as abscesses or fistulas allowed more appropriate surgical planning and, in some cases, avoided unnecessary exploratory laparotomies (2).

The patients' history of prior surgical interventions, especially for breast and abdominal and pelvic malignant tumours, can predispose patients to postoperative adhesions, have an increased risk of tumor recurrence or metastasis, which can manifest with abdominal pain; therefore, the oncological history should be considered in imaging evaluation to ensure an appropriate management (17).

Patients who have coexisting heart conditions, lipothymia, respiratory phenomena have to be carefully monitored by clinicians in order to avoid complications after intravenous administration of iodinated contrast media for CECT examination (13,18). Radiologists have to notice significant subtle diagnoses that need urgent intervention, reducing errors by reviewing all available clinical and paraclinical notes, working in a team, analyzing all medical imaging, especially all CT phases (noncontrast and postcontrast: arterial, portal and late), by having a systematic approach and by using multiple windows and multiplanar reconstruction (19).

Diagnostic discrepancies observed in 24.76% of cases between the ED clinical assessments and CT findings emphasize the inherent limitations of initial clinical judgment, particularly in a high-pressure emergency environment.

The calculated Gini coefficient was 0.45, reflecting a moderate inequality in diagnostic distribution. Most cases were concentrated in two major categories-genitourinary disease (28.47%) and diverticular disease (27.08%).

CONCLUSIONS

This study offers a detailed analysis of CT utility in a real-world emergency setting, confirming that CT plays an important role in the differential diagnosis of LIF pain in the ED, influencing management decisions and improving clinical outcomes. Our data support the implementation of standardized diagnostic algorithms for LIF pain in the ED, incorporating early CT in selected cases in order to ensure a patient-centered approach.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SOLITARY AND MULTIPLE ENCHONDROMAS (OLLIER DISEASE): RARE SKELETAL DISORDERS CAUSING SEVERE BONE DESTRUCTION AND PSYCHO-EMOTIONAL DISTRESS

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SOLITARY AND MULTIPLE ENCHONDROMAS (OLLIER DISEASE): RARE SKELETAL DISORDERS CAUSING SEVERE BONE DESTRUCTION AND PSYCHO-EMOTIONAL DISTRESS (Abstract): Solitary enchondromas represent benign cartilaginous neoplasms originating from residual chondrocytes of the physeal plate. Ollier disease (multiple enchondromatosis) is a rare, non-hereditary skeletal disorder characterized by multiple intramedullary cartilaginous tumors (enchondromas) carry a risk of malignant transformation into chondrosarcoma or atypical cartilaginous tumor (ACT). Both diseases are rare, initially asymptomatic and curative treatment is non-existent. **Materials and methods**: The study included a cohort of 14 patients diagnosed with solitary or multiple asymptomatic enchondromas, classified according to the Takigawa classification. in all cases the diagnosis was established by imaging examination. All patients underwent surgical treatment. The surgical techniques employed consisted of tumor curettage followed by reconstruction of the resulting bone defect using either autologous bone graft or solid bone substitute. In one case, where the enchondromas were multiple and large, with massive digital bone destruction, a necessary amputation was performed. **Results**: Imaging examinations revealed extensive lytic lesions affecting the metacarpals and phalanges in all cases. In the patient with Ollier disease, lesions were also identified in the radius, humerus, scapula, and vomer bone-an exceptional localization. Histopathological analysis confirmed a central atypical cartilaginous tumor (ACT) arising secondarily from an enchondroma in the case of multiple enchondromatosis. The patient with Ollier disease was also diagnosed with psychiatric comorbidities, including anxiety and depression, related to chronic hospitalization and physical deformity. Functional and aesthetic outcomes were evaluated in all cases using the DASH, ROM, and VAS scores. **Conclusions**: The presence of enchondromas is rare, and multiple enchondromatosis-particularly when associated with cranial involvement-represents an extremely uncommon entity. The absence of symptoms often leads to incidental diagnosis. In Ollier disease, onset occurs in early childhood, and the resulting deformities can cause significant psychological and emotional trauma, in addition to the orthopedic and functional burden associated with the disease. **Keywords:** SOLITARY ENCHONDROMA; OLLIER DISEASE; CHONDROSARCOMA; VOMER BONE ENCHONDROMA; SURGERY.

INTRODUCTION

Solitary enchondromas represent benign cartilaginous neoplasms originating from residual chondrocytes of the physeal plate. More than half of the reported cases are localized within the bones of the hand (1).

The true incidence of this pathology is likely underestimated, as enchondromas frequently remain clinically silent. Diagnosis is most often established incidentally during routine radiographic evaluation or in response to clinical manifestations such as localized pain, swelling, or a pathological fracture. Radiographic examination typically demonstrates a well-circumscribed lytic lesion that generally preserves cortical integrity and does not involve adjacent soft tissues, with variable degrees of intralesional calcification (2, 3).

The management of benign osseous tumors of the hand, including enchondromas, typically involves intralesional curettage, with or without subsequent filling of the residual cavity using autologous bone graft or bone substitute material (4). Although autologous bone grafting remains the gold standard for reconstructing bone defects, the use of bone substitutes has become increasingly prevalent in order to minimize donor-site morbidity and associated complications. In cases requiring cancellous bone grafting for hand defects, the distal radius and the olecranon of the ulna are the most commonly utilized donor sites. These locations generally provide sufficient cancellous bone for reconstruction of defects in the carpal bones, metacarpals, and phalanges, and are preferred due to their accessibility within the same operative field and compatibility with the same regional anesthesia (5, 6).

Multiple enchondromatosis also known as Ollier disease, is a rare, non-hereditary skeletal disorder that occurs sporadically and was first described in 1889 by Louis Léopold Ollier (1, 2). It is typically diagnosed in early childhood, manifesting through painful bone deformities, limb asymmetry, and growth disturbances. The estimated incidence of the disease is approximately one case per 100,000 individuals, making it an exceptionally uncommon condition (3). In Ollier disease, multiple enchondromas are irregularly distributed and may appear either unilaterally or symmetrically, varying in both number and stage of development (4, 5). The diagnosis is primarily based on clinical evaluation and imaging studies, with histopathological assessment being essential to identify malignant transformation-most frequently into chondrosarcoma or, less commonly, osteosarcoma (1). The reported risk of malignant transformation ranges between 5% and 50%. Since 2013, the World Health Organization (WHO) has reclassified grade I chondrosarcoma as an atypical cartilaginous tumor (ACT), recognizing it as a locally aggressive neoplasm with limited metastatic potential and classifying it as an intermediate-grade rather than a fully malignant tumor (2, 3).

These lesions can lead to progressive bone destruction, skeletal deformities, and functional disability, often beginning in childhood and persisting into adulthood (6). The visible deformities, coupled with chronic pain and restricted movement, frequently result in psychological distress, including anxiety, low self-esteem, and depressive symptoms, which significantly affect the patient's quality of life (1, 7).

Despite being a rare and debilitating condition with a significant risk of malignant transformation, there is currently no specific curative treatment for Ollier disease (2). Patients presenting with small, asymptomatic enchondromas that do not compromise limb function are typically managed through careful clinical and radiological observation. Surgical treatment is indicated only in cases complicated by limb shortening, severe deformities, pathological fractures, or suspected malignancy (8). Depending on the extent and severity of

bone destruction, surgical approaches may include tumor excision followed by bone grafting, either using autologous bone or synthetic bone substitutes (9). The bone substitute can be solid or injectable, and may also be employed in the absence of an autologous graft (9). When cortical thinning remains significant after tumor ablation, osteosynthesis materials such as Kirschner wires, plates and screws, or external fixators can be used to reinforce bone stability and prevent postoperative fracture. In extensive lesions with severe cortical destruction where reconstruction is not feasible, amputation of the affected segment may be the only viable treatment option (10).

Advanced imaging modalities, including MRI and CT, are critical in determining the extent of the lesions, assessing cortical involvement, and detecting early malignant transformation. Bone scintigraphy may also be used to evaluate metabolic activity and identify lesions with aggressive potential (11, 12). Imaging studies are further valuable in identifying associated systemic abnormalities, as Ollier disease has been linked to conditions such as skull base gliomas, pancreatic neoplasms, and hematologic disorders, supporting the concept of a broader systemic predisposition related to somatic mosaicism (13).

We report a retrospective study including 13 patients diagnosed with unilateral solitary enchondroma of the hand and one patient diagnosed with multiple enchondromatosis (Ollier disease), with lesions also involving the vomer bone and significant psychiatric impairment.

MATERIALS AND METHODS

A total of 14 patients, aged between 23 and 54 years, were included in this study. All were admitted to the Plastic Surgery Clinic of Sf. Spiridon County Clinical Emergency Hospital Iasi between 2016 and 2023.

The study was approved by the institutional ethics committee, and informed consent was obtained from all participants. All patients presented with slow-growing, painless deformities of the metacarpal or phalangeal bones. None of the cases reported pain or sensory disturbances. In five cases, patients presented to the emergency department with pathological fractures resulting from minor trauma, such as direct blows or falls from standing height. One patient was known to have Ollier disease (multiple enchondromatosis), diagnosed at the age of three, associated with psychiatric pathology secondary to physical deformities. The reason for medical presentation in this case was the excessive tumor volume involving both hands, particularly the second finger of the right hand. Radiological imaging was performed in all cases, while computed tomography (CT) was additionally conducted in the patient with Ollier disease. Surgical treatment was indicated in all patients. Surgical interventions were carried out under either WALANT (Wide Awake Local Anesthesia No Tourniquet) or loco-regional infraclavicular block anesthesia. All excised specimens were submitted for histopathological examination. Postoperative outcomes were assessed over a two-year follow-up period, using the DASH, ROM, and VAS scoring systems.

RESULTS

The study included 14 patients aged between 23 and 54 years (mean age 38.5 years), of whom 12 were male (85.7%) and 2 were female (14.3%). Thirteen patients presented with unilateral solitary enchondroma of the hand, while one patient was

diagnosed with multiple enchondromatosis (Ollier disease), representing 7.1% of the cohort (tab. I).

TABLE I.

Baseline Characteristics

Variable	Value
Total patients	14
Age range (years)	23-54
Mean age (approx.)	38.5
Sex: Male	12 (85.7%)
Sex: Female	2 (14.3%)
Lesion type: Solitary	13 (92.9%)
Lesion type: Multiple (Ollier)	1 (7.1%)
Laterality	Unilateral in 13, bilateral in 1 (Ollier)
Anatomic sites (hand)	11 phalanges, 1 metacarpal, 1 multifocal (Ollier)

Among the solitary cases, enchondromas were localized in 11 patients at the level of the phalanges—specifically, two cases involved first phalanx of middle finger of the left hand, five cases first phalanx of the fifth finger of the right hand, three cases first phalanx of the fifth finger of the left hand, and one case third phalanx of the fifth finger of the left hand. One lesion was situated in the second metacarpal.

The patient with Ollier disease presented with multiple lesions affecting both hands (phalanges and metacarpals), bilateral radius, humerus, scapula, and, exceptionally, the vomer bone (fig. 1).

Radiological exam confirmed the presence of well-circumscribed lytic lesions in all cases (fig. 2).

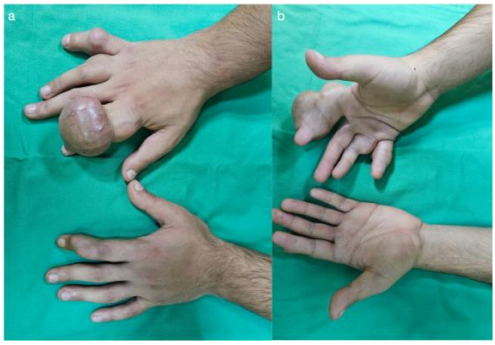

Fig. 1. Clinical aspect of the both hands. a. dorsal aspect, b. palmar aspect

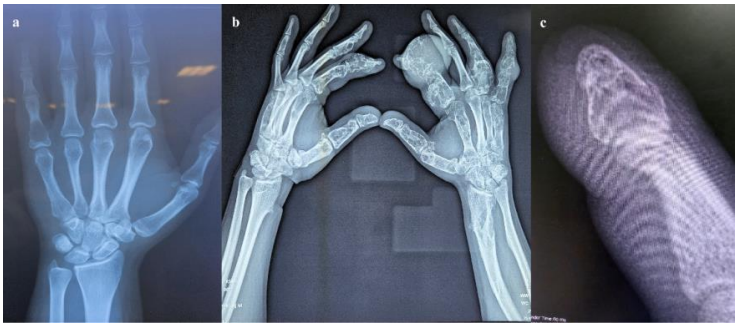

Fig. 2. Radiological aspect of a solitary enchondroma a. first phalanx of the fifth finger, b. distal phalanx of the medius finger, c. multiple enchondromatos

The lesions typically demonstrated cortical thinning without rupture, endosteal scalloping, and occasional intralesional calcifications. No periosteal reaction or soft-tissue extension was observed. These radiographic features were consistent with the benign appearance of enchondromas and supported the indication for conservative surgical curettage.

For the patient with Ollier disease, combined clinical and imaging assessments established the diagnosis of multiple enchondromatosis (Ollier disease), which was subsequently confirmed by genetic testing. Multiple surgical interventions had been performed throughout childhood and adolescence. By the age of 18, the patient's medical history included Ollier disease, a pathological spiral fracture of the distal third of the left femur with displacement (surgically treated in 2010), left genu valgum (surgically corrected in 2024), limb length discrepancy with a 9 cm shortening of the right lower leg, bilateral knee stiffness, and generalized growth delay. Given the cumulative burden of prolonged hospitalizations, repeated surgeries, skeletal deformities and asymmetries, combined with a disrupted family and social environment, the patient was diagnosed with an organic personality disorder, chronic sleep disturbances, generalized anxiety disorder, and severe depressive episodes with anxiety paroxysms. He has been under psychiatric care since the age of 16. Between the ages of 18 and 26, the patient did not seek medical follow-up regarding the progression of his condition. At the time of current admission, clinical examination revealed marked deformities involving the metacarpals and phalanges of both hands, which were asymmetrical and varied in size.

Preserved and symmetrical cutaneous sensitivity was noted. In the right hand, both active and passive opposition movements were impaired due to limited flexion of the 2nd, 4th, and 5th digits, resulting in an inability to perform a cylindrical grasp. Additionally, all previously documented skeletal deformities were clinically confirmed.

The CT scan reveals, in case of multiple enchondromatosis, multiple expansile lytic bone lesions consistent with enchondromas, some exhibiting heterogeneous structure with internal calcifications. Several lesions demonstrate cortical disruption with soft tissue extension and significant bone deformities, particularly in the hand skeleton. No periosteal reactions are noted. The lesions are more numerous in the right upper limb. In the right radius, at the distal third, multiple lytic expansile lesions with cortical breach and mild soft tissue extension are observed, with a maximum size of 12×25 mm.

In the right humerus, a central, expansile lytic lesion with calcifications is seen in the proximal metaphysis, causing cortical thinning without periosteal reaction, measuring 20×34×36 mm. A similar centro-medullary lesion with calcifications (9×13 mm) is noted in the mid-diaphysis, with mild asymmetric cortical thinning. The right scapula (partially included in the scan) shows multiple lytic lesions, particularly in the coracoid process, with the largest measuring 18 mm. The left humerus has a large, central osteolytic lesion extending from the proximal metaphysis into the diaphysis, occupying nearly half its length, with cortical thinning and focal erosions, particularly in the metaphyseal region, with a cranio-caudal diameter of 110 mm. The left scapula (partially included) contains similar lytic lesions, mainly in the coracoid

process, with the largest measuring 14 mm. Additional lesions are seen in the pisiform and triquetral bones, with significant cortical lysis in the pisiform. In the left hand, numerous lytic lesions affect the phalanges, albeit smaller than those on the right. Some exhibit cortical thinning, focal disruptions, and extra-osseous extension, with the largest at the first phalanx of the thumb measuring 16×13×17 mm. Small osteolytic lesions are also present in the carpal bones, some with cortical thinning. In the left radius, a central osteolytic lesion in the proximal metaphysis shows mild expansion and cortical thinning, measuring 13 mm (fig. 3).

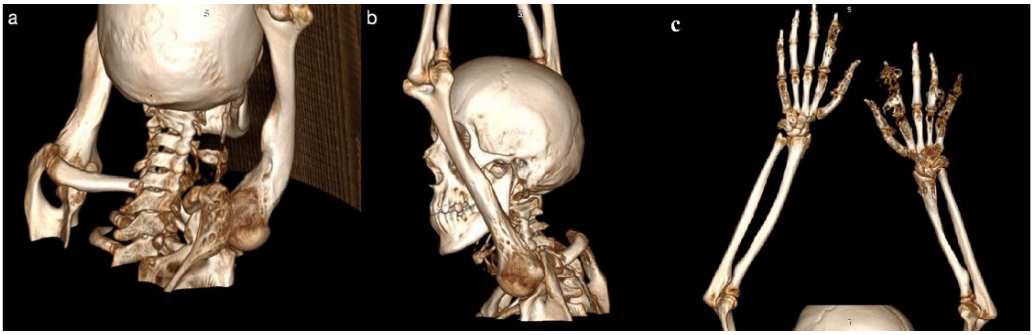

Fig. 3. CT imaging. a, b. Enchondromas located in bilateral humerus and the left scapula.

In the right hand, extensive lytic lesions affect the metacarpal bones and phalanges, sparing only the third digit. Most lesions show cortical thinning or focal disruptions, with a particularly large, expansile lesion in the second digit (notably involving the proximal and middle phalanges), measuring 48×50×39 mm, with gross cortical destruction and extra-osseous extension, c. Enchondromas involving the metacarpals and phalanges of both hands, as well as the distal end of the left radius

Additional findings include an expansile lytic lesion with cortical irregularities and interruptions in the vomer bone, associated with nasal septum deviation to the left (fig. 4).

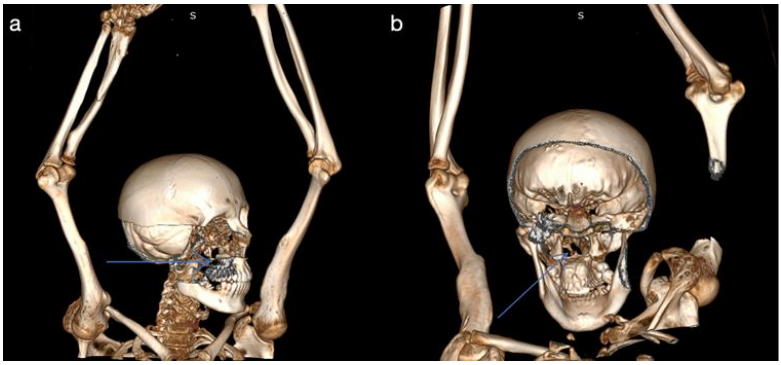

Fig. 4. CT appearance of an enchondroma located in the vomer bone-destruction of the vomer bone: a. lateral view, b. posterior view.

A C2-C3 vertebral block is present, and, despite significant artifacts, cystic areas are noted in the posterior fossa (19×23 mm), suggestive of arachnoid cysts or a mega-cisterna magna. The right thyroid lobe is heterogeneous, containing a hypodense nodule of 9×8 mm, and a similar posterior left thyroid nodule of 9×8 mm is also noted.

Based on the patient's history, clinical findings, and imaging results, surgical intervention was deemed necessary at the level of the second digit of the right hand, which was causing significant functional impairment. This limitation represented the main reason the patient sought medical attention (tab. II).

TABLE II.
Operative Details

Parameter	Findings
Anesthesia	8 loco-regional (infraclavicular block), 6 WALANT (1: 100,000 dilution)
Type of surgical procedure	Intralesional curettage ± cavity filling in 13 cases; amputation in 1 case (Ollier)
Osteosynthesis material	Used in 13/14 cases (92.9%)
Bone grafting	Autologous bone graft in 3 cases (21.4%)
Bone substitute	Solid bone substitute in 10 cases (71.4%)
Fixation stability	Achieved in all reconstructed cases
Fixation stability	Achieved in all reconstructed cases
Amputation (necessity)	1 case - Ollier disease, large tumor (6.5×5×5.8 cm)

Loco-regional anesthesia (infraclavicular block) was used in eight patients (57.1%), while the WALANT technique (1: 100,000 dilution) was used in six (42.9%). Surgical management consisted of intralesional curettage with or without cavity filling in 13 cases (92.9%), and amputation was required in one case due to massive bone destruction secondary to multiple enchondromatosis.

For reconstruction, autologous bone grafts were used in three patients (21.4%), whereas solid bone substitutes were applied in ten (71.4%). Osteosynthesis material was employed in 13 of 14 cases (92.9%) to ensure structural stability (figs. 5, 6).

In the patient with multiple enchondromatosis, amputation was required due to the considerable tumor dimensions (6.5×5×5.8 cm) and the presence of extensive osseous destruction.

All curetted specimens were submitted for histopathological analysis. In patients with solitary enchondromas, no signs of malignant transformation were identified (0/13) (tab. III).

In contrast, histopathological evaluation of the giant tumor in the patient with multiple enchondromatosis revealed a mesenchymal tumor proliferation involving the phalangeal structures of the second digit of the right hand, characterized by a multilobular growth pattern, consistent with a secondary atypical cartilaginous tumor (ACT). The lesion was composed of an abundant cartilaginous matrix, forming multiple lobules of irregular size and shape, separated by fibrous septa with congested vasculature. Areas of both low and high cellularity were observed. The chondrocytes showed minimal to moderate cytonuclear pleomorphism, with well-preserved eosinophilic cytoplasm and irregular, unequal, hyperchromatic nuclei. The nuclei displayed variable morphology, ranging from lymphocyte-like to stellate and fusiform shapes.

Occasional typical mitotic figures were identified. Focally, regions with marked pleo-morphism and increased density of atypical tumor cells were also noted (fig. 9).

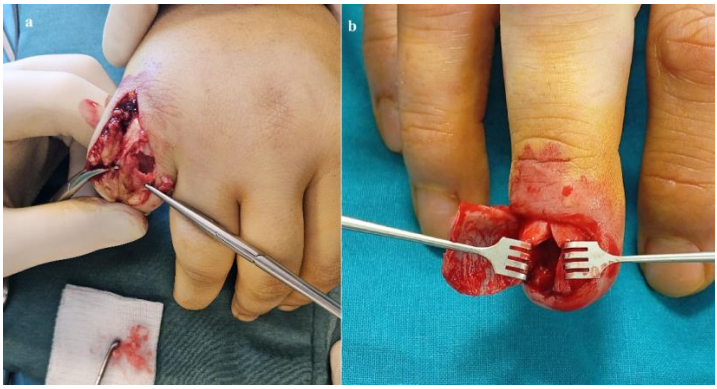

Fig. 5. Some cases of solitary enchondroma - intraoperative view:
a. first phalanx of the fifth finer, b. distal phalanx of the middle finger

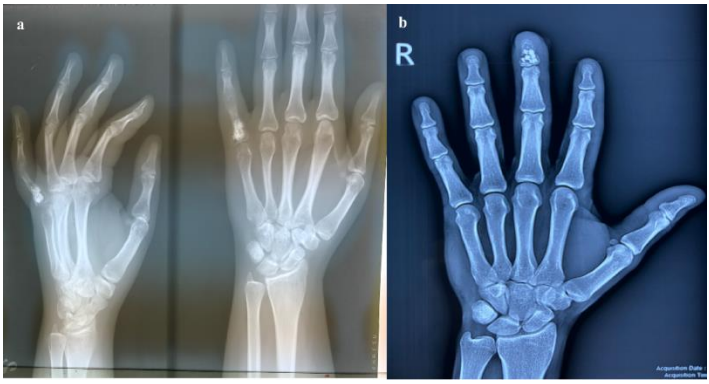

Fig. 6. Postoperative radiological aspect - bone reconstruction with solid bone substitute:
a. First phalanx of the fifth finger, b. Distal phalanx of the middle finger

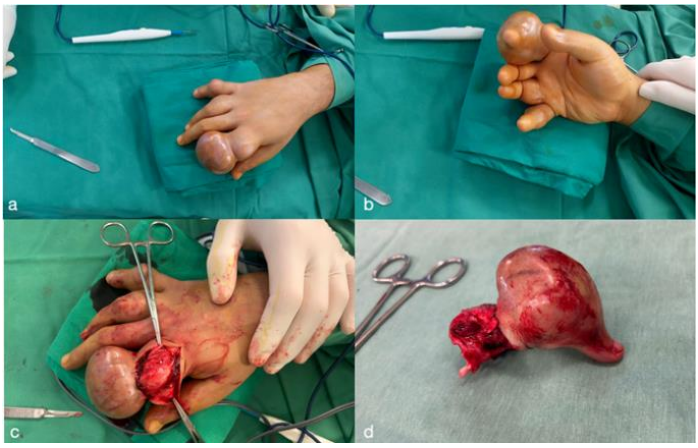

Fig. 7. Preoperative dorsal view: a - dorsal aspect, b.- palmar aspect,
c. intraoperative view, d. amputated segment (the entire right index finger)

TABLE III.
Imaging and Histopathological Findings

Variable	Findings
Imaging findings	Lytic lesions in all 14 cases (100%)
Solitary lesions	No malignant transformation identified (13/13)
Multiple enchondromatosis (Ollier)	Mesenchymal tumor proliferation with multilobular growth pattern, consistent with secondary ACT

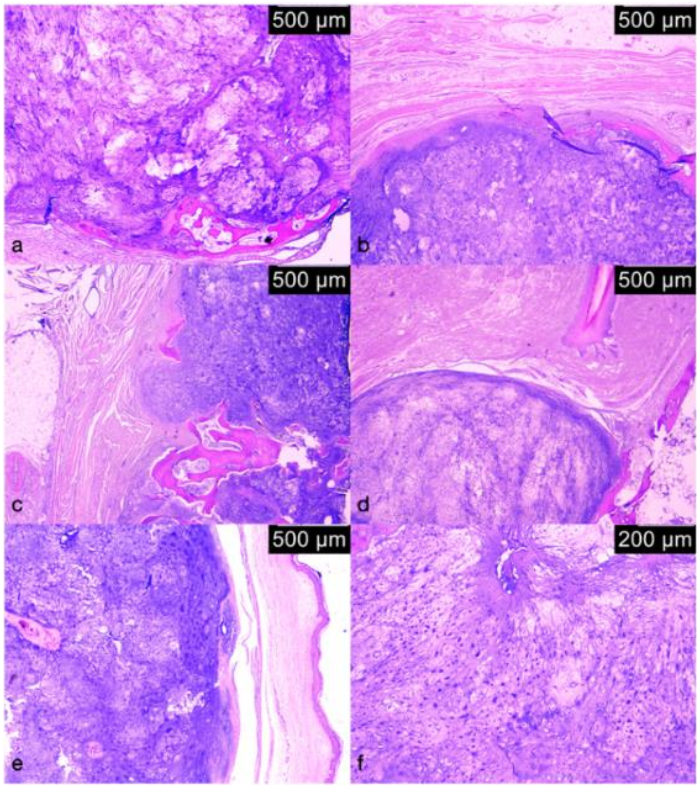

Fig. 8. a. ACT-cortical bone destruction with invasion into adjacent connective tissue (×2.5); b. ACT- cortical bone destruction with invasion into an adjacent ligament (×2.5); c. ACT- cortical destruction with soft tissue invasion (×2.5); d. ACT- invasive nodule in subcutaneous tissue (×2.5); e. ACT-evidence of invasion at the lobular front (×2.5); f. ACT-chondrocytes with moderate cytonuclear atypia (×5).

All patients demonstrated favorable postoperative evolution, with no local recurrence or infection. At three months, complete wound healing and stable osteosynthesis were noted in all cases.

By six months, radiographic evaluation confirmed structural integrity and bone consolidation, with preserved joint mobility and satisfactory aesthetic appearance (tab. IV).

The mean DASH score at final follow-up was 8.4 ± 3.2, indicating minimal disa-

bility. The range of motion (ROM) recovery ranged between 80-100% compared to the contralateral side, and the mean VAS pain score at six months was 1.2/10. Patient satisfaction, both functional and aesthetic, was uniformly high. In the patient diagnosed with Ollier disease, psychiatric therapy was maintained throughout hospitalization under the supervision of the consulting specialist. The patient was discharged with a recommendation for reevaluation after three months; however, follow-up was not achieved due to financial constraints.

TABLE IV.
Outcomes and Follow-up

Outcome parameter	Findings
Follow-up duration	2 years
3-month outcomes	Favorable in all cases; wound healing complete; no infection or recurrence
6-month outcomes	Stable bone structure, no recurrence; good joint mobility
DASH score (mean ± SD)	8.4 ± 3.2 (indicative of minimal disability)
ROM recovery	80-100% compared to contralateral side
VAS pain score at 6 months	Mean 1.2/10
Patient satisfaction (subjective)	High in all cases (no aesthetic complaints)
Psychiatric follow-up (Ollier case)	Maintained during hospitalization; follow-up lost due to financial constraints

DISCUSSION

Solitary enchondroma represents the most common benign cartilaginous tumor of the hand, typically arising from residual chondrocytes of the physeal plate. Although histologically benign, its clinical behavior and radiological appearance may mimic low-grade chondrosarcoma, particularly in cases with cortical expansion or endosteal scalloping. The true incidence of enchondroma is likely underestimated, as many lesions remain asymptomatic and are discovered incidentally during imaging performed for unrelated reasons. In the present series, 13 of 14 patients (92.9%) were diagnosed with solitary enchondroma, most frequently involving the phalanges—an observation consistent with previous reports indicating a predilection for the small tubular bones of the hand (1-3). The phalanges, particularly the proximal and middle segments of the ulnar digits, are known sites of increased enchondral ossification activity, which may explain the predominance of these locations.

Clinically, most patients presented with slowly progressive, painless swelling or localized deformity, in agreement with findings from Kuur and associates (4), who emphasized the insidious nature of the lesion. In this cohort, pain was not a presenting symptom in any case, and functional limitation was minimal prior to surgical intervention. In five cases, however, patients presented to the emergency department with pathological fractures following minor trauma—a typical manifestation of enchondroma-related bone fragility. Radiographically, all lesions appeared as well-defined lytic areas with cortical preservation and variable intralesional calcifications, consistent with the classical imaging features described in the literature (5). Advanced imaging such as CT or MRI is

generally reserved for cases where the extent of cortical involvement or suspicion of malignancy must be clarified. Surgical curettage remains the standard of care for solitary enchondromas of the hand, as it effectively removes the lesion while preserving the structural and functional integrity of the digit. In the present study, all patients underwent intralesional curettage, followed by reconstruction of the residual bone cavity using either autologous cancellous bone graft or a solid bone substitute. Autologous bone grafts were preferred in younger patients with smaller defects, while substitutes were used in larger lesions to minimize donor-site morbidity. These findings align with the observations of Gaulke et al. (6), who reported comparable consolidation times and recurrence rates between autologous grafts and synthetic substitutes. Osteosynthesis was required in nearly all cases to restore mechanical stability after curettage. No postoperative complications or recurrences were recorded during the two-year follow-up period, confirming the efficacy of this surgical approach. The functional outcomes, as measured by DASH and ROM scores, were excellent across the cohort, with a mean DASH score of 8.4 and pain scores approaching zero at six months.

Overall, the outcomes reported in this series reinforce the current understanding that solitary enchondromas of the hand, when managed by meticulous curettage and appropriate reconstruction, have a benign course, excellent functional recovery, and negligible risk of recurrence or malignant transformation. An association between Ollier disease and mutations in the parathyroid hormone receptor 1 (PTHR1) gene, was proposed, particularly the R150C variant, which alters signaling pathways regulating chondrocyte proliferation. Subsequently, in 2011, Amary et al. identified somatic mosaic mutations in the IDH1 and IDH2 genes—encoding isocitrate dehydrogenase enzymes - as central to the disease mechanism (5, 14). These mutations result in aberrant production of D-2-hydroxyglutarate, an oncometabolite that interferes with normal epigenetic regulation, leading to disorganized endochondral ossification, abnormal chondrocyte differentiation, and increased tumorigenic potential (5, 15). Clinically, Ollier disease presents with pain, swelling, and visible deformities, most frequently affecting the hands and lower limbs, often accompanied by limb-length discrepancies resulting from asymmetric skeletal growth. The condition typically progresses slowly; however, the cumulative effects of multiple enchondromas may lead to severe functional impairment, skeletal deformity, and aesthetic disfigurement, profoundly impacting daily activity and psychological well-being (16, 17). Pathological fractures are common, particularly in long bones weakened by extensive enchondromatous replacement (18). The bilateral distribution of lesions in the hands, as seen in this case, is particularly unusual, since Ollier disease typically exhibits unilateral or asymmetrical involvement. Bilateral disease likely reflects a broader embryonic distribution of the somatic mutation, suggesting that the genetic alteration occurred earlier during embryogenesis, giving rise to a wider population of affected mesenchymal progenitor cells. This mechanism may explain the multifocal and systemic pattern observed in this patient, involving not only the hands but also the radius, humerus, scapula, and notably the vomer bone (19, 20). In the present case, the

enchondromatous masses were large, producing extensive bone destruction, with CT imaging revealing near-complete resorption of the phalanges. The disease course is chronic and unpredictable, marked by alternating periods of relative stability and episodes of accelerated lesion growth that may herald incipient malignant transformation. In the reported case, histopathological examination of the amputated segment revealed malignant changes within the enchondroma, consistent with transformation into an atypical cartilaginous tumor (ACT).

The craniofacial localization of an enchondroma in the vomer bone represents an exceptional and clinically significant finding. Enchondromatous lesions in cranial bones are exceedingly rare, with only a handful of cases reported in the literature. Their presence challenges the conventional understanding of the anatomical and embryological boundaries of endochondral ossification, suggesting a wider reach of the somatic mosaicism underlying the disease. The involvement of a flat skull-base bone, such as the vomer, supports the hypothesis that Ollier disease can manifest in nontraditional skeletal regions derived from endochondral ossification centers, including the sphenoid, occipital, and vomer bones. Craniofacial involvement may further complicate the clinical course by producing neurological or otorhinolaryngological manifestations, such as nasal obstruction, sinonasal expansion, or, in rare cases, intracranial extension. In this patient, however, the vomer lesion was entirely asymptomatic, discovered incidentally on CT imaging without associated nasal or sinus manifestations. This rare presentation broadens the current understanding of Ollier disease, reinforcing its multisystemic and mosaic nature and emphasizing the importance of comprehensive imaging evaluation in detecting atypical skeletal localizations that might otherwise remain clinically silent (21, 22).

The surgical management of Ollier disease remains challenging and somewhat controversial. While curettage and bone grafting may be effective for localized lesions, extensive or multifocal disease significantly limits reconstructive potential. Amputation, although radical, may be justified in cases of massive deformity, functional loss, or histologically confirmed malignant transformation. In the present case, amputation of the affected digit provided rapid functional improvement, with no postoperative complications, underscoring the importance of individualized, function-oriented management rather than strict anatomical preservation (23).

Beyond orthopedic considerations, Ollier disease exerts a profound psychological impact. The combination of visible deformities, chronic pain, and recurrent hospitalizations leads to marked emotional distress, particularly in patients diagnosed during childhood (24, 25). The current case illustrates the cumulative psychosocial burden associated with the disease: early-life trauma, together with persistent physical disfigurement and repeated medical interventions, contributed to the development of chronic depression, anxiety, and organic personality disorder. Similar reports in the literature describe the emotional strain of chronic deformity and disability, but few documents psychiatric morbidity of this intensity (26-28).

The literature describes cases of multifocal disease with cranial extension, yet none report such severe psychiatric comorbidity, highlighting the unique multidimen-

sional complexity of the present case—where skeletal deformity, oncologic risk, and psychological decline intersect in a single, rare clinical scenario.

CONCLUSIONS

Ollier disease imposes a profound functional and psychosocial burden on affected individuals. Progressive skeletal deformities of the hands result in substantial loss of dexterity, compromised grasping ability, and a marked decline in overall quality of life. However, the psychological consequences of the disorder often surpass its physical manifestations. Chronic disfigurement, prolonged hospitalization, and persistent social stigmatization contribute to severe emotional distress, manifesting as anxiety, depressive episodes, and long-term psychological exhaustion.

These findings underscore the necessity of a multidisciplinary approach that integrates surgical management with continuous psychological and social support, aimed at optimizing both functional outcomes and mental well-being.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests. No financial support was received for perfecting this article.

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PERINATAL PROFILE OF INFANTS DIAGNOSED WITH CONGENITAL HEART DISEASE: A HOSPITAL-BASED COHORT STUDY

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PERINATAL PROFILE OF INFANTS DIAGNOSED WITH CONGENITAL HEART DISEASE: A HOSPITAL-BASED COHORT STUDY (Abstract): Congenital heart disease (CHD) is the most common congenital anomaly worldwide and remains associated with significant neonatal morbidity. Perinatal factors such as prematurity and low birth weight may influence immediate clinical outcomes. This study **aimed** to describe the perinatal characteristics of children diagnosed with CHD in a level I regional hospital. **Materials and methods:** This retrospective descriptive study included children with confirmed CHD diagnosed between 2017-2023. CHD cases were classified into left-to-right shunt lesions, right-to-left shunt lesions, obstructive malformations, and complex CHD. Extracted perinatal variables included sex, gestational age, prematurity categories, birth weight, Apgar scores at 1 and 5 minutes, and mode of delivery. Data are presented as mean ± SD or number (percentage). **Results:** A total of 138 children were included, 50.72% of whom were male. The mean gestational age was 36.37±2.75weeks, with 49 (35.51%) born preterm. Mean birth weight was 2692.55 ± 865.11 g, and low birth weight was observed in 50 (36.23%). Cesarean delivery occurred in 80 (60.10%) of cases. The highest proportions of CHD subtype were observed for complex lesions (28.99%) and right-to-left shunt defects (30.43%). **Conclusions:** This cohort demonstrates a notable burden of prematurity and reduced birth weight in the context of a high proportion of hemodynamically significant CHD. These findings underscore the importance of coordinated perinatal care and highlight the value of regional epidemiological data for informed healthcare planning. **Keywords:** PERINATAL, CONGENITAL HEART DISEASE, COHORT STUDY.

INTRODUCTION

Congenital heart disease (CHD) is among the most common congenital anomalies, affecting approximately 0.8% to 1.2% of live births worldwide (1, 2). In 2021, over 4.18 million children under five years were living with CHD globally, representing a 3.4% increase since 1990 (3).

Despite significant advances in cardiovascular care and surgery have significantly improved survival, CHD remains the leading cause of mortality among congenital defects and continues to impose a substantial global health burden (1, 4). Early diagnosis and appropriate management are essential, as untreated severe CHD may lead to heart failure, growth impairment, and early death (5, 6), while long-term complications such as arrhythmias or heart failure often require ongoing follow-up (7).

The etiology of CHD is multifactorial, involving genetic and environmental determinants (8, 9). Beyond structural cardiac abnormalities, increasing attention has been directed toward the perinatal profile of affected infants. Previous studies have suggested associations between CHD and adverse perinatal characteristics, including prematurity and reduced birth weight, potentially reflecting altered intrauterine development or shared pathophysiological mechanisms (10, 11).

Most data derive from large international registries, whereas regional evidence remains limited, particularly in Eastern Europe, where socio-economic and parental profiles may differ. Regional analyses are important, as healthcare organization, prenatal screening practices, and obstetric management may influence perinatal patterns.

The **aim** of this study was to describe the perinatal characteristics of children diagnosed with CHD in a level I regional hospital and to examine the distribution of major CHD subtypes within this population.

MATERIALS AND METHODS

This retrospective descriptive study was conducted in the neonatal unit of a level I regional hospital and included all children diagnosed with CHD during the study period 2017-2023. The diagnosis of CHD was confirmed by transthoracic echocardiography performed by the same doctor. CHD cases were classified into four major subtypes: left-to-right shunt lesions, right-to-left shunt lesions, obstructive malformations, and complex CHD. Left-to-right shunt lesions included defects such as ventricular septal defect, atrial septal defect, and patent ductus arteriosus. Right-to-left shunt lesions included cyanotic defects associated with reduced pulmonary oxygenation, such as tetralogy of Fallot and transposition physiology. Obstructive lesions included aortic or pulmonary outflow tract obstruction, while complex CHD comprised multiple structural abnormalities with major hemodynamic impact. Perinatal variables extracted from medical records included sex, gestational age at birth, prematurity status, prematurity categories, birth weight, Apgar score at 1 and 5 minutes, and mode of delivery. Low birth weight was defined as <2500 g, and prematurity categories were defined according to standard obstetric criteria. Data were analyzed using descriptive statistics, with continuous variables presented as mean ± standard deviation (SD) and categorical variables as number and percentage. The study was approved by both the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania (approval number: 640/23.09.2025) and Cuza-Vodă Clinical Hospital of Obstetrics and Gynecology, Iași, Romania (approval number: 5/10.02.2025) before the beginning of the study.

RESULTS

Perinatal characteristics. A total of 138 neonates with CHD were included in

the study. Among them, 68 (49.28 %) were female and 70 (50.72%) were male. Regarding the place of residence, 43 (31.61%) of patients originated from urban areas and 95 (68.84%) from rural areas. The mean gestational age at birth was 36.93±2.59 weeks. Mean birth weight was 2692.55 ± 865.11 g, with a low birth weight (<2500 g) was observed in 50 (36.23%) of patients. The mean Apgar score was 7.05±1.63 (1-9) at one minute, increasing to 7.70±1.40 (0-9) at 5 minutes (tab. I).

TABLE I.
Perinatal characteristics

Perinatal characteristics	Female (n=68)	Male (n=70)	Total (n=138)
Environment			
Rural	48 (70.59%)	47 (67.14%)	95 (68.84%)
Urban	20 (29.41%)	23 (32.86%)	43 (31.16%)
Gestational age (w)	36.37±2.75	37.47±2.31	36.93±2.59
Birth weight (g)	2472.67 ± 855.42	2909.14 ± 825.54	2692.55 ± 865.11
Apgar score			
1 minute	6.76±1.77	7.33±1.41	7.05±1.63 (1-9)
5 minutes	7.44±1.59	7.96±1.11	7.70±1.40 (0-9)

n-number; w - weeks, g - grams

Overall, 89 (64.49%) were born at term, while n=49 (35.51%) were born prematurely. Sex distribution across prematurity categories is detailed in second table.

TABLE III.
Prematurity categories

Prematurity	Female: n=29 (40.8%)	Male n=20 (59.18%)	Total (n=49)
Extremely preterm (<28w)	1 (3.45%)	0 (0%)	1 (2.04%)
Very preterm (28-32 w)	5 (17.24%)	2 (10.00%)	7 (14.29%)
Moderately preterm (32-34w)	2 (6.90%)	2 (10.00%)	4 (8.16%)
Late preterm (34-37w)	21 (72.41%)	16 (80.00%)	37 (75.51%)

n- number, w- weeks

Delivery occurred by cesarean section in 60.10% of cases (n=80), while 39.9% (n=55) were delivered vaginally. Prenatal diagnosis of CHD was established in 20.29% of cases, at a mean gestational age of 24.1 weeks. Most cases were diagnosed postnatally, highlighting the limited prenatal detection rate in the studied population.

CHD subtype distribution. Overall, CHD cases were classified as left-to-right shunt lesions, right-to-left shunt lesions, obstructive lesions, or complex CHD (tab. III).

TABLE IIIII.
Congenital heart disease subtype

CHD subtype	Total
Left-to-right shunt	25 (18.12%)
Right-to-left shunt	41 (30.43%)
Obstructive	32 (23.19%)
Complex CHD	40 (28.99%)

CHD - congenital heart disease

DISCUSSION

The predominance of complex CHD and right-to-left shunt lesions in our cohort suggests a relatively high burden of hemodynamically significant cardiac pathology. These defects are commonly associated with impaired systemic oxygen delivery, respiratory instability, and increased need for specialized neonatal care. In contrast, left-to-right shunt lesions are often better tolerated during the immediate neonatal transition, depending on defect size and pulmonary vascular adaptation.

This study provides a description of perinatal characteristics among children diagnosed with CHD in a level I regional hospital. Given that CHD affects approximately 0.8-1.2% of live births worldwide (12) and remains a leading contributor to congenital morbidity despite advances in care (3), understanding early-life parameters in affected neonates remains clinically meaningful.

Complex congenital heart defects accounted for 28.99%, of cases in our cohort, while right-to-left shunt lesions represented 30.43%, together comprising a substantial proportion of hemodynamically significant anomalies. Such defects are frequently associated with altered systemic oxygen delivery and increased risk of circulatory instability in the early postnatal period.

This subtype distribution provides a physiological context for understanding the modestly reduced mean Apgar scores observed at 1 and 5 minutes, which were slightly below values reported in the general neonatal population according to World Health Organization data (WHO) (13).

Although Apgar score reflects overall neonatal adaptation, rather than isolated cardiac function, transitional challenges are particularly relevant in infants with CHD. The immediate postnatal period requires rapid physiological changes, including functional closure of the ductus arteriosus and *foramen ovale*, a marked decrease in pulmonary vascular resistance, and establishment of effective pulmonary blood flow (14, 15). In neonates with structural cardiac defects, particularly those involving altered shunt physiology or compromised systemic or pulmonary perfusion, these transitional processes may be less efficient. In preterm infants, delayed ductal closure may further aggravate abnormal shunt dynamics, especially when respiratory support such as surfactant therapy or inhaled nitric oxide is required, thereby contributing to transitional hemodynamic instability (10, 16, 17). Impaired systemic oxygen delivery, transient hypoxemia, or reduced cardiac output during this critical transitional period may partially explain the modestly lower early adaptation scores observed in our cohort

(10). Although the descriptive design of this study does not allow for causal conclusions to be drawn, the slightly reduced mean Apgar values may nonetheless reflect a degree of hemodynamic vulnerability during the immediate neonatal transition in infants with CHD.

Prematurity was observed in 35.51% of the cohort, a proportion considerably higher than rates typically reported in the general neonatal population (18)**.** The association between CHD and preterm birth has been previously described and is thought to be multifactorial, involving placental dysfunction, altered fetal hemodynamics, and shared developmental pathways influencing both cardiac morphogenesis and gestational duration (10, 11). In our cohort, the substantial proportion of complex (30.43%) and right-to-left shunt lesions (28.99%) suggests a predominance of hemodynamically significant defects and even early deliver. Prematurity may, in turn, exacerbate postnatal adaptation challenges, as immature myocardial function, delayed ductal closure, and persistent pulmonary vascular resistance can amplify shunt imbalance and transitional instability (14, 16).

Birth weight patterns also align with this vulnerability profile. Infants with CHD are frequently reported to have lower birth weight compared to the general population (11,19). However, while structural cardiac anomalies can influence fetal hemodynamics, potentially influencing intrauterine growth, the relatively high rate of prematurity in our cohort, may have also contributed to the lower mean birth weight.

The cesarean delivery rate observed in our cohort should be interpreted within the broader context of contemporary obstetric practice. Over recent decades, cesarean section rates have increased globally, in many settings exceeding the levels considered optimal by the WHO(13). Pregnancies complicated by suspected or confirmed fetal anomalies, including CHD, are more frequently managed through planned cesarean delivery to allow controlled timing of birth and immediate access to specialized neonatal and cardiology care (14). The relatively low prenatal detection rate observed in our cohort may partially explain the elevated proportion of urgent neonatal evaluations and the variability in delivery practices. Limited prenatal screening accessibility, particularly in rural areas, may contribute to delayed diagnosis and reduced opportunities for planned multidisciplinary perinatal management. While cesarean delivery may facilitate coordinated perinatal management in selected high-risk cases, its increased prevalence also reflects broader trends in modern obstetric practice. As such, the elevated cesarean rate in this cohort likely represents a combination of clinical indication and contemporary delivery patterns.

Beyond their direct clinical implications, these findings also carry important health system relevance. The growing lifetime prevalence of CHD has substantial implications for healthcare planning and long-term resource allocation (20). International scientific statements emphasize the importance of structured, regionally coordinated care pathways to reduce morbidity and improve outcomes (21). However, most epidemiological data derive from large registries(2,6), and regional variability in perinatal patterns is often insufficiently characterized.

Regional cohort-based analyses, such as the present study, provide essential context-specific evidence. Differences in prematurity distribution, birth weight pro-

files, and delivery practices may reflect variations in obstetric management, prenatal screening accessibility, or healthcare infrastructure. Integrating such localized data into larger national frameworks can support differentiated health policy development, including targeted maternal-fetal surveillance programs, optimized neonatal intensive care resource allocation, and regionally adapted referral networks. In this regard, regional epidemiological studies represent a critical component of evidence-based CHD planning.

Strengths and limitations. The strengths of this study include the evaluation of perinatal parameters in a clearly defined cohort and the classification of CHD subtypes. The high proportion of complex and right-to-left shunt lesions provides an important clinical context for interpreting the observed patterns of perinatal vulnerability, including prematurity and reduced birth weight.

However, the retrospective design limits causal interpretation and does not allow differentiation between the independent effects of prematurity and CHD-related intrauterine growth alterations on birth weight. The absence of a control group limits direct comparison with background population rates.

CONCLUSIONS

This study outlines the perinatal profile of children with CHD in a level I regional hospital, highlighting a notable burden of prematurity and reduced birth weight in the context of a high proportion of hemodynamically significant lesions. The coexistence of complex and right-to-left defects with early-life vulnerability parameters suggests increased cardiovascular demands during the immediate neonatal transition. While causal relationships cannot be established, these findings underscore the importance of coordinated obstetric and neonatal management in pregnancies complicated by CHD.

Beyond their clinical implications, regional cohort-based data contribute valuable insight for healthcare planning. As the population living with CHD continues to expand, locally generated epidemiological evidence may support differentiated resource allocation and region-specific perinatal care strategies within broader national health frameworks.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding regarding this scientific research.

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SOCIO-DEMOGRAPHIC CHARACTERISTICS AND REPORTED PSYCHO-MEDICAL SYMPTOMS FOR CHILDREN WITH RARE DISEASES. A COMPARATIVE STUDY BETWEEN PATIENTS WITH PHENYLKETONURIA, CYSTIC FIBROSIS AND HYPOTHYROIDISM

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SOCIO-DEMOGRAPHIC CHARACTERISTICS AND REPORTED PSYCHO-MEDICAL SYMPTOMS FOR CHILDREN WITH RARE DISEASES. A COMPARATIVE STUDY BETWEEN PATIENTS WITH PHENYLKETONURIA, CYSTIC FIBROSIS AND HYPOTHYROIDISM (Abstract). Rare diseases have great impact on both patients and family members' life. **The aim** of the study was to identify socio-demographic characteristics and factors related to psychological and medical condition for pediatric patients diagnosed with phenylketonuria, cystic fibrosis and hypothyroidism. **Material and methods**: 37 children (24 boys and 13 girls) aged M = 7.61 ± 6.37 (1-22-years-old) were included in the research. A total of 44.7% are diagnosed with cystic fibrosis, 31.6% with phenylketonuria and 23.7% with hypothyroidism. Socio-demographic characteristics and psychological and medical data reported by mothers were registered to point the presence of some characteristics related to the diseases and to identify differences between the three categories of patients. Data have been processed using *SPSS Statistics v23.0.0* for MAC.OSX. **Results**: Almost 1/5 of children diagnosed with hypothyroidism and phenylketonuria were found to have depression symptoms and 1/3 of patients with hypothyroidism are having cognitive impairment associated in 40% of cases with language difficulties, and 20% of them present difficulties to adapt to social environment. A total of 18.3% of children with cystic fibrosis presented enuresis and 20% of them has bruxism. **Conclusions**: children with rare disease present several psychological symptoms and social difficulties, psychotherapeutically and educational support for both patients and families are needed. **Keywords**: CHILD, CYSTIC FIBROSIS, HYPOTHYROIDISM, PHENYL-KETONURIA.

A disease is considered rare when it affects one person out of 2,000 or less. They are between 5,000 and 8,000 rare diseases, most of them genetic. A rare disease is, according to the definition of World Health Organization (1), a life-threatening or chronically debilitating condition from which not more than 5% of citizens in the European Community suffer. It is estimated that 30 million Europeans and 25 million Americans are suffering from a rare disease that coincide with 6-8% of the total popula-

tion (2).

As WHO guidelines, there is a strong recommendation to sustain and develop research in this area to help knowledge about rare diseases and to improve quality of life of patients suffering from orphan diseases. Social support should not be neglected also, *EURORDIS Care Survey Program* conducted in 23 countries between 2004-2004 included 12,000 patients with rare diseases (3). The survey concluded that "social security systems are usually designed around common diseases are not flexible enough to take into consideration unprecedented health need" as is an orphan disease.

Some diseases are more frequent and programs are already implemented in many countries.

Phenylketonuria (PKU) is a rare metabolic disease that has consequences on cognitive development. The cognitive impairment appears if the patient is not treated with a strict protein-restricted diet. Even with early diagnosis and treatment of PKU children, these patients present some deficiencies intellectually compared to normal children (4). Following the medical recommendation regarding diet are extremely important for medical development of the disease and for the quality of life of patient. In many cases, diet and its restrictions are the most stressful factor for parents having a child diagnosed with this disease and perceived social support and diagnosis resolutions seems to improve coping strategies and quality of life for caregivers (5, 6). Some studies assessing the occurrence of psychiatric disorders identified in patients with early treatment for PKU a high rate of anxiety disorder and the tendency of withdrawal were mentioned being the most frequent self-reported symptoms. A study of Manti *et al*. (7) showed that patients with good metabolic control in the first eleven years of life proved higher frequency of psychiatric diagnosis comparing to those having more years from diagnosis, meaning that respecting diet represents a distressful situation and children or adolescents are feeling more frustrating. Psychological and psychiatric evaluation is recommended for all children under medical treatment.

Cystic fibrosis (CF) is a genetic disease of the exocrine glands that affects all races but primarily whites. For this reason, since 2001, the American College of Obstetricians and Gynecologists recommends that white women be tested for the gene if they are pregnant or considering pregnancy (8) and three years later, in 2004, the Centers for Disease Control and Prevention (CDC) published their pivotal recommendation that regions "begin newborn screening for CF" (9, 10). A patient with CF and members of the family may have tremendous physical, psychosocial, and educational needs. As the patient get older, some new problems may arise or existing problems may worsen requiring comprehensive, multidisciplinary lifelong care (11). Sometimes patients with CF could be misdiagnosed earlier in life as having asthma or chronic bronchitis.

Hypothyroidism can manifest with different signs and symptoms. In the pediatric population, the prevalence of this thyroid disorder is estimated to be less than 10% (12). This disorder has a wide range of presentation from subclinical hypothyroidism to overt form. Hearing loss is more common in hypothyroid patients than normal population so diagnosing earlier the deafness will help children to be cognitively stimulated. Dental problems and cardiac

manifestations need also to be identified to improve quality of life for these patients.

Living with these rare diseases demand a lot of effort of family members, multidisciplinary team and a strong determination to empower the patient (if possible) to care about own medical and psychological problems. Early diagnosis, adherence to treatment, family, medical and social support are important for a high level of quality of life. The care for the patients must be doubled by the care for the family members to improve life standards for all persons touched by the diseases.

In the majority of cases, mothers are the carrier of children with a rare disease. One of the most common behavior identified in mothers having a child with a rare disease is the hyper-protection, attitude with a negative impact over the years, causing a lot of reactiveness in children during their pre/adolescence period. That is why mothers or caregivers must be well trained to help, supervise, interfere and treat their children.

The aim of this study is to identify psychological and medical symptoms of pediatric patients suffering from these three rare diseases (cystic fibrosis, hypothyroidism and phenylketonuria) as that are declared by their mothers.

MATERIAL AND METHODS

A number of 37 patients aged 1-22 years old were included in the study (M = 7.61 ± 6.37). In Romania, patients older than 18 are continuing to be hospitalized in pediatric clinics till the age of 25 if they are registered in the educational system. The children were diagnosed and under medical supervision in "Saint Mary" Emergency University Hospital for Children in Iasi, Romania with one of the following three rare disease: cystic fibrosis, hypothyroidism and phenylketonuria.

The study has been approved by the Ethical Committee of "Sf. Maria" Emergency Clinical Hospital for Children and questioned mothers and children were informed about the confidentiality and use of data and results and mothers signed an informed consent before filling in the items.

Socio-demographic and family member's information (age for patient and both parents, sex, religion, department, environment and level of education for both parents) were registered. The mothers of the patients had to indicate psychological, social and physical symptoms that they have been identified to their children.

Statistical analysis was performed using *SPSS Statistics version 23.0.0* for MAC. OSX.

RESULTS AND DISCUSSION

Descriptive statistics – general results

The socio-demographic and self-reported data were gathered during their hospitalizations from 2016. For 24 boys and 13 girls hospitalized in 2016, mothers reported psychological and medical symptoms for their children and appreciated their social interactions with peers. More than half of the patients (69.4%) are aged between 2 and 6 years old, 27.8% are aged between 6 and 12 years old, and the rest (2.8%) are older than 12 years old.

The patients are coming from 6 districts of North-eastern part of the country (Bacau, Botosani, Iasi, Neamt, Suceava, Vaslui), 17 of patients (45.95%) living in urban area and 20 (54.05%) living in countryside. The distribution of patients considering their gender and disease is presented in Table I.

TABLE I.

Data referring to the distribution of patients accordingly to disease, gender and age at diagnostics

Disease	Male	Female	Age at diagnostic (M ± SDV)
Phenylketonuria	10 (41.7%)	1 (7.7%)	M = 1.12 ± 1.24 months
Hypothyroidism	3 (12.5%)	6 (46.2%)	M = 1.66 ± 0.70 months
Cystic fibrosis	11 (45.8%)	6 (46.2%)	M = 20.58 ± 34.71 months

Family data

Mothers were asked about their marital situation at the time of diagnosis and 25 of them (67.57%) declared that they were married. Eleven of them (32.35%) are having one child, 18 (52.94%) are having 2 children, 3 (8.82%) are having 3 children and 2 (5.88%) are having 4 children (the number of births/mother is M = 2 ± 0.76).

Data about the religion of the family members revealed that the majority patients are Orthodox-Christian (92.1%), and a small number is Pentecostal (5.3%) and Adventist (2.6%).

The minimum age of mothers is 18 and the maximum is 55 years old (M = 33.05 ± 8.52). For the fathers, the minimum age is 21 and the maximum is 56 years old (M = 37.87 ± 7.79).

Data regarding the educational level of parents was also registered. A total of 6.1% of the mothers have primary education background, 21.2% gymnasium level, 57.6% have reached high school level, and 15.2% graduated college. In what concern the level of education of fathers, 5.7% have primary education background, 22.9% gymnasium level, 68.6% have reached high school level, and 2.9% graduated college.

TABLE II.

The distribution of patients accordingly to their age

Age	1	2	3	4	5	7	8	11	12	13	16	19	20	21	22
N	2	5	6	4	4	1	3	1	3	1	1	2	1	1	1
%	5.6	13.5	16.2	10.8	10.8	2.7	8.1	2.7	8.1	2.7	2.7	5.4	2.7	2.7	2.7

Patients with phenylketonuria

The minimum registered age at diagnosis for this category of pediatric patients is 0 months (3 weeks) and the maximum is 4 months, with a mean of 1.12 ± 1.24 months. In the case of *phenylketonuria* none of the patients (with M =3.36 ± 3.29, minimum age of 1 year old and maximum age of 13 years old) displayed loss of appetite, bowel movement problems, muscular dystrophy, intellectual disability, bradypsychia, language difficulties, memory difficulties, unhealthy behaviors, bruxism, night terrors, bed wetting, behavioral problems, isolation (by others or themselves), panic attacks, or a high caloric diet. 40% of the patients presented nutritional problems and 40% of the patients presented sleep-related problems. Most them (80%) complied with their treatment.

Considering anger outbursts, 25% of the patients had them, and more than half of the patients (60%) were advised on a nutrition diet. 20% of the children had depression, and more than half of the patients (66.7%) followed nutritional recommendation.

Patients with hypothyroidism

The minimum registered age at diagnosis for this category of disease is at birth (more frequent is the third week of life) and the maximum is 3 months, with a mean of 1.66 ± 0.70 months. The age at the time of the study is M =3.62 ± 1.68, with a minimum age of 1 and a maximum age of 7. In the case of *hypothyroidism*, patients did not display bowel movement problems, muscular dystrophy, bradypsychia, and memory difficulties, nutrition problems, bruxism, night terrors, bed wetting, behavioral problems, panic attacks or a high caloric diet. In case of 16.7% we identified a loss of appetite, and 33.3% presented intellectual disability. Almost 40% of the patients had language problems and 20% of the patients displayed unhealthy behaviors. In case of 16.7% of the patients, mothers reported sleep problems.

In case of 20% of the patients, mothers were reported having depressive symptoms. More than half of the patients (60%) complied with their treatment and 83.3% were taking medication. 20% of the patients experienced isolation (by others or themselves) and 20% of the patients displayed anger outbursts. Half of the patients were advised on a diet. Many of the patients (80%) followed their nutritional recommendations.

Our findings are congruent to the scientific data from the literature. Findings published by Rovet and Ehrlich (13) indicated cognitive problems for children with hypothyroidism that persist into adolescence (memory, attention, or visual and spatial processing areas). For these problems, the educational support is extremely important, to supervise medical condition, intellectual achievement and behavior.

In our study, mothers identified in case of 20% that children suffering from hypothyroidism are having depression symptoms, angriness and unhealthy behaviors and isolation. Their physical aspect, the cognitive impairment, language problems and motor skills (14, 15) are factors that influence their social interaction and family members and teachers must be informed about these aspects to diminish the child's distress. Social and educational difficulties should not be underestimated, as the scientific results are proving that physical condition is usually more supervised and cared compared to psychological and social well-being (16, 17). Despite the results presented by many researches referring to the negative cognitive development in case of under-treatment, few studies have been focused on the effects of overtreatment of patients with this disease. A study of Bongers-Schokking (18) shows that overtreatment during the first two years determines lower cognitive outcomes (the study being lead on patients aged 11). The authors suggest also that fast TSH normalization at initial treatment leads to above-normal development scores at a young age but does not affect the quotient of intelligence at the age of 11 years.

Patients with cystic fibrosis (CF)

The minimum registered age at diagnosis for this category of disease is at birth (first month) and the maximum is 132 months, with a mean of 20.58 ± 34.71 months. The age at the time of the study is M =12.23±6.13; the minimum registered age is 5 years old the maximum is 22 years old.

The patients diagnosed with CF have no record of bradylalia, memory difficulties, unhealthy behaviors, night terrors, behavioral problems or panic attacks. 23.1% had

a loss of appetite, 66.7% had bowel movements problems, 30.8% had muscular dystrophy and 9.1% presented intellectual disability and bradypsychia.

Most patients suffering from CF present nutritional problems (25%), 10% had sleep problems, 20% present bruxism and 18.2% were diagnosed with enuresis. A small number of pediatric patients (9.1%) presented symptoms of depression.

Almost half of the patients (45.5%) complied with their treatment and large majority of patients (91.7%) took medication.

In case of 7.7% of patients' mothers declared that children experienced isolation and 8.3% of the patients displayed anger outbursts. A percentage of 41.7% from the patients were on a high caloric diet. More than half of the patients (69.2%) were advised on a nutrition diet, and all the patients followed their nutritional recommendations.

Early medical intervention is essential for these patients so neonatal screening is demanded. Understanding the ethical issues in CF is an important factor for providing optimal care of newborn with CF (19). Our results prove that children with CF have an older age at the moment of their diagnostic comparing to the pediatric patients suffering from the other two diseases.

Comparative statistics

Comparative results for the three diseases are presented in Table III. The higher number of symptoms identified by mother are those having children diagnosed with cystic fibrosis.

TABLE III.

Comparative results for variables in phenylketonuria, hypothyroidism and cystic fibrosis

Variables		Phenylketonuria	Hypothyroidism	Cystic fibrosis
Loss of appetite	Yes	0%	**16.7%**	**23.1%**
	No	100%	83.3%	76.9%
Bowel movement problems	Yes	0%	0%	**66.7%**
	No	100%	100%	33.3%
Muscular dystrophy	Yes	0%	0%	**30.8%**
	No	100%	100%	69.2%
Intellectual disability	Yes	0%	**33.3%**	**9.1%**
	No	100%	66.7%	90.9%
Bradipsychia	Yes	0%	0%	**9.1%**
	No	100%	100%	90.9%
Bradylalia	Yes	0%	**40%**	**0%**
	No	100%	60%	100%
Memory difficulties	Yes	0%	0%	0%
	No	100%	100%	100%
Fertility problems	Yes	0%%	0%	**28.6%**
	No	100%	100%	71.4%
Unhealthy behaviors	Yes	0%	**20%**	0%
	No	100%	80%	100%
Nutrition problems	Yes	**40%**	0%	**25%**
	No	60%	100%	75%

Variables		Phenylketonuria	Hypothyroidism	Cystic fibrosis
Sleep problems	Yes	**40%**	**16.7%**	**10%**
	No	60%	83.3%	90%
Bruxism	Yes	0%	0%	**20%**
	No	100%	100%	80%
Night terrors	Yes	0%	0%	0%
	No	100%	100%	100%
Bed wetting	Yes	0%	0%	**18.2%**
	No	100%	100%	81.8%
Behavioral problems	Yes	0%	0%	0%
	No	100%	100%	100%
Depression	Yes	**20%**	**20%**	**9.1%**
	No	80%	80%	90.1%
Compliance with treatment	Yes	**80%**	**60%**	**45.5%**
	No	20%	40%	54.5%
Medication	Yes	0%	**83.3%**	**91.7%**
	No	100%	16.7%	8.3%
Isolation	Yes	0%	**20%**	**7.7%**
	No	100%	80%	92.3%
Panic attacks	Yes	0%	0%	0%
	No	100%	100%	100%
Anger outbursts	Yes	**25%**	**20**	**8.3**
	No	75%	80%	91.7%
High caloric diet	Yes	0%	0%	**41.7%**
	No	100%	100%	58.3%
Diet	Yes	**60%**	**50%**	**69.2%**
	No	40%	50%	30.8%
Following nutritional recommendations	Yes	**66.7%**	**80%**	**100%**
	No	33.3%	20%	0%

It is important to notice that, regarding the way the patients follow the nutritional recommendations, the rate of affirmative responses is very high, for all three of diseases. This result proves that families understand very well importance of the nutritional diet and respect the medical recommendation to negative effects on child's development. For example, prevention or early treatment of iron deficiency may improve cognitive development and behavior of children with PKU (20). It is important to register from the beginning of the diagnostic family rules for food and nutrition and to identify restriction imposed, for example, by the religious believes. Multidisciplinary counsel is imposed for these medical cases.

Our results reveal psychological symptoms and important factors related to patients suffering from the considered diseases. We found that important rate of pediatric patients questioned are presenting nutritional problems and sleep disturbance (higher rate in phenylketonuria), cognitive impairment (higher rate for hypothyroidism), and enuresis and bowel syndrome (higher rate for cystic fibrosis). Almost 1/5 of children diagnosed with hypothyroidism and phenylketonuria were found to have

depression symptoms, as their mothers declared. The physical and psychological problems seem to be frequent and care for these patients must be done in a multidisciplinary team, as soon as possible after the diagnostic. Personalized educational support plan is required for children having cognitive delay such those with congenital hypothyroidism and hearing tests, memory deficit, speech and visual development must be targeted from the beginning (21).

Study limits. This study has some limitations. The first one is due to the small number of children and comparative study must be treated with precautiousness. The second one is referring to children's age. Some items targeting data, like fertility problems are not available for newborn baby or before the age of puberty. So, comparative results could not be generalized.

CONCLUSIONS

Children with a rare disease present several psychological symptoms and social difficulties that can been identified by the family members, especially mothers who are the most frequent the responsible and dependable caregiver.

The educational support of pediatric patient and psychotherapy intervention for both patients and families are needed as soon as possible after the medical diagnostic. Psychological support must not be neglected or not prioritized due to the medical outcomes.

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NEWS

NANOMEDICINES IN CANCER THERAPY – MICELLAR CYTOCHROME P450 INHIBITORS

Nanomedicines are mainly used as drug delivery systems. Recently was evaluated a new therapeutic possibility of inhibiting a drug's metabolism thereby enhancing its effective dose. Most of the phase I drug metabolism is carried out by the hepatic cytochrome P450 (CYP450) enzymes, which due to genetic polymorphisms leads to high inter-individual variability. Micelles containing the natural furanocoumarin 6′,7′-dihydroxybergamottin (DHB), a known CYP450 inhibitor, were developed and studied for the influence on metabolism and bioavailability of the oncology drug docetaxel. It was observed that the administration of DHB-micelles in mice at 24 h prior to the drug enhanced antitumor efficacy in the tumor xenograft models HT-29 and MDA-MB-231, when compared to the drug alone. The researchers encapsulated in nanocarrier natural compounds, known for their metabolism-blocking capacity and absence of reported toxicity, with intravenous administration for the inhibition of the hepatic metabolism of drugs to enhance the useful dose and/or reduce the ineffective proportion of the dose often responsible for the toxicity of the injected drug. These findings are encouraging in terms of increased efficacy and prolonged overall survival in xenografted murine models with good tolerance to treatment. The preclinical benefit–risk ratio of introducing such a metabolism-blocking agent in association with drugs with a narrow therapeutic window or relying too much on the CYP450 status of patients is under studies. Despite not being optimized in terms of targeting hepatocytes, DHB-micelles represent the first injectable example of nanosized metabolism-blocking agents and open the way for further work on such nanomedicines in man (Paolini M, Poul L, Darmona A, Germain M, Pottier A, Levy L, Vibert E. A new opportunity for nanomedicines: Micellar cytochrome P450 inhibitors to improve drug efficacy in a cancer therapy model. *Nanomedicine: Nanotechnology, Biology, and Medicine* 2017; 13: 1715-1723).

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INTERNAL FIXATION WITH HANSSON TWIN HOOK FOR TROCHANTERIC FRACTURES

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INTERNAL FIXATION WITH HANSSON TWIN HOOK FOR TROCHANTERIC FRACTURES (Abstract): Hansson Twin Hook was recently developed as an alternative to the classical Dynamic Hip Screw. In the present study we aimed at presenting our results obtained by using the HTH in the treatment of trochanteric fractures. **Materials and methods**: Over a 2-year interval, 55 patients with trochanteric fractures were treated by reduction and internal fixation using HTH system. According to the AO fracture classification, we recorded 21 cases of 31.A1 fractures and 34 cases of 31.A2 fractures. **Results**: Clinical assessment at 3 months postoperatively using Merle D'Aubignier scale showed very good results in 41 (82%) patients, good in 7 (14%) patients and poor in 2 (4%) patients (two patients presented fixation failure). In these 2 patients, surgical reintervention was performed -implant removal and its replacement with a Dynamic Condylar Screw with good results at 3 months follow-up. **Conclusions**: Osteosynthesis with Hansson Twin Hook is a good choice in trochanteric fractures. Since it is a new developed implant, more studies are necessary for clinical validation of its value. **Keywords:** TWIN HOOK, TROCHANTERIC FRACTURES, ROTATIONAL STABILITY.

The results of internal fixation in proximal femoral fractures are disappointing in the elderly. Many studies showed a high reintervention rate associated with functional impairment and an increased morbidity of 35 % to 48% (1, 2). Dynamic hip screw (DHS) and short Gamma nail systems are commonly used for internal fixation of trochanteric fractures with favorable clinical outcomes (3). However, 1 to 4% of fractures fixed with DHS and 6-8% of those fixed with short Gamma nail require reintervention for "cut- out" (2).

The Hansson Twin Hook system (HTH) was developed as an alternative to the lag screw in combination with a dynamic plate. It consists of a side-plate combined with a sliding screw from which two apical hooks are deployed in opposite directions - anterior and posterior.

Before clinical use, comparative biomechanical studies of the new Twin Hook system and DHS systems - that rely on lag screw and are now considered "golden standard" in the treatment of trochanteric fractures - were conducted. Both the load

test and angulation and torsion tests revealed that to achieve the same deformation more force is needed for HTH system. These studies recommend HTH system as being biomechanically superior to conventional threaded screw implants. After receiving approval for clinical use, the first study that described its results appeared in 2000 (1); since then, only few studies were published on this topic (4, 5, 6, 7).

In the present study we aimed at presenting our results obtained by using the HTH in the treatment of trochanteric fractures.

MATERIALS AND METHODS

Over a 2-years interval, 55 patients with trochanteric fractures were treated by reduction and internal fixation using HTH system. Age-group distribution showed that most patients (n=20) were in the seventh decade of life (36.3%); mean age was 76 years (range 64-85 years), there was a predominance of female patients (female to male ratio = 36/19) and most patients were from rural areas. According to the AO fracture classification, we recorded 21 (38.2%) cases of 31.A1 fractures (stable trochanteric fractures) and 34 (61,8%) cases of 31.A2 fractures (unstable trochanteric fractures).

Close reduction was performed with the patient positioned on fracture table in all but 6 (10.9%) cases which required open reduction. The femoral head diameter was measured on preoperative X-rays. Incision length, surgery duration, cephalic screw length and femoral plate length were recorded. On anteroposterior and lateral postoperative X-rays, the adequacy of cephalic implant placement was assessed using the quadrants of Baumgaertner and the "tip-apex" distance was measured (8).

Postoperatively, patients were kept in bed for one day, the next day could sit at bedside and the third day resumed walking with the aid of a walker (partial weight-bearing). Patients were assessed clinically and radiographically immediately postoperatively and at 3 months, 6 months, and 1 year. Clinical outcome was assessed using Merle D'Aubignier score.

RESULTS

In our patients the size of femoral head was 42 to 62 mm, with an average of 44.4 mm in women (ranges 42 to 48 mm) and 55.8 mm in men (ranges 52 to 62 mm). The average surgery time was 36 minutes (range 26 to 48 minutes), and average incision length 6 cm (range 4 to 8 cm). The average length of the cephalic implant was 85 mm. Plates with three bicortical screws were used in 41 cases and with 4 bicortical screws in 14 cases (fig. 1).

On anteroposterior and lateral postoperative X-rays, the cephalic implant was in quadrant 5 (quadrants of Baumgaertner) in all cases but one; "tip-apex" distance had an average of 24.3 mm. The one case in which the implant was placed in the inferior quadrant had a tip-apex distance of 39.7 mm (fig. 2), significantly bigger than the average of all others.

Lateral X-ray view was used in all cases for measuring the distance between the tip of the hook and the joint, both in the anterior and in the posterior direction. There were no cases in which the hook penetrated the joint after expandation; the average distance between the tip of the hook and the joint was 9 mm, with a minimal distance of 2 mm.

At the 3-months postoperative follow up, radiological consolidation of fracture

site was seen in all but 2 (3.63%), patients one presenting femoral variation with a tendency for cephalic implant cut-out (fig. 3), and the other one cephalic implant cut-out, these two cases requiring surgical reintervention. Clinical assessment at 3 months postoperatively for 50 (90.9%) patients using Merle D'Aubignier scale showed very good results in 41 (82%) patients, good in 7 (14%) patients and poor in 2 (4%) patients (the 2 patients with fixation failure). In these 2 (3.63%) patients, surgical reintervention was performed - HTH removal and its replacement with a DCS, with good results at 3 months follow-up.

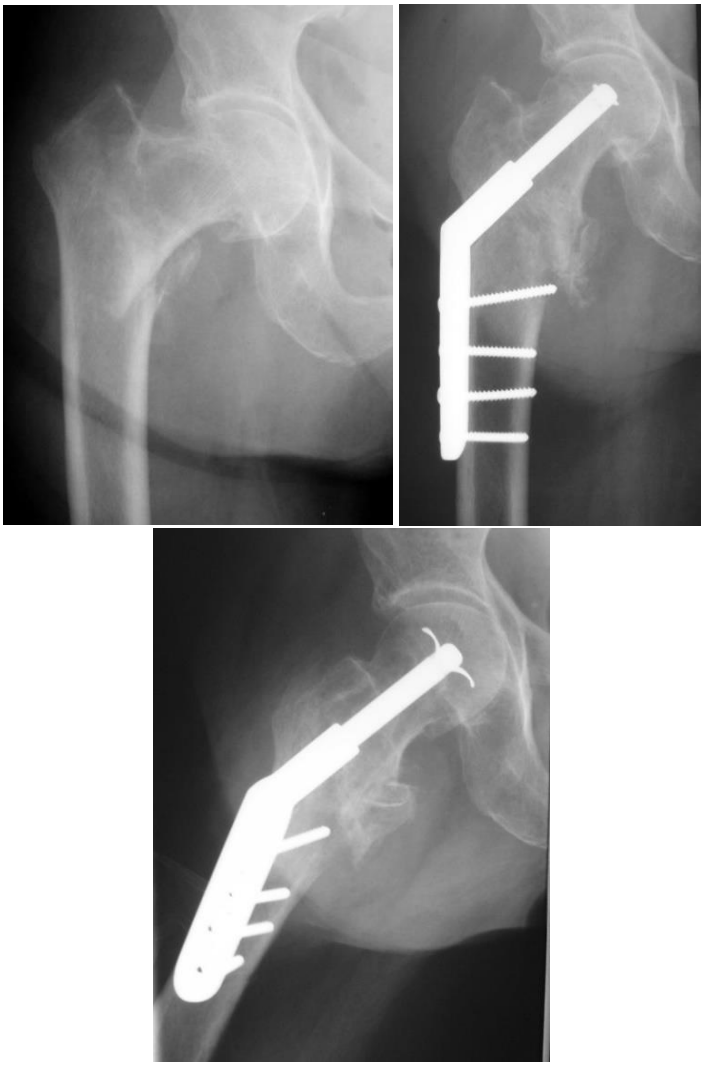

Fig. 1. Internal fixation with Hansson Twin Hook in a trochanteric fracture. Correct positioning of the implant. Preoperative X-ray (A), antero-posterior postoperative X-ray (B), lateral postoperative X-ray (C).

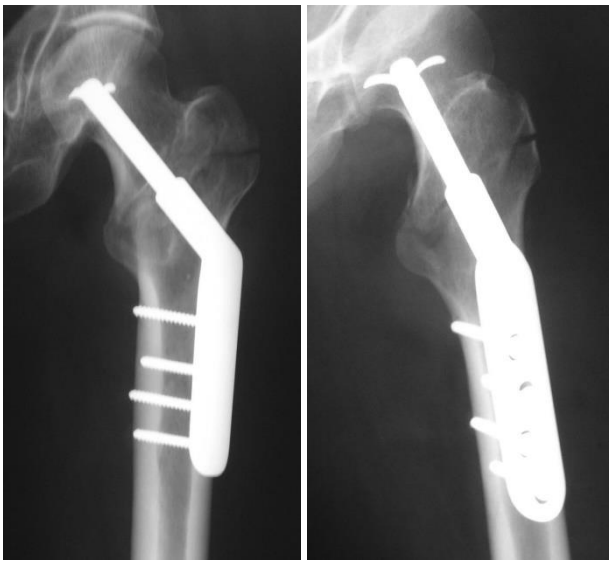

Fig. 2. Internal fixation with Hansson Twin Hook in a trochanteric fracture. Inferior positioning of the implant with a tip-apex distance of 39.7 mm; antero-posterior postoperative X-ray (A), lateral postoperative X-ray (B).

Fig 3. Fixation failure in a 71-year-old patient at 3 months after surgery.

One year after surgery, 37 (67.2%) patients were available for follow-up. For those, the results were very good in 13 (35.1%) patients, good in 20 (54%) patients and poor in 4 (10.9%) patients. In most patients, the results of clinical assessments were not influenced by implant placement - except the two “fixation failure” cases and

fracture type, but rather by general health status and number of associated comorbidities.

DISCUSSION

Osteosynthesis with HTH in trochanteric fractures has recently been proposed and therefore there are very few studies in the literature on the subject. Most studies on this new implant took place in Scandinavian countries where Hansson Hook is traditionally used in femoral neck fractures.

Olsson et al. (5) conducted in 2000 a retrospective study on 102 patients with hip fractures surgically treated using the HTH compared to a classic DHS compression system and Medoff plate (MSP). Technical errors in HTH system insertion occurred intraoperatively in 7 cases, but overall, postoperative failures were equally distributed between the 2 groups. They concluded that HTH system gives adequate fixation comparable to that produced by DHS and MSP. Also in 2000, Olsson (4) conducted a clinical and biomechanical study on 342 trochanteric fractures comparing the resistance and stability of HTH with MSP. He found an enhanced stress resistance with the use of MPS than DHS in intertrochanteric fractures, and comparable stability with the use of MSP and Gamma system in sub trochanteric fractures; in addition, HTH provides enhanced migration resistance under both axial and torsional loading. In 2002, Olsson et al. (6) published another biomechanical study conducted this time in artificial bone comparing HTH and DHS systems; they found that the two implants gave similar fixation in terms of resistance but the degree of impactation at fracture site was superior with HTH.

In 2008, Hagino et al. (9), compared the results obtained by using HTH and DHS systems in terms of technical accuracy: HTH was used in 17 patients, being inserted into the center of femoral with an average tip - apex distance of 22.3 mm; DHS was used in 202 patients with an average tip - apex distance of 14.6 mm. No device failure or cut-out was found. They concluded that using HTH system requires more surgical skill than using DHS, because failure to insert the screw into the center of the femoral head may cause intraarticular perforation by the hooks.

In 2008, Olséen et al. (10) compared the fixation stability of HTH reported by 55 surgeons in 157 elderly patients with trochanteric fractures, 83 % of which were unstable. They found technical intraoperative errors in 7 cases, HTH had not been placed centrally in the femoral head, of these only two presenting fixation failures at the 2-year follow-up; they concluded that HTH system is easy to use, gives adequate fixation in the bone of elderly osteoporotic patients with trochanteric fracture and has a low failure rate.

Roerdink (11) published his study in 2009, starting from the assumption that intracapsular fractures are frequently accompanied by pseudarthrosis and aseptic necrosis of the femoral head performed a comparative biomechanical study in synthetic bone to compare DHS, HTH and dynamic locking blade plate (DLBP). He concluded that HTH system gives a stable fixation with a minimum amount of replaced bone, is a dynamic implant, and its angular and rotational stability is 2 times higher than that of DHS.

CONCLUSIONS

The results of our research have shown that osteosynthesis with Hansson Twin

Hook (HTH) gives a superior biomechanical stability compared to DHS systems and the shorter skin incision results in earlier mobilization, less bleeding and esthetic benefits. HTH is rotationally stable, thus being the ideal implant in basic cervical fractures characterized by an increased tendency to rotate.

In most patients one-year follow-up demonstrated good and very good outcomes. HTH allows less disruption to cancellous bone than conventional DHS systems, thus favoring stability; insertion of cephalic implant is atraumatic, fact of importance in preventing aseptic necrosis of the femoral head. Insertion technique is easy and does not require long-term training especially in surgeons familiar with DHS-type systems; in osteoporotic patients' caution is recommend in using HTH because being less bulky there is a higher risk of articular penetration.

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MULTIPLE RISK FACTORS FOR THROMBOEMBOLISM AND PREGNANCY LOSS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A CASE REPORT

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MULTIPLE RISK FACTORS FOR THROMBOEMBOLISM AND PREGNANCY LOSS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A CASE REPORT (Abstract): Systemic Lupus Erythematosus (SLE) is known to pose difficulties when pregnancy is desired. Pregnancy in patients with SLE remains a high risk situation with an increased frequency of fetal loss, preeclampsia, growth restriction and prematurity. We illustrate the case of a young female referred to our clinic by the Institute of Cardiovascular Diseases with a history of miscarriage at 20 weeks of gestation and an episode of pulmonary embolism resulting in multiple peripheral infarctions in the right lung confirmed by spiral CT angiography of the pulmonary arteries. Whereas clinical examination showed no significant changes, routine laboratory assessment confirmed the presence of hemolytic anemia, thrombocytopenia and renal impairment, as well as non-specific inflammatory syndrome. Immunological tests proved positive for the following: antinuclear antibodies, anti-dsDNA antibodies, anti-mitochondrial M2 antibodies, anti-beta2 microglobulin, anti-cardiolipin and lupus anticoagulants. In addition, TPHA and RPR were positive and the C3 fraction of the complement was below normal. Genetic investigations identified the 677 C/T homozygous mutation of the MTFHR gene (Methylenetetrahydrofolate reductase) as well as 675 4G and 844 A/G mutations of the PAI-1 gene (plasminogen activator inhibitor-1). Cases of young female SLE patients positive for the antiphospholipid syndrome remain challenging for clinicians, especially when pregnancy is desired. **Keywords**: LUPUS, PREGNANCY, MISCARRIAGE, THROMBOEMBOLISM.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease associated with multisystemic involvement and heterogenous clinical manifestations. During pregnancy, it is best that women with SLE have little or no disease activity. However, pregnancy in SLE patients remains a high-risk situation with an increased frequency of fetal loss, preeclampsia, growth restriction and prematurity. The risk for increased disease activity during pregnancy is greater in women who have active SLE in the months prior to conception (1). Pregnant SLE patients also have a greater risk of developing complications. The presence of the antiphospholipid syndrome (APS) was found to associate with pregnancy loss, prematurity, fetal distress,

growth restriction, preeclampsia or eclampsia, fetal neonatal thrombosis, placental insufficiency as well as myocardial infarction, pulmonary embolism and stroke (2).

Epidemiological studies revealed that antiphospholipid (aPL) antibodies are detected in up to 40% of patients with SLE. In aPL-positive SLE patients, pregnancy morbidity is more common (up to 45%) than in aPL-negative women (2). Lupus nephritis is also thought to increase the risk for adverse pregnancy outcome in APS women (1,2).

CASE REPORT

We report the case of a 31-years-old female who was referred to our clinic by the Institute of Cardiovascular Diseases with recent history of pulmonary embolism resulting in multiple peripheral infarctions in the right lung confirmed by spiral CT angiography of the pulmonary arteries. She was diagnosed with APS and treated with both antiplatelet and anticoagulant medication.

Our patient's personal history included active smoker status (7 cigarettes per day for 6 years), miscarriage at 20 weeks of gestation (February 2015) shortly followed by pulmonary embolism. She had no history of substance abuse, alcohol consumption or procoagulant medication.

Clinical examination was normal upon presentation in our service. Routine laboratory tests detected hemolytic anemia, thrombocytopenia (<100,000/mm3) and renal impairment (proteinuria>0,5g/day as well as cellular casts). High levels of inflammatory markers were detected: elevated erythrocyte sedimentation rate (ESR) and C reactive protein (CRP). Immunological investigations identified high antibody titers against β2-glycoprotein I (anti-β2GPI) anticardiolipin (aCL) antibodies, lupus anticoagulants, as well as rheumatoid factor (RF), antinuclear antibodies (ANA), anti-double stranded DNA (anti-dsDNA) and antimitochondrial M2 antibodies (AMA M2) (tab. I). The above-mentioned parameters were measured through ELISA. In addition, Treponema Pallidum Haemagglutination Assay (TPHA) and Rapid Plasma Reagin (RPR) were positive and the C3 fraction of the complement was below normal.

TABLE I

Autoantibody titers detected in our patient compared to normal values provided by the laboratory.

Parameter (ELISA)	Value obtained	Reference interval/ Normal values	Measurement unit
Anti-dsDNA antibodies	246.40	0-40	UI/ml
aPL antibodies (IgG)	63.57	Positive >18	U/ml
aPL antibodies (IgM)	26.75	Positive >18	U/ml
Anti-β2GPI antibodies (IgG, IgA, IgM)	37.99	Positive >24	U/ml
aCL antibodies (IgG)	123.65	0-23	GPL/ml
aCL antibodies (IgM)	45.54	0-11	MPL/min
AMA M2	99.74	Positive >24	U/ml
RF	33.94	Positive >24	U/ml
ANA	32.05	0-20	UA/ml

Genetic investigations identified the 677 C/T homozygous mutation of the methylenetetrahydrofolate reductase gene (MTFHR) as well as 675 4G and 844 A/G mutations of the plasminogen activator inhibitor-1 gene (PAI-1).

The patient was consequently diagnosed with systemic lupus erythematosus according to the American College of Rheumatology (ACR) diagnosis criteria for SLE revised in 1997, as well as secondary antiphospholipid syndrome. She started oral treatment with hidroxycloroquine 400 mg/day, azathioprine 100 mg/day, clopidogrel 75 mg/day and acenocumarol under careful monitoring of INR values.

DISCUSSION

In our patient, the new-onset SLE consisted of a miscarriage followed by an episode of pulmonary embolism, which are both indicators of secondary APS. Hematological changes included thrombocytopenia and autoimmune hemolytic anemia (AIHA). The pathogenesis of the latter is closely related to the presence of anti-erythrocyte antibodies. Studies showed an association between anti-cardiolipin antibodies and anti-erythrocyte antibodies (3). Complement regulatory proteins CD55 and CD59 protect against erythrocyte lysis. However, they are under expressed in patients with SLE (4).

Our patient had thrombocytopenia possibly due to increased peripheral destruction mediated by antiplatelet antibodies. Alongside this mechanism, two other phenomena have an important role in the appearance of thrombocytopenia: cell sequestration in the spleen and impaired production in the bone marrow (5).

Secondary antiphospholipid syndrome is a common discovery in patients with SLE. APS symptoms may be the first to appear in certain cases. Our patient fulfilled the 2006 classification criteria for APS (Sydney Consensus Statement on Investigational Classification Criteria for Antiphospholipid Syndrome) (6). The PROMISSE Study (Predictors of pRegnancy Outcome: bioMarkers in antiphospholipid antibody Syndrome and Systemic lupus Erythematosus) showed that patients having high titers of lupus anticoagulant antibodies exhibit high risk for adverse pregnancy outcome (7). Another study (8) supported the role of all three antibodies for predicting obstetric complications such as: recurrent miscarriage, early delivery, oligohydramnios, prematurity, intrauterine growth restriction, fetal distress, fetal or neonatal thrombosis, pre-eclampsia/eclampsia, HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelet count), arterial or venous thrombosis and placental insufficiency.

SLE is characterized by impaired apoptosis. Patients with SLE may develop autoantibodies such as anti-Ro (SSA) and anti-La (SSB) which can cross the placenta causing neonatal lupus (9). Furthermore, anti-dsDNA antibodies may determine miscarriages by inhibiting the migration and attachment of the trophoblast (10).

Our patient exhibited genetic mutations including the 677 C/T homozygous mutation of the MTFHR gene and the 675 4G and 844 A/G mutations of the PAI-1 gene. The PAI-1 675 4G and 844 A/G mutations are associated with high levels of PAI-1 and impaired fibrinolysis. Recent studies have shown that the 844 A/G polymorphism in the PAI-1 gene identified in our patient may be associated with increased risk for osteonecrosis of the femoral head, acute coronary syndrome and venous thromboembolism (11).

Among other important functions, MTFHR is implicated in DNA methylation and the production of signaling molecules which play a role in embryonic development. Female carriers of the 667 C/T homozygous genotype have low estradiol levels, increased anti-Müllerian hormone concentrations and lower oocyte numbers. Normal MTHFR activity ultimately leads to breaking down the amino acid homocysteine. Increased levels of homocysteine were found to be toxic for embryos (12). The appearance of neural tube defects is associated with both heterozygous and homozygous maternal mutations of the MTHFR gene (13). However, the 677 C/T mutation is not thought to be associated with recurrent pregnancy loss in Caucasian women (14).

The presence of lupus nephritis in our patient is also accompanied by a high risk of fetal loss or complications. Optimal management of lupus nephritis is of major importance to prevent SLE-related adverse pregnancy outcome (15).

CONCLUSIONS

We described the case of a young female diagnosed with SLE after a miscarriage followed by an episode of pulmonary embolism. Clinical examination identified no abnormalities, whereas immunological, hematological and genetic modifications were all present. Our patient expressed her desire for safe successful pregnancy. However, antiphospholipid and anti-dsDNA antibody positivity, as well as our patient's genetic background and the presence of lupus nephritis constitute important risk factors for adverse pregnancy outcome. High SLE activity is a contraindication for pregnancy. Patients in remission for at least 6 months before pregnancy have a better prognosis. The postpartum period as well as the second and third trimesters of pregnancy are associated with high risk for SLE flares. The presence of APS in young female SLE patients pose additional challenges for clinicians, especially when pregnancy is desired.

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NEWS

LIVER SUBCAPSULAR HEMATOMA: A RARE CAUSE OF SUDDEN UNEXPECTED DEATH

The spontaneous subcapsular hematoma of the liver is very rare. There are only a few reported cases in the literature. Most reported cases of liver hematoma often occur during pregnancy as part of the hemolysis, elevated liver enzymes, and low platelet count syndrome. The other causes may be due to amylosis, rupture of hepatocellular carcinoma, adenoma, focal nodular hyperplasia, hemorrhagic cyst, or hemopathy. Idiopathic spontaneous subscapular hematoma is a rare and often fatal condition.A a 43-year-old woman having Steinert disease died because of a fatal spontaneous liver hemorrhage occurring without any traumatism. Was not find any apparent cause that could explain this hemorrhage even after a histological study of the liver (Oualha Dorra, Aissaoui A., Belhaj Meriam, Mesrati, Mohamed A, Moussa Adnene, Salem N, Zakhama A, Chadly A. Am J Forensic Med & Pathol. 2017; 38 (1): 9–10).

Diana Bulgaru Iliescu, A. Knieling

FOCAL BREAST LESIONS - ASSESSMENT WITH DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING

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FOCAL BREAST LESIONS-ASSESSMENT WITH DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING (Abstract): **Aim:** To evaluate breast magnetic resonance imaging for the characterization of the breast lesions and compare to pathology. **Material and methods:** Our study included 64 subjects aged 21 to 76 years with newly discovered breast lesions by mammography and ultrasound who underwent contrast-enhanced MRI at Arcadia Medical Center, Iași between January and December 2016. The following were analyzed: lesion morphology (shape, contour and signal intensity), enhancement pattern and enhancement curve. **Results and discussion:** Of the total of 65 subjects 44 presented benign lesions and 20 malignant lesions. Lesion morphology was consistent with the pathological diagnosis in 59 cases (90.8%). The shape of the masses was mostly oval or round in the benign lesions (29 cases) and mostly irregular in the malignant ones (11 cases). The contour was lobular in 27 benign masses and irregular in 16 malignant lesions. Regarding signal intensity of the lesions we found that the cysts had a water signal intensity and the solid lesions various combinations of signals. **Conclusions:** The rapid upslope of the enhancement curve either the first 2 minutes is associated with malignancy and a plateau in the delayed phase with an uncertain diagnosis of malignancy. Benign lesions were round or oval, with well defined, smooth or lobulated margins, low signal intensity in T1 weighed images, high signal intensity in T2 weighted images, homogenous contrast uptake, persistent enhancement curve (fibroadenomas and fibrocystic changes) and no enhancement (cysts). Breast cancer is an irregular mass, with irregular or spiculated margins, variable signal intensity in T1 and T2 weighted images, heterogeneous contrast enhancement and washout enhancement curve.
Keywords: MASS LESIONS, MRI, ENHANCEMENT CURVE.

Among the breast lesions the most important is breast cancer, which constitutes a major medical, social and economic problem. Although reliable estimates are lacking in many countries, the incidence of breast cancer in Romania was 46.2 per 100,000 population in 2000 and was the main cause of death in women over 40 years old (28.2 per 100,000 population) (1, 2). The incidence of breast cancer increases with age, with two peaks at 45-50 and 55-65 years. Over 250,000 new cases of breast cancer are diagnosed every year in the European Union and 172,000 new cas-

es/year in USA (3, 4).

The symptoms related to the disease, its complications, the anxiety given by the diagnosis alone and the treatment have a great negative impact on quality of life. The long-term prognosis of breast cancer is worse than for other cancers; the 5-to 10-year mortality rate is approximately 35-50%. The cost of medical care is measured in billions of euro (5, 6).

Breast cancer is a complex disease with a variable combination of symptoms and signs. Many breast cancers occur in women without any identifiable risks; none of the risk factors is determinant and most of them have expression in women over 50 years of age (7, 8, 9). The early diagnosis of the tumor is highly important, because it reduces mortality and morbidity, increases the quality of life and makes possible a conservative surgical treatment (10, 11).

Dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) of the breast is an important new tool for the detection and characterization of breast lesions. The use of DCE- MRI for breast cancer detection is based on the concept of tumor angiogenesis or neovascularity. Tumor-associated blood vessels have increased permeability, which leads to prompt uptake and release of gadolinium leading to a pattern of rapid enhancement and washout on MRI. This dynamic rapid enhancement pattern helps to distinguish breast cancers from benign lesions. Literature reports have shown that not all malignant lesions show enhancement at DCE-MRI with enhancement absent in up to 12-16% of known malignant lesions, mainly due to small tumor size and diffuse parenchymal enhancement. Diffusion-weighted imaging (DWI) sequences and apparent diffusion coefficient (ADC) can help the differential diagnosis between benign and malignant lesions; ADC is a quantifiable parameter of the molecular movement of water; studies depicted high ADC values in benign lesions and normal fibro-glandular breast tissue, and low values in malignant lesions (12).

MATERIAL AND METHODS

Our study included 64 female patients aged 21 to 76 years old, with newly discovered breast lesions by mammography and ultrasound who underwent contrast-enhanced MRI at the Iasi Arcadia Medical Center between January and December 2016. The following were analyzed: lesion morphology (shape, margins, signal intensity), enhancement pattern (homogenous, heterogeneous, thin or thick rim, dark or enhanced septations), enhancement curve (Kuhl curve): type III (washout) – malignant, type II (plateau) – undetermined and type I (progressive) – benign.

MRI examinations were performed using a Philips Achieva 1.5 T equipment; gadolinium contrast-enhanced breast MRI protocol included:

- patients were asked to sign the informed consent for MRI assessment and fill out a breast questionnaire;
- an intravenous line was placed; the patient was scanned in prone position, and each breast was centered within the aperture of the dedicated radiofrequency breast coil
- axial T1-weighted spin-echo sequences for localization;
- sagittal fat-suppressed T2 weighted sequences, with repetition time (RT) 4,600 ms and echo time (ET) 84 ms, field of view (FOV) 18 cm, matrix 256 x 192, contiguous 2-mm sections;
- 3D axial 3D fat suppression fast spin echo (FSE) 3D axial T1-weighted fast spin-

echo (FSE) fat-suppressed sequences before and every minute after gadolinium injection (0.1 to 0.2 mmol/kg, injection rate 2 mL/s), for 5 minutes, RT/ET 11.0/4.2, FOV 18 cm, matrix 256 x192, contiguous 2-mm sections;

RESULTS

Of the total of 65 subjects, breast MRI discovered in 44 (56.6%) cases benign lesions (fibroadenomas in 18 cases (27.6 %), fibrocystic changes in 14/21.5 %, cysts in 11/16.9%, and intra mammary lymph node in 1/1.5%) and malignant lesions in 21 (32.3 %).

The shape of the masses was mostly oval or round in the case of benign lesions (29 cases/65.9%) and mostly irregular in the malignant ones (11 cases/52.4 %). The contour was lobular in 27 (61.4%) benign masses and irregular in 16 (76.1%) malignant lesions.

We had studied also the signal intensity of the lesions: the cysts had water signal (low signal intensity in T1-weighted images and high signal intensity in T2-weighted images), but the solid lesions showed a heterogeneous signal, given by the components of the mass (solid, necrosis, mucin) and the most frequent was:

- isointense signal on T1- weighted and high signal intensity on T2- weighted images (16- 36.4%) in case of benign lesions;
- isointense signal on T1- and T2-weighted images and low signal intensity on T1-weighted and high signal intensity on T2-weighted images (6/ 28.5 % lesions respectively) in case of malignant lesions.

In our study the pattern of malignant tumors was as follows (fig. 1):

- irregular mass (11/52.4% cases), with spiculated or irregular margins (19/90.5%);
- the signal intensity was–low signal intensity on T1-weighted images and high signal intensity on T2-weighted images (6/ 28.5 % cases) and signal intensity on T1- and T2- weighted images like parenchyma (6-28.5% cases);
- heterogeneous contrast enhancement was the most frequent pattern (12/57.1%), followed by homogeneous (5/23.8%) and clumped enhancement (4/19%); thick rim, central enhancement, enhancing internal septations were not encountered;
- type III (washout) enhancement curve was demonstrated in most cases (12/ 7.4%)

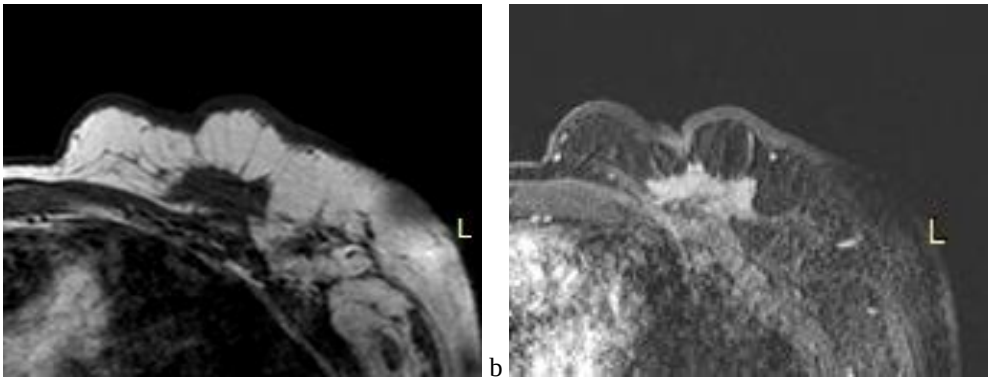

Fig. 1. Breast MRI, breast cancer with chest wall and nipple invasion - irregular mass, with spiculated margins, low signal intensity on T10 weighted images (a), homogeneous contrast enhancement (b)

• chest wall and nipple invasion or lymph node enlargement were additional features which confirmed the malignancy, but were found only in 4 cases with advanced disease.

Fibroadenomas were oval, with well-defined, smooth or lobulated margins, isointense on T1-weighted images, high signal intensity on T2-weighted images, homogeneous enhancement (17/ 94.4%) or dark (unenhanced) internal septations (1-5.5%), with a progressive enhancement curve (fig. 2).

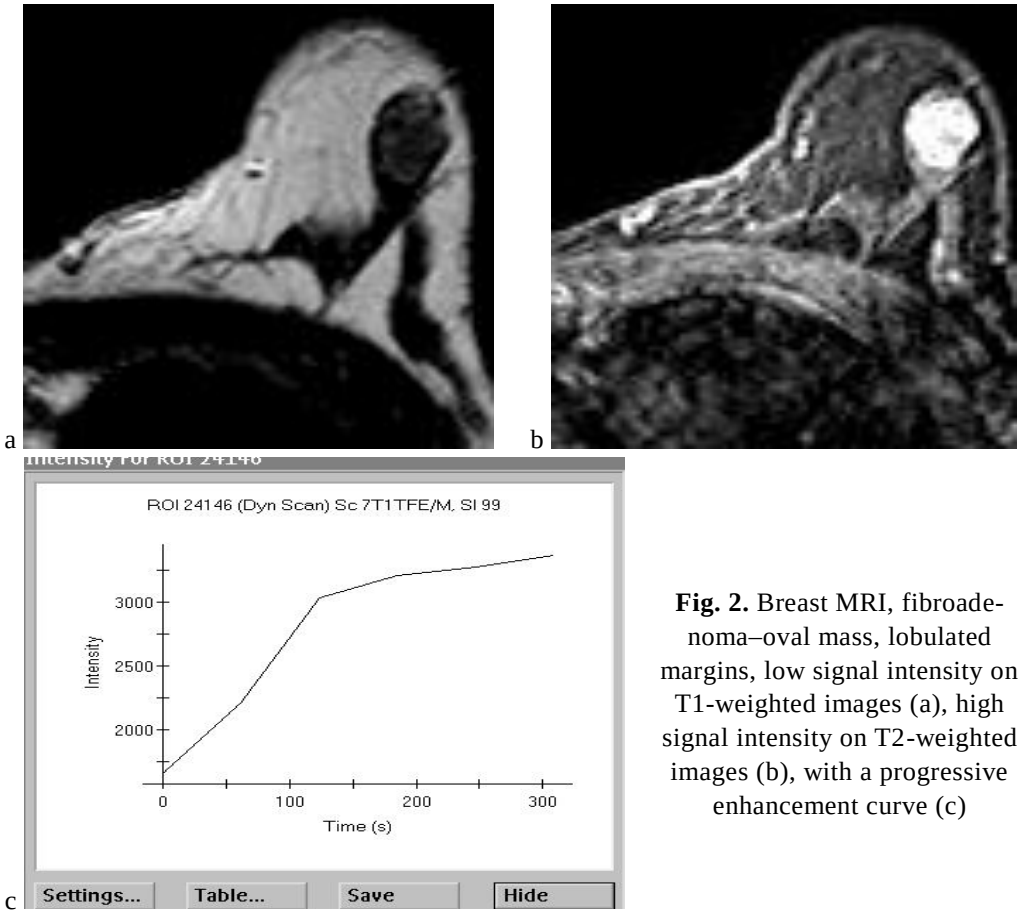

Fig. 2. Breast MRI, fibroadenoma–oval mass, lobulated margins, low signal intensity on T1-weighted images (a), high signal intensity on T2-weighted images (b), with a progressive enhancement curve (c)

All the cysts were oval or round, had smooth margins, maximum 35 mm in size, water signal intensity; 2 (18.2%) patients had cysts with thin-rim enhancement (inflammation around the cysts) and in 9 (81.8%) patients there was no contrast uptake.

At MRI, the fibrocystic parenchyma is difficult to distinguish from normal breast on non-

contrast-enhanced sequences; on post-contrast T1 weighted sequence stippled regional or heterogeneous contrast uptake was seen, which varies with the menstrual cycle. Enhancement curve had continuous (11/78.6%) or plateau (3/21.4%) pattern.

Single and clustered cysts of 3 mm to 23 mm in size, with no contrast enhancement were discovered, signal intensity being homogeneous in 12 (85.7%) cases (simple cysts) and heterogeneous, with fluid - fluid level in 2 (14.3%) cases (complicated cysts).

In one patient with ultrasound suspicion of breast nodule MRI detected an intramammary lymph node, which had an oval shape, smooth margins, central fat content and peripheral uptake of the contrast medium with progressive enhancement curve.

The morphology of the lesions was confirmed in 59 (90.8%) cases by the pathologic diagnosis.

DISCUSSION

In malignant tumors, we encountered various aspects:

- in our study the shape and margins of the lesions were like those reported in the literature: most of them had irregular shape, spiculated or irregular margins;
- signal intensity in our study was – low signal intensity on T1-weighted images and high signal intensity on T2-weighted images and signal intensity on T1- and T2-weighted images like parenchyma, compared to the data in the literature (signal intensity on T1-weighted image like that of parenchyma, and iso- to low signal intensity compared to parenchyma on T2-weighted image;
- heterogeneous contrast enhancement was the most frequent pattern, finding in agreement with the literature data, and thick rim or central enhancement, enhancing internal septations were not encountered;
- in agreement with the literature data, type III (washout) enhancement curve was demonstrated in most cases.

The pattern of most fibroadenoma cases in our study was like that described by preexisting studies: oval or round, with well-defined, smooth or lobulated margins, isointense on T1-weighted images, high signal intensity on T2-weighted images, homogeneous enhancement with a plateau enhancement curve.

Our study confirms that irregular shape is consistent with the diagnosis of malignancy (it was not encountered in any benign lesion). The oval or round shape was a pattern of benign lesion in 29 cases but was also found in 6 cases of malignant tumors; the oval or round shape had a relative risk (RR) of benign mass of 1.55 and an attributable risk (AR) of 0.59, so it is a pattern of benign lesions.

The irregular and spiculated margins were found only in the malignant tumors, but the lobular margins were encountered in 27 benign and 2 malignant masses; lobular margins had a RR of benign mass of 1.27 and an AR of 0.46, so it is a pattern of benign lesions.

Washout type of enhancement curve was found only in case of malignancy, but the progressive type was encountered in 25 benign and 7 malignant lesions, plateau type in 19 benign and 2 malignant cases. The progressive type of contrast uptake had a RR of benign mass of 1.27 and an AR of 0.21, so it is a pattern of benign lesions and the plateau type a RR=0.5 and AR = -21 so it is not consistent with a benign or malignant lesion (tab. I).

We found that the rapid upslope of the enhancement curve within the first 2 minutes is associated in 96% of cases with malignancy and a plateau in the delayed phase (minutes 2 to 5) can be associated with a 45% chance of benign and a 55% chance of malignant lesions.

TABLE I
MRI pattern of malignant and benign breast lesions

MRI pattern	Malignant lesion	Benign lesion
Shape	irregular	Round or oval
Margins	irregular or spiculated	Smooth or lobular
Enhancement	heterogeneous	homogeneous
Enhancement curve	washout	progressive
Signal intensity	isointense on T1, high signal on T2-weighted images	Variable signal intensity

CONCLUSIONS

The most notable finding of our study is that the rapid upslope of the enhancement curve within the first 2 minutes is associated with malignancy and while a plateau in the delayed phase is a cause of uncertain diagnosis of malignancy.

Benign lesions were round or oval, with well-defined, smooth or lobulated margins, low signal intensity on T1-weighed images, high signal intensity on T2-weighted images, homogeneous contrast, with a persistent enhancement curve (fibroadenomas and fibrocystic changes) and no enhancement (cysts).

Breast cancer is an irregular mass, with irregular or spiculated margins, variable signal intensity in T1- and T2-weighted images, heterogeneous contrast enhancement and washout enhancement curve.

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CAN BIOELECTRICAL IMPEDANCE ANALYSIS HELP US IN MANAGING, EASY AND FASTER OUR PATIENTS' HEALTH PROBLEMS?

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CAN BIOELECTRICAL IMPEDANCE ANALYSIS HELP US IN MANAGING EASY AND FASTER OUR PATIENTS' HEALTH PROBLEMS? (Abstract) Bioelectrical impedance principle it is a new way of detecting abnormal cellular response. Wegamed devices use this method to discover in a holistic manner a view of the man in all dimensions, body, emotions and intellect, and correlate's the treatment with the characteristics of the individual at all these levels. The aim of the study was to see if using alternative medicine methods can we evaluate correct and noninvasive the patients. **Material and methods:** A retrospective study was conducted to investigate the correlations between the clinical and paraclinically diagnoses and the diagnoses offered by the Wegamed devices. The study includes 440 patients who referred to our clinic between January 2013 and January 2016, who were thoroughly examined through evidence-based medicine methods and then undergo the Test expert plus analyze. **Results:** This retrospective study showed a concordance for some pathologies and diagnostics. The concordance was 100% for acute or oncological pathologies and for other pathologies, mostly chronic, degenerative; the most important is the doctor, who must interpret and correlate, based on his/her experience. **Conclusion:** The classical diagnostic methods can provide accuracy, but a personalized treatment requires viewing the man in all dimensions and specific correlations with the characteristics of the individual. **Keywords**: ELECTRODIAGNOSTIC STUDY, HOLISTIC MEDICINE, WEGAMED DEVICES.

Bioelectrical impedance analysis (BIA) is based on the principle that electric current flows at different rates through the body depending upon its composition. The body is composed mostly of water with ions, through which an electric current can flow (1, 2, 3). The water in the body is localized in two compartments: extracellular water (ECW, approximately 45%) and intracellular water (ICW, approximately 55%) (4, 5, 6). On the other hand, the body also contains non-conducting materials (body fat) that provide resistance to the flow of electric current (7). Adipose tissue is significantly less conductive than muscle or bone (8, 9, 10, 11). The principal of BIA is that electric current passes through the body at a differential rate depending on body composition (3, 12). Hence, there is a direct relationship between the concentrations of ions and the electrical conductivity and an indirect relationship exists between the ion concentration and the resistance of the solution (6, 13, 14, 15).

Nowadays the scientists want to discover a perfect, cost efficient, fast and noninvasive method for a correct diagnose. A mixture between the principles of alternative medicine and the science based medicine may be the answer (16, 17).

For this study, we used 2 types of diagnostic devices from Wegamed based on the bioelectrical impedance characteristics (14). The first one, Text expert plus is a highly – sensitive skin-resistance measuring device designed for use on humans to register physical skin characteristics with respect to their normal, reactive and regulative magnitudes. Investigational and therapeutic options include:

- metabolic regulation testing & therapy program (using homeopathic test and therapeutic data);
- energy screening-automatic resistance measurement of predefined body segments;
- investigational and therapeutic application of pulse currents;
- in addition, by means of special electrodes, a current measurement cam be made to localize galvanic buccal currents.

The Test expert plus device makes causal indication investigations possible by means of holistic capture of the bioenergetics functional state of the human body, its organs and organ systems under normal conditions, as well as monitoring the course of diseases and therapies.

The second device, check medical sport, measures the whole-body. There are 6 electrodes attached to the patient's feet, hands and head. The electrodes are used to apply very low-intensity 13 Hz pulse currents to specific body regions. The current flowing between the two electrodes automatically seeks out the path of least resistance via the body's fluids. The condition and constitution of the tissue affects the system's resulting electrical conductance. For safety reasons, when using this device there are some exclusion criteria, such as: pregnancy, epilepsy, pacemakers, severe cardiac arrhythmia and general acute ailments (7, 14).

MATERIAL AND METHODS

A retrospective study was conducted to investigate the correlations between the clinical and paraclinically diagnoses and the diagnoses offered by the Wegamed devices.

The study includes 440 patients who referred to our clinic between January 2013 and January 2016, who were thoroughly examined through evidence-based medicine methods and then undergo the Test expert plus analyze. The cases consist of 37% men and 63% women, range, 14-97 years, with an average age of 57.8 years, all of them Caucasians.

Designed and developed as an electro acupuncture test device, it provides the prerequisite for bioelectronics regulatory diagnostics using the Wegatest method, including homeopathic remedy test. Known electromagnetic test signals are applied to an acupuncture point via the device's measurement circuit, which then lead to resonance phenomena with the organism and thus to resistance changes at the acupuncture point. In accordance with the applied test information, these resonance phenomena provide investigational information to the trained and qualified users.

The data obtained was processed with the purpose to investigate the relationship between the clinical diagnoses and the information offered by the Test expert plus analyze.

RESULTS AND DISCUSSION

The clinical diagnoses found are divided in 32 main pathology groups. In this article

are reviewed the first 4 major diseases found in the selected group: common lombosciatic syndrome, spondyloarthropathy, knee osteoarthritis and one major group that contains all cardiovascular pathologies.

Test expert plus analysis showed that the main organs with abnormal electrical response are the head (more than 50% of the patients), the large intestine and the lumbar area (fig. 1).

TABLE I.

Clinical diagnosis.

Clinical diagnosis	No. %
Common lombosciatic syndrome	30.00%
Spondyloarthropathy	18.86%
Knee osteoarthrosis	16.14%
Cardiovascular pathology	11.14%
Arthralgia	10.00%
Osteoarthrosis	9.77%
Cervicobrachial neuralgia	9.77%
Herniated disc	7.95%
Gastroenterological pathology	7.05%
Chronic lumbago	6.82%
Neurological pathology	6.36%
Coxarthrosis	5.68%
Osteoporosis	5.23%
Periarthritis scapula humeralis	4.09%
Endocrinological pathology	3.41%
Trauma	3.18%

TABLE II

Clinical diagnosis.

Clinical diagnosis	No. %
Discopathy	2.95%
Otolaryngology pathology	2.27%
Arnold / Luschka neuralgic	1.82%
Temporomandibular arthrosis	1.82%
Calcaneal spur	1.82%
Cervical arthropathy	1.59%
Vertebrobasilar insufficiency	1.14%
Tendinitis	0.91%
Rheumatoid arthritis	0.91%
Ankylosing spondylitis	0.68%
Anemic syndrome	0.68%
Algoneurodystrophy	0.45%
Pulmonary pathology	0.45%
Lumbar spinal stenosis	0.23%
Gout	0.23%
Brahial plexus injury	0.23%

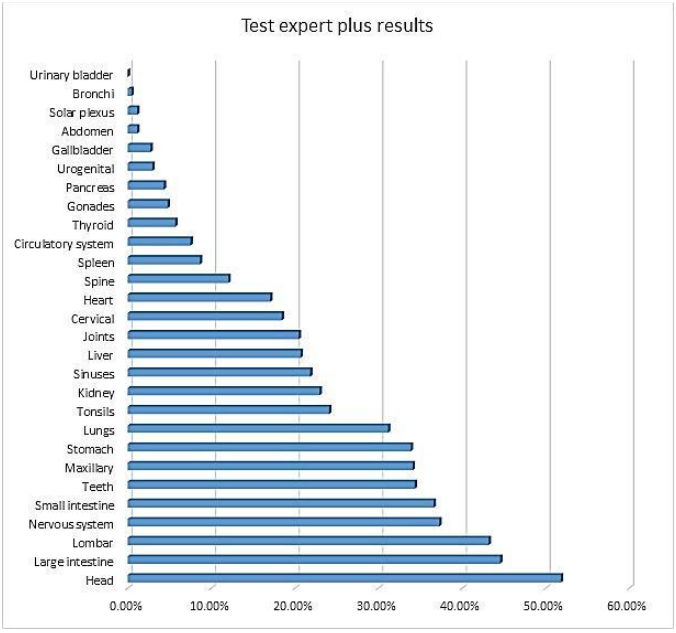

Fig. 1. Test expert plus results.

A. **Common lombosciatic syndrome**. In the selected group were diagnosed 132 (30%) patients with common lombosciatic syndrome. 73 (55%) were women and 59 (45%) men. These patients also presented other pathologies represented in figure 3. 27 (20.45%) cases suffered from spondyloarthropathy, 25 (18.9%) patients were diagnosed also with knee osteoarthritis and 18 cases (13.63%) had herniated disc problems. At the evaluation of the entire body with the Test expert plus, 67 (50.75%) patients had the lumbar region affected. The next organ that presented modifications was the large intestine, found abnormal in 62 (46.96%) cases.

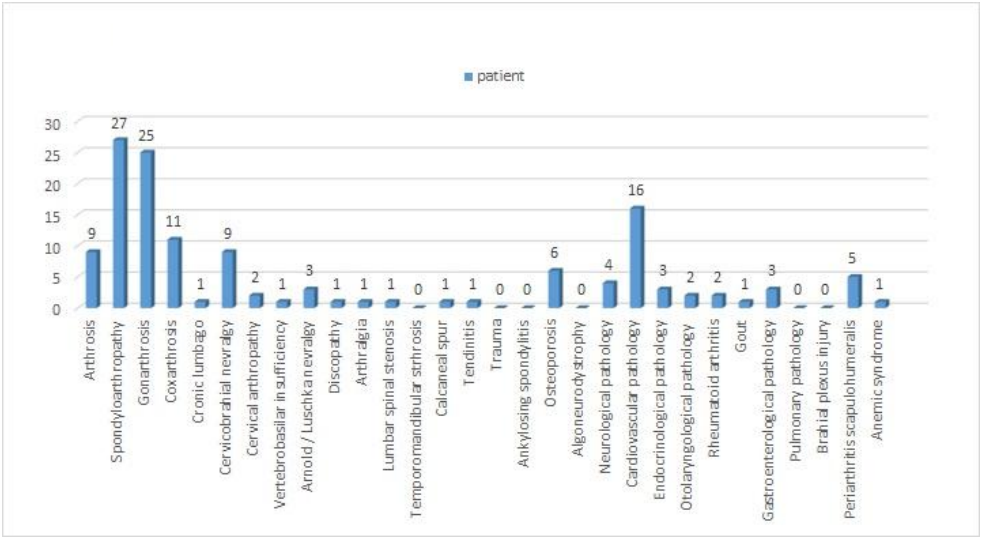

Fig. 2. Clinical diagnosis of the patients with common lombosciatic syndrome.

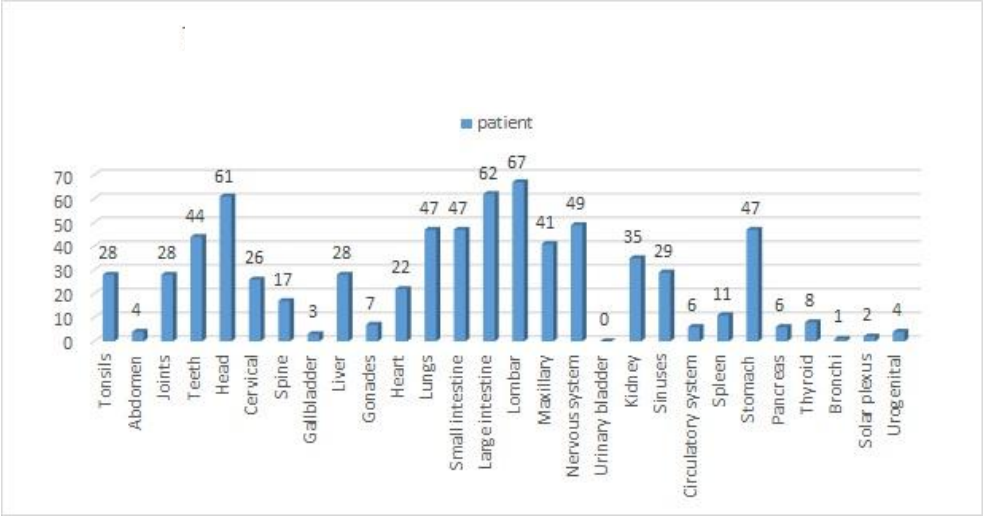

Fig. 3. Test expert plus results from patients with common lombosciatic syndrome.

B. **Spondyloarthropathy**. There were diagnosed clinical and paraclinically 83 (18.86%) patients with spondyloarthropathy. In 27 (32.53%) cases, this disease was associated with common lombosciatic syndrome and with knee osteoarthritis in 17 (20.48%) cases (fig.4).

C. The results from Test expert plus analyze showed that 47 (56.6%) patients presented alteration of the electrical signal in the region of the head. The large intestine was affected in 42 (50.6%) cases. The number of the patients with digestive problems at any level exceeds the number of the patients with spondyloarthropathy, therefore is a strong correlation of the spondyloarthropathy and digestive system alteration (fig. 5).

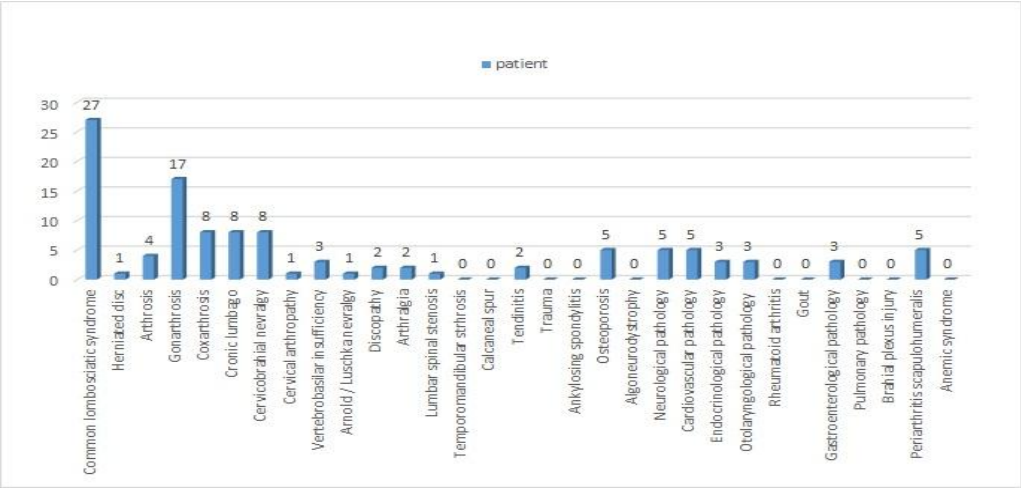

Fig. 4. Clinical diagnosis of patients with spondyloarthropathy.

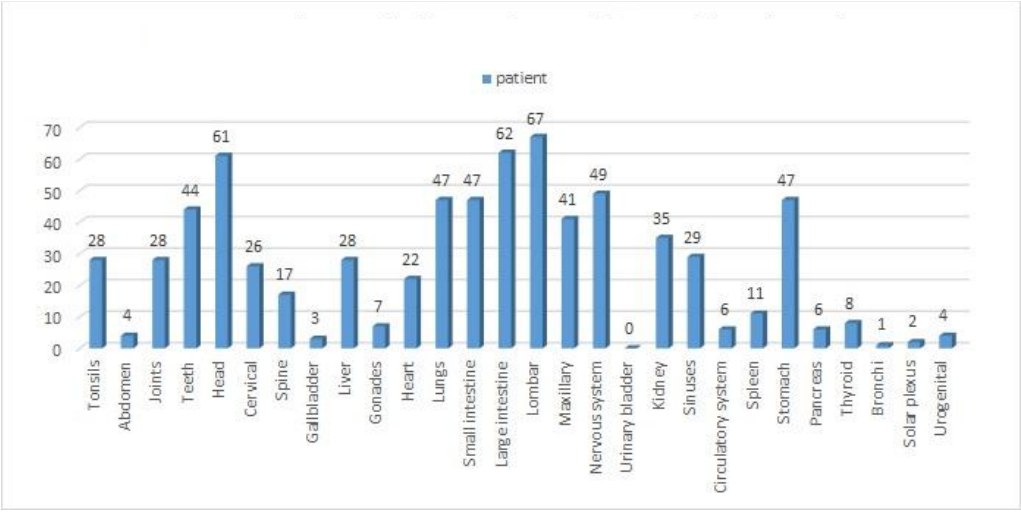

Fig. 5. Test expert plus results from patients with spondyloarthropathy.

D. **Knee osteoarthritis**. From the selected group, 71 (16.14%) patients were diagnosed with knee osteoarthritis. 24% of those patients had older degenerative osteoarticular pathologies. 14 (19.71%) cases had associated cardiovascular diseases (fig. 6).

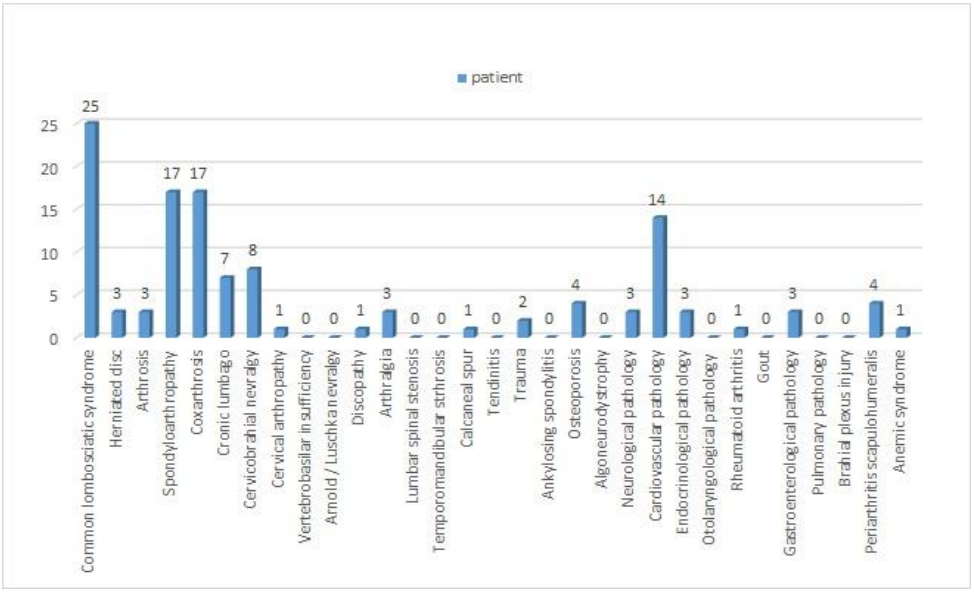

Fig. 6. Clinical diagnosis of patients with spondyloarthropathy.

Test expert plus evaluation results are similar with the ones mentioned above. Thus, 38 (53.3%) of patients presented large intestine modifications. The head region had the electrical signal altered in 35 (49.2%) cases. For this disorder, also exists a strong correlation with one or more digestive system problems (fig. 7).

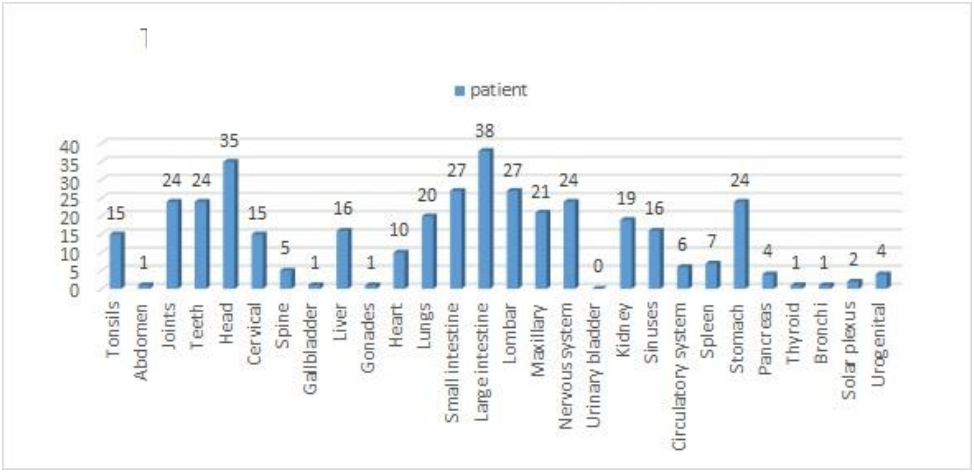

Fig. 7. Test expert plus results from patients with knee osteoarthritis.

E. **Cardiovascular pathologies**. 45 (11.14%) cases were initially diagnosed with cardiovascular pathologies. 16 (33.5%) associated common lombosciatic syndrome and 14 (31.1%) suffered from knee osteoarthritis (fig. 8). Test expert plus investigation showed that 24 (53.3%) of patients presented modifications of the electrical response at the head level and 22 (49%) of cases at the nervous system level (fig. 9).

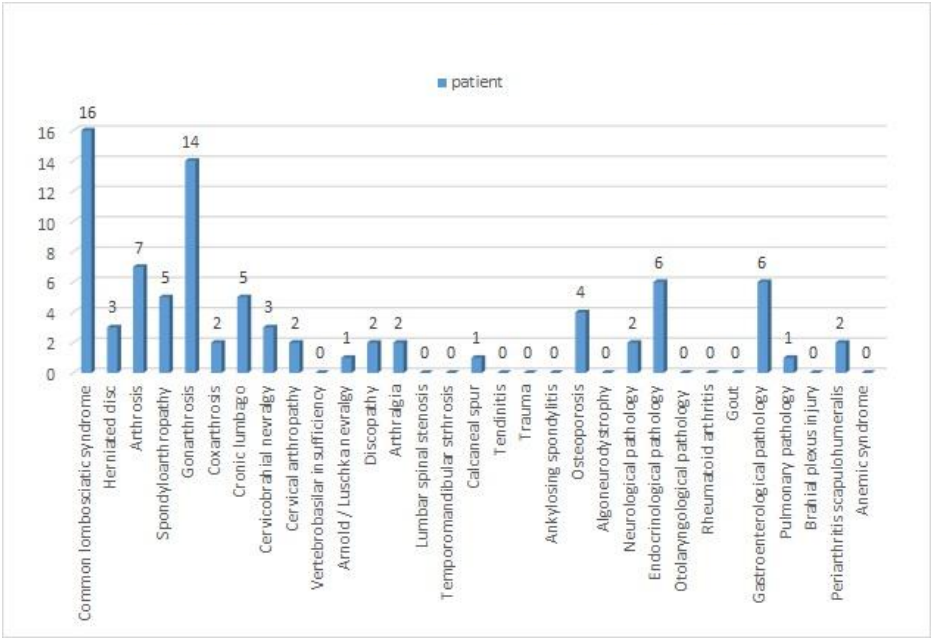

Fig. 8. Clinical diagnosis of patients with cardiovascular pathology.

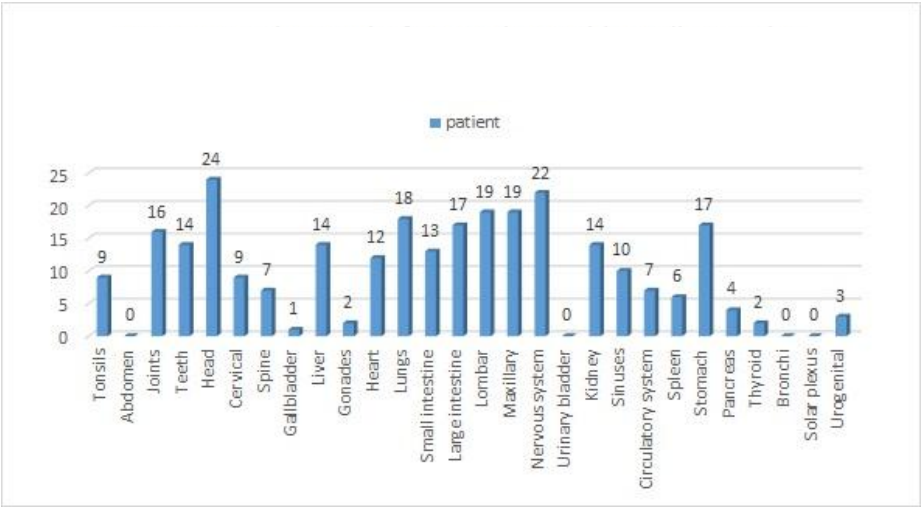

Fig. 9. Test expert plus results from patients with cardiovascular pathology.

Next, we will illustrate 2 cases that reveal the benefits and the limitation of this holistic method of diagnose.

Case study 1: female patient, 60-years-old, with family history of colon cancer, presented for cervical spondylosis. The Wegamed electro diagnostic revealed an important energetic anomaly concerning the inferior digestive system. Clinical and paraclinically diagnostic did not show any pathological signs at that level and based on the Wegamed study, we recommended a colonoscopy with biopsy, which revealed numerous polyps in the large intestine in a pre-malignant stage (fig. 11).

Case study 2: male patient 60-years-old, diagnosed with ankylosing spondylitis stage IV. The patient has a good functionality overall but his physical impairments affect his daily living activities. The Wega test showed moderate to severe impairment in all major organs affected by the disease. Also, there were low energy resources.

The dental examination showed minimal-moderate inflammation (fig. 12).

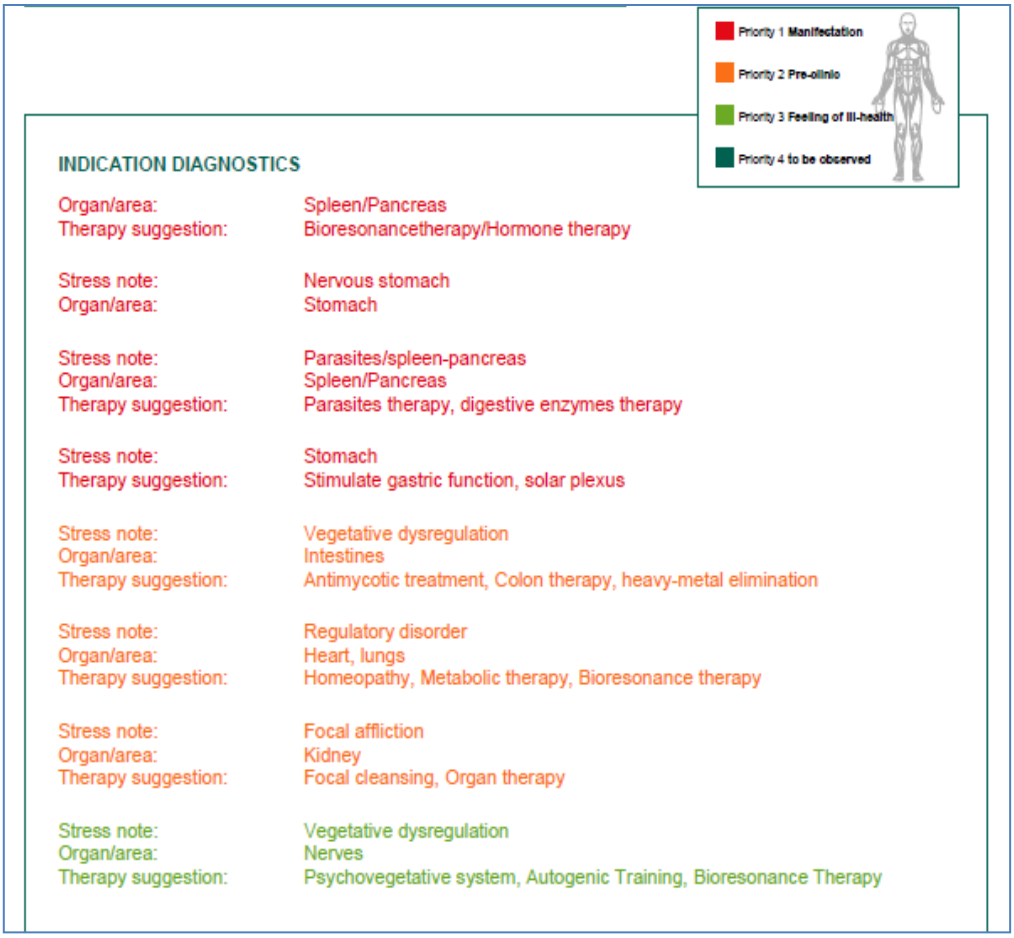

Fig. 10. Wegamed analyze of case study 1 revealing the inferior digestive system.

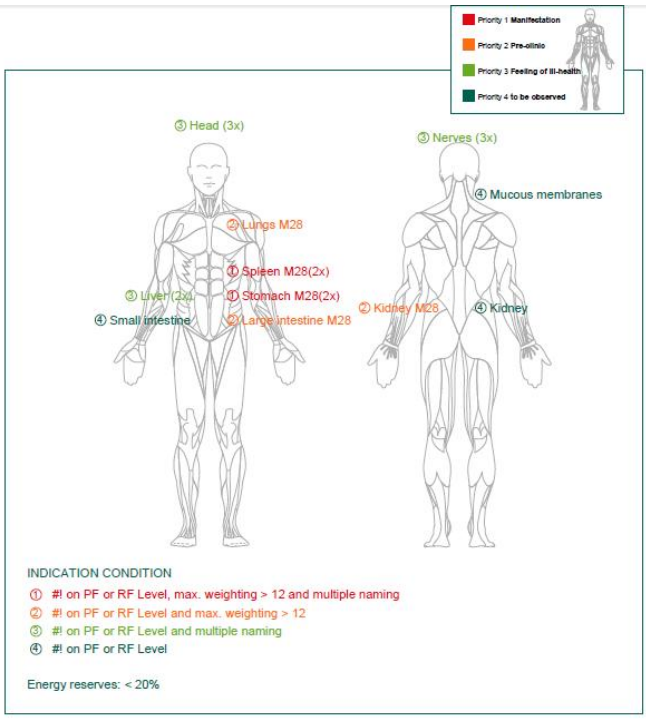

Fig. 11. Wegamed analyze of case study 2 showing moderate to severe impairment in all major organs affected by the disease.

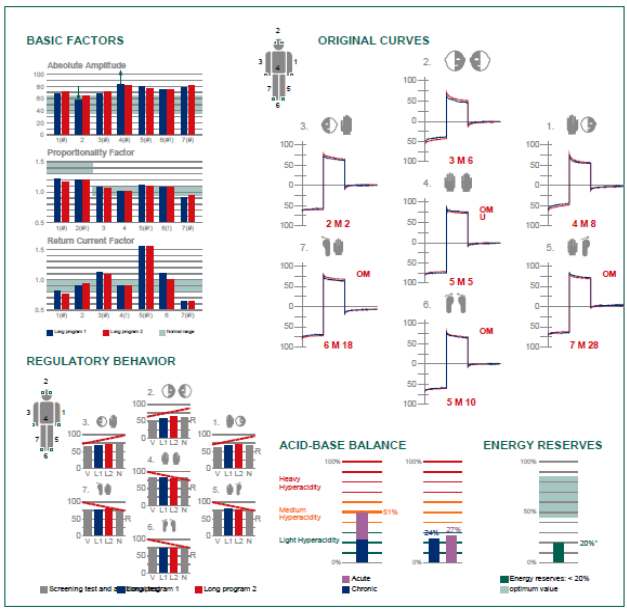

Fig. 12. Wegamed analyze of case study 2 that reveals low energy resources.

CONCLUSIONS

In our research the statistical analysis based on the correlation between primary and secondary diagnostics of the cases and Wegamed devices analyze was done to find a concordance between these two methods of diagnostic. This showed that for some pathologies and diagnostics the concordance is 100% and for other pathologies, mostly chronic, degenerative, the most important is the doctor, that must correlate based on his/her experience.

Based on the obtained results we should view in our medical practice the patient in all dimensions, body, emotions and intellect, and to correlate the treatment with the characteristics of the individual at all these levels.

The classical diagnostic methods provide accuracy to the molecular level, but it doesn't correlate in functionality over time. Bioelectrical impedance analysis (BIA) could be cost efficient, fast and noninvasive method associated with the classical diagnosis methods.

Further studies are needed to understand the intimate correlation mechanism between classical diagnostic and bioelectrical impedance analysis (BIA).

ACKNOWLEDGMENTS

The study was performed with the help from Wegamed GMBH (Am Zehnthof 189 D-45307 Essen, Germany). The work would not have been possible without the help and support from Dr. Peter Moloney (CEO GMBH).

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NEWS

HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY – A CUTTING EDGE THERAPHY FOR ADVANCED OVARIAN CANCER?

Nowadays, it is well known the importance of hypertermic intraperitoneal chemotherapy in colo-rectal cancer, pseudomyxoma peritoneii and malign mesothelioma, but it's significance in advanced ovarian cancer isn't so certain so far. There were published the results of a phase II study performed at the Unit of General, Emergency and Transplant Surgery at Sant'Orsola-Malpighi Hospital, University of Bologna, Italy, the Unit of General Surgery and Gynaecologic Surgery of Papa Giovanni XXIII Hospital, Bergamo and the Unit of Gynaecology of the Jena University Hospital, Germany. Patients with primary, advanced (FIGO stage IIIC to IV), or recurrent EOC were eligible for the present study to be treated with cytoreductive surgery and HIPEC with Cisplatin (100 mg/m^2) and Paclitaxel (175 mg/m^2). It seems that using cytoreductive surgery and HIPEC, using cisplatin and paclitxel it is feasible and comparable with the other drugs regimens with similar mortality and morbidity rate. The benefit for the overall survival and for the morbidity and mortality rate, it seems that it's a statement for the treatment in ovarian cancer (Federico Coccolini, Luca Campanati, Fausto Catena et al. Hyperthermic intraperitoneal chemotherapy with cisplatin and paclitaxel in advanced ovarian cancer: a multicenter prospective observational study. *J Gynecol Oncol*. 2016 Jan;26(1):54-61).

Mihaela Buna-Arvinte

INFLAMMATORY AND GENETIC MARKERS (APO B100 AND ANGIOTENSIN-CONVERTING ENZYME GENE) IN THE CORONARY ARTERY DISEASE

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INFLAMMATORY AND GENETIC MARKERS (APO B100 AND ANGIOTENSIN-CONVERTING ENZYME GENE) IN THE CORONARY ARTERY DISEASE (Abstract) **Aim**: To analyze the correlations between inflammation markers and ApoB100 and angiotensin converting enzyme (ACE) gene polymorphism and the severity of coronary artery disease (CAD). **Material and Methods**: We conducted a study in 58 patients with acute coronary syndromes (ACS) who underwent coronarography at the Iasi 'Prof. Dr. George I. M. Georgescu' Institute of Cardiovascular Diseases. The patients included in the studies were selected from those who needed a coronarography for unstable angina or acute myocardial infarction. The data were uploaded and processed using the statistical functions in SPPS 18.0 at a 95% materiality threshold. **Results**: Elevated inflammation markers were found in all study patients, with small differences in distribution. None of the study patients presented ApoB100 gene mutations. As to ACE polymorphism, a predominance of genotype II in unicoronary patients and ID and DD genotypes in bicoronary and tricoronary patients was found. **Conclusions**: The results of our study confirm the role of genetic and epigenetic factors in the severity and progression of the coronary disease, leaving room for larger and more comprehensive studies and new research perspectives. **Keywords**: CORONARY ARTERY DISEASE, APOB100, ANGIOTENSIN CONVERTING GENE, STENOSIS, ACUTE CORONARY SYNDROME

Acute coronary syndromes (ACS) are the most common cause for hospitalization among patients with coronary artery disease (CAD). The term acute coronary syndrome refers to a large spectrum of diseases including unstable angina (UA), acute myocardial infarction without ST segment elevation (NSTEMI) and acute myocardial infarction with ST segment elevation (STEMI) (1). More than 90% of the cases are caused by coronary atherosclerosis. The pathophysiological mechanism consists of an interaction between plaque rupture, platelet activation and thrombus formation, endothelial dysfunction, spasm, vascular remodeling (2). Risk factors for CAD were officially de-

scribed based on the discoveries of the Framingham study: age, smoking, high total cholesterol level, hypertension, obesity, diabetes, metabolic syndrome, C reactive protein, homocysteine. The latest discovery is represented by genetic factors: polymorphisms in the angiotensin converting enzyme (ACE) gene and ApoB100 gene (3).

ACE gene is located on the chromosome 17q23. It has 21 kb and comprises 26 exons and 25 introns (4). The gene polymorphism is due to the insertion (I) or deletion (D) of an alu sequence in intron 16 that leads to the generation of three genotypes: insertion/insertion (II), deletion/deletion (DD) or insertion/deletion I/D) (5, 6). I/D polymorphism is associated with the circulating and tissue ACE levels. Homozygotes DD have a higher level compared to the homozygotes II or heterozygotes (7). ACE polymorphisms explain many of the physiological variations of the cardiovascular system (8). Therefore, these genetic differences have been associated with many diseases such as arteriosclerosis (9), arterial hypertension (10) and CAD. In its genetic variants, ACE may be associated with the cardiovascular risk factors in predicting CAD development and severity (11,12).

The human ApoB100 is located on the short arm of the chromosome 2 (13) and it encodes the main apolipoprotein of chylomicrons and LDL-cholesterol, the mutations contributing to the occurrence of dyslipidemia, risk factor of the coronary disease (14). The polymorphism is multiple. One of the mutations is the substitution of adenine with guanine at the level of codon 3500 Apo B100 (15).

MATERIAL AND METHODS

We conducted a study in 58 patients with ACS who underwent a coronarography at the Iaşi 'Prof. Dr. George I. M. Georgescu' Institute of Cardiovascular Diseases. The patients were divided into two subgroups depending on the type of disease: AMI subgroup (30 patients with acute myocardial infarction) and UA (18 patients with unstable angina).

The experimental protocol was approved by the Ethics Committee, the procedures being consistent with the Declaration of Helsinki II, and all patients signed an informed consent before enrollment into the study.

A thorough physical examination was done and a detailed personal and family history was obtained. The severity of coronary disease was assessed according to the number of diseased coronary arteries with above 70% stenosis (no, one, two, or three coronary arteries = 0, 1, 2, 3 C).

Also evaluated was the presence of conventional risk factors (1. hyperlipidemia, 2. hypertension, 3. diabetes, 4. family history of ACD, 5. smoking, 6. obesity), their analysis being performed according to well-defined criteria.

For gene dosage analysis, 200 ml peripheral venous blood EDTA samples were collected from each patient, and DNA was automatically extracted. After checking DNA purity, we performed the gene amplification by the polymerase chain reaction (PCR). The result was the determination of polymorphism in the ACE (DD, ID, II) and apoB100 genes (pro/pro, pro/pat, pat/pat).

The data were uploaded and processed using the statistical functions in SPPS 18.0 at the materiality threshold of 95%.

RESULTS

Erythrocyte sedimentation rate (ESR) ranged from 2 to 130 mm/h (fig. 1) in 62.1% of the patients, the individual values

exceeding the upper limit of reference range (<20 mm/h). Depending on the coronary disease, a slightly higher average ESR value was found in the subgroup with unstable angina (31.63 *vs* 35.07 mm/h; p=0,660).

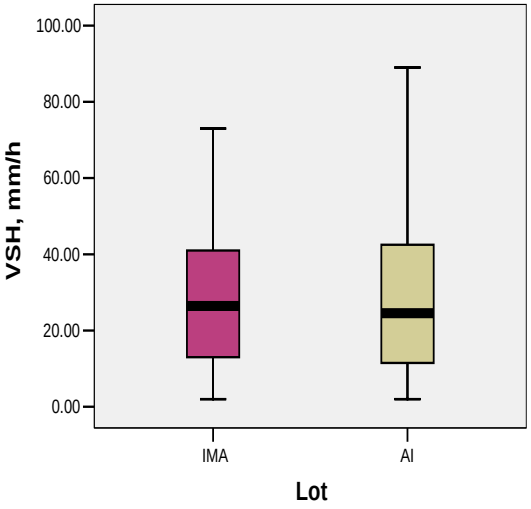

Fig. 1. Mean ESR values

The individual *C reactive protein (CRP)* levels were in the range 0.30-77 mg/dl, 53.4% of the values being above the reference range (<4 mg/dl). The average CPR level in the AMI group was slightly higher than in the UA group, but without significant differences (8.61 *vs* 7.57 mg/dl; p=0.715) (tab. I).

TABLE I

Descriptive indicators of CRP (mg/dl) depending on the epidemiological characteristics

Subgroup	N	Average	SD	SE	Confidence interval 95%		Min	Max	Test F (ANOVA) p
					-95% CI	+95% CI			
AMI	30	8.61	13.85	2.53	3.44	13.78	0.30	77.00	0.715
UA	28	7.57	5.69	1.08	5.37	9.78	1.10	25.00	
Total	***58***	***8.11***	***10.64***	***1.40***	***5.31***	***10.91***	***0.30***	***77.00***	

SD = standard deviation; SE= standard error

Fibrinogen The individual values of this parameter varied in the range 276-1012 mg/dl, 51,7% being over the reference limit (<500 mg/dl) (fig. 2). The average values of fibrinogen did not register significant differences depending on the coronary disease (554 *vs* 545 mg/dl; p=0,845) (tab. II).

In this study, most patients (41.4%), had significant (over 75%) stenosis of one coronary artery (1 CA) but together patients with 2 or 3 over 75% stenosis coronary arteries accounted for 57% of the patients (fig. 3).

TABLE II

Descriptive indicators of fibrinogen (mg/dl) depending on the epidemiological characteristics

Subgroup	N	Average	SD	SE	Confidence interval 95%		Min	Max	Test F (ANOVA) p
					-95% CI	+95% CI			
AMI	30	554.00	143.39	26.18	500.46	607.54	374	959	0.845
UA	28	545.00	202.96	38.36	466.30	623.70	276	1012	
Total	58	549.66	173.19	22.74	504.12	595.19	276	1012	

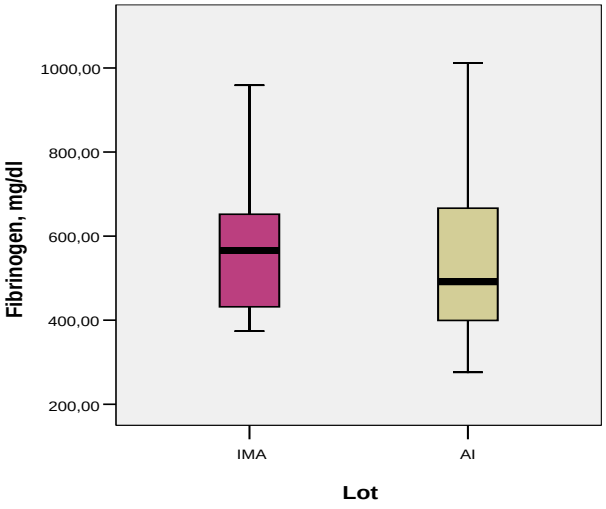

Fig. 2. Mean fibrinogen values

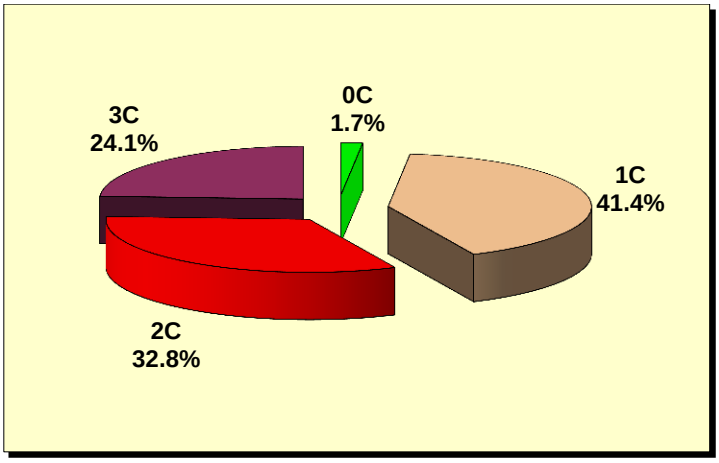

Fig. 3. Distribution of cases according to coronary status

Regarding the genetic polymorphism, no mutations in ApoB100 gene were identified.

Only normal fragments were noticed, healthy homozygote 140 bp (base pairs), without 140/110 bp (mutant heterozygote) or 110 bp (mutant homozygote) (fig. 4).

Regarding the ACE gene, all three genotypes (II, ID, DD) were identified: 3 II, 5 DD and 11 ID.

There are noticed the characters of homozygote II (480 bp), heterozygote ID (480/190 bp) and homozygote DD (190 bp) (fig. 5).

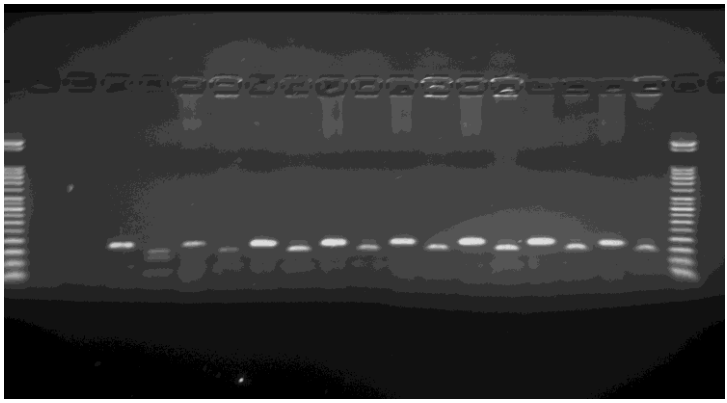

Fig. 4. ApoB100- detection of amplified and digested DNA

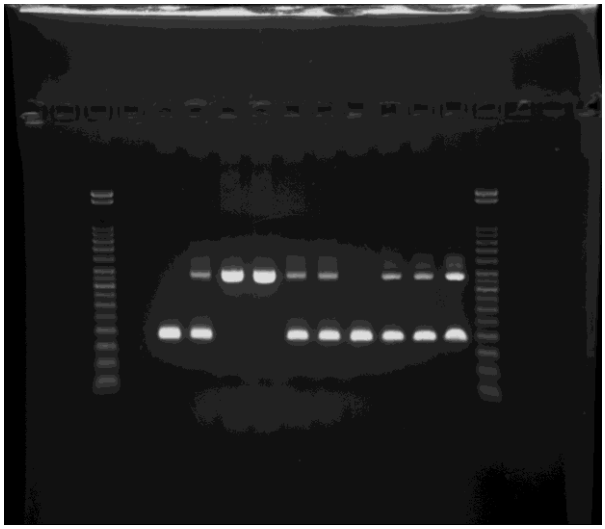

Fig. 5. ACE gene-detection of amplified and digested DNA

DISCUSSION

Similar to the literature (16, 17), inflammation was present in all enrolled patients, with small differences in the distribution of inflammatory markers in the two subgroups: ESR slightly increased in the UA subgroup, CRP slightly increased in the AMI subgroup, while the mean fibrinogen

levels did not show significant differences depending on the coronary artery disease.

In our study we did not identify patients with mutations of ApoB100 gene. Therefore, in our study subgroups, the ApoB100 gene polymorphism is not a risk factor.

As to the ACE polymorphism, there was a predominance of genotype II in unicoronary patients and of ID and DD in the bicoronary and tricoronary ones.

Our results are consisted with other studies in the literature. For example, Dhar et al. (16) studied 217 cases diagnosed with CAD and 255 healthy, age-and sex-matched controls with negative stress test. There were performed medical tests, such as electrocardiography, echocardiography, stress test, in addition to the laboratory analysis across all studies and mentioned at the beginning of the analysis. The results showed that both DD [OR: 2.16; 95%CI: (60.60e67.40)] and ID [OR: 1.48; 95%CI: (93.28e97.72)] genotypes of the ACE gene had a significant influence on the development of CAD. Coexistence of diabetes and hypertension proved to be risk moderating. Tobacco use increased the risk of disease in those with predisposing genotypes. DD and ID genotypes proved to be predisposing factors for CAD cases in their study population.

Other studies were negative. Poorgholi et al. (17) conducted a crossover study of 1950 patients referred for coronary angiography. They determined the genotype ACE in 767 CAD-positive patients (documented angiographycally and by Gensini score over 6) and 374 patients without CAD. The results showed that 504 patients (74.6%) in the group CAD positive were men, and the average age of this group was 60 (60 ± 10). In the group without CAD, the average age was 56 (56 ± 10) and 196 of them were men (52.4%). The analysis of groups and subgroups by gender did not objectivized significant differenced regarding the genotypes (p for the frequency of genotypes and allele being 0.494 and 0.397). Therefore, the genetic polymorphism of ACE was not associated with the severity of the coronary disease in the population of Iran.

However, other studies do not support this association. Thus, Poorgholi et al. (17) conducted a crossover study of 1050 patients referred for coronary angiography. ACE gene I/D polymorphism was determined in 676 CAD-positive patients (documented angiographically and by Gensini score over 6) and 374 CAD-negative patients. The results showed that 504 (74.6%) of the CAD-positive patients were men, and the mean age of this group was 60 (60 ± 10). In the CAD-negative group the mean age was 56 (56 ± 10) and 196 of them were men (52.4%). The analysis of groups and gender subgroups did not show significant differences (p values for genotype and allele frequency being 0.494 and 0.397, respectively). Therefore, ACE gene I/D polymorphism was not associated with the severity of CAD in the population of Iran.

CONCLUSIONS

The results of our study confirm the role of the genetic and epigenetic factors in the severity and progression of the coronary artery disease, leaving room to larger and more comprehensive studies and new research perspectives.

ACKNOWLEDGEMENTS.

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MEDICAL PRACTICE IN CHILDREN PRESENTING FEVER WITH PETECHIAL RASH TO AN EMERGENCY DEPARTMENT

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MEDICAL PRACTICE IN CHILDREN PRESENTING FEVER WITH PETECHIAL RASH TO AN EMERGENCY DEPARTMENT (Abstract) **Introduction:** The association of fever and petechiae in children is one of the most alarming findings for a paediatrician. To quickly distinguish between benign and life-threatening conditions is challenging in many cases. We aimed to evaluate the clinical practice of children presenting with fever and petechiae as initial symptoms. **Methods:** 41 patients (age 3 months-11 years) presenting with fever and petechiae were identified in an Emergency Paediatric Assessment Unit over a period of 9 months. General data, symptoms and signs were assessed for each patient. The work-up consisted in: complete blood count, inflammatory tests, coagulation tests, Monospot test, nasopharyngeal rapid tests, blood culture, and cerebrospinal fluid culture where appropriate. **Results:** Most children were <5 years of age (70.7%). Female to male ratio was 1:2.4. The most common clinical diagnoses were: viral respiratory illness (48.8%, 20/41) and upper respiratory tract infection (17.1%, 7/41). Meningococcal disease was found in one case. CRP>6 mg/l was poorly correlated with serious illness. The following variables were strongly associated with serious illness: ill appearance, shivering, lethargy, back rigidity, ESR>50 mm/h and prolonged capillary refill time. 59%(24/41) of children were treated with antibiotics, however, at discharge 42%(10/24) of them, did not have a work-up suggestive for a bacterial illness. **Conclusions:** Screening for low prevalence but high morbidity conditions, as the meningococcal disease, with an extensive work-up is time and resource consuming and may lead to unmotivated antibiotic use. Larger studies are needed to change the emergency practice for management of fever and rash. **Keywords:** FEVER, RASH, MENINGOCOCCAL DISEASE, CHILDREN.

The evaluation of febrile children presenting with petechial rash poses major management predicaments, especially in the emergency department and has been a topic of much debate(1–5). . Back in 1989, Baker at all published a study involving 190 patients admitted for fever and petechial rash and concluded "no laboratory test or physical finding was sufficiently sensitive to detect all patients with serious

disease". In 2000, Brogan and Raffles (6) proposed the "ILL" criteria. Irritability, lethargy, prolonged capillary refill and white blood count (WBC) outside the range 5–15 × 10^9/l, and C-reactive protein (CRP) greater than 5 mg/l, were considered risk factors for detection of serious illness. The authors proposed that children with fever and petechial rash who have none of the "ILL" criteria and normal WBC and CRP, to be observed for 4 hours and reassessed. If no deterioration occurred, then outpatient care would be recommended. Children who were not "ILL" but had abnormal WBC and/or CRP could be treated for meningococcal disease with a minimum of 48 hours antibiotics pending blood cultures, with notification of "suspected" meningococcal disease. The children, who presented one or more of the proposed criteria, plus abnormality of the WBC and/or CRP, would be treated as meningococcal sepsis, with consideration of early intensive care referral.(6)

Emergency department physicians, when faced with a child presenting with petechial rash and fever need to rule out meningococcemia or other causes of bacterial sepsis and often request extensive work-ups, (including blood cultures, cerebrospinal fluid (CSF) analysis), recommend antibiotics and hospital admission (5,7,8). Serious bacterial infections are the cause of substantial mortality and morbidity in children and paediatricians need to identify these children so appropriate treatment can be initiated (9–11). However, the efficiency of this approach needs to be optimized in order to avoid unnecessary investigations and treatments.

We aimed to evaluate the medical practice, in children presenting with fever and petechial rash in an emergency paediatric assessment unit.

MATERIAL AND METHODS:

Study group and data collection:

Consecutive children presenting to the Emergency Department with fever and petechial rash as initial symptoms between August 2011 and April 2012 were eligible for the study. The Hospital is a Regional Hospital in the midlands of Ireland.

The study involved 44 patients (age 3 months- 11 years) presenting with fever and rash as initial symptoms. Three children were excluded from the analysis as they presented 10-15 petechiae above the nipple line considered a result of coughing/vomiting. They had no blood work done, were followed for 4 hours and discharged.

The following variables were assessed by the treating physician: general data (referral, age in years, onset in days, treatment before presentation, treatment during and after admission); Symptoms and signs (General: ill appearance, refusing food, headache, shivering, capillary refill time, hypotension, tachycardia, cold limbs; Neurologic: irritability (inconsolable crying or screaming), lethargy, altered mental state, bulging fontanel, photophobia, positive Kerning sign, positive Brudzinski sign, abnormal pupil, seizures, unconsciousness, paresis; Gastroenterological: vomiting, abdominal pain, diarrhoea; Musculoskeletal: muscle pain; Respiratory symptoms and signs; Ear nose throat symptoms). The work-up consisting in: complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), polymerase chain reaction assay (PCR), prothrombin time (PT), activated partial thromboplastin time, Monospot test, nasopharyngeal rapid tests and blood culture. Cerebrospinal fluid (CSF) culture where performed in 6 children with the clinical suspicion of meningitis.

The protocol used to guide the management of a child presenting fever and petechial rash presenting in our Emergency Department is presented in fig. 1.

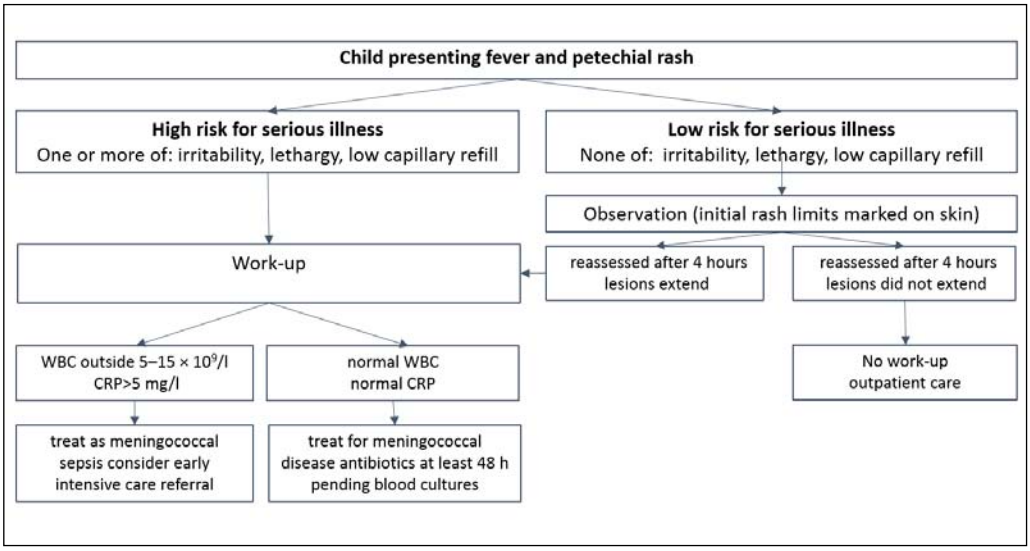

Fig. 1. Management of children presenting with fever and petechial rash in the emergency department

Definitions

Children were classified in two categories: serious illness; non-serious illness. 'Serious illness 'was attributed when children with positive urine/blood/CSF cultures, positive PCR tests from CSF, or focal lesions on chest X-ray. The classification of 'non-serious illness 'was assigned to children whose reference standard tests for serious bacterial infection (chest radiograph, urine / blood/CSF culture) were negative or the tests were not done.

Ethical consideration

Ethical approval was obtained from the Hospital's Ethics Committee. As study procedures consisted of an interview that enabled chart completion by the treating physician, the parent/legal guardian of each participant provided verbal consent. The study respects the Helsinki Declaration regarding confidentiality of data collected.

Statistical analysis

All analyses were conducted using *SPSS 19.0* for Windows. Statistical significance between continuous variables was assessed using student T-test. The $p < 0.05$ was considered statistically significant. We used Spearman rank correlation method. We analysed the sensitivity and specificity of signs, symptoms and testing results for the diagnosis of serious illness. We used Cohen's kappa coefficient to measure inter-rater agreement between variables and the diagnosis of serious illness. We performed binary logistic regression analysis (serious illness=1; non-serious illness=0) for each variable. Variables that reached statistical significance were included in a group model. Binary logistic regression analysis was done for the group model. We calculated the area under the curve for the model to estimate its accuracy.

RESULTS

Out of 7327 children addressing the hospital's Emergency Department, from August 2011 to April 2012, 44 children (0.6%) associated fever with petechial rash at presentation. Table I presents baseline characteristics of 41 children assessed according to the study protocol.

Most children presenting with fever and petechial rash were under 5 years of age (n=29; 70.7%), with a peak between 2 and 5 years (n=17; 41.5%). Female to male ratio was 1:2.4. The most common clinical diagnoses in children presenting with fever and petechial rash were: viral respiratory illness (n=20; 48.8%), upper respiratory tract infection (n=7; 17.1%), pneumonia (n=3; 7.3%); asthma exacerbation (n=3; 7.3%); and gastroenteritis (n=3; 7.3%). Meningococcal disease was found in one case (2.4%). One child presenting with urinary tract infection (UTI) also had infectious mononucleosis.

Spearman correlations between the diagnosis of serious illness and referral (from self/general practitioner/ambulance); onset of rash (day before presentation/day of presentation) were not significant.

TABLE I.

Baseline characteristics, for those with and without a serious illness

	Serious illness n=8	Non serious illness n=33	p value
Age in years, mean (SD)	3.77 (2.5)	3.2 (2.7)	0.958
Gender Male (%)	7 (87.5)	22(66.6)	0.003
Onset day before (%)	3(37.5)	8(24.2)	0.750
Onset presentation day (%)	5(62.5)	25(75.8)	0.750
ATB before presentation (%)	2(25)	6(18.2)	0.160
Referral from GP (%)	7 (87.5)	22 (66.6)	0.066
Self-referral (%)	1(12.5)	10 (30.3)	0.000
Ambulance (%)	0(0)	1 (3)	0.000
WBC / mm^3 mean (n/total)	12220 n=8/8 >15000/mm^3 n= 0/8 < 5000/mm^3	11170 n=2/3315000/mm^3 n= 1/33 < 5000/mm^3	0.929
Platelet count / mm^3 mean (n/total)	360111 n=0/8 <50000/mm^3	325437 n=0/33 <50000/mm^3	0.727
CRP mg/l mean (n/total)	4.05 n=2/8 > 6 mg/l	2.28 n=4/33 > 6 mg/l	0.946
ESR mm/h mean (n/total)	64.11 n=8/8 >15 mm/h n=6/8 >50 mm/h	30.03 n=25/33 >15 mm/h n=4/33>50 mm/h	0.916
Prothrombin time sec mean (n/total)	13.74 n=0/8 >15 sec	13.33 n=0/33 >15 sec	0.380
APTT sec mean (n/total)	29.24 n=0/8 >70 sec	30.76 n=0/33 >70 sec	0.069

SD=standard deviation; sec=seconds; ATB=Antibiotics;
CRT=capillary refill time; APTT= Activated partial thromboplastin time

Workup and diagnosis:

Ten children presented with leucocytosis, while one case presented with leucopenia. The anomalies were not correlated with the diagnosis of serious illness.

Erythrocyte sedimentation rate (ESR) was>15 mm/h in 32 children; however, it was poorly correlated to serious illness diagnosis. If using the cut-off ESR=50 mm/h (area under the curve =0.814, p=0.006), 89% of serious illnesses would be correctly identified as such, and 6.1% of non-serious illness would be incorrectly identified as positive. CRP above 6 mg/l was recorded in 6 children (4 in the non-serious illness group and 2 in the serious illness group) and was poorly correlated to serious illness diagnosis. The area under the curve for CRP (area =0.783, p=0.010), using the cut-off of 6 mg/l, shows 33.3 % of all serious illnesses would be correctly identified as such, and 9.4% of all non-serious illness would be incorrectly identified as positive.

Monospot test was positive in a single child. Prothrombin time ranged between 12 and 16.5 probably due to mild dehydration. The activated partial thromboplastin time was in normal range in all children. Platelet count was in normal range for all children.

Table II shows the blood and CSF cultures and PCR in regards to diagnosis.

TABLE II.
Diagnosis based on work-up (CSF, blood culture, PCR)

Total	Class	Initial Diagnosis	CSF culture/PCR	Blood Cultures	Blood PCR	Anti-biotic
Fever and petechiae as initial symptoms n=41	Serious infection n=8	Meningitis n=2	N. Mening. Gr B n=1; enterovirus n=1	N. Mening. Gr B n=1; Negative n=1	N. Mening. Gr B n=1; Negative n=1	n=2
		Pneumonia n=3	-	Negative n=3	Negative n=3	n=3
		Bacteremia n=2	-	gram negative diplococci n=1; Gr A Strep n=1	Negative n=2	n=2
		UTI, mononucleosis n=1	-	Negative n=1	Negative n=1	n=1
	Non-serious infection n=33	Viral respiratory illness n=20	Negative n=5	Negative n=20	Negative n=20	n=10
		URI n=7	-	Negative n=7	Negative n=7	n=6
		Gastro-enteritis n=3	-	Negative n=3	Negative n=3	n=0
		Asthma exacerbation n=2	-	Negative n=2	Negative n=2	n=0
		Physical Exertion n=1	-	Negative n=1	Negative n=1	n=0

n=number of patients, URI diagnosis was established after positive results of nasopharyngeal rapid antigen detection tests for group A strep, nasopharyngeal rapid tests for viruses or nasopharyngeal cultures. The antibiotic treatment refers to acute treatment after discharge from emergency department; N. Mening. Gr B= Neisseria Meningitis Group B; Gr A Strep= Group A streptococci

One third of children showed at least 2 signs of the "ILL" criteria. We used Cohen's kappa coefficient to measure inter-rater agreement between variables and the diagnosis of serious illness. Six variables were in significant agreement with the diagnosis of serious illness (kappa value statistically significant - tab. IV): ESR above 50 mm/h, ill appearance, shivering, lethargy, prolonged capillary refill time and back rigidity. These were included in a group model. The area under the curve for the model was=0.907 (CI 95%: 0.765 to 1).

TABLE IV
Sensibility and specificity of significant variables.

Variable	Kappa	p	Sensitivity % Sensitivity CI %	Specificity % Specificity CI %	PPV% PPV CI %	NPV % NPV CI %
Lethargy	0,21	0,05	87,5	50	28	94,74
CI %			47.4 to 97.9	32.9 to 67.1	12.1 to 49.4	73.9 to 99.1
Ill appearance	0,29	0,04	62,5	75	35,7	90
CI %			24.7 to 91.0	57.8 to 87.8	12.9 to 64.8	73.4 to 97.8
Shivering	0,27	0,05	50	80,6	36,36	87,9
CI %			16.0 to 83.9	63.9 to 91.8	11.2 to 69.1	71.8 to 96.5
CRT	0,19	0,03	12,5	100	100	83,7
CI %			2.0 to 52.6	90.2 to 100	16.6 to 100	69.3 to 93.2
Back rigidity	0,19	0,03	12,5	100	100	83,7
CI %			2.0 to 52.6	90.2 to 100	16.55 to 100	69.3 to 93.2
ESR >50 mm/h	0.57	0.00	75	87.9	60	93.5
CI %			35.1 to 96.1	71.8 to 96.5	26.4 to 87.6	78.5 to 99.0

Significant variables were selected if they had a significant Cohen coefficient and reached both specificity and sensitivity above 50%. PPV=Positive Predictive Value; NPV=Negative Predictive Value; CRT= Capillary refill time.Positive Brudzinski sign; seizures; unconsciousness; paresis were not seen in any the patients, thus sensitivity and specificity analysis could not be performed.

Treatment

Most children (58.5%) were discharged from emergency department after a maximum of twelve hours, while 41.4% were admitted in the hospital. Ten children (24.4%) were treated with antibiotics before presentation, but this factor was not significantly correlated with the diagnosis of serious illness. In the emergency department, antibiotics were prescribed acutely in all the children with a diagnosis of serious illness: meningitis (n=2), pneumonia (n=3); bacteraemia (n=2), urinary tract infection (n=1). However, 28.6% (8/28) of children without bacterial infection were also prescribed antibiotics.

DISCUSSION

The majority of children with fever and petechiae in our study who did not have an identified cause for the rash were consid-

ered to be presumably due to viral infections. We documented that from those children addressing the emergency care department in the regional hospital, for fever and petechial rash, 18% had serious illness, while Meningococcal meningitis was found in only 2% of children included in the study. However, Baker et al had found a higher prevalence of meningococcal disease of 7% among children with fever and petechiae (12). Although the prevalence of meningococcal disease was low, the need for triage is high as acute illnesses in children are extremely common(1). Nevertheless, recognition and early treatment of the child with meningococcal disease is vital.

Most of children presenting with fever and rash in our study were males under the age of 5 years. Young age is reported in other studies (13,14), but we did not find other reports showing higher prevalence in males. Clinical signs and laboratory investigations determine management and treatment. In many situations, unnecessary tests or treatments are initiated which could be detrimental to the patient and cost efficient. The significant criteria for diagnosing serious illness, included in our model were: ESR above 50 mm/h, ill appearance, shivering, lethargy, prolonged capillary refill time and back rigidity. This model had good accuracy for predicting the diagnosis of serious illness in our data.

The "ILL" criteria model is largely used; however, this model has several limitations and needs further improvement and revalidation. The accuracy of the ILL criteria in our study was poor. We have found that the 'ill appearance' was a strong predictor for serious illness, nonetheless, this is a subjective interpretation of symptoms/signs. Where physicians need to triage patients and refer them to secondary care if necessary, this 'gut feeling' of 'something is wrong' could prove very useful, summarizing physician's experience (15,16).

In our analysis the C-reactive protein had poor specificity in detecting serious illness in our study. Other studies found that CRP provides moderate information for ruling in/out serious bacterial infection in children with fever at presentation. Asseray et al concluded that the cost generated by CRP examination is not justified (17). The cost of using superior tests, like procalcitonin, for decision making in the association of fever and petechiae in the emergency department, might be more specific but it was not evaluated in our study (18). Our data suggests that using a different cut-off for the ESR might provide higher specificity in diagnosing serious illness.

Antibiotic treatment was prescribed in all cases suspected of bacterial infection, however, at discharge 41% of children, did not have a work-up suggestive for a bacterial illness. Antibiotic overuse promotes the development of drug-resistant bacterial strains, increase treatment failure rates and costs (19,20).

Strengths and limitations of the study

The paper addresses a particular topic of interest to paediatricians and emergency department physicians, as there is a paucity of useful literature regarding clinical decision-making in children presenting with fever and petechial rash. The relatively small number of patients decreases the statistical power of the study. The study did not record the follow-up; thus we were unable to establish the evolution under treatment. Although we did not follow up all patients, we assumed they did not have adverse outcomes, as no patient died and no patient returned to hospital. Details

regarding localization or number of petechial lesions were not noted, which might produce higher predictive value.

CONCLUSIONS

Most children presenting with fever and rash in the paediatric emergency department were under 5 years of age. The most common clinical diagnoses were: viral respiratory illness and upper respiratory tract infection. Meningococcal disease was found in one case. CRP>6 mg/l was poorly correlated with serious illness. The following variables were strongly associated with serious illness: ill appearance, shivering, lethargy, back rigidity, ESR>50 mm/h and prolonged capillary refill time. Most children were treated with antibiotics, however, at discharge almost half of them, did not have a work-up suggestive for a bacterial illness.

Screening for low prevalence but high morbidity conditions, as the meningococcal disease, with an extensive work-up is time and resource consuming and may lead to unmotivated antibiotic use. Larger studies are needed to change the emergency practice for management of fever and rash.

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NEWS

EVALUATE THE DIFFERENT RAPID SCREENING TESTS FOR DIAGNOSIS OF LEPTOSPIROSIS

Leptospirosis is a worldwide zoonosis caused by spirochetes of the genus Leptospira. The disease is endemic in some tropical and subtropical region and exposure to infection is widespread. Leptospirosis is characterised by wide clinical variability, ranging from a mild flu-like illness to an acute life threatening condition, but only patients with the symptomatic forms of the disease are hospitalised. Leptospirosis is a common cause of acute febrile illness in tropical climate and must be differentiated from other infection like typhoid, malaria, dengue, scrub typhus, viral hepatitis etc. Early diagnosis of Leptospirosis is important since mortality rate is high in patient with severe Leptospirosis. Diagnosis of Leptospirosis is often made by serological tests. The MAT is the serological test used in reference laboratories because of its high degree of sensitivity and specificity. However, MAT is a complex test that requires significant expertise and large panel of live-cell suspensions, as well as, antibody levels detectable by MAT usually appear after day 6 or 7 of symptoms. Hence, interpretation of the results is difficult and results are usually not available quickly enough for patient management. The early diagnosis of Leptospirosis is now possible by using different serological methods which are available commercially in the market such as an IgM ELISA, an IgM dipstick assay (LDS), latex agglutination test, lepto lateral flow test and the indirect hemagglutination assay (IHA). Therefore, rapid and easy to perform tests have emerged in recent years for the diagnosis of Leptospirosis. Majority of these rapid tests are immunochromatographic or particle agglutination tests. The introduction of such tests in the market needs their evaluation by comparing their results with the gold standard MAT or other tests like IgM ELISA. The present study was to evaluate the usefulness of the diagnostic test kits (leptocheck WB, Latex agglutination test and SD leptospira) for the diagnosis of Leptospirosis by comparing their results with the 'gold standard' test, MAT and IgM ELISA (Panwala T, Rajdev S, Mulla S, et al. To Evaluate the Different Rapid Screening Tests for Diagnosis of Leptospirosis. *Journal of Clinical and Diagnostic Research*. 2015, Vol-9(2): 21-24).

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PLATELET INDICES AND LIVER FIBROSIS EVALUATION IN CHRONIC HEPATITIS C

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PLATELET INDICES AND LIVER FIBROSIS EVALUATION IN CHRONIC HEPATITIS C (Abstract): Recently, several studies have reported that the mean platelet volume and platelet distribution width may give information about liver fibrosis severity in chronic hepatitis C. **The aim** of the present study was to evaluate whether platelet indices correlate with hepatic fibrosis measured by transient elastography in patients with chronic hepatitis C. **Materials and methods:** Patients with chronic hepatitis C were prospectively enrolled. Samples for complete blood count and routine biochemical parameters were collected and analyzed in the same day with liver fibrosis assessment by transient elastography. Mean platelet volume, platelet large cell ratio and platelet distribution width were compared with stages of liver fibrosis. Statistical analysis was carried out using SPSS 17.0 software. A P-value of less than 0.05 was considered statistically significant. **Results:** There were 139 patients with chronic hepatitis C (70.5% males, mean age 54.8±16.7 years). Compared with mild/moderate liver fibrosis patients, those with advanced liver fibrosis had an increased mean platelet volume (10.4±0.7vs. 10.9±0.9, p<0.002), platelet large cell ratio (28.5±5.3 vs. 32.5±7.2, P<0.0001), and platelet distribution width (12.8±1.5 vs. 14.1±2.7, P=0.003). **Conclusions:** Increased platelet indices were associated with advanced liver fibrosis stages evaluated by transient elastography in patients with chronic hepatitis C. **Keywords:** PLATELET INDICES, LIVER CIRRHOSIS, HEPATITIS C, FIBROSIS, TRANSIENT ELASTOGRAPHY.

Today, chronic hepatitis C virus (HCV) infection affects more than 180 million individuals worldwide, being among the world's leading causes of death and the major reasons for the increasing prevalence of liver cirrhosis and hepatocellular carcinoma (1).

Staging of liver fibrosis is important for the estimation of prognosis, for surveillance and for treatment decisions in patients with HCV. Liver biopsy is still considered "gold standard" for the staging of liver fibrosis in spite of its several shortcomings such as intra- and inter-observer variability in histopatologic interpretation, sampling errors, variable accessibility, and high cost; in addition, it is an invasive procedure, sometimes associated with rare but potentially life-threatening complications (2,3). Therefore, noninvasive methods such as serum fibrosis markers and ultrasound-based elastography have been inten-

sively evaluated for the assessment of liver fibrosis over the past two decades and many of them have proven to be excellent tools for staging fibrosis in patients with chronic HCV (4). Serum fibrosis markers may be used singly (5) or in combination, some of which are patent-protected and commercially available: FibroTest® (6), Fibrometer® (7), ELF® (8), and Hepascore® (9), with no risk for the patient and no contraindication, with interlaboratory reproducibility. The advantage of serum markers is that they are largely available and reproducible; the disadvantage is that they can be influenced by the comorbidities and medications. A simple, cost-free, readily available test in all hospital setting is APRI (AST/platelets ratio) (10). Transient elastography (TE) measures liver stiffness and has highest level of performance among noninvasive methods for diagnosis of advanced fibrosis and cirrhosis (11).

Over the last few years, several studies have evaluated the platelet indices for the assessment of liver fibrosis in patients with chronic hepatitis B virus (HBV) (12,13) and HCV (14). Platelet indices include mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR). Some studies have reported that MPV and PDW may give information about liver fibrosis severity in HBV and HCV (12-14), although the relationship between these and other platelet indices and liver fibrosis is still controversial (13,15). Recently, Purnak et al. demonstrated that increased MPV is an independent predicting factor for liver fibrosis in patients with chronic HCV (14). However, to our knowledge, the association between platelet indices and liver fibrosis in hepatitis C virus related liver disease remains mainly unknown.

The aim of the present study was to evaluate whether platelet indices correlate with hepatic fibrosis measured by TE in patients with chronic HCV.

MATERIAL AND METHODS

One hundred and thirty-nine patients with known chronic HCV naïve to antiviral treatment and followed-up in a tertiary care center, who have undergone TE examination from January 2013 to June 2014 were prospectively enrolled in this study. Exclusion criteria involved all conditions that are known to modify platelet indices such as chronic liver diseases other than HCV, type 2 diabetes mellitus, hypertension, hypercholesterolemia, obesity, malignancy, and hypochromic anemia (16,17). None of the patients included have received anticoagulant or antiaggregant medications, nonsteroidal antiinflammatory drugs (NSAID) and oral contraceptives before hospital admission because all these drugs could influence platelet aggregability.

The study protocol was approved by the local Ethics Committee, and all patients were consecutively enrolled to the study after providing written informed consent.

Liver fibrosis assessment. All patients had liver stiffness measured by TE (FibroScan®, Echosens, Paris, France). At least 10 valid measurements, a 60% success rate, and an interquartile range of less than 30% of the median elasticity were required for a measurement to be valid. The liver stiffness was expressed in kiloPascal (kPa), and values range from 2.5 to 75 kPa, with normal values <5.5 kPa (18). According to Metavir score, a cutoff value of 8.7 kPa defines mild (5.6-7.1 kPa=F1) or moderate (7.2-8.7 kPa=F2) fibrosis, while values ≥ 8.7 kPa defines advanced fibrosis (8.8-12.5 kPa=F3; ≥12.5 kPa=F4) [19]. In this study

patients were divided in two groups: mild/moderate fibrosis (F1-F2) and advanced fibrosis (F3-F4).

Serum measurements. Samples for complete blood count analysis were obtained by phlebotomy using minimal pressure and collected into a vacutainer tube, containing ethylenediaminetetraacetic acid (EDTA). These blood samples were analyzed within 2 h on automatic blood counter Sysmex XT-4000i (Kobe, Japan). All tests were performed according to the standardized protocols recommended by the manufacturers (17).

For the determination of platelet indices we used an automated hematology analyzer that could overestimate platelet count especially in thrombocytopenic samples compared with the immunological reference method. The platelet indices were derived from the platelet distribution curve obtained from impedance method. It includes MPV that describes the average platelet size reported in femtolitre (fL), PDW defined as the distribution width at 20% frequency level calculated as the standard deviation of platelet volume divided by MPV, and measures the heterogeneity in platelet size. Derived platelet indices are highly specific to the individual technologies, with different analyzers having different reference ranges. As separate feature, the platelet large cell ratio, P-LCR, can be reported specifically on Sysmex analyzers and is the percentage of platelet larger than 12 fL.

Statistical analysis was carried out using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as median and interquartile ranges and categorical data as percentage. Mann-Whitney U test was used to compare groups, with the chi-square test used for categorical variables. A P value of less than 0.05 was considered statistically significant.

RESULTS

Patient characteristics. In the study period there were admitted 658 patients with chronic liver disease, 226 (34.4%) were diagnosed with HCV, and 139 (61.5%) met the inclusion criteria. There was no difference regarding age, sex or severity of liver disease between patients included and those excluded from the study.

Clinical and biochemical characteristics of the patients are summarized in tab. I. There were 139 patients with chronic HCV (70.5% male, mean age 54.8±16.7 years).

The fibrosis spectrum in patients with chronic HCV was as follows: 25 (17.9%) with stage 1, 39 (28.1%) with stage 2, 15 (10.8%) with stage 3, and 60 (43.2%) with stage 4. All the patients with stage 4 liver fibrosis had compensated liver disease, Child-Pugh class A. Of these 9 (15%) were diagnosed with esophageal varices.

Significant increases in serum alanin aminotransferase, aspartate aminotransferase, alkalin phosphatase, and gamaglutamyl transpeptidase, and significant decreases in albumin, hemoglobin, leucocytes and platelet count were observed in patients with advanced fibrosis (F3-F4).

Comparison of platelet indices between the study groups. There was a significant difference regarding the MVP, PDW and P-LCR values between the patients with mild/moderate fibrosis and those with severe fibrosis ($P=0.002$, $P=0.003$, $P<0.0001$). latelets counts were significantly lower in patients with advanced fibrosis compared with those with mild/moderate fibrosis (240.8±52.1 vs. 156.7±75.8, $P<0.0001$) (tab. II).

TABLE I

Baseline clinical and biochemical characteristics of study population

Variable	F1-F2 median (IQr) (n=64)	F3-F4 median (IQr) (n=75)	P
Age, yrs	58 (31)	65 (16)	**0.002**
Leucocytes,/mm^3	7150 (1950)	5210 (1680)	**<0.0001**
Hemoglobin, g/dl	13.9 (1.7)	13.7 (2.45)	**0.030**
Platelets, 10^9/L	233 (76)	142 (161)	**<0.0001**
ALT, UI/L	24 (20)	69 (288)	**<0.0001**
AST, UI/L	22 (4)	65 (133)	**<0.0001**
Alkalin phosphatase, UI/L	84 (35)	108 (57)	**<0.0001**
GGT, UI/L	37 (21)	73 (108)	**<0.0001**
PT, sec	11.4 (1.3)	11.9 (2.05)	0.086
Albumin, g/l	4.5 (0.5)	3.5 (0.4)	**<0.0001**
Total bilirubin, mg/dl	0.66 (0.46)	0.74 (0.48)	**0.001**
CRP, mg/L	0.12 (0.17)	0.10 (0.16)	0.617
Fibrinogen, mg/dl	367 (98.75)	326 (78.25)	0.464
Spleen size, mm	114 (24)	126 (21)	**<0.0001**
Portal vein diameter, mm	9 (1)	13 (2)	**<0.0001**

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CRP* C-reactive protein, *GGT* gamaglutamyl transpetidase, *IQr* interquartile ranges, *PT* prothrombin time.

TABLE II

Platelet indices in relation to the fibrosis stages in patients with chronic HCV

Variables	F1-F2 median (IQr) (n=64)	F3-F4 median (IQr) (n=75)	F	P
MPV, fL	10.5 (0.90)	11.0 (1.7)	9.820	**0.002**
P-LCR, %	28.8 (6.70)	32.5 (13.90)	13.762	**<0.0001**
PDW, %	12.65 (2.10)	13.9 (4.75)	9.362	**0.003**

IQr interquartile ranges, *MPV* mean platelet volume, *PDW* platelet deviation width, *P-LCR* platelet large cell ratio

When all of the patients were considered in the analysis, the strongest and most inverse correlation was found between liver stiffness and platelet count (rs =-0.607, P <0.001), followed by serum albumin (rs =-0.566, P <0.001), and prothrombin time (rs =-0.505, P <0.001). There was also a strong correlation between liver stiffness and spleen size (rs = 0.504, P <0.001), followed by serum ALT, and then by total bilirubin (rs=0.358, 0.335, respectively, P <0.001).

There were no correlations between platelet indices and liver stiffness value measured by transient elastography.

DISCUSSIONS

Liver biopsy is still considered gold standard for the staging of liver fibrosis. However, its diagnostic sensitivity is limited by significant variability of the sampling and histological assessment (small sample size not representative to the entire

liver) (2,3). Furthermore, severe complication such as bleeding, hemoperitoneum, or even death could occur in some patients (20). All these disadvantages make it impractical to assess disease progression by serial biopsies in routine clinical practice (21).

Transient elastography (Fibroscan®) is reproductible, non-invasive and validated test for the assessment of liver fibrosis (11,22). Along with this imagistic method, there are also several direct and indirect markers used alone or in combination, some patent-protected, which have proven excellent methods for staging fibrosis in patients with chronic HCV and HBV (4-7). The advantages of all noninvasive methods include no risk for the patient and no contraindication, widespread availability, easy to perform and repeat in outpatient clinic and at the bedside, well accepted by the patients and thus, can be repeated for longitudinal disease monitoring which is difficult, if not impossible, with liver biopsy (22,24). However, both Fibroscan® and some patent-protected serum markers are still expensive. It was this context that assessment of platelet indices triggered much interest as possible non-invasive, widely available, practically costless serum markers of liver fibrosis in patients with chronic HBV or HCV (12-15, 25, 26). Recently, there have been published several studies on chronic HBV patients, concluding that MPV might be a marker for assessment of liver fibrosis (12). Some studies have suggested that elevated MPV levels may be an independent predictor of cirrhosis (25) or associated with liver disease activity (26) in patients with chronic HBV or portal vein thrombosis (27).

Data on platelet indices and chronic HCV are scanty. Purnak et al (14) demonstrated that MPV is increased in patients with advanced fibrosis, and this marker along with other serological markers could give information about liver fibrosis severity in chronic HCV. The authors demonstrated that patients with HCV had significantly higher levels of MPV as compared to the control group, despite the same number of platelets, concluding that high levels of MPV could represent an indirect early marker of advanced liver fibrosis in such patients.

Our study showed that high platelet indices (MPV, PDW and P-LCR) could differentiate between mild/moderate and advanced fibrosis in HCV patients. These results confirmed some previously mentioned studies including patients with chronic HCV (14). It has been reported that increased platelet indices are directly related with thrombocytopenia, which is a direct reflection of platelet destruction by hypersplenism (28). In advanced stages of fibrosis thrombocytopenia is a multifactorial condition attributed to the sequestration of platelets by the spleen secondary to portal hypertension, reduced thrombopoetin which regulates megakaryocyte maturation and platelet production, and bone marrow suppression (28). All these mechanisms could influence platelet indices, because thrombocytopenia is not only a reflection of platelet destruction, but also of platelet production, especially in advanced chronic liver disease.

Our study has some advantages: such as being the first prospective study that evaluated the role of all platelet indices in patients with chronic HCV, using transient elastography for liver fibrosis quantification. It has also some limitations as relatively small sample size, and lack of evaluation of dynamics of platelet indices. Our

results need to be confirmed by further investigations on larger groups of patients.

CONCLUSIONS

In our study the platelet indices, MPV, PDW and P-LCR are associated with fibrosis stage measured by transient elastography in patients with chronic HCV, and they might be used as markers to evaluate liver fibrosis stage.

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NEWS

IMPACT OF BACTERIAL CELLS AND SECRETOMES ON WOUND HEALING

According to Brothers KM *et al.*, bacterial cells and secretomes from *Serratia marcescens (*95%), *Pseudomonas aeruginosa* (71%) and *Staphylococcus aureus* (29%) strains inhibit human corneal epithelial cell migration *in vitro* and *ex vivo*, thus affecting the process of wound healing. Lipopolysaccharide (LPS) seems to be a key factor in this inhibitory mechanism, since LPS core biosynthetic genes are required to inhibit corneal epithelial cell migration. Purified LPS from *S. marcescens* inhibits epithelial cell migration *in vitro* and wound healing *ex vivo*, while after LPS depletion of *S. marcescens* secretomes, migration of epithelial cells was no longer inhibited, suggesting that *S. marcescens* LPS is sufficient for inhibition of epithelial wound healing (Brothers KM, Stella NA, Hunt KM, Romanowski EG, Liu X, Klarlund JK, et al. Putting on the brakes: Bacterial impediment of wound healing. *Sci Rep*. 2015;5:14003. doi: 10.1038/srep14003.)

Teodora Vremera

INFORMED CONSENT IN PSYCHIATRIC MEDICAL CARE: A PROSPECTIVE QUANTITATIVE STUDY

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INFORMED CONSENT IN PSYCHIATRIC MEDICAL CARE - A PROSPECTIVE QUANTITATIVE STUDY (Abstract): Respecting fundamental ethics principles entails the process of obtaining an informed consent, which is a necessary requirement for offering good psychiatric medical care and conducting research studies on human subjects, who are regarded as the vulnerable population. Presently, in Romania, the informed consent regarding medical interventions is covered by the law of the patient's rights, Law 46/2003, chapter 3. **The aim** of this study is to evaluate the medical staff's perception of the need of the informed consent in the practice of psychiatric medical care. **Material and methods**: This is a quantitative, inquiry based study, with prospective evaluation, based on the responses of 217 medical staff members involved in treating patients with psychiatric disorders. The study unfolded between July 2012 and July 2013, and the questionnaires were answered by medical staff members (psychiatrists, general practitioners, other clinical specialists, psychologists) from Iasi, Botosani, Vaslui, Suceava counties. **Results**: The age group distribution of the medical staff involved in the medical care shows statistically significant differences between the groups questioned (χ^2=227.14; df = 5; p=0.001). Concerning the answers recorded at question no. 2, regarding informing the psychiatric patient, and comparing the studied groups of medical staff, a majority of affirmative answers was noted. **Conclusions**: The medical staff members involved in the patient's treatment recognizes the necessity of a full informed consent when discussing about psychiatric medical care. In psychiatry, the variation of competence raises multiple ethics discussions **Keywords:** INFORMED CONSENT, PSYCHIATRIC PATIENT, MEDICAL STAFF.

The notion of "consent" is frequently encountered in medical records and in specialized literature as "informed consent"; this clarification has the role of emphasizing the informational component of this valid document (1). The informed consent (2) is a very important concept in the relationship between the medical staff and the patient (3). The informed consent is a concept that has developed significantly in the last few years (4). It represents the legal way of validating the respect of personal

autonomy in medical practice and research (5). From an ethics point of view, the informed consent is a necessary stipulation when conducting research and in medical care (6, 7, 8). The consent is a specific legal document, required in written form after the patient has been provided with the complete set of information. The patient must receive information on the necessity of hospitalization, treatment, diagnosis, benefits and on risks involved. The doctor has to use a language that is adequate to the patient's educational level (9). The informed consent is the legal and ethics way by which a patient decides to join clinical research, after having received, understood and analyzed the given information. The decision to participate in the research must be uncorrupted and the informed consent must be signed before the study proceeds (10). Being an ethical and legal request, the informed consent is a documented requested both nationally and internationally, both in research and in medical care (7). The elements of an informed consent are: competence, communicating information, analyzing information, understanding information, voluntary decision (10).

This concept does not have old historical origins, and the written agreement, for both research and medical care, belongs to the modern ideologies of the 20th century (11, 12). Even though these regulations regarding the consent in clinical research have existed since the 19th century, the medical staff was either unaware of them, or ignored them (13, 14).

MATERIAL AND METHOD

This is a prospective questionnaire based study, applied to the medical staff involved in the healthcare of psychiatric patients. The study was conducted between July 2012 – June 2013 and had a research population of 271 members of the staff involved in the therapy of the psychiatric patient: psychiatrist, psychologists, general practitioners, doctors from other specialties (neurologists, neurosurgeons, emergency doctors) from medical centres in Iasi, Suceava, Botosani, and Vaslui. The questionnaires contain questions about the Mental Health Law, the informed consent, providing information, stigmatization, discrimination, confidentiality. The questionnaires followed all the steps from pretesting, revision, validation and application in the final form. The results were statistically processed for each item, subsequently making the correlation of the answers according to different characteristics of the study groups. The Cronbach's alpha value was 0.730, which offers an acceptable result when compared with the threshold of 0.70, and validates the use of the questionnaire to other categories of doctors and psychologists involved in monitoring people with psychiatric disorders.

Amongst the groups of doctors, the frequency distribution analysis according to the specialty highlights the prevalence of resident doctors in the group of doctors with other specialties, and of specialist doctors in the group of general practitioners, while in the group of psychiatrists, a homogeneous distribution can be noticed. The study uses the Kruskall-Wallis nonparametric method for highlighting statistical differences.

RESULTS:

In doctor groups, the distribution analysis according to specialty points out the predominance of resident doctors (54%) for the group of doctors from other specialties and specialist doctors (66%) for the group of general practitioners, whereas the psychiatrists group shows an even distribution. The

application of the Kruskall-Wallis intergroup non-parametric test points out statistically significant differences between the specialty of the doctors who answered the questionnaire (Chi-Square = 15.22; df = 2; p = 0.001).

The groups distribution according to sex shows the predominance of females, sex ratio F/M = 2.3/1. The smallest percentage of females is found in the group of psychiatrists, and the peak frequency is found in the group of doctors with different specialty than general practitioners, but the distribution does not show statistically significant differences when compared to the other groups (χ^2=0.38; df=3; p=0.944).

Concerning age groups, there can be distinguished a higher percentage of subjects questioned with ages between 30 and 39 years of age (49.8%). The distribution by age of the medical staff involved in medical care shows statistically significant differences between the groups questioned (χ^2=227.14; df = 5; p=0.001):

- In the psychiatrists group, most of the subjects have ages between 30 and 39 years old (56.1%), and 12.3% have ages over 60 years of age;

- In the group of doctors with different specialties, subjects in the age group 30 – 39 years old prevail (46%), followed by the under 30 years group (32%);

- 46% of the general practitioners had ages between 30 and 39 years, and 36% had ages in the 40 – 49 years interval;

- 50% of the psychologists had ages between 30 and 39 years and 38.3% between 40 and 49 years.

Concerning answers recorded at question no. 2, affirmative answers in all studied groups were noted when comparing the groups that were studied.

"Do you consider that patients with psychiatric disorders must be informed about the benefits and side effects of the treatment before initiating it?:

a. Yes, the psychiatric patient has the right to an adequate informed consent that has to be followed;

b. In the case of patients with psychiatric disorders, I would provide a minimum of information;

c. The patient with psychiatric disorders must not be informed;

d. I do not know" (tab. I).

TABLE I

Distribution of answers to question no. 2 on the groups studied

Question no. 2	Psychiatrist		Different specialties		General practitioner		Psychologist	
	n	%	n	%	n	%	n	%
Yes	55	96.5%	41	82.0%	41	82.0%	51	85.0%
Minimum info.	2	3.5%	7	14.0%	7	14.0%	8	13.3%
No					2	4.0%		
I don't know			2	4.0%			1	1.7%
Mean rank	98.22		114.20		114.10		110.66	

The mean rank of the answers to question no. 2 did not show any statistically significant differences between the questioned groups (Chi-Square = 6.88; df=3; p=0.076):

- Only 3.5% of the psychiatrists consider that they would provide the minimum of information regarding the therapeutic approach;

- Providing minimum information is

found in 13-14% of each of the other groups questioned;

- In the general practitioners group, 4% do not consider that patients with psychiatric disorders must be informed about the benefits and side effects of the treatment, while in the psychologists' group 1.7% does not know what to answer to this question.

DISCUSSION

The purpose of the study was to assess the perception of healthcare professionals with regard to the need and importance of informed consent in medical care. The process of obtaining a valid informed consent is extremely important in both medical research and psychiatric healthcare. Transposing the informed consent in the medical practice is frequently hampered by difficulties in informing the patient in such a manner for him to understand the information, by spoiling its voluntary character etc. (7). The study shows statistically significant differences between the specialty of the doctors who answered the questionnaire (Chi-Square = 15.22; df = 2; p = 0.001). In psychiatry, the variation of competence creates multiple ethical discussions. An informed consent is valid only when the study's subject or the patient receives all the relevant information and comes to a decision freely (15). The significant impairment of the cognitive functions, especially in patients with certain psychiatric disorders, compromises the minimal conditions necessary for a valid informed consent in most of the cases. Also, there are cases when the informed consent cannot be obtained or the informed consent is invalid for minors, some patients with dementia, patients with mental retardation, patients considered a vulnerable category (16). The ethical controversies in psychiatry regarding the admission, respectively the treatment, are channelled especially on the situations in which the person with the psychiatric disorder cannot give his/her consent (17, 18).

In psychiatric healthcare, the medical staff assigned to a patient with psychiatric disorders should try and obtain the free consent of the patient; however, most of the times, they feel compelled to limit the patient's freedom and use constraint (19, 20). This study shows that for the group of general practitioners, 4% do not consider that patients with psychiatric disorders must be informed. The mental capacity of patients with psychiatric disorders is an issue that raises multiple ethical and legal concerns, especially in cases where coercive measures are required (21, 22, 23). In cases of dementia, depending on the type: Alzheimer disease, vascular dementia or any other form of dementia, severe cognitive impairment is always correlated with incompetence (24). Studies show that for patients with psychiatric disorders, like schizophrenia and depression, at least one of the components of the informed consent is impaired (25). All elements of an informed consent are impaired in patients with schizophrenia (26). In schizophrenia, obtaining a valid consent is a continuous challenge, especially in medical research (27).

Diminished mental capacity is more frequent in patients with psychotic disorders rather than in patients with non-psychotic disorders (28). According to studies, the most important predictor for reduced mental capacity in psychiatric disorders is the absence of awareness of the disease and of the necessity of the psychiatric treatment (29). In the last 20 years a great focus has been directed towards developing tools for assessing mental capacity (31).

According to some studies, factors strongly associated with diminished or absent mental capacity are non-voluntary admission, compulsory treatment, severity of symptoms and certain types of psychoses (22). Patients with psychiatric disorders, who were hospitalized non-voluntarily in psychiatry hospitals, are more frequently associated with reduced or absent mental capacity, when compared to patients who were hospitalized voluntarily (23). Multiple studies demonstrated that the severity degree of the psychiatric pathology (30) is associated with reduced or absent mental capacity (32, 33).

CONCLUSIONS

In this study, the majority of staff members involved in therapy recognize the psychiatric patient's right to be informed. However, a small percentage of the participants, more precisely of the general practitioners, invalidate this right.

In psychiatry, the variation of competence raises multiple ethical discussions. The impairment of cognitive functions, especially in some psychiatric disorders, undermines the necessary conditions for a valid informed consent. The issue of informed consent in psychiatric care is extremely complex.

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HUMAN PAPILLOMA VIRUS GENOTYPING IN FRESH HEAD AND NECK TUMORS - OUR FIRST EXPERIENCE

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HUMAN PAPILLOMA VIRUS GENOTYPING IN FRESH HEAD AND NECK TUMORS - OUR FIRST EXPERIENCE (Abstract): A growing number of research papers on the role of HPV in the evolution of head and neck cancers have been published in recent years. In these cancers, the prevalence of HPV DNA varies greatly by study, cancer site and geographic location. The most consistent finding relates to the oropharyngeal cancers, where HPV DNA has been found in 35–50% in developed countries in contrast with the rest of the oral cavity, where HPV/DNA was found in 5–15% of the cases. **Aim**: To implement the HPV genotyping method of fresh head and neck cancer samples in our laboratory. **Material and methods**: Fresh tumor samples 3/3 mm in size were collected from 25 patients with tumors of the head and neck: tongue, lips, base of the tongue, oropharynx, and hypopharynx admitted to different surgical clinics in NE Romania. DNA was purified with *Wizard Genomic DNA Purification Kit*. DNA purity (260/280: 1.86 – 1.98) and concentration (227.5 – 1640.1 ng/µl) were measured using a Nanodrop. HPV genotyping was performed using *Linear Array HPV Genotyping* test (*Roche*) in accordance with manufacturer's recommendations. **Results**: Four (16%) of the 25 patients in the study group were women and for all patients the median of age was 67 (range: 58 – 88 years). More than half of the patients recognized being chronic alcohol and tobacco users. All patients were negative for all 37 HPV genotypes tested with *Linear Array*. We validated our results because on all test strips beta globin (low/high) was detected, and the results for negative and positive controls were within the limits indicated by the manufacturer. **Conclusions**: The genotyping method was validated according to kit instructions. To get a general idea on the prevalence of HPV genotypes in head and neck cancer in our region we tested a very small number of samples. It would be useful to continue this study by testing a larger number of patients and using more sensitive methods, such as HPV E6mRNA, cytology, viral load, and HPV16. **Keywords**: HPV, PREVALENCE, HEAD AND NECK CANCERS, RISK FACTORS, DE-ESCALATION THERAPY.

According to GLOBOCAN 2012 (the project of International Agency for Research on Cancer which provides contemporary estimates of the incidence, mortality and prevalence from major types of cancer, at national level, for 184 countries of the world) Romania ranks second in mortality from larynx, lip, oral cavity and nasopharynx cancer in all-age males (32.4/100,000) among 20 European countries (1). Human papillomavirus (HPV) infection is considered a major prognostic and predictive marker in head and neck squamous cell carcinoma (HNSCC). In these cancers, the prevalence of HPV DNA varies greatly by study, cancer site and study location. The most consistent finding relates to the oropharyngeal cancers, where HPV DNA has been found in 35–50% in developed countries in contrast with the rest of the oral cavity, where HPV DNA is found in 5–15% of the cases (2).

As in cervical cancers, for which many HPV testing techniques have been developed, molecular methods for HPV detection in HNSCC are being evaluated with regard to their sensitivity, specificity and clinical relevance. HPV detection in these tumors is relevant to prognosis even in an already-developed tumor, whereas in cervical cancer, where HPV is the cause of almost all tumors, this information has less clinical relevance (3). The study by van Houten VM et al. showed the "missing" biological link, which clearly demonstrated that high-risk HPVs are indeed responsible for a number of HNSCCs by the detection of HPV DNA in tumor samples, presence of viral E 6 oncogene, detection of wild-type p53 gene, and also by the detection of HPVDNA and E6 in the corresponding lymph node metastases (4).

In NE Romania the burden of head and neck cancers is high as between January 2010 – September 2014 we detected 226 such cases: 119 oral cavity cancers – lips and tongue (age range 39-80 years), 1.68% (12/119) women; 56 larynx cancers (age range 47-58 years), 3.57% (2/56) women; 15 hypopharynx cancers (age range 54-67 years), 26.66% (4/15) women; 36 oropharynx cancers (age range 41-71 years), 5.55% (2/36) women. Most of these patients were diagnosed with keratinizing and nonkeratinizing squamous cell carcinoma (5). Testing of the corresponding FFPE samples for HPV DNA presence is subject of an ongoing research study.

In this study we aimed at optimizing the HPV genotyping method in fresh head and neck tumors, in order to further evaluate the prevalence of HPV in head and neck cancers.

MATERIAL AND METHODS

In the interval July 2014 – May 2015 we collected 25 fresh tumor samples 3/3 mm in size from patients treated for different tumors of head and neck (tongue, lip, base of the tongue, oropharynx, hypopharynx) in different surgical department: ORL, OMF and Plastic Surgery.

The DNA was purified with Wizard Genomic DNA Purification Kit. The procedure involved one step of overnight incubation at 55º C/300 rpm of the tumor sample with EDTA 0.5 M, nucleic lysis solution and proteinase K. The next steps consisted in successive addition of specific reagents (protein precipitation solution, ethanol 70%, rehydration solution) and centrifugation at 14.000 g for 1 and 4 minutes, respectively. After the rehydration solution was added, the DNA was kept in the fridge at 4ºC, and the quantification was performed the next day. DNA purity (260 / 280: 1,86 – 1,98) and concentration (227,5 - 1640,1 ng/µl) were evaluated with the Nanodrop. HPV

genotyping was done with *Linear Array HPV Genotyping* test (*Roche*).

Linear Array HPV Genotyping test was described previously, and it is based on PCR amplification (*Applied Biosystems GeneAmp PCR* System *9700 Thermal Cycler*) and strip hybridization in twincubator (6). At the time of hospital admission, the patients were informed about the study and written informed consent was obtained from all participants. The study protocol was approved by the Research Ethics Committee of the Iaşi University of Medicine and Pharmacy.

RESULTS

Four of the 25 participants (16%) were women and the median of age was 67 years (range 58 – 88 years). More than half of the patients recognized being chronic alcohol and tobacco users. All tested patients were negative for all the 37 HPV genotypes tested with Linear Array. We validated our results because on all test strips beta globin (low/high) was detected, and the negative and positive controls yielded results within the range specified by the manufacturer.

DISCUSSION

This pilot study fulfilled its objective of validating the strips of all tested patients. We have experience with the *Linear Array HPV Genotyping* test, as we correctly used it for genotyping over 1000 women with abnormal Pap test, and were declared "proficient" at the 2010 WHO HPV genotyping proficiency panel. However, when we first attempted to genotype fresh head and neck tumor samples we used *High Pure PCR Template Preparation Kit for DNA* purification. With this kit very low amounts of DNA and impure forms of DNA were obtained, making the validation of the strips impossible. With the *Wizard Genomic DNA Purification Kit* high amounts of pure DNA were obtained.

TABLE I

Comparison of different studies on HPV prevalence according to HPV testing method used (7-16)

Author	Year, country	Type of samples	Method	HPV prevalence / no of testes samples
Garnaes E,	2015, Denmark	tonsillar cancers	next-generation sequencing	58% / 632
Hernandez BY	2014, USA	invasive laryngeal cancers	Linear Array	21% / 148
Tsimplaki E	2014, Greece	oral tongue squamous cell carcinoma	HPV E6/E7 mRNA expression	11.3% /53
Rodrigo JP	2014, Spain	oropharyngeal cell carcinomas	GP5+/6+-PCR	6.1% / 248
De Stefani A	2013, Italy	oropharyngeal squamous cell carcinoma	Linear Array	25% / 52
Ribeiro KB	2011, France, IARC	head and neck cancers	HPV16 E7 DNA,	3.1% /196
Shanesmith R	2011, USA	oropharyngeal squamous cell carcinoma	Linear Array	82.4% / 131
Kingma DW	2010, USA	oral and oropharyngeal squamous cell carcinoma	Linear Array	71.9% / 68

The same method for HPV genotyping (*Linear Array*) was also used by other authors who found differences in the prevalence of HPV in head and neck cancers (7-11). The same great variation in HPV prevalence was found when other molecular techniques were used. As can be seen in Table I, HPV prevalence appears to be higher in the US than in European countries, except for a Danish study which reported a high HPV prevalence using a high sensitivity assay: next-generation sequencing (12-16).

In our study we detected no HPV genotype of all 37 genotypes tested with *Linear Array*, which is a PCR based technique, known to be highly sensitive. A possible explanation for this result could be the epidemiological characteristics of our patients, who are old (mean age – 67 years, range 58 – 88 years) and more than half recognized being chronic alcohol and tobacco users. As no HPV genotype was detected, we could not perform any statistical analysis of the possible risk factors. By comparison, patients with HPV-positive HNSCCs tend to be younger, have a history of less tobacco use and previous exposure to marijuana, and had more oral sex partners as compared to those with HPV-negative carcinomas (17). Also, another possible explanation can be the small number of tested patients, only 25, from 4 surgical departments. In our area, the research on HPV involvement in HNSCCs is quite new and in some situations it is difficult to get fresh samples from surgeons. As in cervical cancer testing, in HNSCC many HPV testing methods are available (PCR/Real Time PCR/Reverse Transcriptase from fresh/frozen/FFPE samples/oral cavity brushing, *in situ* hybridization for HR HPV types, p16 immunostaining, DNA/RNA microarray) (3), and there is a need for a standardized assay to assess the full clinical relevance of the obtained results. Data from recent studies suggest that detection of E6 and E7 mRNA expression in carcinomas is a reason to assume that the virus has been etiologically involved (17). Improved survival in patients with a HPV-positive carcinoma may be related to the type of treatment. Preliminary data from small studies indicate that HPV-positive tumors are more likely to respond to induction chemotherapy and radiation therapy (18, 19). Recent studies found increased survival in HPV/ p16-positive oropharyngeal cancer patients treated with postoperative radiation therapy (21) and suggest de-escalation treatment protocols in these cases (22).

CONCLUSIONS

In this study we aimed at optimizing the HPV genotyping method in fresh head and neck tumors, in order to further evaluate the HPV prevalence in head and neck tumors. With *Linear Array* technique we could continue to genotype head and neck tumor sample and have a significant number of tested patients. Also, younger patients with no history of alcohol and tobacco use should be included in the study. The aim of this testing is to reduce chemotherapy and radiation therapy in case if HPV positive tumors, according to current therapeutic protocols. Also, the use of more sensitive biomarker tests (i.e., E6*I mRNA, p16INK4a, UBC9, microRNA expression profile) would help to correctly classify the different types of head and neck cancers.

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CHARACTERISTICS OF RESPIRATORY MECHANICS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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CHARACTERISTICS OF RESPIRATORY MECHANICS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): In chronic obstructive pulmonary disease (COPD) the changes in respiratory thoracoabdominal mechanics result from both hyperinflation and functional and structural skeletal muscle alterations. **Aim:** Evaluation of the particularities of respiratory movement in COPD and identification of inter-relations between respiratory thoracoabdominal mechanics and clinical and functional respiratory status. **Materials and Methods**: This study comparatively analyzed the respiratory thoracoabdominal mechanics recorded by polygraphy, using piezoelectric film belts, in 34 COPD patients and 30 healthy subjects, during rest, and during 6-minute walk test. **Results:** In COPD patients, phase angle between direction of thoracic and abdominal respiratory movements presented significantly higher values compared to the control group, for supine (p=0.023) and sitting position (p=0.001), revealing the presence of paradoxical respiratory movements. Furthermore, phase angle dynamics increased significantly during walking test compared to sitting position (p=0.001). Quantitative difference of end-expiratory pulmonary volume (qDEEL) correlated with pack-years (r=0.372, p=0.03) in supine position, and with body mass (r=0.338, p=0.05) and St. George activity score (r=0.353, p=0.041) during walking test. Rib cage inspiratory contribution to tidal volume ratio (%RCi) inversely correlated with pack-years (r=-0.417, p=0.014) and body mass index (r=-0.344, p=0.047) in supine position, and with St. George activity score (r=-0.345, p=0.046) in sitting position. **Conclusions:** In COPD patient thoracoabdominal mechanics is characterized by paradoxical respiratory movements, more prominent during exercise. The alteration of thoracoabdominal mechanics correlated with St. George activity score, pack-years and body mass. **Keywords:** COPD, RESPIRATORY MECHANICS, PLETHYSMOGRAPHY.

Muscle dysfunction is an important co morbidity in chronic obstructive pulmonary disease (COPD), found in interrelation with bronchial obstruction and lung emphysema, together with these causing alterations in exercise capacity through complex mechanisms, including change in thoracoabdominal respiratory mechanics (1).

Hyperinflation caused by emphysema results in lung ventilation inefficiency, use of accessory respiratory muscles, muscle fatigue and altered respiratory mechanics. On an alternative pathogenic pathway, systemic inflammation from COPD causes structural and functional muscle alterations, skeletal muscle dysfunction, involving intercostals

muscles and diaphragm. These are reflected in clinical, respiratory functional and respiratory mechanical effects (2).

The aim of this study was to analyze the pattern of respiratory movements in COPD and to identify interrelations between respiratory thoracoabdominal mechanics, and clinical and functional respiratory status.

MATERIAL AND METHODS

This prospective, case-control study consisted in a comparative assessment of the respiratory movement patterns in two groups: a group including patients with COPD admitted to the Clinic of Pulmonary Diseases Iasi, and a control group including healthy volunteers.

The subjects in the two groups were thus selected as to match for age, gender and body mass index. Considering the small number of female patients with COPD admitted to the clinic, we limited our research to males.

COPD was diagnosed according to GOLD guidelines (3). Ventilatory function was measured by spirometry, including bronchodilator response assessment. The clinical evaluation included dyspnea assessment using Modified Medical Research Council Dyspnea Scale, mMRC (4), body mass index (BMI) calculation, assessment of quality of life in COPD by *St George's Respiratory Questionnaire* (SGRQ) (5), exercise capacity assessment by 6 minute walking test (6MWT) (6). Smoking status was assessed by calculating *pack-years* index.

Thoracoabdominal respiratory movements were recorded by polygraphy, with the polygraph *SOMNO-check effort Weinmann* (SOMNOcheck® Effort, Weinmann®, Germany), and two piezoelectric film belts, a thoracic belt and an abdominal belt, as follows: 6 minute recording in supine position; 6 minute recording in sitting position; 6 minute recording during 6 minute walking test.

The signals, represented by filtered and scaled respiratory abdominal (AB) and thoracic (RC) wave forms, were processed and analyzed using the software *VivoSense Complex Respiratory Analysis Version* 2.0, from Vivonoetics (Vivonoetics, San Diego, California) (7). This allowed the analysis of the following parameters of the respiratory mechanics:

- Inspiratory tidal volume *(Insp Vol)* and expiratory tidal volume *(Exp Vol);*
  - *Tpef/Te: Time to reach peak expiratory tidal flow over expiration time;*
  - *%RCi, %RCe: Percent Rib Cage Inspiratory/Expiratory Contribution to Tidal Volume Ratio,*
  - *Ph Angle: percentage agreement between direction of RC and AB movements during breath;*
  - *|Ph Angle|: Absolute value of AB-RC Phase Angle;*
  - *Ph Angle (Area): AB-RC Phase Angle* - was computed from Lissajous loops between RC and AB excursions. The sign of the phase angle indicates whether AB is ahead of RC (Ph Angle>0) or behind (Ph Angle<0) (7);
  - *qDEEL: quantitative difference of end-expiratory pulmonary volume;*
  - *Work of breathing.*

Statistical data analysis was done using *Statistical Package for the Social Sciences* (SPSS 16.0) and MedCalc 12.1. Statistical significance threshold was set at p=0.05.

RESULTS

Our study included a group of 34 patients with COPD (mean age 65.4 ± 8.6) admitted to the Clinic of Pulmonary Dis-

eases Iasi and 30 healthy men which served as controls (mean age 61.4 ± 8.5).

- *Assessment of estimated respiratory tidal volumes: expiratory volume (Exp Vol), inspiratory volume (Insp Vol)*

In supine position, respiratory volumes were significantly lower in the COPD group than in the control group, both for expiratory volumes (357.1±142.6 ml in COPD group and 506.2±269 ml in control group, p=0.004) and inspiratory volumes (357.1±143.1 ml in COPD group and 505.7±268.6 ml in controls, p=0.004), respectively.

Respiratory volumes inversely correlated with body mass index in COPD group (r=-0.436, p=0.01). Respiratory volumes dynamics in COPD patients showed a significant increase at transition from supine to sitting position (p=0.027), followed by a significant decrease during walking test (p<0.001).

- *Assessment of the absolute value of AB-RC Phase Angle |Ph Angle|*

In supine position, the percentage value of AB-RC Phase Angle presented significantly higher values in COPD group than in the control group (58.7±43.7 in COPD group and 36.9±32.1 in the control group, p=0.023), revealing the presence of paradoxical respiratory movement in COPD patients. The same was found for the sitting position (44.3±33.4 in COPD group, and 24.1±17.4 in control group, p=0.001).

The phase angle difference between COPD and control group became insignificant during walking test (78.5±38.2 in COPD group, and 60.4±29.9 in the control group, p=0.071).

Phase angle dynamics presented a mild decrease, below statistical significance (p=0.278) at the transition from supine to sitting position, followed by a significant increase, compared to sitting position, during walking test (from 44.3±33.4 to 78.5±38.2, p=0.001) (fig. 1).

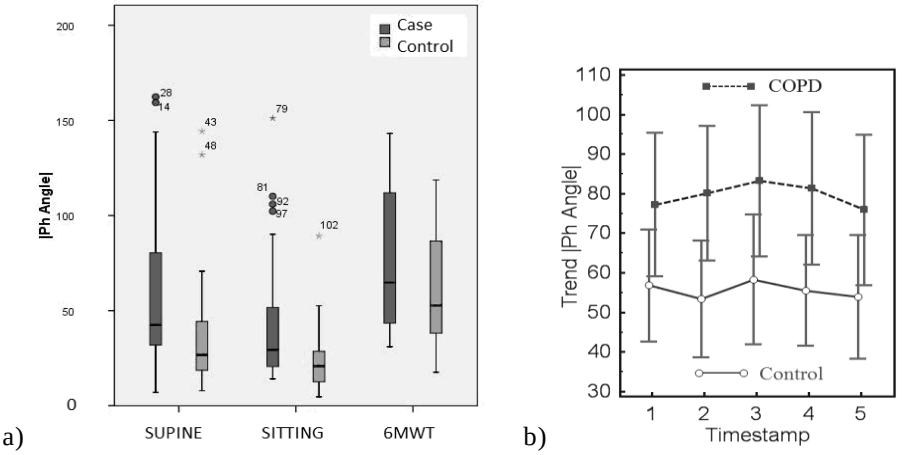

Fig. 1. Phase angle |Ph Angle| in COPD and control group: a) at rest and during 6 minute walking; b) trend during walking test

- *AB-RC Phase Angle assessment: Ph Angle (Area) and |Ph Angle (Area)|*

The parameter |Ph Angle (Area)| showed significantly higher percentage values in COPD group than in the control group, in supine position (59.3±45.6 in

COPD group and 36.5±32.3 in the control group, p=0.03) and in sitting position (43.5±33.5 in COPD group and 23.5±18 in the control group, p=0.001). During walking test, COPD subjects presented significantly higher values of Ph Angle (Area) compared with the controls (19.4±54.2 in COPD group and -5.3±30.4 in the control group, p=0.026). These results revealed the presence of paradoxical respiratory movements in COPD patients.

|Ph Angle (Area)| dynamics in COPD patients presented a mild decrease, statistically insignificant (p=0.244) at the transition from supine to sitting position, followed by a significant increase during walking test, compared with sitting position (from 43.5±33.5 to 77.5±42.3, p=0.001).

- *Quantitative difference of end-expiratory pulmonary volume qDEEL*

The dynamics of qDEEL (expressed in milliliters) in COPD patients showed a mild increase, below statistical significance (p=0.308) during transition from supine to sitting position, followed by a significant increase during walking test, compared to both supine (73.1±34 compared to 32.7 ±27.8, p<0.001) and sitting position (73.1±34 compared to 43.3±27.2, p<0.001). However, an increase in qDEEL during walking test was also found in the control group, the difference between COPD and control group being insignificant (p=0.59).

The value of qDEEL assessed in supine position correlated with pack-years (r=0.372, p=0.03); value of qDEEL assessed in sitting position correlated with body mass in COPD group (r=0.361, p=0.036). The value of qDEEL assessed during walking test correlated with body mass (r=0.338, p=0.05) and *St. George activity score* (r=0.353, p=0.041).

- *Percent Rib Cage Inspiratory Contribution to Tidal Volume Ratio % RCi*

Percent rib cage inspiratory contribution to tidal volume ratio (% RCi) assessed in supine position in the COPD group inversely correlated with pack-years (r=-0.417, p=0.014) and body mass index (r=-0.344, p=0.047).

%RCi ratio assessed in sitting position inversely correlated with *St. George activity score* in the COPD group (r=-0.345, p=0.046).

- *Respiratory rate*

Respiratory rate presented significantly higher values in COPD group than in the control group in supine position (23.3±3.8 min^{-1} compared to 18.9±5.4 min^{-1}, p=0.002), sitting position (24.4±4.9 min^{-1} compared to 18.9±4 min^{-1}, p<0.001) and during walking test (29±4.5 min^{-1} compared to 26.7±6.5 min^{-1}, p=0.017). The transition from resting to walking test resulted in a significant increasing of respiratory rate (p<0.001).

Respiratory rate assessed in sitting position correlated with body mass index (r=0.363, p=0.035). Respiratory rate assessed during walking test correlated with pack-years (r=0.373, p=0.030) in COPD patients.

- *Assessment of inspiratory time: Ti*

Inspiratory time corresponding to supine position correlated to pack-years in COPD group (r=-0.374, p=0.029). Inspiratory time corresponding to sitting position inversely correlated with body mass index in COPD group (r=-0.342, p=0.048). Mean inspiratory time during 6 minute walking test correlated with pack-years in COPD group (r=0.419, p=0.014).

- *Time to reach peak expiratory tidal flow over expiration time: Tpef/Te*

The dynamics of Tpef/Te values in COPD subjects proved similar mean values for supine and sitting position (0.46±0.05 and 0.45±0.03, p=0,918), with a significant increase during walking test, compared with both supine (p=0.001) and sitting

position ($p<0.001$). During walking test the mean value of Tpef/Te was significantly higher in COPD group than in the control group (0.5±0.02 compared to 0.48±0.02, $p=0.002$). Mean Tpef/Te value in supine position correlated with both body mass ($r=0.382$, $p=0.026$) and height ($r=0.620$, $p<0.001$), in COPD patients.

- *Assessment of work of breathing*

Work of breathing presented significantly higher values in COPD group, compared with the control group in supine ($p<0.001$) and sitting position ($p=0.003$), the difference becoming insignificant during walking test ($p=0.174$).

The dynamics of the work of breathing in COPD subjects showed a mild, insignificant decrease during transition from supine to sitting position ($p=0.228$), followed by a significant increase during walking test compared with sitting position ($p<0.001$).

The work of breathing correlated with body mass in COPD patients: in supine position ($r=0.355$, $p=0.039$); in sitting position ($r=0.376$, $p=0.028$); during exercise ($r=0.401$, $p=0.019$).

DISCUSSION

The dynamics of respiratory tidal volume in COPD patients presented a significant increase at the transition from supine to sitting position. This improvement may be explained by a more efficient use of respiratory muscles in sitting position and by a more efficient functioning of the diaphragm.

Exercise represented by 6 minute walking test resulted in a significant decrease of inspiratory/expiratory volumes compared to resting values ($p<0.001$) in both COPD and control group. Tidal volume corresponding to supine position inversely correlated with body mass in COPD group.

Phase angle between thoracic and abdominal movements presented significantly higher values in COPD than in the control group in both supine and sitting position ($p=0.023$ and $p=0.001$, respectively).

Phase angle dynamics in COPD patients showed a mild, insignificant decrease ($p=0.278$) at the transition from supine to sitting position. This decrease of thoracoabdominal asynchrony could explain the tidal volume improvement.

The mean phase angle value progressively increased during walking test in COPD subjects, insignificantly compared to supine position ($p=0.092$) and significantly compared to sitting position ($p<0.001$), but compared to control group the difference became insignificant ($p=0.071$). This observation could be explained by the phenomenon of paradoxical abdominal motion amplification during exercise in COPD patients.

Brennan *et al.* demonstrated paradoxical movements of the lower ribcage in COPD patients, explaining these by an increase in intercostals and accessory muscles activity, in addition with a decrease in diaphragm respiratory activity. These mechanisms seem to be favored by hyperinflation in COPD (8).

From this point of view, Vogiatzis *et al.* have identified two distinct profiles of patients with COPD: one characterized by early hyperinflation during exercise, completely reversible at the end of exercise, and another characterized by late hyperinflation during exercise (9).

Bloch *et al.* showed that lung volume reduction surgery in COPD patients significantly increases abdominal contribution to tidal volume and decreases the duration of paradoxical abdominal movement during inspiration.

The improvement of thoracoabdominal synchronization and the increase of the abdominal contribution to tidal volume dynamics during the respiratory cycle have the effect of decreasing muscle labor during inspiration by a greater participation of

diaphragm in breathing (10).

Quantitative difference of end-expiratory pulmonary volume (qDEEL) did not show significant differences between COPD and control group in supine and sitting position. During walking test, qDEEL mean value progressively increased in COPD group, being significantly higher than in supine ($p<0.001$) and sitting ($p<0.001$) position. However, case-control differences became insignificant ($p=0.59$).

These results confirmed the data obtained by Aliverti *et al*. in a study using optoelectronic plethysmography. These proved an immediate increase of the end-expiratory pulmonary volume in COPD subjects associating lower ribcage inspiratory paradox, and a late increase of the end-expiratory pulmonary volume in COPD subjects without paradoxical movements of the lower ribcage. However, during exercise, dyspnea increased similarly in the two categories of COPD patients. In addition, paradoxical movement did not correlate with ventilatory function and exercise capacity; leg effort increased more markedly in the patients without paradoxical movements (11).

CONCLUSIONS

Thoracoabdominal mechanics in COPD is characterized by paradoxical respiratory movements. In our study the trend of phase angle between thoracic and abdominal respiratory movements revealed a significant increase of thoracoabdominal asynchrony during exercise. At rest, the transition from supine to sitting position resulted in a mild improvement of the thoraco-abdominal synchronism. Thoraco-abdominal breathing movements parameters significantly correlate with smoking (pack-years), physical activity score and body mass in COPD patients: *quantitative difference of end-expiratory pulmonary volume* correlates with pack-years when assessed in supine position, with body mass when assessed in sitting position, and with *St. George activity score* when assessed during walking; rib cage inspiratory contribution to tidal volume ratio inversely correlates with pack-years and body mass when assessed in supine position and inversely correlates with *St. George activity score* when assessed in sitting position; mean inspiratory time during walking correlates with pack-years. Although further research is needed, dual band respiratory plethysmo-graphy by polygraphy could be a helpful and accessible method which can bring valuable information defining the clinical phenotypes of COPD patients.

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NEWS

RELATION OF SERUM HAPTOGLOBIN LEVEL TO ERYTHROPOIETIC ACTIVITY IN BETA THALASSEMIA CHILDREN

Serum haptoglobin (Hp) is a reliable marker for hemolysis. A team of researchers led by Ragab et al investigated the possible relation between Hp depletion and hemolysis severity, hepatitis C virus (HCV) infection and iron load in β-thalassemia children. The study included 22 β-thalassemia major (TM) and 20 β-thalassemia intermedia (TI) children, as well as age and sex matched healthy controls. Pre-transfusion hemoglobin level, serum ferritin, Hp and transferrin receptor levels (sTfR), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed. Markers of hepatitis C virus (HCV) were determined using PCR. Both patient groups had significantly lower Hp levels compared to the controls, with significant lower levels in TM children. Significant inverse correlations were found between serum Hp and sTfR levels in thalassemia children and among HCV infected patients. The study found that STfR was the only significant independent predictor for serum Hp level. No significant correlation was found between serum Hp and serum transaminases in HCV infected children. The study concluded that serum Hp depletion in thalassemia has a significant relation to the severity of the disease and is correlated with the erythropoietic activity (Ragab SM1, Safan MA2, Badr EA2. Study of serum haptoglobin level and its relation to erythropoietic activity in Beta thalassemia children. *Mediterr J Hematol Infect Dis*. 2015 Feb 15;7(1):e2015019. doi: 10.4084/MJHID.2015.019. eCollection 2015.)

Teodora Vremeră

CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF GOUT, A DYSMETABOLIC DISABLING DISORDER

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CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF GOUT, A DYSMETABOLIC DISABLING DISORDER (Abstract): Gout is a disease caused by disturbances of uric acid metabolism and it manifests as rheumatic pain with various clinical and developmental issues, but without any major diagnosis problems; it might unfavorably interfere with other metabolisms, especially with carbohydrate and lipid metabolism that interact and erode each other. **Aims**: To provide clinical and laboratory data and to follow the development of gout in patients treated in the clinic. **Material and methods**: The study included 28 patients (25 male and 3 female patients) diagnosed with gout, admitted to the First Clinic of Rheumatology of the Clinical Rehabilitation Hospital Iasi during 2012 - 2013. **Results and discussion**: A new diagnostic method, dual energy computed tomography, was effective in some selected cases of gout, as it may reveal uric acid crystals with specific densities in the damaged joints and periarticular soft tissues. **Conclusions**: Gout is a disorder that occurs when the uric acid produced by the body is stored in the form of crystals in joints and / or soft tissues. In joints, uric acid crystals precipitate and cause inflammatory arthritis that leads to swelling, redness, heat, pain and joint functional impotence. **Keywords**: GOUT, MONOSODIUM URATE CRYSTALS, METABOLIC SYNDROME, DISABLING DISORDER.

Although gout is a disease with a long history (it was described by ancient Egyptians and later by Hippocrates), the relationship between the disease and uric acid was shown in the nineteenth century by Sir Alfred Baring Garrod and it was seen as an acquired metabolic disorder only in 1909 (1,2).

Gout is a chronic disorder closely related to the metabolism of uric acid and to the release of monosodium urate crystals, identified by McCarthy and Hollander in the synovial fluid of inflamed joints (this became the gold standard in the diagnosis of gout). These crystals are deposited in the joints, causing episodic or persistent joint inflammation. They have a key role in understanding the disease. Consequently, local temperature rises, with pain and increased sensitivity of the joint (most often in the joint of the hallux), associated with very intense nocturnal pain (3, 4).

Gout is not merely a metabolic disease where uric acid is deposited in joints and

tissues. It can also induce over time diabetes, atherosclerosis as well as major renal complications (5). Increased production of uric acid is usually caused by a purine-rich diet, by food containing a large amount of fructose or by excessive alcohol drinking, as well as by an increased production, by reduced renal excretion or a combination of these two mechanisms (6, 7). The occurrence of gout is influenced by factors such as gender (gout is more common in men than in women), age (the risk increases after the age of 65), race or diet.

There are some hematological lymphoproliferative and myeloproliferative disorders, hemolytic anemia, cutaneous psoriasis which are characterized by major cell damage associated with hyperuricemia and gout. Gene mutations in enzymes involved in purine metabolism (hypoxanthine-guanine-phosphoribosyltransferase deficiency or increased activity of phospho-ribosyl pyrophosphate synthetase) are known genetic causes of hyperuricemia and gout (7, 8).

Predisposing genetic factors were identified in order to evaluate gout in the general population, with possible therapeutic implications (polymorphism of genes involved in the synthesis of urate transporters in renal proximal tubules) (8). These transport proteins might be seen as pumps for urate, providing the renal excretion of uric acid (ABCG2 - ATP-binding cassette transporter isoform G2; Q141K variant, leading to a two-fold decrease of the urate efflux of and to its intracellular accumulation; URAT1 – one of the essential mechanisms of uric acid reabsorption; NPT1 - sodium phosphate transport protein 1, the protein involved in the tubular secretion of uric acid) (9, 10).

Gout has three stages: *asymptomatic hyperuricemia* - uric acid crystals deposit in tissues; *acute attacks* - crystals deposited in joints cause inflammation, usually in one single joint; *chronic gout* - persistent joint pain and swelling, uric acid deposits in soft tissues (called tophi) are usually located intra or periarticularly in the elbows, fingers and toes or ears. In addition to joint manifestations and tophi formation, renal impairment is one of the most common complications of hyperuricemia (11,12).

Gout is a common rheumatic disease with various clinical and developmental aspects, but it has a quite typical clinical picture, theoretically without major diagnosis problems.

MATERIAL AND METHODS

The study determined the clinical evolution of gout in 28 patients (25/89.28% men and 3/10.72% women) diagnosed with gout, (highly significant difference: $p < 0.005$), admitted to the First Clinic of Rheumatology of the Clinical Rehabilitation Hospital Iasi during 2012 – 2013. The aim was to provide clinical and laboratory data and to follow the development of gout.

The data was gathered using clinical and epidemiological survey forms that recorded age, sex, place of origin, eating and occupational behaviors, co morbidities and associated treatments, family history of gout in relatives of the last four generations, onset signs and development trends etc.

The processing and interpretation of collected data was performed using the Epid SPSS13.0 software and statistical and mathematical tests.

RESULTS AND DISCUSSION

Gout is a common disease in the general population and it is caused by hyperuricemia, which is a major risk factor in the

development of the disease and it is however easy to detect. If hyperuricemia levels are higher than 7 mg / dL, the risk of joint and kidney damage increases also. An attack of gout is caused by the precipitation of uric acid crystals in the synovial fluid. It is a slow process, resulting from persistent hyperuricemia.

The highest frequency of the disease was found in the age range of 51-60 years in men and above 60 years in women, with a sex distribution of 89.28 % male to 10.72 % female.

Differences in age and sex of patients with hyperuricemia are likely related to variations in renal *clearance* of uric acid that are influenced by estrogen and androgen levels (12) (fig. 1).

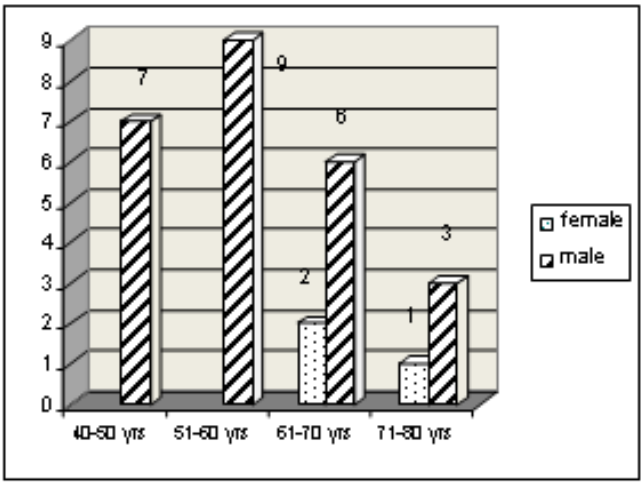

Fig.1. *Sex and age distribution of gout study patients*

Hyperuricemia was positively correlated with male gender, obesity, body size, body mass index, dietary intake of proteins, social status. 6 of the 28 cases (21.42%) had a body mass index BMI of > 25 kg/m^2.

17 of the 28 patients (60.71%) were from urban areas and 11 patients (39.29%) were from rural areas (fig. 2).

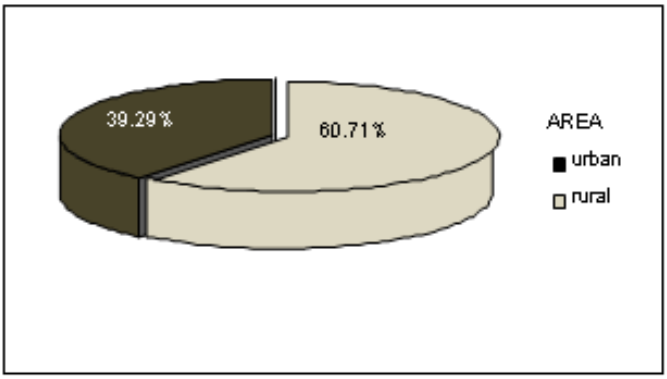

Fig. 2. *Rural / urban distribution of gout study patients*

The epidemiology of gout is closely related to that of hyperuricemia, which is defined as a serum urate concentration higher than two standard deviations above the normal maximum values related to sex, namely, over 7.0 mg / dL (0.42 mmol / l) in men and above 6.0 mg / dL (0.36 mmol / l) in women.

Epidemiological studies have shown a strong correlation between serum urate levels and chronic alcohol ingestion, due to increased production of urate and suppression of the renal excretion of uric acid caused by hyperlactatemia (8,12). In our study, 35.71% of gout patients were chronic consumers of ethanol.

The average duration of the disease at admission was: less than 1 month in 2 cases (10.71%); 1-2 months in 7 cases (25.00%), 2-5 years in 13 cases (46.42%); over 5 years in 6 cases 21.42%.

Our data have also shown that gout has four clinical pictures corresponding to four stages of the disease: asymptomatic hyperuricemia, acute attacks of gout, intercritical period and chronic tophaceous gout.

Rarely, the gout patients did not have a second acute attack. Usually, acute episodes reoccurred between six months and two years after the first attack.

In untreated patients, the acute attacks occurred more often and with more severe symptoms, for long periods, often associated with fever and having a polyarticular character.

Joints were damaged at the onset of gout, as follows: metatarsal phalangeal (MTP I) and proximal interphalangeal (PIP I), bilateral in 3 patients (10.71%); median region of tarsus in 2 patients (7.14%); knees in 9 patients (32.14%) and polyarticular damage in 14 patients (50.00%). The typical attack of gout with sudden onset of intense pain was found mainly in one joint, namely, the metatarsal phalangeal joint; the objective examination showed purple shiny skin, especially in the affected joint; local temperature was increased and cutaneous hyperesthesia was present, increased sensitivity to gentle palpation of the area as well as attempts to mobilize the damaged joint. Signs of lymphangitis were found in some cases (32.14%) and cellulite effusion was rare (7.14%).

In some cases the onset was polyarticular, with damage of knees (26 patients; 92.85%), ankles (23 patients; 82.14%), MTP hallux (14 patients; 50.00%), PIP (8 patients; 28.57%) elbow (12 patients; 42.85%), hand (11 patients; 39.28%), shoulder (3 patients; 10.71%), hip (3 patients; 10.71%).

Usually, the clinical onset of primary gout occurred in a state of full health, either as an attack of acute arthritis or due to a renal colic as the result of uric acid lithiasis. In some cases, the gout of the joints was acute, with extreme paroxysmal pain caused by inflammations. Chronic arthritis was preceded or not by an acute attack.

Recurrent episodes of acute arthritis in MTP are characteristic of gout, but there may be also other causes for this location of arthritis, such as infections, psoriasis and other crystal deposition arthropathies (7, 8).

Over 50% of patients may have arthritis in another location, which is less specific for the first attack of gout – temporomandibular, acromioclavicular, sternoclavicular, manubriosternal, sacroiliac, pubic symphysis, femoral hip; it may be accompanied by flexor tenosynovitis associated with carpal tunnel syndrome (9,10).

The less acute polyarticular manifesta-

tions are not rare. Therefore it is important to detect monosodium urate crystals in the synovial fluid of the affected joints in order to diagnose gouty arthritis when there is no clear cause of the disease.

Musculoskeletal ultrasound shows good results in the ability to detect crystal deposits and other characteristics of gouty arthropathy. Recently, specific elements of gouty arthropathy were described in the literature: hyperechoic image of the external surface of hyaline cartilage - the so-called sign of "double contour" (12), hyperechoic areas in the synovial fluid, with the appearance of "soft" or "hard" tophi (13).

A new diagnostic method, dual energy computed tomography, was effective in some cases of gout, as it may reveal uric acid crystals with specific densities in the damaged joints and periarticular soft tissues (3).

Extraarticular manifestations are present in structures rich in proteoglycans, as they have a high susceptibility to develop tophi, due to their high affinity for sodium urate. Thus, 6 patients of the study group (21.42%) had gouty tophi localized in the pinna, fingers and toes, knees, olecranon bursa, Achilles tendon. In 2 patients with chronic tophaceous gout (7.14%) - pseudorheumatoid form – the skin covering superficial tophi was pressed and ulcerated, leading to the drain of a white pasty substance composed of sodium urate crystals.

Renal lithiasis was identified by ultrasound in 9 patients (32.14%), who had hyperuricemia for long periods of time (> 9 mg %) and high levels of uricosuria.

In the 28 cases, laboratory examinations showed during the acute episode the following data: uric acid values between 6-7 mg % and 9 mg; uricosuria values < 0.4 g / 24 h (14.28%); 0.4-0.8 g / 24 h (17.85%); 0.8-1 g / 24 h (3.57%); > 1 g / 24 h (28.57%); these examinations were not carried out in 10 cases.

Dyslipidaemic syndrome was found in 7 cases with high cholesterol levels (25.00%); in 11 cases with high triglyceride levels (39.28%), in 7 cases with high levels of total lipids (25.00%) and in 3 cases with diabetes mellitus type II (10.71%).

Serum uric acid during the acute episode may be normal and measuring hyperuricemia levels is not a prerequisite for diagnosis. Hyperuricosuria was present in 32.14% of patients.

Radiological changes in the acute stage of the disease included the swelling of the periarticular soft tissue, juxtaarticular osteoporosis, joint space narrowing. In 9 patients (32.14%), radiological lesions indicated the development of secondary arthrosis, as osteophytes, subchondral osteosclerosis or significant clearing of joint space were found.

Treatment is aimed at eliminating the monosodium urate crystals. Joint inflammation resolves after their removal and the disease can be considered cured. Serum uric acid levels should be maintained indefinitely within normal limits, in order to prevent the formation of new crystals and the relapse of gout attacks (9).

It is important to treat arthritis because it can occur at any time, even after the treatment started and it can persist for as long as the crystals in the joints remain undissolved. The prevention of episodes of arthritis may be achieved using anti-inflammatory drugs that reduce mild subclinical inflammation caused by the presence of monosodium urate crystals in joints.

The presence of gout may indicate also the presence of metabolic syndrome or of some of its components (hypertension, dyslipidemia, obesity, glucose intolerance), which should be also recognized and treated (14).

The treatment of evolving gout aims at stopping the acute attacks and preventing the relapses, reducing the levels of hyperuricemia and treating complications and comorbidities.

Patients in the study group received early treatment of acute gouty arthritis that solved the inflammatory phenomena as rapid as possible by delivering a non-steroidal anti-inflammatory drug for the remission of the inflammatory phenomena (0.5 mg, 1-3 times / day). The treatment of recurrent episodes of arthritis, gouty tophi or nephrolithiasis was made, in order to avoid lesions of the cartilage and bone and the development of tophi.

Uricase theray is an option for the treatment of hyperuricemia in gout, but its use is limited due to side effects. In humans, the uricase may decrease the level of uric acid by 0.78 mg/dL after 4 hours of administration. Rasburicase, a recombinant uricase, was successfully used in severe cases of gout. It is delivered in a monthly single dose, due to the development of tolerance after multiple doses.

All non-steroidal anti-inflammatory drugs (NSAIDs) were effective for the treatment of gouty attacks. Studies show that naproxen (500 mg two times a day) has similar effects as prednisone 35 mg / day (32, 33). Gastrointestinal, cardiovascular and renal adverse effects are the main reasons for limiting the use of NSAIDs in the treatment of gouty arthritis. A short course of 30-40 mg prednisone for 2-4 days proved to be effective in the treatment of gouty arthritis (8).

The most common drug used to lower serum uric acid is allopurinol, an inhibitor of xanthine oxidase, which is an enzyme involved in the metabolism of purines into uric acid (11).

Febuxostat is used in patients intolerant to allopurinol and in gout patients with renal failure, as it is a selective inhibitor of xanthine oxidase, which is metabolized in the liver.

Pegloticase is a new drug approved in the USA in 2010 for the treatment of gout resistant to conventional therapies. It is a drug obtained from a porcine recombinant enzyme.

CONCLUSIONS

The study group showed multiple comorbidities, especially diabetes mellitus, hypertension, dyslipidemia, obesity.

Hyperuricemia was positively correlated with male gender, obesity, body size, body mass index, dietary intake of protein, social status.

Our data have also shown that gout has four clinical pictures corresponding to four stages of the disease: asymptomatic hyperuricemia, acute attacks of gout, intercritical period and chronic tophaceous gout.

The risk of developing articular manifestations of gout or tophi is related to duration of hyperuricemia, sex and age of the patient. Both gouty arthritis and tophi are minor manifestations which are fully treatable and reversible; therapy should be initiated on onset; however, renal impairment is more severe and sometimes irreversible, so that early diagnosis and treatment is required.

A better evolution and prognosis of

gout may be achieved through close cooperation between patient and doctor, by providing early and effective treatment, in order to prevent osteoarticular and renal lesions.

All these correlations found in practice and also reported in the literature show that gout is a complex metabolic nosological entity, characterized mostly by rheumatic manifestations.

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OUTCOMES OF PULMONARY REHABILITATION IN SEVERE PULMONARY HYPERTENSION – CASE REPORT

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OUTCOMES OF PULMONARY REHABILITATION IN SEVERE PULMONARY HYPERTENSION - CASE PRESENTATION (Abstract): Chronic pulmonary artery hypertension (CPAH) is characterized by progressive increase in vascular resistance and pulmonary artery pressure, leading to right heart failure and death. Diagnosis algorithm is complex, and the underlying cause often remains undefined. We present the case of a 74-year-old male patient, whose diagnosis was disputed for several months between cardiologists and pulmonologists, being eventually diagnosed with severe pulmonary hypertension and hypoxemic respiratory failure of chronic thromboembolic etiology intricate with diffuse interstitial lung disease. The new therapeutic approach with riociguat failed and the treatment was stopped, then the patient addressed Iasi Pulmonary Rehabilitation Clinic for an inpatient pulmonary rehabilitation program, comprising functional assessment, nutritional and psychological counseling, mild inspiratory muscle training sessions, low workload endurance exercise, followed by relaxation and appropriate breathing techniques. After two weeks, oxygen saturation, shortness of breath, fatigue, sleep, anxiety and depression symptoms had improved while 6-minute walk test distance did not change significantly. **Keywords**: PULMONARY HYPERTENSION, RESPIRATORY FAILURE, PULMONARY REHABILITATION.

Chronic pulmonary artery hypertension (CPAH) is a debilitating disease of the pulmonary vasculature, characterized by progressive increase in vascular resistance and pulmonary artery pressure, leading eventually to right heart failure and death. Diagnosis algorithm is complex, and the underlying cause often remains undefined.

The current treatment strategy for CPAH patients includes three main steps, the initial approach including physical activity, supervised rehabilitation, and psychosocial support while pharmacological therapy is indicated only as a second step, according to the vaso-reactive or non-reactive type of CPAH and determinants of prognosis (1, 2, 3, 4). Pulmonary rehabilitation and supervised exercise, added to

pharmacological treatment options increase exercise capacity and quality of life of these patients, although there are small cohort trials proven otherwise (5).

CASE REPORT

We present the case of a 74-year-old male patient, with a complex medical history, who was admitted in November 2016 to the Pulmonary Rehabilitation Clinic of the Iasi Rehabilitation Hospital for an inpatient pulmonary rehabilitation program, his major complaints being severe dyspnea, fatigue, sleep disturbances, and an overall diminished quality of life and physical function while under long-term oxygen therapy at home. The patient was a former mild smoker (5PY) and not exposed to respiratory hazards.

Clinical examination revealed oral cyanosis, telangiectasis, splitted second heart sound left parasternal 2nd interspace, severe regurgitation murmur in tricuspid area, BP = 100/60 mmHg, arrhythmic heart sounds (HR = 95/min), oxygen saturation SpO_2 = 88% in ambient air, with no sign of pulmonary or systemic congestion, no finger clubbing.

His past medical history has recorded a diagnosis of prostatic adenocarcinoma Gleason 5, eight years ago, under hormone therapy for seven years, discontinued by the urologist due to occurrence of progressive shortness of breath, fatigue, and weight loss (10 kg in 9 months), leading to repeated hospitalizations in pulmonology and cardiology services.

The case was disputed for several months (May-September 2016) between cardiologists and pulmonologists, being eventually diagnosed with severe pulmonary hypertension (precapillary type) and hypoxemic respiratory failure, following the recommended algorithm (1) (tab. I).

TABLE I

Diagnostic approach and results

Investigation	Results
Echocardiography	Dilated right chambers, PAPs estimated = 86 mmHg; mild systolic dysfunction RV, moderate functional and pulmonary tricuspid regurgitation, normal LV, mild to moderate mitral regurgitation, without hemodynamic significance
ECG, Holter	Atrial flutter with variable conduction
Bodypletismography, Spirometry & DLCO	FVC = 120%, FEV1=123%, FEV1/FVC = 78,47%, MEF50 = 130,5%, TLC = 121,8%, RV = 134,5% (normal values), DLCO = 42,7% from predicted values (fig.1)
Chest X-ray	Bilateral diffuse interstitial opacities
CT of the chest	Bilateral ground-glass opacities, fine subpleural reticular opacities, several mediastinal adenopathies, and three nodular peribronchial opacities
Lung perfusion scan	Multiple segmental perfusion deficits (moderate-severe) (fig. 2)
Right heart catheterization	PAP = 70/27/42 mmHg, PCP = 9/5/3 mmHg, PVR 6,34uW, CI = 3.7l/min/m^2
Immunology	c-ANCA, p-ANCA, ANA, anti-La/SS-B, anti-Ro/SS-A, anti-SM, anti SCL70, anti-RNP = negatives
Tumor markers	CEA, CEA 19-9, NSE, Cyfra 21-1, PSA (under hormone therapy) within normal range
Others	BNP = 207pg/ml; 400 pg/ml

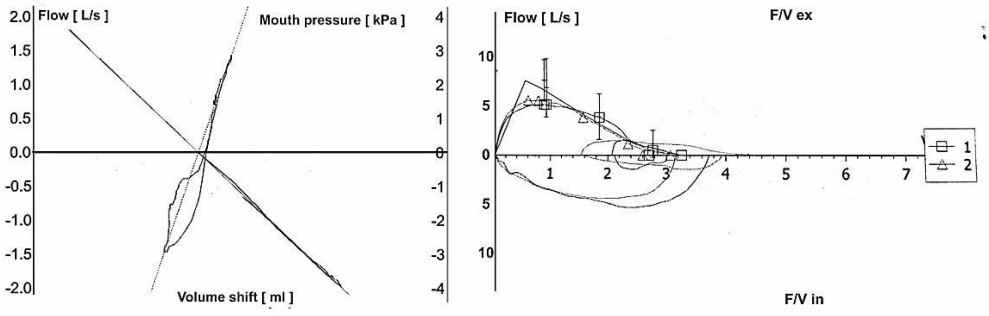

Fig. 1. Body pletismography and flow-volume loop showing normal values for pulmonary volumes and flows

Etiology of CPAH was supposed to be chronic thromboembolism possibly intricate with diffuse interstitial lung disease (idiopathic pulmonary). Due to the diagnosis of post embolic pulmonary hypertension distal type, associated with inoperability, in June 2016 the patient has started treatment with pulmonary vasodilator riociguat 1 mg tid for 2 weeks, then 1.5 mg tid but after one week he has developed paroxysmal nocturnal dyspnea, leading to reassessment of treatment and etiology of PAH, the pulmonary interstitial lung disease being now considered the leading culprit and only diuretic and anticoagulant treatment was continued (August 2016).

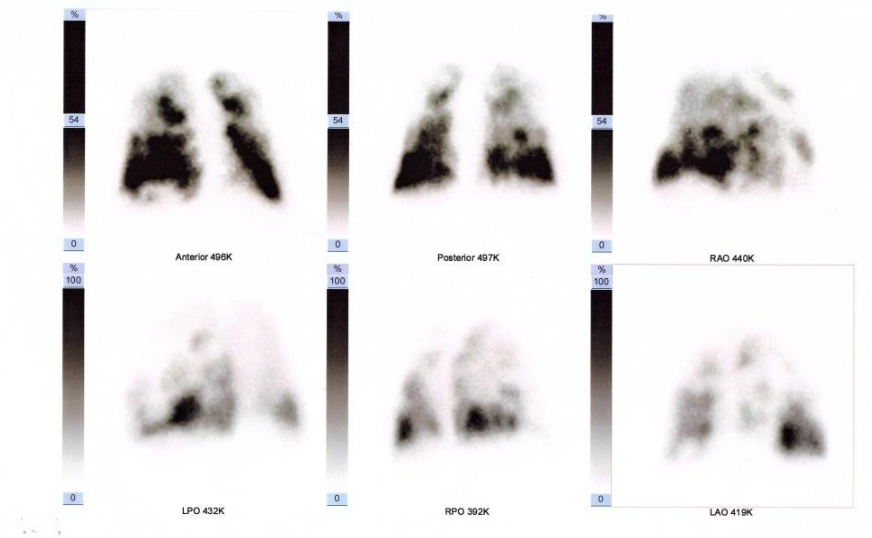

Fig. 2. Multiple segmental perfusion deficits (moderate-severe) in lung perfusion scan (chronic thromboembolism intricate with diffuse interstitial lung disease)

During admission in Iasi Respiratory Rehabilitation Clinic, the patient has followed a comprehensive respiratory rehabilitation program, comprising functional assessment, nutritional and psychological counseling, mild inspiratory muscle train-

ing sessions, low workload endurance exercise, followed by relaxation and appropriate breathing techniques (6, 7, 8, 9). After two weeks, oxygen saturation, shortness of breath (assessed through Borg and mMRC scales), fatigue, sleep, anxiety and depression symptoms had improved while 6-minute walk test (6MWT) distance had no significant improvement.

DISCUSSION

This case is particular through diagnosis difficulties of CPAH and as a consequence etiologic treatment had not the anticipated beneficial results. Prognosis indices are represented by clinical evidence of right ventricle failure, the progression of symptoms, WHO class and 6MWT distance (10, 11). Unsolved thromboembolic obstruction in the pulmonary arteries leads to group 4 pulmonary hypertension, according to World Health Organization. Pulmonary endarterectomy is the treatment of choice for chronic thromboembolic pulmonary hypertension as potentially curative therapy, but about one-third of patients, including our case, are not suitable candidates for the surgery, either due to distal and inaccessible nature of the lesions or comorbid conditions, requiring pharmacologic treatment options (12).

Riociguat is a member of the therapeutic class of soluble guanylate cyclase stimulators, associated with significant improvements in 6-minute walk distance, pulmonary vascular resistance, and time to clinical worsening (13, 14), being the only agent approved for treating both chronic thromboembolic hypertension and pulmonary arterial hypertension. The novel mechanism of riociguat lies in its ability to restore the homeostatic and therapeutic effects of nitric oxide that are diminished because of phenotypic alterations associated with pulmonary hypertension (14) but in the presented case it seemed to have no positive results, questioning the vascular etiology of CPAH.

On the other hand, several small size randomized controlled trials reported that trained CPAH patients have decreased fatigue and dyspnea, higher level of physical exercise, improved distance to 6MWT, quality of life, and cardiorespiratory function (15, 16) but the optimal method of exercise, intensity, and duration of the training are still under debate. Because of the positive outcomes, pulmonary rehabilitation was introduced in the recent guidelines of treatment and management of PAH. A low workload endurance exercise and weightlifting training under close medical supervision, thorough patient education regarding his disease, nutrition, and the safe level of exercise were recommended (1, 17).

In the clinical case discussed above, the initiation of pulmonary rehabilitation was relatively late, and outcomes were negatively affected by the unclear cause of the PAH, rapid evolution of the symptoms, and the negative prognosis of the main underlying disease.

Still, features demonstrating an improved quality of life as the diminished severity of dyspnea, improved sleep pattern, and psychiatric symptoms of depression and anxiety were displayed after two weeks of a comprehensive rehabilitation program.

CONCLUSIONS

Our study highlights that the core issue of the multidisciplinary approach to complex clinical cases of pulmonary hypertension remains the close cooperation between cardiologists and experienced pulmonary rehabilitation experts, to improve outcomes and ultimately, survival of these severely impaired patients.

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CUTANEOUS MANIFESTATIONS ASSOCIATED WITH THYROID DISEASE

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CUTANEOUS MANIFESTATIONS ASSOCIATED WITH THYROID DISEASE (Abstract): **Aim:** To present the clinical and laboratory features of patients with dermatologic disorders associated with thyroid diseases, diagnostic criteria, type of administered treatment and its effectiveness. **Material and methods**: This study is a retrospective study based on the medical records of patients with thyroid diseases admitted to the Dermato venereology Clinic of the Iasi "Sf. Spiridon" University Emergency Hospital between January 1, 2012-December 31, 2013. Data on clinical manifestations, methods of investigation, therapeutic approach, and associated dermatologic and systemic diseases were reviewed. **Results**: A total of 38 patients were enrolled in this study of which 36 females and two males. An high incidence of cases with autoimmune thyroiditis (63%), followed by polynodular goiter (26.3%) and hypothyroidism (10.7%) was found during the study. The identified dermatologic disorders associated with thyroid diseases were in order of frequency *alopecia areata* (22%), followed by *lichen planus* (18%). **Conclusions:** This study demonstrates that there is a significant association between certain dermatologic disorders and thyroid diseases, requiring periodic thyroid function tests. **Keywords**: THYROID DISORDERS, CUTANEOUS MANIFESTATIONS, AUTOIMMUNE THYROIDITIS

Thyroid disorders are recognized for their involvement in all systems of the human body, and the skin is no exception. Some skin changes and dermatologic disorders may be the first symptoms of thyroid disease.

Some studies have suggested that thyroid hormones play a pivotal role in the embryonic development of the skin, as well as in maintaining normal skin function in adult skin (1).

Skin changes occur both in hyperthyroidism and in hypothyroidism. In hyperthyroidism the skin is soft, moist, and

warm associating hyperpigmentation (diffuse, localized or Addison disease) or palmar erythema. Heat is due to increased skin blood flow and peripheral vasodilatation, which can also lead to flushing and palmar erythema. Hyperhidrosis more marked on the palms and soles may be present. In hyperthyroidism hair is described as soft, fine with or without non-scarring alopecia. The presence of thyroid hormone receptors in dermal papilla and external epithelial sheath has been demonstrated (2).

In hypothyroidism, the skin tends to be pale, both because of dermal mucopolysaccharides and dermal water content. The increased dermal carotenoid levels cause a yellow discoloration of the palms, soles and nasolabial sulcus. The most characteristic clinical sign of hypothyroidism is generalized myxedema resulting from the deposition of hydrophilic mucopolysaccharides in the dermis, especially hyaluronic acid and chondroitin sulfate. The skin may appear edematous, dry, pale as wax and firm to touch. Hair in hypothyroidism is coarse, dry, brittle, with reduced diameter, showing an increased tendency to fall, often resulting in partial alopecia (2, 3).

The term of thyroid ophthalmopathy is currently used but the disease process involves the orbital and periocular soft tissues with secondary effects on the eye. Thus, Thyroid-associated orbitopathy (TAO) is a more appropriate definition. The ocular manifestations of TAO can coincide or even precede other systemic complications and include superior eyelid retraction, periorbital edema with secondary proptosis, conjunctival injection and chemosis and impaired ocular motility. While ocular involvement is usually not threatening vision excepting the very severe cases of exposure keratopathy, compressive optic neuropathy and ocular hypertension almost 20% of the patients consider the ocular morbidity in TAO more troublesome than the systemic complications of dysthyroidism (4).

Some skin conditions and diseases with predominantly cutaneous manifestations were associated with thyroid dysfunction or presence of thyroid antibodies. These conditions can be grouped into connective tissue disease (systemic lupus erythematosus, scleroderma, Sjogren's syndrome, dermatomyositis), bullous dermatoses (bullous pemphigoid, pemphigus vulgaris, pemphigus foliaceus, herpetiform dermatitis), pigmentation disorders (melasma, *vitiligo*) and other diseases (*alopecia, lichen planus, granuloma annulare*) (5).

The aim of this study was to present the clinical and laboratory features of patients with dermatologic disorders associated with thyroid diseases, diagnostic criteria, types of administered treatment and their effectiveness based on the study of the patients admitted to the Iasi Dermatology Clinic in the interval January 1, 2012- December 31, 2013.

MATERIAL AND METHODS

This is a retrospective study of the patients with thyroid diseases admitted to the Dermatology Clinic of the Iasi "Sf. Spiridon" University Emergency Hospital in the interval January 1, 2012- December 31, 2013. Data on clinical manifestations, methods of investigation, therapeutic approach, and associated dermatologic and systemic diseases were reviewed.

A total of 38 patients, 36 females and 2 males, were included in the study.

The study was based on patient medical records and photos. Based on the obtained data the patients were grouped according to distribution by age, sex, area of residence, socioeconomic status, clinical features,

association with other diseases, type of administered treatment and its effectiveness.

RESULTS

Between January 1, 2012-December 31, 2013 at the Dermatology Clinic of the Iasi "Sf. Spiridon" University Emergency Hospital of the total of 7672 admissions, 38 patients were admitted for dermatologic disorders associated with thyroid diseases. Thus, the prevalence of thyroid diseases was 0.5%. Based on the obtained data we found that the prevalence of thyroid diseases had increased each year as follows: from 28 in 2012 to 34 in 2013. In the study group thyroid diseases were more common in the rural population (58% *vs.* 42%), corresponding to a ratio of 1: 1.36. A higher frequency of thyroid disorders was found among female patients (95%).

The age of the study patients ranged between 14 and 79 years, most patients belonging to age groups 50-60 years (31.8%) and 60-70 years (21%) (fig. 1).

During the study an increased incidence of the cases with autoimmune thyroiditis (63%), followed by polynodular goiter (26.3%) and hypothyroidism (10.7%) was noted. Of the 24 cases with autoimmune thyroiditis, 11 cases presented hypothyroid status and 13 cases euthyroid status, no cases of autoimmune thyroidopathy with hyperthyroid status being recorded.

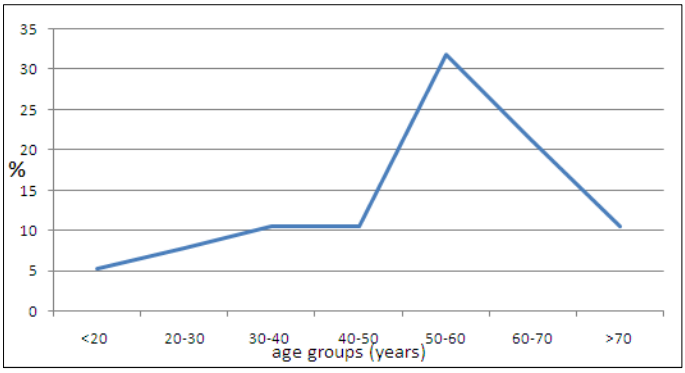

Fig.1. Age group distribution

Thyroid diseases are accompanied by various skin manifestations. In this study these manifestations were: xerosis, xanthelasma, keratoderma, oncho-dystrophy and oral candidiasis. Given the predominance of thyroid dysfunction with hypothyroid status, xerosis was the most common manifestation (45.8%) (fig. 2).

In the study certain dermatologic disorders associated with thyroid pathology were identified. There was an increased incidence of *alopecia areata* (7 cases, accounting for 22% of all skin disorders associated with thyroid diseases) followed by 6 cases (18%) diagnosed with *lichen planus* and 4 cases each (12%) with chronic urticaria and psoriasis (12%). There were 3 cases (9%) of *lupus erythematosus*. Also, *vitiligo* and scleroderma were found in a similar percentage as *lupus erythematosus*, 9% respectively. The lowest incidence of skin diseases associated with thyroid disease was recorded for *pemphigus vulgaris* (1 case), *pemphigus foliaceous* (1 case) and *granuloma annulare* (1 case), accounting for 3% of the cases.

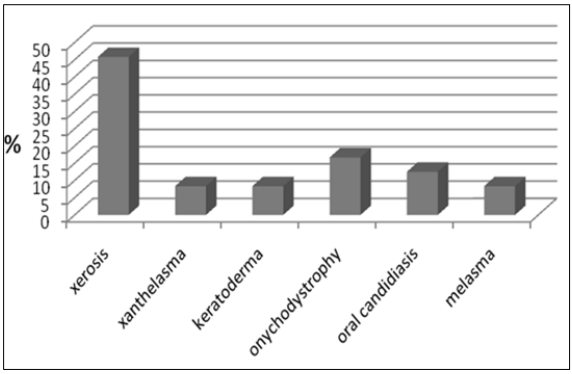

Fig. 2. Skin manifestations associated with thyroid disease

The youngest age at onset of *alopecia areata* in our patients was 19 years and the oldest age at onset 68 years, the age group over 40 years being most commonly affected (71%). There was a higher frequency of alopecia in women (86%) (fig. 3).

Fig. 3. One female patient with alopecia universalis

The association of *lichen planus* with autoimmune diseases is well-known; in this study 6 cases with different clinical forms and anatomical locations being found. The most common anatomical sites were the oral and anogenital regions. The role of autoimmune pathogenesis is supported by the association with numerous autoimmune diseases; in our study of the 6 cases of *lichen planus* associated with thyroid diseases 3 cases were associated with *vitiligo*. The youngest age of patients with *lichen planus* was 39 years and the oldest age 65 years, most cases in age group 40-60 years and with a female predominance (86%). Of the 6 cases of *lichen planus*, 3 cases were associated with autoimmune thyroid disease with euthyroid status, 3 cases with polynodular goiter and 2 cases with autoimmune thyroid disease with operated nodules.

Of the 38 study patients only 3 were diagnosed with *vitiligo* associated with autoimmune thyroiditis. The age ranges were 39-65 years, with a female predominance. In the 3 cases diagnosed with *vitiligo* the associated thyroid diseases were: euthyroid autoimmune thyroiditis (2 cases) and autoimmune thyroiditis with hypothyroid status (1 case).

Age of patients with scleroderma ranged from 53 to 73 years. All three patients were females. Thyroid diseases associated with scleroderma were autoimmune thyroiditis with hypothyiroid status (2 cases) and polynodular goiter (1 case).

Of the 38 cases with thyroid disease anti thyroid antibodies were positive only in 5 of the 7 patients with *alopecia areata*, 2 of the 3 cases with *vitiligo*, 1 of the 4 cases with psoriasis, 1 of the 3 cases with scleroderma, 2 of the 6 cases with *lichen planus* and one case diagnosed with *granuloma annulare*. Antithyroid antibodies were positive in all patients diagnosed with systemic *lupus erythematosus* and chronic urticaria, and in patients with pemphigus antithyroid antibodies were not measured.

All patients were systematically evaluated by an ophthalmologist and none of the signs mentioned above were noticed. A degree of mild conjunctival injection and dry eye symptoms could be noticed and were treated with topical medication.

The treatment was individualized for each patient depending on associated diseases. As there are no cases of hyperthyroidism, only hypothyroid patients were treated. Levothyroxine replacement therapy was initiated depending on thyroid hormone levels, no treatment being administered to euthyroid patients. Of the 38 patients, 18 were received levothyroxine. To the treatment of thyroid disease was associated the treatment of skin disease.

DISCUSSION

The prevalence of thyroid diseases at the Iasi Dermatology Clinic is 0.5%, much lower compared with literature data according to which the prevalence is estimated to be 2-5% (6, 7). Our data are in agreement with the data in the literature according to which all ages are affected by thyroid disease, with a peak incidence between 35 and 65 years.

The prevalence of *lichen planus* (16%) is similar with that reported in the literature. Kreuter et al. (8) conducted a retrospective study and found that 15.4% of patients with atrophic *lichen sclerosus* presented an associated autoimmune disease. 16.3% of patients with atrophic *lichen sclerosus* had a thyroid disease of Hashimoto thyroiditis and Graves's disease type and positive anti thyroid antibodies in 9.4%.

It has been reported that patients with chronic spontaneous urticaria also have associated autoimmune diseases, particularly autoimmune thyroid disorders such as Hashimoto thyroiditis. Several studies have shown that a significant number of patients (33%) had elevated anti thyroid antibody levels (9, 10), whereas in our study group this percentage was of only 12%. Compared to the literature, the incidence of cases with of psoriasis vulgaris at the Iasi Dermatology Clinic was higher than that reported in a study on 1236 patients, 12% *vs.* 1.77%, respectively (11).

CONCLUSIONS

This study demonstrates that there is a significant association between certain dermatologic and thyroid disorders, requiring periodic thyroid function tests.

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NEWS

INCREASED THICKNESS OF ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE OCCURS MORE FREQUENTLY IN STEATOHEPATITIS THAN IN SIMPLE STEATOSIS

The incidence of obesity is increasing and contributes to the rising incidence of fatty liver. Body mass index (BMI) is used to assess the degree of obesity but does not take into account the pattern of body fat distribution. The incidence of fatty liver disease, both nonalcoholic steatohepatitis (NASH) and simple steatosis, is increasing in the Western world. In a large autopsy study of obese patients and controls, a 6.2% incidence of NASH was found. The need to distinguish between NASH and simple steatosis is essential; as patients with NASH can go on to develop portal hypertension and cirrhosis. Body mass index (BMI) is commonly used to define overweight and obesity but does not predict the presence of NASH or simple steatosis. Despite a normal BMI, patients may still develop nonalcoholic fatty liver disease (NAFLD), especially if they have a more prominent abdominal distribution of their adipose tissue. Thicker abdominal subcutaneous adipose tissue is associated with steatohepatitis, but not simple steatosis, also for patients with BMI below 25 kg/m2. There is good correlation between abdominal subcutaneous adipose tissue thickness and BMI. A trend noted in this study is that patients with NASH died at a younger age. Fibrocongestive splenomegaly, despite the absence of cirrhosis, may occur in some patients with steatohepatitis (Fiel MI, Sima HR, Desman G, et al. Increased Thickness of Abdominal Subcutaneous Adipose Tissue Occurs More Frequently in Steatohepatitis than in Simple Steatosis. *Arch Pathol Lab Med*. 2013; 137: 642–646).

Doina Butcovan

PACHYDERMODACTYLY- ROLE OF LOCAL CORTICOTHERAPY

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PACHYDERMODACTYLY- ROLE OF LOCAL CORTICOTHERAPY (Abstract): Pachydermodactyly is a rare skin disease, defined as a benign form of digital fibromatosis. It is clinically characterized by painless hypertrophy of the skin around the proximal interphalangeal joints of the fingers, more common in males. This rare condition can affect both hands and is often associated with mechanical injury of the skin. Although there is no specific therapy, cessation of mechanical trauma associated with topical corticosteroid therapy can lead to satisfactory results. We present 3 sporadic cases of classical pachydermodactyly, anatomopathologically confirmed by the presence hyperkeratosis and acanthosis, which responded favorably to topically applied corticosteroids under occlusive dressings. **Keywords**: FIBROMATOSIS, PACHYDERMODACTYLY, STEROID THERAPY

Included in the category of superficial fibromatoses, pachydermodactyly defines a skin condition characterized by painless swelling of the tissues around the proximal interphalangeal joints of the fingers, as shown by its name (gr. *pachy* = thick, *derma-atos* = skin, *dactylos* = finger).

Pachydermodactyly is more common in males (male/female ratio 3/2). Clinically it is characterized by hypertrophy of the dorsolateral aspects of the fingers (especially the index, middle, and ring fingers), most often bilateral and symmetrical. The hypertrophic changes are progressive and limited to the skin around the proximal interphalangeal joint, the affected skin segment presenting no other changes (1).

Pachydermodactyly was first described by Bazex in 1973, and until 2014 only 150 cases have been reported worldwide, but the total number is certainly higher, many of them not being yet reported (2).

The medical literature describes cases of pachydermodactyly associated with carpal tunnel syndrome, Dupuytren's contracture, gynecomastia, varioliform macular atrophy, thyroid dysfunctions, Ehlers-Danlos syndrome, but the most common association is with local microtrauma. Based on these findings, Bardazzi *et al.* (6) described five clinical forms of pachydermodactyly:

- classic - associated to mechanical trauma and affecting more fingers;
- localized or monopachydermo-dactyly;
- familial;
- extended to the metacarpophalangeal joints and dorsal aspect of the hand;
- symptomatic - associated to tuberous sclerosis;

CASE REPORT

We present 3 sporadic cases of classic pachydermodactyly in male children aged 8, 11 and 13 years, two of them living in rural and one in urban area. The disease was diagnosed one year earlier, and the patients were monitored at the Dermatology Clinic of the Iasi "Sf. Spiridon" University Emergency Hospital (fig. 1).

Fig. 1. Skin changes around the proximal interphalangeal joints

The children presented to the clinic with asymptomatic, diffuse thickening of the skin around the proximal interphalangeal joints of fingers II-IV, bilateral in two cases and unilateral in one case. Physical examination revealed the absence of joint pain and local warmth.

Case history showed that the changes occurred many years ago, being caused by repetitive micro trauma related to farm work in which rural children take active part and also to the habit of rubbing the hands together associated especially with emotional states, behavior noticed by their parents before the occurrence of symptoms.

Laboratory tests ruled out an autoimmune-mediated inflammatory disease, the biological, biochemical and immunological tests being within normal ranges. Also, X-rays of the hands showed no joint abnormality and secondary nuclei normal for age (fig. 2).

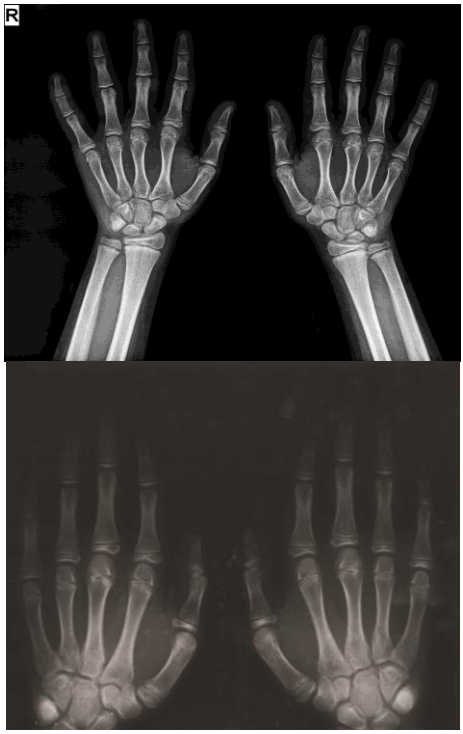

Fig. 2. X-ray image

Comparative ultrasound assessment of the hands denied the presence of any abnormal collections within the radiocarpal, metacarpophalangeal, and proximal interphalangeal joints, and appearance of normal extensor tendons, without tenosynovitis.

The clinically evident deformities around the proximal interphalangeal joints were solely due to skin thickening. At this level, skin thickness was 3.4 mm, compared to 0.7 mm in the unaffected areas on the dorsal aspect of the wrist (5-fold higher). The thickness of the scalp was also measured, and as it was normal a diagnosis of pachydermoperiostosis (Touriaine-Solente-Gale syndrome) was ruled out.

Together, the clinical and imaging findings made the diagnosis of pachydermodactyly, subsequently confirmed by histopathological examination, which revealed irregular thickening of the skin with slightly elongated dermal papillae, acanthosis and discrete spongiosis (fig.3); the corneous layer exceeded the thickness of the epidermis and the granular layer was prominent (fig. 4); there was extensive collagenization of the dermis, and fat glands and lobules were divided by thick connective tissue septa (fig. 5, 6).

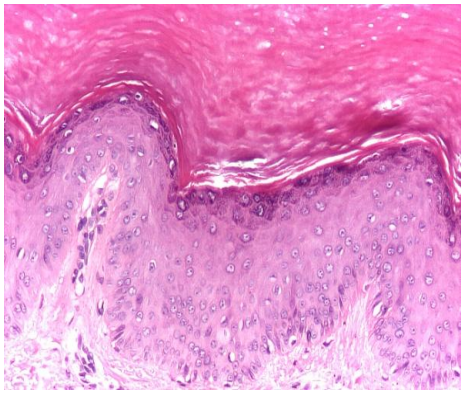

Fig. 3. Irregular epidermis with prominent granular layer, HE, x 4

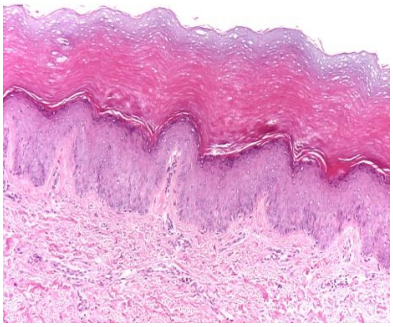

Fig. 4. Keratin crust thicker than epidermis, HE, x 4.

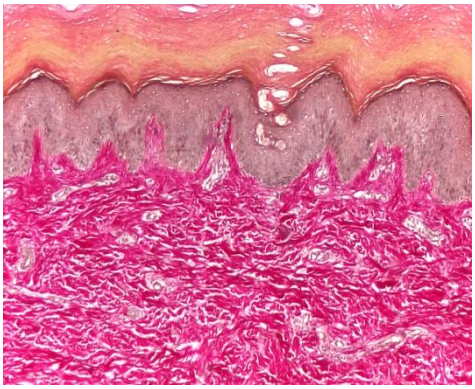

Fig. 5. Marked collagenization of dermis, VG, x 4

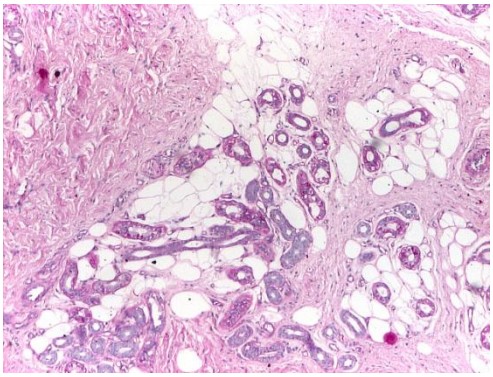

Fig. 6. Fat lobules divided by connective tissue septa, PAS, x 4

In all three cases, the performed investigations, particularly pathological examination, allowed us to differentiate the lesions from lesions with similar symptomatology, such as pachydermoperiostosis, juvenile chronic arthritis, rheumatoid arthritis, gout (gouty tophi), lichen myxedematosus, psoriatic acropachy-dermodactyly, acromegaly, fibrosarcoma, arthritis of the fingers (Heberden nodes), and joint involvements associated with endocrinopathy.

Caught in an early stage of disease, the patients had a positive response to topical corticosteroids, other invasive therapeutic procedures not being necessary. The described changes were classified as having the same degree of soft tissue injury even if in children with bilateral involvement they were present in a greater number of fingers. Potent topical corticosteroids under occlusive dressings (8-10 hours/day for 3 weeks, then a decreased frequency of applications) were used.

Psychological counseling (both child and parents), focused on the discontinuation of the habit of rubbing the hands together in situations of mental or emotional distress and teaching new relaxation techniques aimed at correcting this behavior, was also recommended.

The course was favorable, improvements being obtained after the first weeks of local corticosteroid therapy (fig. 7).

Fig.7. Hand appearance after local corticosteroid therapy

DISCUSSION

Pachydermodactyly is regarded as a localized form of superficial fibromatosis that mostly affects young adult with a mean age of 21.2 years according to Sagransky. Most commonly, the changes occur on the dorsal and lateral aspects of proximal phalanges of the index and middle fingers, with symmetrical involvement of both hands (3, 4, 5).

The mechanisms involved in the pathogenesis of this disease are not fully understood, some authors claiming that it is induced by repetitive minor local trauma. In this respect, Sagransky *et al*. (4) reported 2 cases of pachydermodactyly in poultry processing plant workers handling a significant amount of chicken per days at a fast pace. Other authors suggest as a possible mechanism the habit of rubbing the hand together, which in time leads to diffuse skin thickening and hyperpigmentation along the metacarpophalangeal joints.

The clinical features and especially a history of repetitive trauma are enough to make a diagnosis of pachydermodactyly, but histopathologic findings are useful in excluding other diseases with similar manifestations.

Male gender, and childhood or adolescence, are major contributing factors in the clinical expression pachydermodactyly.

Thus, histopathologic examination is not necessary for making the diagnosis since the diagnosis of pachydermodactyly is clinical; however it can support the diagnosis by revealing an increase in dermal collagen with variable degrees of hyperkeratosis and acanthosis. The dermis is thickened, with coarse collagen bundles, slight fibroblast proliferation, increased fibroblastic activity, and sometimes collagen deposition around sweat glands. The association of acanthosis with dermal thickening is not specific but characteristic of pachydermodactyly and is a useful criterion to distinguish pachydermodactyly from other similar diseases such as keratoderma, where dermal thickening is not present, or fibromatosis, where epidermal changes are usually absent (7).

Caught in the early stages, the patients responded favorably to local corticosteroid therapy, the dermatocorticosteroids proving their efficacy through their anti-inflammatory effects and ability to inhibit cell proliferation and collagen synthesis (8).

The anti-inflammatory effect of corticosteroids is due to the inhibition of the release of phospholipase A2, enzyme responsible for the synthesis of prostaglandins, leukotrienes and other arachidonic acid derivatives; corticosteroids also inhibit transcription factors such as activator protein-1 and nuclear factor-*kB*, involved in the activation of proinflammatory genes (lipocortin and *p11CBP* (calpactin binding proteins). Their immunosuppressive properties consist in the suppression of the synthesis and effects of hormonal factors involved in the inflammatory response, thus inhibiting the migration of leukocytes to the site of inflammation and interfering with the functions of endothelial cells, granulocytes, mast cells, and fibroblasts. The antiproliferative role is exerted through inhibition of DNA synthesis and mitoses, partially explaining the therapeutic action of these agents in dermatological diseases (9).

The efficacy of topical corticosteroids depends on their potency and degree of skin penetration, their activity being is closely related to the ability to bind to a specific receptor in the cytosol, and also to the vehicle into which it is incorporated.

In addition to local action, important is the systemic absorption of topical corticosteroids. Systemic absorption depends on the pharmacokinetic properties of the molecule, as well as on the integrity of stratum corneum and the presence/absence of inflammation; thus, topical agents applied on the skin or mucous membranes have increased absorption if the skin surface is injured or covered by an occlusive dressing (10).

Skin absorption occurs via diffusion of the dermatocorticoid in the stratum corneum. Hydrophilic steroids have a higher penetration rate through the stratum corneum than the hydrophobic ones, while keratinocytes are penetrated more rapidly with lipophilic agents, which explain the multitude of clinical effects of dermatocorticosteroids. After diffusion in keratinocytes, corticosteroids bind to the glucocorticoid receptor in the cytoplasm, inducing the cascade of reactions from which the mediators of messenger RNA synthesis result. The binding capacity of the glucocorticoid receptor in the cytoplasm is greater the lipid solubility of the dermatocorticosteroid is higher. In the human body there are many cells that contain receptors for glucocorticoids, thus explaining the receptor-mediated effects of

glucocorticoids (11).

In the use of local corticosteroids, a rather important problem is the phenomenon of tolerance, which could require a discontinuous drug administration schedule, using a double dose every two days. Another problem is withdrawal, which can be avoided, as in the case of systemic corticosteroids, by progressively spaced applications, or maintaining everyday applications but using a lower potency topical corticosteroid. Also to keep in mind are the complications, occurring especially when potent local corticosteroids (classes 3 and 4) are used; these include skin atrophy, stretch marks, purpura, delayed wound healing, rosacea, pigmentation disorders, and the most frequent systemic complications are hypothalamic-pituitary-adrenal axis suppression and Cushing's syndrome.

CONCLUSIONS

This case report presents the management of three cases of pachydermodactyly, rare entity, with an favorable outcome at local corticotherapy. Its purpose is to attract attention to the importance of clinical findings and minimal non-invasive investigations that can make the diagnosis of pachydermodactyly, and could avoid unnecessary costs incurred by sophisticated imaging investigations or inappropriate therapies.

ACKNOWLEDGEMENTS

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CHARACTERISTICS OF CO INFECTION WITH HEPATITIS B VIRUS AMONG ROMANIAN PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS

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CHARACTERISTICS OF CO INFECTION WITH HEPATITIS B VIRUS AMONG ROMANIAN PATIENTS INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS (Abstract). **Aim:** To determine the epidemiological and viroimmunological features and outcome of HIV/HBV-co infected patients cared in the Iasi HIV/AIDS Regional Center. **Material and Methods**: This retrospective study included 252 patients diagnosed with HIV infection and associated hepatitis B virus (HBV) infection assessed at the Hospital of Infectious Diseases in the interval 2000-2013 and treated with antiretroviral drugs active against both HIV and HBV. **Results**: The prevalence of HIV/HBV co infection was 19.9%. A slightly higher frequency of this co infection was found among males (53.2%); most patients belonged to age group 20-29 years (86.5%), mean age was 25.56 years. The predominant route of transmission was parenteral (58.5%), followed by heterosexual transmission (40.1%). The mean CD4 cell count was 246.20 cells/mm^3, in over 41% of cases CD4 count ranging from 200 to 499 cells/mm^3. The mean HIV plasma viral load was 142,906 copies/ml. ALT levels varied between 10-323 IU/l, average 49.90 IU/l, over 65% of subjects having pathological levels. In 21.8% of the cases, total cholesterol was very high, and in 16.8 % of the patients the serum triglyceride levels were below the reference range (160 mg %). **Conclusions**: Our results suggest that HIV-positive patients, chronic hepatitis B infection has a high incidence, especially in younger age groups and is correlated with significant degrees of immunosuppression. **Keywords:** HIV/HBV CO INFECTION, VIROLOGICAL RESPONSE, IMMUNE RESPONSE, ART.

Infection with human immunodeficiency virus (HIV) continues to be a major health problem, the average number of people infected worldwide by the end of 2011 being approximately 34 million. Although the introduction of effective antiretroviral therapy resulted in a significant decrease in the number of deaths related to acquired immunodeficiency syndrome (AIDS), with the extension of life expectancy liver disease has become the most common cause of death among HIV-infected patients, accounting for 14 to 18% of all deaths in this population (1, 2, 3).

Worldwide, hepatitis B is the leading cause of chronic liver disease, accounting for up to half of all cases of cirrhosis and hepatocellular carcinoma; it is estimated

that approximately 400 million people are infected with hepatitis B virus (HBV), most cases occurring in regions in Asia and Africa, where the virus is endemic (4).

Due to the common routes of transmission, HBV and HIV infections coexist in about 3-6 million people worldwide, the prevalence of chronic HBV infection being 10 times higher in HIV-infected people than in the general population (5). HBV/HIV co infection rate mostly depends on the affected community and HBV transmission route, which differs depending on the geographic area (6,7). The prevalence of HIV/HBV co infection in Europe is about 9% (8), while in Romania it reaches about 35-37%, much higher than the European average (9, 10).

HBV infection is considered an opportunistic infection in HIV-infected patients, the co infection with these two viruses causing complex interactions. HIV infection modifies the natural history of chronic hepatitis B by enhanced replication of HBV and higher serum HBV DNA levels, lower rates of HBsAg and HBeAg seroconversion, increased fibrosis, and faster progression to liver cirrhosis (11, 12). HBV may accelerate immunodeficiency and the course of HIV infection by a direct activation of HIV replication and induction of a state of persistent immune activation (13).

This study aimed to describe the epidemiological and viroimmunological features and the course of chronic HBV infection and to determine its prevalence in the HIV-infected patients receiving care and treatment in the Iasi Regional HIV-AIDS Centre.

MATERIAL AND METHODS

This retrospective study (2000-2013) included 252 patients (female 46.8%, male 53.2%, mean age 25.6 years) with HIV/HBV co infection receiving care and treatment in the Iasi Regional HIV/AIDS Centre.

The epidemiological, clinical, and treatment data were collected retrospectively from patients monitoring and clinical records.

The followed parameters were: epidemiological (mode of transmission, age, gender) viroimmunological (dynamics of CD4 and HIV plasma viral load), metabolic (transaminase, total cholesterol and triglyceride levels), and therapeutic. CD4 count was determined by flow cytometry according to CDC classification, and differentiated into < 200, 200-499, and ≥ 500 cells/ml. The evolution of HIV viremia was followed, the undetectable HIV viral load (viral suppression) being based on the analysis of the lower limit of quantification (HIV RNA 50 copies/ml).

Co infection with HBV was defined as the persistence of positive HBsAg in at least two determinations at a minimum interval of 6 months, including the cases of chronic infection and inactive HBV carrier status. The patients with HCV infection or HBV/HCV and HBV/HDV co infection were excluded to avoid possible interactions.

Highly Active Antiretroviral Therapy (HAART) was defined as a combination of at least three antiretroviral (ARV) drugs, including the association of two nucleoside reverse transcriptase inhibitors with either a protease inhibitor, or with a non-nucleoside reverse transcriptase inhibitor. Particular attention was paid to the therapeutic history of drugs active against HBV replication used to treat HIV infection: lamivudine (3TC), emtricitabine (FTC), tenofovir (TDF).

The evaluation and interpretation of the

results was based on the frequency and structure indicators processed with *SPSS* statistics using Student t-test, χ^2 test, Pearson correlation coefficient and the linear trend. P values 0.05 were considered significant.

RESULTS

Over the 14-year study period, the 1358 HIV-infected patients cared and treated in the Iasi Regional HIV/AIDS Centre had periodic serologic testing for HBV; HBsAg was present in 252 of them; thus, the incidence of chronic hepatitis B was 19.19%.

In our series, HIV/HBV co infection was slightly more common among males (53.2%); in terms of age group distribution, as expected, most patients were in the age group 20-29 years (86.5%), the proportion of patients under age 19 and over age 50 being lower. Mean age was 25.56 years, range 6-59 years, with a wide coefficient of variance (40.07%) (tab. I).

In most patients (58.5%) HIV infection was transmitted by parenteral routes, followed by heterosexual transmission (40.1%); 2.4% of HIV infections occurred through homosexual contact, and transmission through injecting drug use was not reported.

TABLE I

Gender and age -group distribution

Characteristic	Number	%
Male sex, *n* (%)	135	53.2%
Age years ≤ 19	1	0.4
20-29	218	86.5
30-39	15	6.0
40-49	15	6.0
50-59	3	1.1
Median age (yrs.)	25.56	

According to CDC classification, most of the study patients were in advanced stages of HIV infection: 46.82% stage C, 34.9% stage B, and 18.25% stage A.

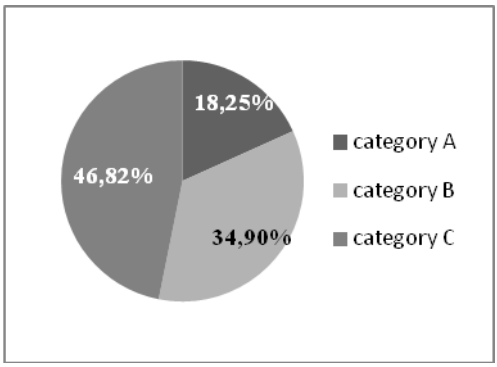

Fig 1. Baseline CDC clinical category of HIV infection

A large number of patients had a significant degree of immunosuppression with a CD4 lymphocyte count < 200cells/mm³ (48.4%) while > 500 cells/mm^3 were found in only 10.3% of the study patients. The individual CD4 count ranged from 3 to 1069 cells/mm^3, with an average CD4 count of 246.20 cell/mm^3.

HIV viral load ranged from undetectable, in 18.3% of the subjects, to a maximum of 4,600,000 copies/ml. About 26.2% of the cases had plasma levels above 100,000 copies/ml, the average viral load being 142,906 copies/ml (tab. II, fig. 2).

Hepatic cytolysis syndrome was a parameter constantly followed throughout the study: 65% of subjects had alanine aminotransferase (ALT) levels above the reference limit (32 IU/ l) and 36.1% exceeded the mean value of 49.9 IU/l in the study group.

The following metabolic abnormalities, common in HIV-infected patients, were found in the study patients. Thus, total cholesterol levels above the reference limit were found in 21.8% of subjects, while the

individual levels of serum triglycerides ranged from 11 to 256 mg%, average 130.72 mg%.

There were a small percentage of cases that had no indication for treatment, while over 98% of patients required antiretroviral therapy, fact accounted for by the age of the study group, most of them being multiple treatment-experienced patients. It is worth mentioning that all HIV/HBV-co infected patients received antiretroviral therapeutic agents with dual action against HIV and HBV, 92.3% receiving lamivudine alone or in co formulations, in only 3.6% of the cases the therapeutic option being tenofovir (fig.3).

TABLE II

Characteristics of patients with HBV/HIV co infection

Characteristic	Number	%
Transmission, *n* (%)		
Heterosexual	101	40.1%
Homosexual	6	2.4%
Nosocomial	145	57.5%
Baseline CDC classification		
Class A	46	18.25%
Class B	88	34.9%
Class C	118	46.82%
CD4(cells/ml)		
<200	122	48.4%
200-499	104	41.3%
> 500	26	10.3%
CD4 mean (cells/ml))	246.2	
Viral load copies/ml>100 000	66	26.2%
10,000-100,000	81	32.1%
0-10,000	59	23.4%
undetectable	46	18.3%
Viral load, mean (copies/ml)	142,906	

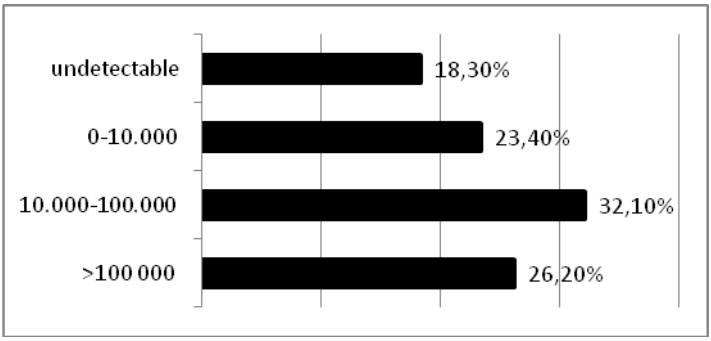

Fig. 2 Baseline virological status

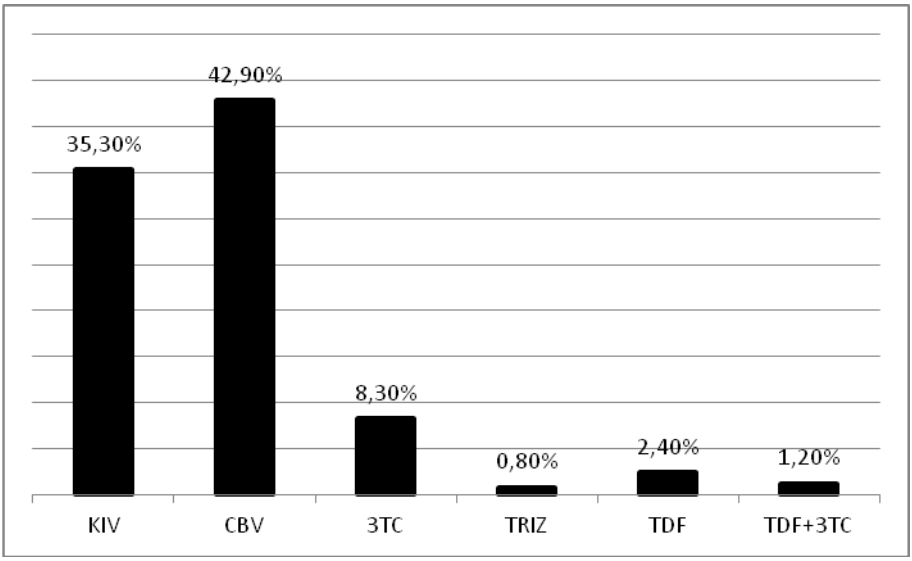

Fig. 3. ART regimens used in HIV-HBV co-infected patients
Kivexa KIV)= abacavir+lamivudine, Combivir (CBV)=zidovudine+lamivudine, 3TC=lamivudine,TDF=tenofovir, FTC=emtricitabine, Trizivir(TRZ)=abacavir +lamivudine+zidovudine

DISCUSSION

High rates of HBV infection have been described among HIV-infected population worldwide, and the risk of HBV-associated end-stage liver disease appears to be increased in HIV-co infection; thus, for a specific serological surveillance and proper initiation of treatment very important is to estimate its regional prevalence (14).

Our study provides the first information on hepatitis B co infection among the population infected with the HIV in Northeastern Romania. Our data show a prevalence of 19.19%, lower than that reported by two previous studies conducted in our country (10, 15), possibly because they included a selected population of HIV-infected children and adolescents, respectively. Our study emphasizes that HIV-infected patients are at a 2.5 - 3 times higher risk for HBV infection compared to the general population of Romania.

The epidemiology of HBV/HIV co infection is complex and incompletely elucidated, and co infection rates depend on the prevalence of HBV infection in the general population and the dominant transmission routes of HIV infection (16, 17). The epidemiology of HIV infection in Romania is unique in Europe as almost all cases are attributed to horizontal transmission, nosocomial, of the virus during early childhood. In our study population study, co infection was mainly associated with this mode of transmission, sexual transmission being prevalently recorded during the last 5-7 years of the study. Our study showed that intravenous drug use, a known risk factor for co infection is virtually absent in the cohort of HIV-infected patients in Northeastern Romania (17). Surprisingly, this did not lead to a significantly lower rate of co infection compared to other HIV populations in Europe with a much higher rate of

intravenous drug use (18).

A statistical association regarding the risk factors for HBV infection showed a higher prevalence in the age group 20-29 years, a known and observed fact in our country, due to the higher risk of exposure to the two viruses by parenteral route. The predominance of male gender was also observed, as described previously in many population groups, probably because of more frequent exposure to risk factors involved in transmission, especially sexual contact with a greater number of partners.

The significant percentage of patients in advanced stages of disease compared with other studies can be explained on one hand by the epidemiological characteristics of HIV infection in our country, most patients being part of the cohort of long-term survivors, on the other hand by the possible influence of HBV on the acceleration of HIV disease progression (19,20).

The lack of a significant immune response against HBV is reflected in the average levels of transaminases which showed pathological levels in more than half of HIV/HBV- co infected patients in the study group.

A limitation of our study is the lack of a complete set of serological markers of HBV and other important parameters such as HBV viral load and HBV genotype. Due to limited financial resources, in many countries HBsAg remains the only widely available test for the diagnosis of chronic hepatitis B in HIV-infected patients. Another limitation may be due to the retrospective design of the study that enrolled mostly compliant patients with good adherence to ARV therapy, coming for regular checkups.

CONCLUSIONS

In this study provides new insight into the current epidemiology and clinical features of HBV/HIV co infection in Northeastern Romania. Current data reveal a prevalence of chronic HBV infection of 19.19% in the HIV-infected population, indicating a significantly increased risk compared with the general population.

Our findings highlight the importance of proper management of patients infected with human immunodeficiency virus which has to include prophylaxis, regular screening for HBV infection, and administration of potent anti-HBV therapy in co infected patients according to current guidelines.

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CLINICAL FEATURES AND EVOLUTION OF ORGAN DYSFUNCTIONS IN SEPSIS

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CLINICAL FEATURES AND EVOLUTION OF ORGAN DYSFUNCTION IN SEPSIS (Abstract): In sepsis, the systemic inflammatory response is adapted to the etiologic agent and the increase in the level of mediators is associated with organ dysfunction. Currently, a rapid assessment of patient ability to develop an adequate immune response is not possible, the response mechanisms being similar in the context of different etiological agents. **Aim:** To find statistical arguments for the evolution of laboratory parameters in sepsis patients. **Material and methods:** This retrospective study included 90 patients diagnosed with sepsis. The clinical, etiological, and laboratory data, and Carmeli and APACHE II prognostic scores were analyzed. The data were processed using SPSS version 16.0. **Results:** The causative agents was identified in 16 cases; organ involvement and systemic response varied, and no statistical correlations were found between the inflammatory syndrome parameters and Carmeli or APACHE II prognostic scores or identification of the causative agent. **Conclusions:** Statistical correlations were found between maximum blood glucose levels and the presence of organ dysfunction in the studied sepsis patients. No correlations were found between sepsis severity and the presence of anemia or thrombocytopenia, or between fever syndrome and inflammatory syndrome. **Keywords***:* DATA ANALYSIS, ORGAN FAILURE, SYSTEMIC INFLAMMATORY RESPONSE SYNDROME.

The notion of sepsis had been under intense debate and considerable efforts have been made over the past 20 years to identify therapies that reduce mortality (1,2). There is such an extraordinary heterogeneity in patients with sepsis characterized by different conditions, infection site, microbial agents with large variations in virulence and concentration, as well as a different inflammatory response and immunological adaptation, that limiting the concept of sepsis to a surge in inflammation may be considered no more than a simplistic approach (3, 4, 5).

Inflammatory response is often adapted to the etiological agent or toxinic aggression or, on the contrary, a series of microorganisms may lead to an increase in the level of mediators that cause aggressions in the infected organism, associating acute respiratory dysfunction, multiple organ dysfunctions, and refractory hypotension (6, 7). At present, a quick evaluation of patients' capacity to show an adequate immune response is impossible and it is considered that mechanisms related to or-

gan dysfunction and those involved in evolution towards death could be similar in the context of different etiological agents, while variable response in sepsis may be related to infection distribution at organ level (7, 8).

MATERIAL AND METHODS

This 7-month retrospective study (January-July, 2013) aimed to determine clinico-epidemiological and evolutive correlations under specific therapy based on the analysis of the data in patient records. Ninety patients with sepsis of known or unknown etiology admitted to the Iasi Hospital of Infectious Diseases were included in this study. The following were analyzed: social status, length of hospital stay, duration of febrile state, etiology, Carmeli prognostic score, APACHE II score, and some biological parameters based on which we tried to establish correlations or differences in terms of statistical significance.

Inclusion criteria were: suspected or proven infection associated with systemic inflammatory response syndrome (at least two criteria: fever/hypothermia, tachycardia/tachypnea, leukocytosis/leucopenia), and severe sepsis diagnosed by the presence of such associated organ dysfunctions as respiratory, renal and cardiac failure, neurologic impairment, disseminated intravascular coagulation or shock.

Biological samples were processed according to the standards for bacteriology, hematology and biochemistry laboratories, and the database created in Excel was processed using SPSS 16.0, $p<0.05$ being considered statistically significant. When quantitative parameters did not show a normal Gauss-Laplace distribution and *t-student* test could not be applied, nonparametric tests were used (*Mann-Whitney Test*).

RESULTS

The 90 patients included in this study were aged 3 to 84 years, mean age 58.5 years, median age 62 years. The mean age of patients associating organ dysfunctions was 60.4 years, while the median age of those classified according to APACHE II score was 59 years. No statistically significant age differences were found between the patients associating organ dysfunction and those without dysfunctions (*calculated* $p = 0.292 > 0.05$).

The causative agents could be identified in 16 cases, and organ and systemic affectation varied (tab. I).

TABLE I

Etiology and organ damage in the study sepsis patients

Microorganism	No. of cases	Isolation site	Organ damage	Carmeli score	APACHE II score
E. coli	4	2 HC 2 HC+UC	kidneys	1 in class 1 2 - 2 1 - 3	2 < 20 (I) 2 between 20-29 (II)
Staphylococcus aureus Meti-R	5	5 HC	1: heart+ skin 3: skin + lungs 1:heart+skin+lungs	2 - 2 3 - 3	2 < 20 (I) 3 between 20-29 (II)

Microorganism	No. of cases	Isolation site	Organ damage	Carmeli score	APACHE II score
Coagulase-negative staphylococcus	1	HC	lungs	3	24 (II)
Streptococcus spp.	1	HC	heart	2	22 (II)
Proteus mirabilis	2	HC	1: liver 1: kidneys	2	20-29 (II)
Enterococcus faecalis	1	HC	lungs + liver	2	26 (I)
Bacilli Gram negative	1	HC + UC	CNS + liver + kidneys	3	28 (II)
E. coli + Enterococcus spp.	1	HC + UC	kidneys	3	34 (III)

HC= hemoculture; UC= uroculture; CNS= central nervous system

In all study patients the parameters of inflammatory syndrome (white blood cells (WBA), fibrinogen, erythrocyte sedimentation rate (ESR), and CRP were determined upon admission and we aimed at determining both the possible correlations between these parameters and between them and blood glucose levels. Significance level was below 0.05, so no correlations between these parameters were found in our sepsis patients.

Blood glucose levels were higher in patients associating organ dysfunction, the statistically significant difference confirming a correlation between blood glucose level and the presence of organ dysfunction in the study patients (fig. 1).

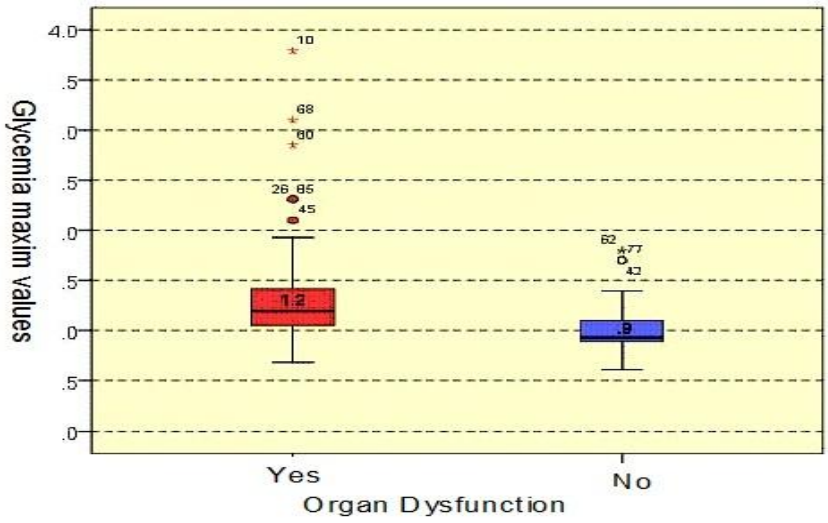

Fig.1. Correlations between blood glucose levels (g/l) and organ dysfunction in the study sepsis patients

DISCUSSION

The statistical analysis of the avriables of systemic inflammatory syndrome has shown no correlations between high blood sugar, fibrinogen, ESR, and CRP levels and high body temperature in sepsis patients with or without associated organ dysfunction.

Anemia syndrome, defined as a serum hemoglobin concentration of less than 10 g%, and thrombocytopenia as a platelet count below 100.000/mm³, were more common in the patients with APACHE II severity classes II and III than class I, but no statistically significant differences were found. Thus, the *t-student* test was used for hemoglobin, and the *Mann-Whitney test* for thrombocytes, the data not being normally distributed.

The possible correlations between organ dysfunction and the identified cause of sepsis were analyzed using the *Chi-Square* test, no statistically significant correlations being found.

CONCLUSIONS

In the study sepsis patients we have found statistical correlations between maximum blood glucose level and the presence of organ dysfunctions.

Anemia and thrombocytopenia, frequently associated with the systemic inflammatory response in these patients, did not correlate with the severity of their sepsis progression. Also, no statistical correlations were found between febrile syndrome and inflammatory syndrome in patients with or without organ dysfunctions.

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ENDOSCOPIC THERAPY WITH CURATIVE INTENT FOR NEOPLASTIC EPITHELIAL LESIONS: INDICATIONS REWIEW AND THE ROLE OF "WATER-JET" TECHNOLOGY

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ENDOSCOPIC THERAPY WITH CURATIVE INTENT FOR NEOPLASTIC EPITHELIAL LESIONS (Abstract): Neoplastic lesions of the gastrointestinal tract have a large prevalence worldwide and in Romania. Their therapy is tailored to their TNM stage, which can be estimated using non invasive or minimally invasive imaging studies. Precursor lesions and superficial carcinomas are amenable to endoscopic therapy, providing that their endoscopic and/or endoscopic ultrasound aspect suggest a maximal extension to the submucosal layer. Polypectomy, endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) are ablative techniques used for their curative intent treatment. Large superficial carcinomas are to be removed by ESD as "en bloc" resection is essential to post procedure histological appraisal of the curative oncology nature of their resection. ESD complications rates and duration are nowadays reduced with the aid of "water-jet" technology, by combining submucosal fluid jet injection and electro-resection in a single knife. **Keywords:** ENDOSCOPIC, NEOPLASTIC, "WATER-JET".

NEOPLASTIC EPITHELIAL DIGESTIVE LESIONS

Digestive tract cancers have high prevalence worldwide, colorectal cancer ranking 3rd in all cancers (after breast and lung cancer), gastric cancers ranking 4th and esophageal cancer ranking 8th. In Romania, for 2008, the incidence values are 22.8/100000 for colorectal cancer (2nd in Romania), 10.3/100,000 for gastric cancer (6th) and 2/100,000 for esophageal cancer (20th) (1).

Most digestive tract cancers have epithelial origin, being carcinomas. Transition process from normal epithelium to carcinoma passes through pre-neoplastic lesions with different degrees of dysplasia. Carcinomas invade the digestive wall, from the inner to the outer strata: first are limited to the epithelium (Tis, T0), then invade mucosa and submucosa (T1), next the muscular layer (T2), then the serous membrane (T3) and finally they may invade the adjacent organs (T4), T being the stage in the TNM classification (2).

If a certain level of the wall invasion is reached, neoplastic cells may migrate through the lymphatic vessels to the regional lymph nodes (lymph node metastasis, N1, TNM) or through the blood vessels to distant organs (distant metastasis, M1, TNM). It has been proved that the risk of metastasis is null if the submucosal invasion is less than 200 μm for the esophagus,

500 μm for the stomach and 1000 μm (1mm) for colorectal carcinomas (3,4). There is a direct relation between the T stage and the metastasis risk, with the cut-off between null to positive located at a certain level of the submucosa.

High risk pre-neoplastic and neoplastic lesions are to be treated, if possible, with curative intent by endoscopic or surgical excision, with resection limits in normal tissue. Therapy decision is taken depending on the TNM pre-therapeutic stage, which cannot be precisely known, but only predicted with maximum probability: T stage depending on the endoscopic and endoscopic ultrasound aspect (see forward); N and M stage depending on the endoscopic ultrasound and CT scan aspect and sometimes by other imaging methods (magnetic resonance imaging MRI, position emission tomography PET scan, bone scintigraphy, etc)

Therapy result will only be known after the pathological exam of the resected specimen which will ascertain if the neoplastic tissue has been totally resected with healthy tissue limits.

ENDOSCOPIC THERAPY WITH CURATIVE INTENT

Endoscopic therapy with curative intent aims to excise mucosal and submucosal layer including the neoplastic tissue, with normal lateral and profound resection borders, leaving the muscular layer and serous membrane in place. This can only be achieved for pre-neoplastic lesions and carcinomas staged T0, T1m and T1sm. For these lesions endoscopic therapy is less invasive than surgery.

Post endoscopic therapy pathological exam specimen resection will assess whether the neoplastic tissue has been entirely resected with normal safe lateral and profound limits, as well as the relation between the depth of neoplastic invasion and the metastatic submucosal risk level previously mentioned. If the resection is curative (all neoplastic tissue has been resected, with normal limits, and the depth of invasion is lesser than the metastatic submucosal risk level) then no complimentary therapy is necessary. Otherwise, if the resection is not curative, a complementary treatment is needed, endoscopic or more often surgical. This histological assessment can only be done is the lesion has been resected in a single specimen.

PRE-ENDOSCOPIC THERAPY STAGING

Pre-neoplastic and neoplastic lesions with invasion limited to mucosa or submucosa (T0, T1m or T1sm) are considered "superficial" neoplasm, type 0 in the Japanese classification of digestive tract carcinomas (5). These lesions can be further subdivided according to Paris classification in polypoid and non-polypoid (3,4), each subtype having a certain risk of submucosal invasion (tab I).

Moreover, for colorectal lesions, the submucosal invasion risk can be estimated depending on the shape of the mucosal crypts at high resolution endoscopy, the "pit-pattern" analysis defined by Kudo (6,7). Type I (*round*) and type II (*stellar*) pertain to non-neoplastic lesions (hyperplastic polyps), type III_S (*short tubular*), type III_L (*long tubular*) and type IV (*branched*) correspond mainly to adenomas or at most Tis carcinomas, while type V (*irregular and non-structured*) corresponds to carcinomas, frequently invasive into submucosa. The submucosal invasion probability is 4% for type III_S, 0% for type IV and 41% for type V.

TABLE I

The risk of submucosal invasion for superficial carcinomas (type 0 in Japanese classification) according to their endoscopic aspect in Paris classification

Type	Subtype		Esophagus	Stomach	Colorectal
Polypoid	0-I		79%	57%	
	0-Ip	Pedunculated			5%
	0-Is	Sessile			34%
Nonpolypoid	0-IIa	Superficial, elevated	48%	29%	4%
	0-IIb	Superficial, flat	15%	20%	0%
	0-IIc	Superficial, depressed	27%	40%	61%
	0-III	Excavated	84%	-	-

The accuracy of endoscopic ultrasound in superficial carcinoma staging is 69-76% for esophageal lesions (8, 9, 10), 86% for gastric lesions (11) and 60-80% for colorectal location (12).

CURATIVE INTENT ENDOSCOPIC RESECTION AND METHODS

Classical indications are pre-neoplastic lesions and superficial carcinomas staged uT0 or uT1m in endoscopic ultrasound with an endoscopic aspect suggesting submucosal invasion absence.

To these, extended indications add carcinomas staged uT1sm in endoscopic ultrasound and carcinomas with possible submucosal invasion. The explanation for these extended indications lays on 2 factors: *first*, the frequent over-staging by endoscopic ultrasound of these lesions and *second*, on the fact that even if there is submucosal invasion, the resection may still be curative if the metastatic submucosal risk level has not been reached.

In all cases, but especially for extended indications, the post resection histological assessment is essential, which makes the *"en bloc"* resection imperative.

The polypoid pedunculated (0-Ip) lesions are resected by polypectomy, while the polypoid sessile (0-Is) and non-polypoid lesions are resected by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD).

For superficial gastric cancer, in tab.II are mentioned the classical and extended indications for EMR/ESD and surgery, as formulated by the *Japanese Gastric Cancer Association*, by Gotoda (13, 14). To note that besides the endoscopic aspect, the size and differentiation are factors to be taken into account when establishing the therapeutic method.

EMR versus ESD

EMR and ESD use fluid injection into the submucosa to make lesions became protrusive and distance them from the *muscularis propria* so as to avoid its thermal injury with consequent perforation. While for EMR the lifted lesion is resected in one or multiple fragments (in case of large lesions) by the polypectomy snare, for ESD the lesion is dissected around and beneath in the submucosal space, in a similar fashion to surgical dissection, thus obtaining a one piece resection specimen.

TABLE II

Therapeutic indications for superficial gastric carcinomas. ❖– classical indication for EMR. ☒– extended indication for ESD. ⛆– relative indication for surgery. ★– absolute indication for surgery.

Histology	T1m carcinoma				T1sm carcinoma	
	Non ulcerated (0-IIa, 0-IIb)		Ulcerated (0-IIc, 0-III)		Sm1	Sm2
	≤20mm	>20	≤30	>30	≤30	Any size
G1, G2, differentiated	❖	☒	☒	★	☒	★
G3, undifferentiated	⛆	★	★	★	★	★

The ESD technique is as follows: marking of the lesion using coagulation dots around the lesion is firstly done, next fluid is injected into the submucosa beneath the lesion so as to lift it, then a circumferential incision around the dots is performed and finally the submucosal space under the lesion is carefully dissected. Dissection is done using specially designed knifes (fig. 1). The procedure is usually long, needs repeated fluid injection and preventive or therapeutic hemostasis of submucosal vessels.

(a) (b) (c)

Fig. 1. ESD knifes. (a) Dual knife, (b) Hook knife, (c) IT Knife 2

ESD has emerged as an alternative to multiple fragments "piece-meal" EMR, the advantages of ESD being:

- it respects the principles of surgical oncology - a one piece resected specimen allowing for histological assessment of its curative or non-curative nature. If lateral and profound borders are clear of the disease and there is no risk of distant metastasis the resection is curative. If the borders are not clear of the disease or the invasion depth is deeper then the submucosal metastasis risk level, then the resection is not curative and a complementary therapy is needed;
- the local relapse rate is minimal because there is theoretically no residual tissue;

In the mean time, ESD has some potential disadvantages:

- the bleeding and perforation risk is higher than for EMR. In a French ESD

register published in France in 2011, on the 118 cases realized in more than 2 years, the bleeding rate was 11.2%, while the perforation rate was 18.1%. The complications rate was 18.5% for the esophagus, 24% for the stomach and 36.5% for colon and rectum (15)

- a longer procedure time, which can reach several hours for lesions sized 10 to 15 cm diameter.

"WATER-JET" TECHNOLOGY IN EDS

Many ESD knifes have been designed to lessen its disadvantages. Some late generation knifes have multiple capabilities, both for submucosal dissection and fluid injection. The aim is to lower perforation rates and shorten procedure time.

The simplest of such knife is Flush Knife developed by Fuji Corporation (fig. 2). Apart its dissection role, it also has fluid injection capability, but only for washing the operating field during procedure improving visibility, and not for submucosal elevation. This knife has already entered in endoscopy practice, with very good results in clinical trials – one case series with 100% "en bloc' resection rate, no complications and no local 12 months relapse rate (18).

The second knife with mixed capabilities has been developed by the ERBE Corporation, the Hybrid Knife (fig. 3). It allows both submucosal injection and dissection, shortening the procedure time by avoiding the frequent instrument exchange in the operating channel during the procedure (dissection knife and injection needle). Consequently, the perforation risk is minimized because the operator may inject fluid into the submucosa at any time during procedure, keeping the muscular layer at distance and avoiding its thermal injury. It has been initially developed for neurosurgical interventions (19). Initial in vivo animal studies have shown good results: it lowers complications rates on esophageal ESD (20), it decreases complications rate and procedure time in the stomach (21) and it diminishes perforation rate for colorectal ESD (22).

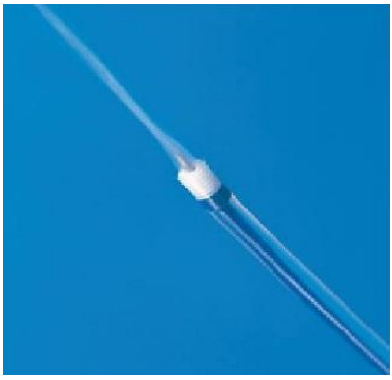


Fig. 2. Flush Knife.

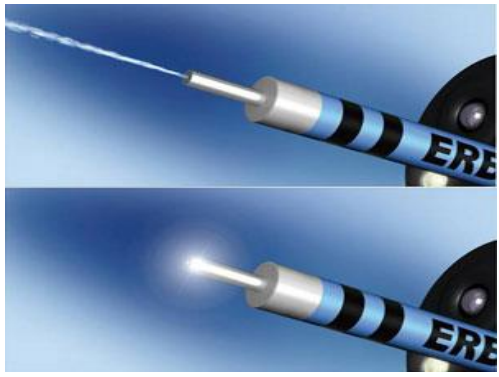
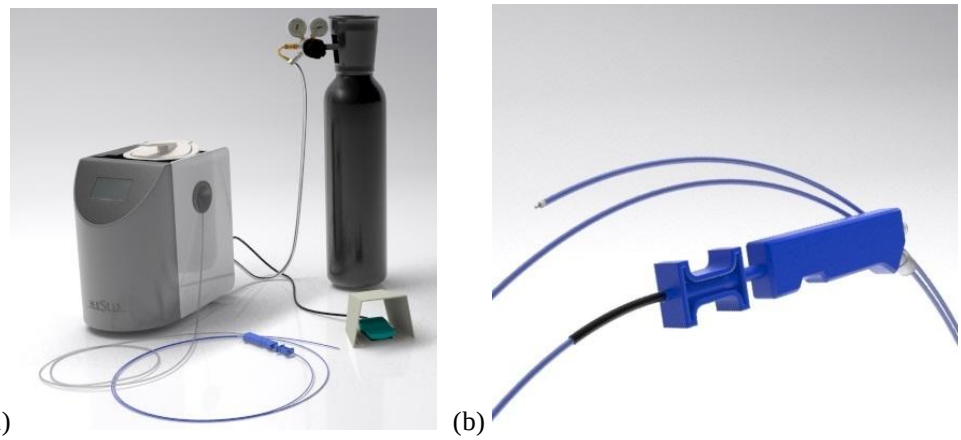


Fig. 3. Hybrid Knife

The two abovementioned instruments are already in clinical practice; in the French paper previously mentioned 16% and 12.8% of the ESD procedures have used Flush Knife and Hybrid Knife respectively (15).

The last mixed knife capability is ENKI-2 developed by NESTIS Corporation (fig. 4). The principle of a single submucosal dissection and injection instrument is kept, as for Hybrid Knife, but the difference is in the way of achieving fluid injection pressure: for Hybrid Knife an electrically driven fluid pump is used, while for ENKI-2 the injected fluid lays in a liquid nitrogen pressure chamber. The difference in fluid injection is significant, because high pressures are obtained with a constant pressure during injection, without an ascending and descending phase (fig. 5).



(a) (b)

Fig. 4. ENKI-2 system. (a) ENKI-e dissection knife connected to the pressure chamber, itself connected to a liquid nitrogen canister; (b) details of the EKNI-2 knife

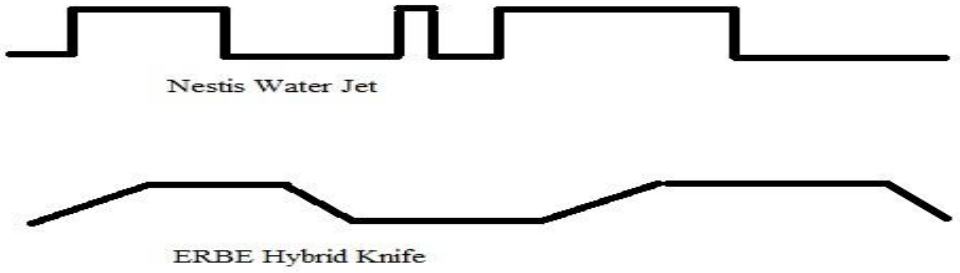


Fig. 5. Fluid injection pressure variation, comparison between Hybrid jet and ENKI-2

OUR EXPERIENCE

We have been part of the team testing the ENKI-2 system in vivo animal models. The results show that ENKI-2 fluid injection increases dissection speed of gastric ESD, but it will not be faster than knife electro-dissection, even with a pulsed injection pattern (25). ENKI-2 diminishes the perforation rate and the procedure time for an experienced ESD operator, while for an inexperienced one the decrease is not statistically significant (26). Here are some images from these in vivo experiments on colorectal pig model, from our collection (fig. 6).

ENKI-2 has CE marking; it is already used for human cases and is available in many centers throughout Europe.

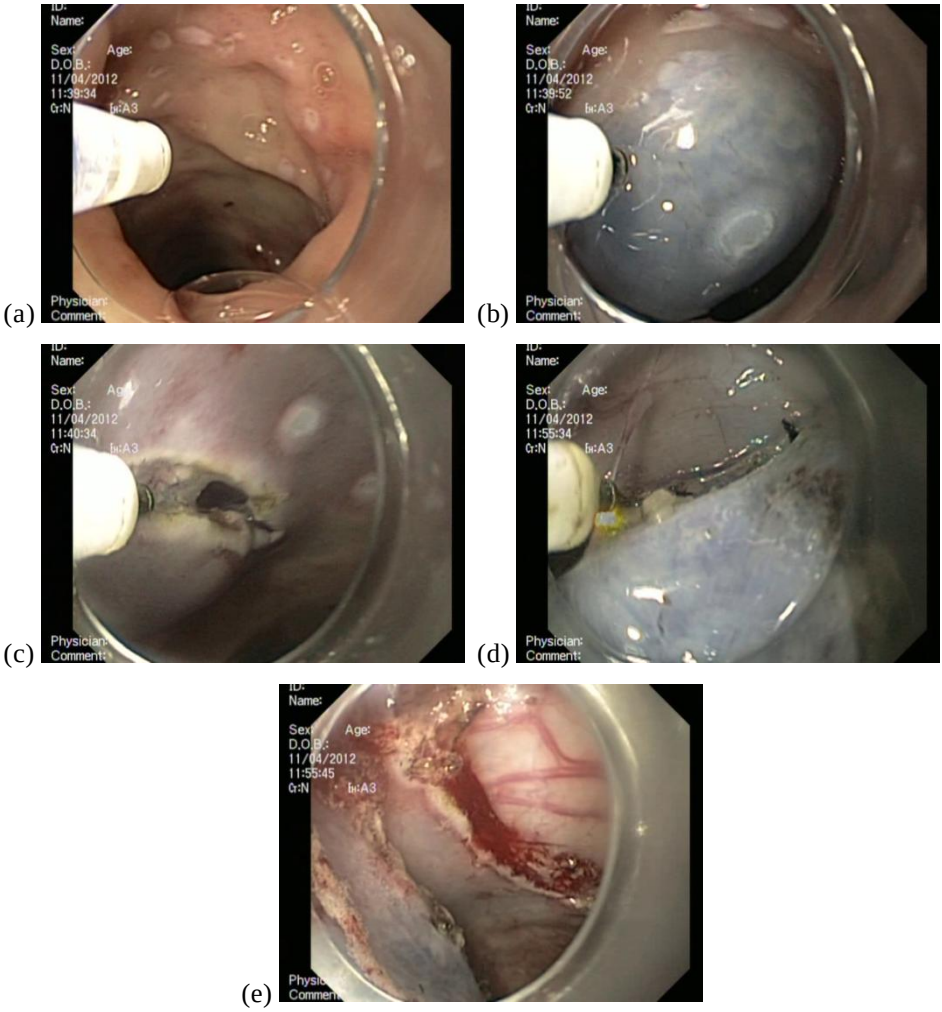


Fig. 6. ESD using ENKI-2 in pig colon and rectum, in vivo (our collection). (a) an artificial 30mm diameter lesion is delineated using coagulation dots on the pig's colon and rectum mucosa; (b) high pressure fluid injection with epinephrine and indigo carmine(c) circumferential incision; (d) submucosal dissection with preventive hemostasis on submucosal vessels; (e) the post resection ulcer borders at the end of the procedure

In conclusion, endoscopic resection of pre-neoplastic and neoplastic superficial lesions may be done endoscopically by ESD, respecting the principles of surgical oncology and "water-jet" technology may facilitate this procedure.

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AUTISM, AN OVERWHELMING CONDITION: HISTORY, ETIOPATHOGENESIS, TYPES, DIAGNOSIS, THERAPY AND PROGNOSIS

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AUTISM, AN OVERWHELMING CONDITION: HISTORY, ETIOPATHOGENESIS, TYPES, DIAGNOSIS, THERAPY AND PROGNOSIS (Abstract): Autism is defined as a neurologic developmental disorder affecting brain and behavior, becoming usually apparent before 3 years of age, with stable evolution and no remission. No neurologic morphologic abnormality was associated with the disease. Several types of disease being described, autism is part of a larger spectrum known as autism spectrum disorders (ASD), or pervasive developmental disorders (PDD). The disease was first described long before it was defined and it has received its modern name. Main cause in the development of autism is considered to be genetic, up to 90 %. However, environmental factors could be incriminated, sometimes. The five types included in ASD are: Asperger syndrome, pervasive developmental disorder-not otherwise specified (PDD-NOS), typical autism, Rett syndrome and childhood disintegrative disorder (CDD). The classical triad of symptoms includes: social interaction impairments, communication impairments and repetitive, stereotype behavior. Diagnosis is based on interview of the parents and specialized observation of the suspected children. Main tools used in therapy are the family and the educational system. Well established, specialized programs of therapy were developed in time. Prognosis of autism is severe, since no cure is possible; nevertheless spontaneous recoveries do occur, in some cases. **Key words**: AUTISM, AUTISM SPECTRUM DISORDERS (ASD), PERVASIVE DEVELOPMENT DISORDERS (PDD)

Some cases were described long before the disease has received its name and its characteristics were established. The earliest well-argumented case of autism is that of Hugh Blair of Borgue, which is described in a law report from 1747, where his brother tried to revoke Blair's marriage, in order to obtain the inheritance.

The Swiss psychiatrist Eugen Bleuler first used the word autism (from Latin, autismus) to describe symptoms of schizophrenia. In fact, the word is deriving from the Greek *autós* (self) and was used to define abnormal self-admiration and autistic isolation of the patients in their fantasies, in which outside influence became intolerable (1).

In 1938, Hans Asperger of the Vienna University Hospital used the term autistic psychopaths in a lecture, thus the term autism found its modern sense. Asperger then described the entity now known as the Asperger syndrome, although the condition was not recognized as a separate diagnosis

till 1981.

In its modern sense, in English language, the term autism was first used by Leo Kanner (Johns Hopkins Hospital), when he reffered in 1943, to „early infantile autism"; he was then describing 11 cases of autism. His description was so accurate that is still typical for the autism spectrum disorders (2).

In the late 1960s, autism was recognized as a syndrome with life-long duration, distinct from other development disorders, such as mental retardation or schizophrenia; it was also widely accepted the benefit brought up by the active therapy programs. Although in 1970, the genetic etiology of the autism it was still not clear, now the disease is considered one of the most heritable of all psychiatric diagnoses (3).

ETIOPATHOGENESIS

The genetics of autism is complex and is not yet clear if autism can be explained by genetic mutations, or by peculiar multigene interactions of common genetic subtypes. Its complex features develop from interactions between multigene, environment and factors which are able to influence the gene expressions. Genetic factors are estimated to represent over 90 %, in the genesis of autism. Studies on twins have shown that many of the non-autistic siblings do manifest however, social and/or learning disabilities (4).

The risk of hereditary transmission in the families with autistic individuals was estimated at about 30 %. Linkage and association genetic analyses were unconclusive. It seems that more than one gene is involved; more such genes were located, but the specific mutations could not be identified. Recently was accepted that several different causes might finally lead to autism, as it is the case with the mental retardation, when different types of brain abnormalities would have the same final result (5,6).

The number of autism diagnoses increased dramatically, especially after 1980, and even more in 1990 and 2000. It seems that this is due, at least in part, to modification of the diagnosis procedures. It is not yet established if the prevalence of the disease increased in the same measure (2008 – 11.3 children/1000, 2012 – 20 children/1000, in U.S.A) (7).

Environmental factors may rather aggravate than trigger, the development and the evolution of the disease. Among such factors, considered to be involved are: infectious diseases, solvents, heavy metals, phtalates and phenols from plastic products, drugs, pesticides and vaccines (8).

In a certain way, autism seems to be directly opposite to schizophrenia, since in autism are expressed paternal genes which are regulating the overgrowth of the brain, while in schizophrenia are expressed maternal genes, which are inducing the underdevelopment of the brain (9).

Advanced paternal and maternal age at the time of the procreation are also incriminated in the genesis of the autism. From the point of view of the paternal age, two theories are suggested: mutations accumulated in time, which would affect the quality of the spermatozoa, and the autistic traits of personality, which would determine such male individuals to marry and procreate later; the two hypotheses are in fact completing each other. It is difficult to appreciate to what extent, paternal or maternal age, or both are important in the genesis of the disease (8).

Prenatal viral infections which are clearly involved are rubeolic and cytomeg-

alic virus infections. The rubeolic congenital syndrome is the most striking proof of the involvement of non-genetic factors in the genesis of the autism. Teratogens, such as thalidomide, valproic acid/misoprostol are involved as well, in the development of the disease. The alcoholic fetal syndrome is also increasing the risk for autism.

The gestational diabetes was found as a significant risk factor, in a 2009 study, demonstrating a risk for having an ASD child, two times higher than in the case of the non-diabetic mothers. In a study conducted in april 2012, metabolic syndrome, associating diabetes, obesity and high blood pressure was more frequent among mothers of ASD children; in the same study, the interrelation between obesity and pregnancy, as a single risk factor for having children with autism was also found strongly positive (7,8).

The opiate theory was advanced in 1979 by Jaak Panksepp, who noticed that small amounts of opiate substances injected in young laboratory animals, produced symptoms similar to those found in autism. He postulated that a digestive abnormality present at birth, could lead to the transformation of the gluten and of the caseine into opiate substances – gliadorphine and caseomorphine, which would interfere with the capacity of sensory reception in these children. Thus, they will show a need for constant stimulation, at the visual level (obsession for rotating objects, characteristic movement of the fingers in front of the eyes, etc.), auditory level (screaming), vestibulary level (swinging, spining, rotatory movements) and tactile level (hand clapping, self-injury) etc., which would explain at least partially, the symptoms of autism. Yet, the scientific data are not validated to exclude these type of nutrients from diet (10, 11).

Another factor incriminated in the development of autism is the prenatal exposure to important stress. The theory of the fetal testosterone is stating that the increased levels of testosterone in the amniotic fluid would force the brain development toward an increase in the complex analysing and synthetic capacities, in the detriment of the communication abilities and empathy (an overdevelopment of the so-called „male traits" and a decrease of the „female traits" of personality); the theory was controversial and contradicted by other studies. Other perinatal incriminated factors are: the small birth weight, the gestational age and hypoxia at birth. Vaccines were incriminated, especially because numerous parents became aware of the autistic symptoms of their children, concomitantly with a routine vaccination. An impressing number of well-validated studies have infirmed this wrong belief (12, 13).

Pathophysiology is supporting the influence of the amygdala neurons which are involved in the development of the social skills and in the capacity to learn social behavior patterns, and the involvement of the noradrenergic system of the loccus ceruleus, a neuronal system known to be involved in excitation and attention, and thus influencing the learning abilities and the capacity to adapt to the environment. The pathophysiologic theories are involving the cortical structures and their interrelation with behavior (14).

The overdevelopment of the brain of the autistic subjects early after birth would be responsible for an increased number of neurons, an overdevelopment of the synapses in certain key cortical zones, an unbalanced functioning of the neuronal excitatory, versus inhibitory networks. The ab-

normal development of brain synapses is involved as well in the genesis of the epilepsy, thus explaining the frequent association between the two conditions (15).

The theory concerning the mirror neuronal system: this system is activated when a laboratory animal is achieving a task, or is observing another animal achieving the same task. This system would be involved in the capacity of imitating activities, intentions and emotions, through the observation of other individuals. The abnormal development of the mirror neuronal system would interfere with the capacity of imitation, which is important in creating behavioral patterns. The delay in the activation of the mirror neurons was noticed in the Asperger syndrome (16).

A reduced functioning of the deficit neuronal network was also incriminated in the pathogenesis of autism; this network is involved in social and emotional processing, and consequently, in attention and concentration abilities. It has extended connections with the excitatory neuronal networks and it seems that in autism the two networks are not efficiently interconnected, with a deficit in the capacity to rapidly change one circuit, for the other; this could be responsible for a disorder in the capacity of thinking about the self. In ASD individuals was evidentiated a peculiar type of signals in the cingulated cortex, in an imagistic study from 2008. The theory of the reduced interconnections is presuming the presence in autism of an overdevelopment of the neuronal interconnections, but which are underfunctioning and with a syncronization deficit, with an excess of low-level processing. In autism are present reduced functional connections between the frontal lobe and other cortical areas. Imaging studies and brainwave studies which have analyzed cortical reactions according to the events the subject had been exposed at, showed the preference of ASD subjects for the non- social stimuli (17).

TYPES

Autism spectrum disorders are including five types of autistic developmental disorders.

Asperger syndrome is the most frequent and fortunately the least severe form of autism. It is characterized by disabilities of social interaction and communication. Usually, the subjects do not accept changes in the daily routine and they show a form of learning disability. Motor skills of these subjects are underdeveloped and they are clumsy: they show usually a hypersensitivity for powerful visual and auditory stimuli. Frequently such subjects are intelligent, with high IQ scores, and they may demonstrate a peculiar talent in a domain, in which they are unusually gifted. They have the habit to learn everything about a single subject in which they became obsessively interested, and afterwards they spoke continuously only about this subject. Sometimes, Asperger syndrome and high functional autism are superposed, although this is not clear established by the specialists. Adults with the diagnosis of Asperger syndrome are at higher risk for the development of anxiety and depression (18).

Pervasive Developmental Disorder not otherwise specified (PDD-NOS). The large majority of the ASD cases are receiving this diagnosis. Usually, these subjects show autistic symptoms more severe than the Asperger syndrome, but they do not meet entirely the diagnosis criteria for typical autism. In such cases the diagnosis is that of PDD-NOS. These patients show im-

pairment of the social function, language abilities better developed than in the typical autism, but not so good as in Asperger syndrome and fewer repetitive behaviors, than in the Asperger syndrome, or typical autism. The disease usually has a later age of development (19).

Typical autism is characterized by clear diagnosis criteria; social function and language abilities are severely impaired and repetitive behaviors are characteristic. Such cases frequently associate mental retardation and convulsive symptoms.

Rett syndrome is affecting almost exclusively the female sex; it is very rare – 1/10-15,000 children. Usually at an age between 6 months and one year and a half, the child cease to respond to social stimuli, a characteristic hand grasping reflex is appearing and the language abilities are rapidly lost. Motor coordination problems may sometimes evolve severely. The syndrome is the result of a single gene mutation, which is not inherited, but the result of the hazard (20).

The childhood disintegrative disorder (CDD) is the most severe form of the autism spectrum disorders, fortunately also the rarest of all. After a period of normal development, usually between 2 and 4 years of age, such children are rapidly losing social, intellectual and language abilities. Frequently, a convulsive disorder is associated and the severe symptoms cannot be recovered. Male sex is more often affected; less than 2 children/100,000 affected by autism spectrum disorders are developing this syndrome (19, 20).

DIAGNOSIS

The diagnosis of autism is based upon the classical triad of symptoms: social development impairment, communication disabilities and the repetitive, stereotype behavior. Other symptoms, such as atypical alimentation are frequent as well, but are not essential for the diagnosis.

It is important to stress, the fact that the common symptoms of ASD are present in the general population, wthout frequent association, and there is no clear border to separate the severe pathology and the ordinary personality traits (21).

The social function impairment is the most striking characteristic of the disease, distinguishing it from other syndromes. These children are smiling and are looking at others more rarely, when they are called by their name. A striking characteristic is that they establish much more rarely visual contact with the others. At 3-5 years of age, they show a poor understanding of the social activities and a reduced capacity to spontaneously contact other people, to imitate, to respond emotionally and to infer the emotional estate of other people.

Communication impairment is affecting 1/3, up to ½ of the autism cases, in such a degree that these subjects do not develop communication skills enough to satisfy their usual needs. In the first year of life delayed babbling, unusual gestures and vocal manifestations are common. They ask more rare different things, they manifest echolalia and the capacity to point or to indicate something, in order to communicate is constantly affected. Autistic subjects show impairment of the complex, abstract and deductive language, proved by poor results at such tests.

The repetitive behavior is classified under The Repetitive Behavior Revised Scale of 2007: stereotype movements (hands flapping, body swinging etc.), compulsive behavior is following rigid rules (such as the arrangement of different objects in precise

position); resistance to change, ritual behavior, restrictive behavior (obsession for one game, one toy or one TV program etc.); self-injury is reported to affect approximately 30 % of the autistic children (22).

Other symptoms are including sensory abnormalities (more often hypo reactivity), motor impairment (in coordination, reduced muscular tonus), alimentary behavior abnormality (affect up to ¾ of the cases, for that it was once used as a diagnosis criterion; selectivity is the most frequent problem, but ritual at table and food refusal are also common).

Imagistic techniques, MRI and diffusion tensor imaging are used to identify characteristic features for autism, visible on brain scanning.

Important clues for the diagnosis are:

- no babbling till 12 months
- no gesture till 12 months (like to point with a finger)
- no word till 16 months
- no two words proposition (spoken spontaneously and not echolalia) till 24 months
- any regress in what concerns social or language skills, at any age of the child

Diagnosis standards impose at least 6 symptoms, including at least two symptoms of the quality of social interaction, at least one symptom of language disability and at least one of repetitive and restrictive behavior.

Diagnosis instruments that are frequently used are: the Autism Diagnosis Interview Revised, which is a partially structured parent interview and the Autism Diagnosis Observation Schedule, which uses observation and interaction with the child. The Childhood Autism Rating Scale is used in specialized clinic environment, to evaluate the severity of autism through children observation (23).

Differential diagnosis includes the mental retardation, auditory problems, language impairment syndromes, as the Landau-Kleffner syndrome, which can be diagnosed on EEG.

Although an early diagnosis is important for the initiation of the therapy and good chances of recovery, unfortunately this is rarely done in practice (25).

A 2009 American study showed that the ASD diagnosis was established at an average age of 5.7 years, which is far below the recommendations (5). There are cases not diagnosed till adult age, and also cases that are over diagnosed based on uncertain symptoms (21).

Frequently associated co-morbidities are: mental retardation (reports are ranging from 25 to 70 %, a large variation reflecting the difficulty of a correct evaluation of the intelligence of the autistic individuals); anxiety is common among autism spectrum disorder subjects, there are studies which report a frequency of up to 84 % of the autism cases; epilepsy, metabolic syndromes (phenylketonuria), ADHD (attention deficit and hyperactivity disorder) Tourette syndrome are also commonly associated in the ASD patients. Sleep troubles are affecting approximately 2/3 of the autistic patients, representing a reason for initiation of therapy, besides the autism symptoms (23, 24, 25).

THERAPY

Main goals of the therapy of autism are to diminish the deficits associated with the disease, and to relief the stress endured by the family, as well as the increase of the life quality and the degree of autonomy of the patients.

A standard therapy does not exist, the

treatment is addressed strictly to the needs of the respective child. Main resources used in the therapy are the family and the educational system. Studies which tried to evaluate the efficiency of different therapeutically methods were inconclusive; however, the psychosocial methods used do show positive effects, suggesting that whatever form of therapy is preferable than none. The applied therapies are improving the adaptive capacity and are decreasing the severity of the symptoms and of the behavior troubles. Most used methods are: the Applied Behavior Analysis (ABA), developmental models, structured learning techniques, speech therapies, social functions therapy and occupational therapy (26).

The opportunity of including ASD subjects with different degrees of severity in the mass educational system is still debated. Many types of medications are used; most frequent antidepressants, stimulatory drugs / psychotropes and antipsychotics. Besides antipsychotics, there are few studies to support the safety of the use of such medications, in autism. The risk of atypical reactions and the high risk of development of side effects are difficult to estimate, in the case of autistic individuals. Till this moment, there is no known drug capable to cure or to diminish the symptoms of autism (27, 28).

In time, numerous unconventional therapies were used in the treatment of autism, such as: acupuncture, antifungal therapy, art therapy, the Denver model, therapy using dolphins, aerobics (interactive computer programs), facilitated communication, music, contact therapy, homeopathy, neuro-feedback, rhythms therapy, yoga etc (19).

PROGNOSIS

Autism is not curable; spontaneous recoveries may occur, revoking the autism diagnosis. The recovery may appear sometimes after intensive therapy, but not always. The reported rates of recovery in U.S.A. are situated between 3 and 25 % of the total number of cases. Most autistic children do learn to speak around 5 years of age, some earlier, but some later.

Most ASD adults tend to manifest milder symptoms, than during childhood. Studies to evaluate the evolution of the ASD subjects, above medium age do not exist.

Usually, learning to speak before age of six and an IQ score above 50 are meaning a better future potential.

Different studies show that a high level of autonomy was reached in 4-12 % of the cases. At the opposite pole, 12 % needed hospitalized specialized care, all lifelong. The large majority (46 %) needed continuously specialized residence and achieved only limited autonomy (20).

The costs of autism are very important; in U.S.A., a child born in 2000, needs for his life-duration 3.99 million dollars (adjusted according to the inflation rate for 2013) (7, 29).

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COXARTHROSIS – DISEASE OF MULTIFACTORIAL ETIOLOGY METHODS OF PREVENTION AND TREATMENT. THE ROLE OF KINESITHERAPY IN COXARTHROSIS

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COXARTHROSIS – DISEASE OF MULTIFACTORIAL ETIOLOGY. METHODS OF PREVENTION AND TREATMENT. THE ROLE OF KINESITHERAPY IN COXARTHROSIS (Abstract): Coxarthrosis is a disorder of the physiological balance between the strength of the articular cartilage and articular bone, and between the pressures exerted on the joint. It is a disease with a slow progressive and long course. Its insidious onset and progression, marked by variable periods of stagnation lasting months or even years make it difficult to establish the time of onset. **Material and Methods**: The study was conducted between January 2012 and December 2012, on a series of 27 coxarthrosis patients, diagnosed at the Rheumatology Clinic of the Iasi Rehabilitation Hospital. **Results and Discussion**: Of the 27 patients, 11 (40.74%) had primary bilateral coxarthrosis, 8 (29.63%) early coxarthrosis, right, 6 (22.22%) coxarthrosis, left, secondary to aseptic femoral head osteonecrosis, and 2 (7.41%) patients had bilateral coxarthrosis secondary to acetabular insufficiency. Kinesitherapy proved to be highly effective in the rehabilitation of coxarthrosis patients by alleviating pain, mechanical protection of the hip joint, walking rehabilitation in total hip arthroplasty, and social and professional integration of the patients. The massage besides its analgesic effect also acted as a muscle relaxant. Once the objectives have been met, the kinesiology program becomes global and functional rather than analytical, as it aims at reintegrating the coxofemoral joint into normal movement patterns. **Conclusions**: Kinesitherapy has been shown to be a physical treatment that can not be replaced by other rehabilitation methods and is crucial in the recovery of lost functions. **Keywords**: COXARTHROSIS, KINESIOTHERAPY, REHABILITATION.

One of the main characteristics of living matter is movement. Throughout its long evolution, movement has continuously improved. It has gradually evolved from the simplest movement of unicellular organisms to the movement performed by a specialized apparatus, namely the locomotor system in animals (1).

The coxofemoral joint (Fig. 1) can be compared with a mechanical system that from an anatomical point of view displays three degrees of freedom of motion: *flexion-extension* (along the sagittal plane), *adduction and abduction* (the lower limb moves away respectively toward the other limb, in the frontal plane of the body,

around the anteroposterior axis); *internal and external rotation* (performed horizontally around a vertical axis) (Fig. 2).

The coxofemoral joint is of great importance for the static position (in unipodal and bipodal support that distributes weight from the pelvis to the lower limbs) and for the locomotion (it provides the swing of the lower limb during the swing phase) (2). Hip stability is due to bone factors, providing vertical stability, ligament factors, providing anterior stability, and muscle factors, particularly influencing anterior stability (3,4).

Fig. 1. Coxarthrosis

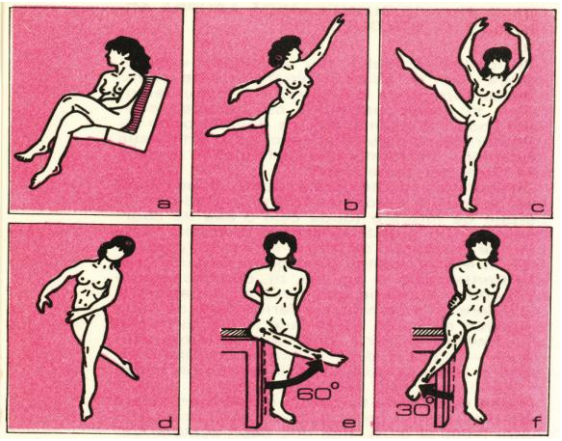

Fig. 2 The coxofemoral joint can be seen as a cardanic articulation

Typical to the physiology of joint movement is a quite low friction coefficient (between 0.005 and 0.01). The pressure regime also varies and the movement is sometimes made under the high pressure of body weight and sometimes just under the lower pressure of muscle contraction (4). Such a small friction coefficient is due to the extremely low roughness of joint surfaces, and the viscosity of the synovial fluid varies inversely with the speed of joint movement (5). The type of synovial lubrication changes depending on the exerted pressure and the speed with which the movement is made.

The biomechanical approach of the locomotor system allows a deeper understanding of how the premature joint wear occurs and develops and, in case of the hip, how coxarthrosis evolves (5, 6).

Just like any other joint, the hip is always under the influence of the permanent static pressure generated by the contraction of different muscle groups. In addition, there is an intermittent dynamic pressure caused by body weight during standing and walking. The circumstances under which the hip joint acts in the unipodal stance justify why a force four times one's body weight is exerted on the articular surface. In order to maintain the equilibrium of the basin towards the femoral head, the action of body weight must be counterbalanced by muscle strength. As the muscle force lever arm is three times lower than that of body weight, a resultant force with a value about four times that of body weight measured above the hip is exerted on the hip. This

force is evenly distributed over the entire articular surface (6).

This congenital or acquired deficiency, more or less pronounced, that affects the supportive joint tissues, can lead to an imbalance (secondary coxarthrosis) (7). An abnormal increase in articular pressures caused by the decrease in the contact area between the articular surfaces leads to the disruption of the normal balance (primary coxarthrosis).

If the pressure exerted on the joints exceeds the resilience of supportive tissue, a series of pathological changes occur that induce major changes in joint configuration during the development of coxarthrosis.

Genes that are involved in most topographical variations of arthritis are most probably those coding vitamin D receptors. They influence bone mass, the proximity of loci for type II collagen, a major component of hyaline cartilage, the insulin-like growth factors 1, the cartilage oligomeric proteins, and the human leukocyte antigen (HLA) system (8). It has been suggested that the disease loci could be placed on chromosome 2q and / or 11q. There is evidence that the different locations of arthritis are genetically encoded in distinct loci.

In the early stages, the number and activity of chondrocytes increases, followed by their progressive decrease. The synthesis of proteoglycan (PG) in chondrocytes decreases and the amount of lytic enzymes and matrix metallo-proteinases (MMP) increases (9). Their production is stimulated by pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and by mechanical factors. Moreover, plasminogen activates latent MMPs. Tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitor-1 (PAI-1) has an inhibitory effect on MMPs and plasminogen, respectively.

The arthritic damage progresses under the influence of proinflammatory and proteolytic biological systems, of which the most important are IL-1 (active mediator of inflammation, with a major role in tissue destruction), PG, nitric oxide (NO) (the latter stimulates the production of proinflammatory cytokines - especially IL-1 and TNF-α - and induces vasodilatation and increased permeability) and MMPs.

Diagnosis and clinical management. The diagnosis of coxarthrosis is facilitated by history taking that may reveal the onset of the disease, its location, the rhythm and radiation of pain. Coxarthrosis has two fundamental aspects: increased mechanical stress on joints (excessive loading, congenital or acquired defects of body architecture) and alteration of cartilaginous tissue, leading to the development of osteoarthritis. The secondary cartilage changes are due to metabolic, infectious or inflammatory factors. The disease manifests itself by the gradual and progressive occurrence of pain in the hip, a mechanical type of pain that occurs in motion and relieves itself or disappears at rest.

In most cases, pain occurs in the groin and radiates to the anterior side of the thigh and further to the knee. In other cases, pain occurs in the buttock area. At the disease onset, pain is triggered by position changes and in the more advanced forms of disease pain becomes permanent. Pain arises from small efforts, is more difficult to alleviate and requires a longer resting period. Later, pain occurs at the first steps, but it disappears when the joint warms up and it reappears if the effort is prolonged (10). Pain becomes more frequent over time and it is associated with limitation of joint mobility. As pain worsens and the limitation of joint

mobility occurs, lameness becomes permanent and pronounced, progressively reducing the functionality of coxarthrosis patient. Bone crepitus reported by patients and revealed by the clinical examination indicates the severity of the bone lesion.

Diagnosis should be made at an early stage of the disease so that treatment to be as effective as possible. Short term joint stiffness (<30 min) occurs as a result of the thickening of the synovial membrane and articular capsule (11). The limitation of joint mobility is a consequence of the retraction of the articular capsule, of the osteophytes and of the incongruence of the articular surfaces. Joint instability occurs as a result of weakening periarticular muscle strength and deformities of the bone ends that make up the joint.

The radiologic examination is the preferred method for diagnosing coxarthrosis and assessing the femoral head shape, the appearance of the joint space, the presence of osteophytes and changes in femoral head structure.

A new type of radiographic procedure, a technical version called micro focal radiography, with high spatial resolution, can detect early changes invisible to standard techniques.

Scintigraphy with methylene or hydroximethylene diphosphonate shows details about blood flow in the bone and soft tissue and bone turnover. Ultrasound can assess the arthritic process and the degenerative changes. Lesions that remain invisible on radiographs are identified by the high sensitivity and specificity of nuclear magnetic resonance imaging.

Therapeutic guidelines. Coxarthrosis treatment aims at decreasing pain intensity and increasing stability, mobility, coordination and balance during walking.

Currently, the therapeutic regimen for patients with coxarthrosis is a long term process that takes place in two stages: a medical conservative stage that combines a program of joint hygiene, with drug therapy (analgesics and chondroprotective agents) and kinetic physical therapy, which is a maintenance therapy or a functional rehabilitation (12) and a surgical stage.

Treatment aims to reduce pain and the functional discomfort of the patient and at the same time to stop the development of joint damage. Conservative treatment is effective in less advanced forms. Pain disappears, joint mobility improves, but the conservative treatment may not always stop the development of the disease. Of particular importance are hygienic measures aimed at fully unloading the hip joint and avoiding any strain. Working conditions should change, sport activities that involve stress on the hip joint should be stopped and, just in case, a walking cane should be used (13). A walking cane carried in the hand opposite the affected hip and on which the patient rests associated with the arthritic hip support release approximately two-thirds of the pressure exerted on the articular surface. Fighting obesity and overweight is also important in order to avoid hip joint overload. This hygienic treatment should be associated with anti-inflammatory and analgesic medication.

Kinesitherapy aims at maintaining a good joint and general mobility and tonic muscles, prerequisites for a successful rehabilitation. Kinesitherapy is associated with periodical balneophysical therapy (14).

Modern surgery offers multiple ways to stop the development of osteoarthritis. Conservative and functional surgical procedures (osteotomies and changing relationships between the articular surfaces)

give the possibility of changing the way the mechanical factors act upon the hip (15). They manage to increase the contact area between the two joint ends and thereby to lessen to a large extent the pressure exerted on this surface. As a result of the biological effect of improving local circulation, osteotomies relieve pain.

Conservative surgery (osteotomy, arthroscopic debridement, arthrodesis, partial arthroplasty) or total hip replacement surgery must be associated with a prolonged conservative and rehabilitative treatment.

MATERIAL AND METHODS

The study was conducted between January 2012 and December 2012, on a series of 27 coxarthrosis patients diagnosed at the Rheumatology Clinic of the Iasi Rehabilitation Hospital. The patients were admitted for 10-15 days and were recruited based on the type of joint damage, age, degree of patient compliance, and other factors observed at individual assessments.

RESULTS AND DISCUSSION

Of the 27 patients, 11 (40.74%) had primary bilateral coxarthrosis with pain and functional decompensation. Muscle testing, the testing of the muscles that stabilize the coxofemoral joint, showed a value of 4. In these patients, pain diminished or alleviated at rest and they were walking with a limp. The degrees of coxofemoral joint mobility were affected by the arthritic process, especially in internal and external rotation movements, and they were associated with contracture of adductor and pelvitrochanteric muscles (Fig. 3).

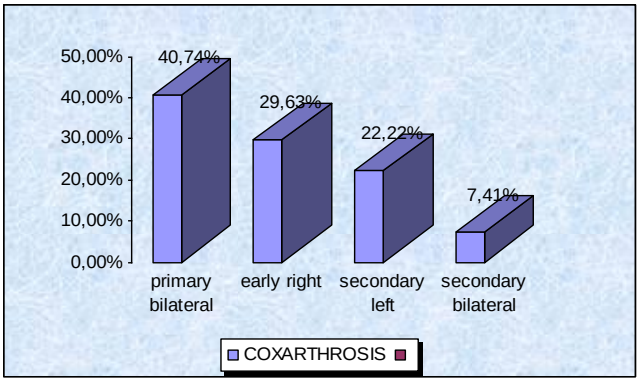

Fig.3. Types of coxarthrosis in the study group

Kinesitherapy and massage were effective in relaxing the coxofemoral joint and correcting or preventing the incorrect body position. Medical gymnastics resulted in restored mobility of the lower limb and the progressive increase in muscle strength was due to practicing passive mobilization, self-mobilization, active mobilization and movements for toning the lower limb muscles. A cane was used when walking, the cane being held in the hand corresponding to the healthy hip.

Analgesics and anti-inflammatory drugs, vasodilator agents and anti-contraction medication were used in fighting pain, controlling muscle contracture, and preventing vascular disorders.

Of the 27 coxarthrosis patients, 8

(29.63%) had early coxarthrosis, right hip, presenting mild or moderate pain and instability in motion. Testing of the muscles that stabilize the coxofemoral joint showed a value of 5, with a mild contracture of adductor and pelvi-trochanteric muscles that limits the mobility of the hip. Without cane, they were walking with a limp when tired or on longer distances. The treatment methods used were kinesitherapy, passive mobilization, self-mobilization and active mobilization by progressive resistance exercises, toning the lower limb muscles if hypotonic, toning the hamstring and gluteal muscles through isometric or isotonic exercises. The stage objectives were to adapt to effort and to resume walking. Physiotherapy, thermotherapy, hydrotherapy and, if possible, hydro-kinesitherapy have also been recommended.

Six (22.22%) of the 27 patients had coxarthrosis, left, secondary to aseptic femoral head osteonecrosis with incorrect position of the lower limb and sometimes with pain in the lumbosacral area. Often the patients were forced to use walking cane. Muscle testing showed lower values in extensor abductor and internal rotator muscles. This accounted for the altered stance in flexion and adduction.

Of the 27 coxarthrosis patients, 2 (7.41%) presented bilateral coxarthrosis secondary to acetabular insufficiency. Patients reported mixed arthralgia in the coxofemoral joints, often associated with back pain or knee pain. Isometric contractions for the quadriceps muscle, correcting the trunk and pelvis position by means of exercises for paravertebral and abdominal muscles, hydro-kinesitherapy, active and passive mobilization, massage, toning muscles aimed to control pain, maintain mobility and muscle strength. Patients had a good outcome and regained stability, mobility and muscle strength in both lower limbs.

The kinesitherapy program conducted during our study was individualized and aimed to relieve pain (through hydrotherapy, electrotherapy and thermo-therapy) by achieving a good stability of the hip joint, providing functional mobility angles, and avoiding incorrect body position.

The analysis of the data on the achieved joint and muscle balance showed an improvement in joint mobility, normal muscle strength values, and a good social and professional integration of the patients.

CONCLUSIONS

Researchers have shown that early use of kinesitherapy had good results in coxarthrosis patients for the functional recovery of the affected area due to the individualization of the kinesitherapy procedures according to patient's response to treatment.

The proper assessment of joint function impairment, pain, radiological injury quantification, patient compliance has allowed an accurate decision making on therapy and long-term rehabilitation methods.

The complex therapeutic, educational, and physical kinetic and drug regimen associated with close doctor-patient cooperation has been shown to manage this disease from its onset.

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NEWS

A CLINICAL AND HISTOLOGICAL REPORT OF A TOOTH WITH AN OPEN APEX TREATED WITH REGENERATIVE ENDODONTICS USING PLATELET-RICH PLASMA

Regenerative endodontic procedures are biologically procedures which aim to regenerate the damaged pulp-dentin complex, resorbed root, cervical or apical dentin by stimulating of the proliferation of remaining vital tissues under favorable conditions. The conventional method consisted of inducing bleeding into the pulp canal by mechanically irritating the periapical tissues. The created blood clot operated as a matrix for the growth of new tissues into the pulp canal. However, this procedure implied discomfort for the patient because of the irritation of the periapical tissues. Understanding of the physiological roles of platelets in wound healing has led to the idea of using platelets as a scaffold for regenerative endodontic therapy. Platelet-Rich Plasma (PRP) consists of a limited volume of plasma enriched with platelets, obtained from the patient. The purpose of this case report was to present the clinical, radiographic, and histological findings of a regenerative procedure using platelet-rich plasma (PRP) 14 months after the procedure. A 12-year-old boy whose maxillary second premolar tooth had been treated with regenerative endodontics was seen with a chief complaint of pain and sensitivity to cold. After clinical and radiographic examinations, a pulpal diagnosis of reversible pulpitis and normal periapical tissues was made for this tooth. Because of the patient's complaint and his guardian's insistence on either a root canal treatment or an extraction, a root canal treatment was performed in this tooth. After entry into the root canal, the soft tissue present in the canal was removed with the aid of a large barbed broach and examined histologically. Examination of the tissue removed from the root canal of this tooth revealed the presence of a vital pulp-like vital connective tissue. There was no evidence of bone in the specimen. Very few inflammatory cells were noted in the periphery of the specimen. Based on these findings, the authors concluded that pulp-like tissue could be generated in human teeth with the use of PRP as a scaffold in regenerative endodontic procedures **(**Torabinejad M, Faras H. A clinical and histological report of a tooth with an open apex treated with regenerative endodontics using platelet-rich plasma *J Endod* 2012; 38(6); 864-8).

Gianina Iovan

STRESS: FROM SYMPTOM TO DISEASE

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STRESS: FROM SYMPTOM TO DISEASE (Abstract). **Aim:** To evaluate the presence and role of stress in causing diseases. **Materials and methods**: We conducted a 6-month study in patients who presented for the first time with a series of symptom and tried to look for an organic cause of those symptoms. We wanted to see the impact of stress in everyday life and its role in causing various diseases. **Results:** The obtained results showed a female predominance in terms of seeking medical advice and that the most affected age group was 20-30 years, accounted for by the start of socio-professional issues. The psycho-behavioural symptoms were the most prevalent, followed by the gastro-intestinal ones. The presence of stress had left its mark on these events, fact proven by the lower rate of organic pathology. **Conclusions:** Stress awareness must guide us in medical practice as to avoid under- or over-diagnosis. **Keywords:** STRESS, HYPERTENSION, METABOLIC DISORDERS.

Hans Selye (1) used the term „stress" in 1950 to designate a set of body reactions to an external action exerted by a wide range of causative agents: physical agents (trauma, burns), chemical agents (chemical substances from food, air, water, work environment, drugs), and biological agents (external - viruses, bacteria, parasites etc. - or internal - endocrine, immune and mental factors, personality traits etc.) resulting in various morphofunctional changes.

Depending on its action upon the body, stress is classified into two categories: eustress and distress. Eustress is produced by stress factors that have beneficial action on the body and does not generate harmful reactions (2). This stress is positive and moderate, and helps a person perform at a higher level and achieve his goals. Distress is caused by stress factors that cause overexertion and generate negative effects. This is the intense and prolonged stress which exceeds the adaptation level, leading to poor performance. It may result in diminished normal functions or even diseases (3).

Stages of stress are: alarm, resistance, and exhaustion (4).

Potential effects of stress can be grouped into five broad categories, namely (5):

• *Subjective effects*: anxiety, aggression, apathy, boredom, fatigue, malaise, low self-esteem, anxiety, loneliness;

• *behavioural effects*: predisposition to accidents, alcoholism, abuse of coffee, tendency to eat and/ or to smoke excessively, impulsive behaviour;

• *cognitive effects*: decrease in the ability to take rational decisions, poor concen-

tration, decreased attention, hypersensitivity to criticism, mental blocks;

• *physiological effects*: increased blood sugar, pulse, blood pressure, dry mouth, cold sweat, dilated pupils;

• *organizational effects*: absenteeism, low productivity, isolation, job dissatisfaction, reduced responsibilities, reduced loyalty to the organization, resignations.

If a person already has a disease such as cardiovascular disease or a form of diabetes, the increased muscle tension and increase blood sugar, caused by stress, can aggravate the suffering. Studies have shown that prolonged stress is correlated with the onset of illness or disease. The degree to which stress contributes to disease development is still unclear (6).

I. CORRELATIONS OF STRESS WITH SOME DISEASES

1. Hypertension (6, 7). Because blood pressure sometimes increases in response to a stressor, researchers have suspected a possible link between stress and hypertension. Chronically elevated blood pressure levels - which can lead to cardiovascular disease - are thought to be stress-related. Studies showed that individuals who work under great psychological pressure (e.g. airline pilots) or in a high-stress environment (high workplace noise levels) tend to develop higher blood pressure than those who live and work in an atmosphere less fraught with tension.

2. Forms of diabetes and (digestive) ulcer (6, 7). Both diabetes and ulcer are directly linked to stress. In diabetes, when stress raises blood glucose level, pancreatic cells react by producing insulin. Chronic stress can damage these cells, which cannot be replaced, severely affecting the ability of the pancreas to produce the insulin needed to control blood glucose. This results in an increased risk for the onset of diabetes especially in those individuals who are genetically predisposed to this disease (9). Digestive ulcer is caused by excessive secretion of gastric acid, producing severe pain and often leading to gastrointestinal bleeding or perforation. Nonetheless, we do not know the real cause of digestive ulcer, but we are sure that stress can aggravate this condition.

3. Mental disorders (9, 10, 11). Stress generates emotional struggles, which may aggravate existing emotional disorders.

4. Bulimia nervosa and anorexia nervosa (12). Ten-times times more common in women and with a prevalence of 1-3% (bulimia) and 0.5% (anorexia), these two represent extreme forms of eating behavior disorders, the causes of which are not known precisely; beside heredity, stress and emotional disturbances play an important role in developing bulimia and anorexia. Many times, stress can lead to depression which is frequently characterized by reduced appetite and weight loss.

II. ROLE OF INFORMATION IN STRESS

Anything that overwhelms the adaptation mechanisms can cause a stress reaction. But this always happens through information. Whether physical, chemical or psychological factors are involved, the information generated by these must reach the reticular substance, the cortex, the limbic system and the hypothalamus, in order to trigger stress reaction (5).

Information is the one that influences the synthesis of neuro-pituitary hormones, which will influence, in their turn, the secretion of peripheral hormones, which will then act on different tissues. This applies

both to psychological stress and physical stress when the information is triggering, ultimately, the neuro-endocrine stress response. The only difference is the fact that in psychological stress the information is coming from outside, and in physical stress, the information is coming from inside the body, due to damage caused by stress factors. Therefore, the information itself can be a stressful factor. Mental stress is actually a stress information because it is made of the information provided by stress factors. After being evaluated as a harmful or potentially harmful factors, stress causes adrenal activation through cognitive and affective processes (13). R.S. Lazarus emphasizes the fundamental role of cognitive state in the stress process, which is evaluated as a harm/loss, a threat, or a challenge. The anticipatory nature of psychological stress links it more to the feedbefore mechanism, unlike physical stress which involves more feedback mechanisms. Psychological stress occurs when feedbefore mechanism is outdated and cannot find in its repertoire an appropriate strategy to preserve stability in the conditions that generated the information (14).

MATERIAL AND METHODS

Attached to the theoretical data, we conducted a 6-month prospective study to determine the impact of stress in everyday life and its role in causing various diseases. The study included patients presenting a series of symptoms and we tried to find the organic cause of these symptoms.

Inclusion criteria: $\geq$ 18 years; consent to participate in the study; onset symptoms; absence of previously diagnosed organic condition; presence of stress.

Patients underwent a thorough medical history, during which no attempt at inducing stress was made but was carefully looked for it in their medical records. Then patients were asked to fill out a questionnaire specifically developed into a language that respondents understand, as a way to find out whether they experienced any kind of stress.

The questionnaire was based on Holmes and Rahe stress scale. A score of 0-150 was interpreted as no significant problems; 151-200 – mild damage; 201-300 – moderate damage; over 301 sever damage.

After filling out the questionnaire, depending on the findings, patients were referred to a laboratory for full investigations aimed at determining the organic cause responsible for their complaints.

The series included 246 patients presented with the following symptoms: digestive symptoms (epigastric pain, heartburn, loss of appetite, abnormal transit); cardiovascular symptoms (palpitations, high blood pressure, and headache); psychiatric symptoms (disturbances of attention and concentration, nervousness, emotional labiality, depressive state, agitation, restlessness); weight gain.

In patients presenting digestive symptoms, biochemical and hematological tests, including Helicobacter Pylori testing, were performed. If the results were inconclusive, a contrast barium enema in Trendelenburg position and upper endoscopy were also performed.

For those with cardiovascular symptoms blood pressure and heart rate were repeatedly measured, and an electrocardiogram and echocardiography were performed to identify a possible cardiovascular abnormality. The patients with psychiatric symptoms were referred to a psychiatrist and psychologist for assessment.

RESULTS AND DISCUSSION

The study series included 182 women

(73.98%) and 64 men (26.01%) and age distribution can be seen in table I. The distribution of patients according to symptoms is presented in table II.

The results of the stress questionnaire are presented in table III.

TABLE I

Age-group distribution

Age groups	Years					
	21-30	**31-40**	**41-50**	**51-60**	**≥ 61**	**Total**
Number	163	61	19	2	1	246
Percent	66.26	24.79	7.72	0.8	0.4	100

TABLE II

Distribution of patients according to symptoms

Area	Subjective (No)	Objective (No)	Diagnosis
Digestive	66	13	Gastroduodenal ulcer
Psychiatric	129	0	-
Cardiovascular	51	9	Hypertension

TABLE III

Results of stress questionnaire

Results	Patients	
	Number	**Percent**
Without significant problems	83	33.73
Mild damage	86	34.95
Moderate damage	54	21.95
Severe damage	23	9.34

Of the 246 patients who presented various complaints, in only 22, less than 10%, an organic cause was found.

From the obtained results, it can be seen that there is a female predominance in terms of seeking medical advice and that the most affected age-group is 20-30 years, explained by the start of socio-professional problems. It can be clearly noticed the prevalence of the psycho-behavioural symptoms followed by the gastro-intestinal ones, and that the presence of stress has left its mark on these events, fact proven by the low rate of organic pathology.

The relationship between different diseases and stress is known and proven for many diseases. Clinicians and scientists have studied and debated the contribution of stress to the inflammatory process in bowel disease (IBD) since 1930 but no definitive conclusion has been reached. Patients with inflammatory bowel disease are near reaching a consensus, with 74% believing that psychological factors have played a role in triggering the onset of disease or relapse of their illness (15).

Stressful life influences the cardiovascular system and the atherosclerosis process. As part of the reaction to stressful life situations, notably those associated with tension, frustration, conflict, anxiety and depression the cardiovascular apparatus exhibits both hyper and hypo dynamic responses. These include alterations in the rate, rhythm, force and magnitude of cardiac contraction, change in the configuration of heart action potential, and modification in the peripheral circulatory resistance (16). Carissa A.L. et al (17) showed in their study that the adolescents exposed to chronic, negative stressors that worsen over time may show heightened cardiovascular reactivity that puts them at risk for subclinical atherosclerosis.

Reviews of research on life stress and depression unanimously conclude major life events precede the onset of many, if not the majority of depressive episodes (Kessler, 1997; Mazure, 1998; Tennant, 2002 ; Paykel, 2003; Hammen, 2005) (18).

CONCLUSIONS

This study proves that about 10% of the patients presenting to an ambulatory unit and which have suffered a stress whatever its nature have an organic cause of symptoms. We do not want to underestimate the presence of diseases among the population, but over-diagnosis is a problem of attitude. An additional laboratory investigation does nothing else but to confirm or infirm a diagnosis. The presence of a disease is an additional stress for anyone, as is uncertainty. Stress factors are everywhere and stress is not sparing anyone. We must be aware of its existence and act accordingly.

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NEWS

DERIVATIVES OF IBUPROFEN IN PREVENTING COLON CANCER

The cancer chemopreventive properties and gastrointestinal toxicity of ibuprofen are well documented. Ibuprofen and a modified derivative (phosphor-modified ibuprofen or p-ibuprofen) were used in a chemically induced model of colon cancer by Ouyang et al. Fisher 344 rats were injected with azoxymethane and treated with either ibuprofen (500 ppm) or p-ibuprofen (900 ppm) for 20 weeks to observe aberrant crypt foci (ACF) or 40 weeks to evaluate tumor incident and multiplicity. β-catenin and p65 were measured in colonic tissues by immunofluorescence staining. Equal molar doses of ibuprofen (75 and 670 mg/kg) and p-ibuprofen (135 and 1215 mg/kg) were administered to rats for 7 days to assess acute toxicity. At week 20, p-ibuprofen and ibuprofen significantly reduced the multiplicity of ACF compared with the control ($p < 0.05$) by 31.2 and 37.9%, respectively. At week 40, p-ibuprofen and ibuprofen reduced the multiplicity of colon tumors compared with control ($p < 0.05$) by 47.2 and 56.6% respectively. Equal molar concentrations of ibuprofen (670 mg/kg) and p-ibuprofen (1215 mg/kg) resulted in stomach ulceration in 85.7% and 14.3% of rats, respectively ($p < 0.05$). Immunofluorescence staining and western blot analysis demonstrated that both ibuprofen and p-ibuprofen suppressed β-catenin nuclear translocation in colon cancer cells. In addition, p-ibuprofen, but not ibuprofen inhibited NF-kB activation in colon cancer cells. Collectively, these results suggest that p-ibuprofen is a potential effective novel drug for long-term use in colon cancer prevention (Ouyang N, Ji P, Williams JL. A novel NSAID derivative, phospho-ibuprofen, prevents AOM-induced colon cancer in rats. *Int J Oncol* 2013; 42(2): 643-650).

Maria Drăgan

EVALUATION OF TUMOR RESPONSE TO SYSTEMIC THERAPY IN HEPATOCELLULAR CARCINOMA

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EVALUATION OF TUMOR RESPONSE TO SYSTEMIC THERAPY IN HEPATOCELLULAR CARCINOMA (Abstract): Evaluation of tumor response (the change in tumor volume) represents the basis for assessing therapeutic efficacy. Furthermore, objective response and progression are endpoints in clinical trials. *Response Evaluation Criteria in Solid Tumors* (RECIST) is an assessment tool derived from the conversion of quantitative imaging observations. Version 1.1 has been validated and its criteria are useful in all studies intended to assess objective response, disease stabilization and tumor progression, or time to progression analysis. In addition to changes regarding the disease assessment criteria of measurable and non-measurable lesions, the new system integrates volumetric assessment and functional imaging. The RECIST's utility system follows from the applicability in clinical trials and the possible use in clinical practice. However, adapting it to assess liver tumors requires evaluation in prospective trials. **Keywords**: HEPATOCELLULAR CARCINOMA, IMAGING, TUMOR RESPONSE

Primary liver tumors are the 5th most common human cancer and the third cause of cancer mortality worldwide. These data do not only indicate a high frequency, but also emphasize their extreme lethality. Of the primary liver tumors, *hepatocellular carcinoma* (HCC) is the most common, accounting for 80-90% of the total (1).

The highest incidence is observed in East Asia, followed by Central Africa, Southeast Asia, Pacific Islands, East Africa, West Africa, Southern Europe, South Africa, Eastern and Western Europe, North America and South Australia, Northern Europe and Central America. The incidence in Europe is below 10 per 100,000 populations (2). In Romania, HCC is the 10th most common cancer and the 7th in terms of mortality (3).

About 80% to 90% of cases develop from underlying chronic liver disease. Cirrhosis is a late stage of many liver diseases, including viral hepatitis, alcoholic liver disease, nonalcoholic steatohepatitis, hemochromatosis, primary biliary cirrhosis, autoimmune liver diseases, and metabolic

disorders. However, primary biliary cirrhosis and autoimmune liver diseases rarely lead to HCC (2).

Regarding the biology of HCC, currently three main altered signaling pathways responsible for the appearance and progression of this cancer are known. Data provided by Spangenberg and Liu (4, 5) show that the main pathways are mediated by TGF-β (transforming growth factor-β) and Wnt, centered on Akt signaling cascades and pathways, expressed in indolent cancers initiated by β-catenin gene mutations. Unfortunately, blocking Wnt-β-catenin pathways is not possible at present. However, the Ras-Raf-MAPK and Akt pathways can be targeted with such pharmacological agents as mTOR (mammalian target of rapamycin) inhibitors.

Clinical studies have evaluated the cytotoxic chemotherapy alone or in combinations and have not demonstrated significant improvements in overall survival and time to progression. The research in the molecular pathogenesis of HCC was directed to identify intercellular signaling pathways, because the neoplastic disease arises from aberrant mechanisms of gene control and abnormal protein expression. New techniques for assessing the tumor phenotype may be used for tailoring new therapeutic approaches. HCC is a highly vascularized tumor, with an increase in microvascular density and increased levels of VEGF. As a consequence, various trials have used sorafenib (antiangiogenetic predominant mechanism) or bevacizumab alone or in combination with other drugs (6, 7, 8, 9, 10, 11, 12, 13, 14).

Evaluation of tumor response following the use of such antiangiogenetic therapies became a major issue. Unlike cytotoxic chemotherapy, whose effectiveness can be assessed by reduction (one or two-dimensional) in tumor size, the tumor response to antiangiogenetic therapies is usually expressed by extending tumor necrosis without a significant impact on tumor size. Moreover, the combination of the two above mentioned types of medication makes the evaluation of therapeutic efficacy difficult (15, 16).

To standardize tumor response to molecular therapies and to simplify the evaluation methods, Eisenhauer et al. (17) proposed changes in RECIST criteria (Response Evaluation Criteria in Solid tumors). RECIST 1.1 criteria aimed at assessing primary tumor response in clinical trials (less useful in practice) where the primary endpoint is the objective response. It defines four main categories of response for measurable lesions: complete response - CR (disappearance of all tumor lesions), partial response - PR (30% reduction of the measured maximum diameter), progressive disease - PD (20% increase in the maximum diameters but not less than 5 mm – fig. 2), and stable disease - SD (criteria for partial response or progressive disease are not met).

Major changes included in version 1.1 were:

- the number of lesions required to define tumor response was reduced from 10 to 5 (total) and from 5 to 2 (per organ);

- to define complete response in lymph nodes, it is possible to evaluate them by pathological exam;

- progression of disease requires an increase of target lesion of at least 5 mm;

- the lymph nodes are to be measured in the short axis and if the size is greater than 15 mm it may be considered a target lesion;

- the nodes with short axis decreased under 10 mm after treatment are to be con-

sidered as normal lymph nodes .

Also, the new guide has introduced information on the evaluation by functional imaging. These criteria, although universal, can be modified and adapted to each trial individually (17).

Initial assessment (baseline) aims to establish tumor or lymph node lesions and to fit them into measurable lesions (target) or non-measurable (non-target). The criteria necessary to define target lesions are: minimum size of 10 mm assessed on a CT scan (fig. 1) with sections of 5 mm or less, 10 mm for clinically measurable lesions, 20 mm if the assessment is done by chest radiograph, and 15 mm in short axis for nodes (measured by CT). Non-measurable lesions consist of all the tumors that do not meet the above criteria and truly non-measurable lesions like ascites, pleural or pericardial effusion, leptomeningeal metastases, inflammatory breast cancer, carcinomatous lymphangitis, and purely blastic bone metastases. Cystic lesions or previously treated lesions by local procedures should be avoided. For a correct assessment, the same imaging method should be used from baseline throughout the study (17).

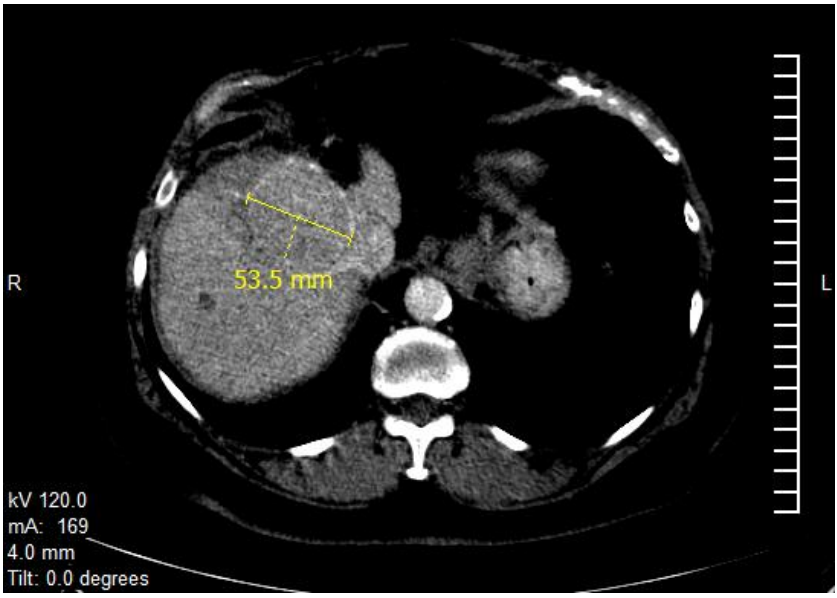

Fig. 1. Initial assessment of a patient with liver cancer (T1=53. 5 mm)

The course of non-measurable lesions can be classified into 3 categories:

- complete response - CR - defined as disappearance of all lesions and reduction in axis diameter of lymph node below 10 mm;

- incomplete response / stable disease - IR/SD - defined as the persistence of non-target lesions or high levels of tumor markers;

- progressive disease - PD - defined as unequivocal progression (17).

The time interval between assessments should be about 6-8 weeks and coincide with the completion of a treatment cycle. To define the clinical benefit, the response should be maintained over a period of time of at least 16 weeks (progression free survival PFS16). To evaluate the effectiveness

of therapies in terms of PFS, all patients in the study must have measurable disease. In particular situations, assessing the response may be impossible: premature discontinuation of treatment due to toxicity, death, patient refusal. The study results should be based on reporting the results for all eligible patients and not only based on the selective processing of the "evaluable" patients (17).

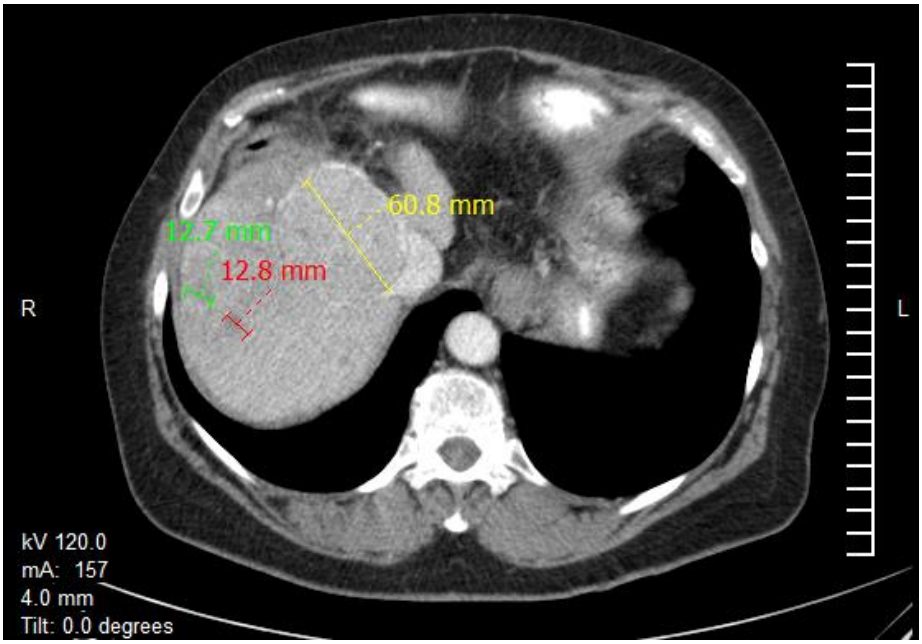

Fig. 2. Progression of disease – new lesions

Since RECIST criteria were originally designed to evaluate tumor response in cytotoxic treatments, *European Association for the Study of Liver* (EASL) recommended the use of mRECIST (modified RECIST) criteria in clinical trials (18, 19). Thus, the measurement of lesions is done only to viable tumor tissue, which captures the contrast substance in the arterial phase, and pleural effusions and peritoneal cytology require confirmation for progression of disease to be declared, portal lymph nodes are considered involved if the short axis is greater than 2 cm, portal vein thrombosis is considered non-target lesion, and a new lesion defines progression of disease if the maximum diameter is at least 1 cm and captures the contrast agent. The study by Ahsun Riaz et al. proved a better match between mRECIST and pathologic response than RECIST and WHO systems. If WHO and mRECIST are combined, the results have better value than any method individually. Patients previously treated by local methods should be evaluated carefully, taking into account the possibility of artifacts induced by lipiodol, clips (18, 19).

Ultrasonography (US) is the initial examination performed in most patients with suspected liver masses because it is faster, has satisfactory accuracy, and is a noninvasive method. Moreover, US provide real-

time imaging and can be used for guided biopsies. By contrast enhanced US (CEUS), focal liver lesions can be diagnosed based on their specific vascular characteristics. CEUS has a sensitivity of 94% and a specificity of 93% for the diagnosis of HCC. The main disadvantage is the dependence on operator and patient, because obese patients can be difficult to scan and the reproducibility is limited. Based on these drawbacks of US, EASL guidelines recommended the use of CT or MRI in clinical trials. However, during the follow-up period, after local treatment with curative potential, the US can be used after the first year without evidence of recurrence. Still, during the first year after treatment, CT or MRI needs to be performed every 3 months and if no relapse is observed, US may be used (2, 18, 19).

Currently, CT is still the gold standard for liver imaging. Therefore, in clinical trials, as well as in daily practice, the major goals of liver imaging remain:

- to assess the number and size of lesions;
- to locate the lesions;
- to suggest the nature of lesions (malignant vs. benign);
- to identify the origin of lesions (primary vs. secondary);
- to evaluate the damage to liver parenchyma;
- to assess the extrahepatic disease;
- to evaluate the response to treatment (2).

Moreover, with the new techniques of liver resection and partial transplant, there is a need for an accurate assessment of the liver graft.

In conclusion, there is no absolute consensus regarding the optimal strategy for the evaluation of liver tumors in clinical trials. The way we choose to evaluate the response is based on clinician's requirements, the availability of equipment, patient's status, and the experience of the staff.

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NEWS

PYRIDOMYCIN A NEW DRUG FOR TUBERCULOSIS

Tuberculosis is a serious public health issue. Increased frequency of cases caused by first-line antituberculosis drugs resistant strains requires identification of new therapeutic alternatives. First described in 1953, pyridomycin is a bactericidal agent against mycobacteria without action on other bacteria. It is a "natural isoniazid" produced by *Streptomyces pyridomyceticus* or *Dactylosporangium fulvum*. A recent study by Ruben et all. revealed the mechanism by which this antibiotic kills *Mycobacterium tuberculosis*. Like isoniazid, pyridomycin binds NADH dependent enoyl (ACP)-reductase InhA and inhibits InhA which results in the inhibition of mycolic acid synthesis in *M. tuberculosis*. Unlike isoniazid, pyridomycin action does not depend on KatG catalase-peroxidase. Thus isoniazid-resistant strains (due to alteration of KatG activity) retains sensitivity to pyridomycin, thus opening new avenues for drug development. (Ruben C. Hartkoorn, Claudia Sala, João Neres et al. Towards a new tuberculosis drug: pyridomycin - nature's isoniazid. *EMBO Molecular Medicine*, 2012, 4(10):1032–1042).

Cătălina Luncă

CLINICAL FEATURES AND COURSE OF BACTERIAL MENINGITIS IN CHILDREN

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CLINICAL FEATURES AND COURSE OF BACTERIAL MENINGITIS IN CHILDREN (Abstract): **Aim**: To analyze the clinical features and course of and to define the risk factors for bacterial meningitis in children. **Material and Methods**: Retrospective study of 100 cases of bacterial meningitis in patients aged 0-18 years admitted to the Iasi Infectious Diseases Hospital between 2005 and 2010. **Results**: We found a clear prevalence in male children (58%) from rural area (67%), with the highest incidence in the age group 2-5 years. A significant percentage of patients (43%) had previous hospitalization, condition which is known as predisposing factor for bacterial meningitis, the most common being ear infections (20%) and height and weight deficit (9%). 71% of patients were admitted within the first 48 h. The most common onset clinical manifestations were fever (84%), vomiting (70%), signs of meningeal irritation (59%), somnolence (23%), loss of appetite (19%), and coma in 5% of patients. In 36% of cases CSF was opalescent with moderate pleocytosis (35%); in 29% of patients CSF albumin level ranged between 0.7-1.0 g, the majority presenting normal glycorahia (71%). In only 21% of cases the microbial agent was identified (pneumococcal and meningococcal etiology, 8% and 6%, respectively). The course was generally favorable, and mortality rate was low (5%). Complications occurred in 3% of patients consisting in hydrocephalus and brain abscess. **Conclusions**: Bacterial meningitis remains a disease with potentially severe course. Clinical onset, most commonly atypical in children, requires differential diagnosis at the time of admission in order to initiate the most appropriate antibiotic therapy.
Key words: CHILDREN, BACTERIAL MENINGITIS, PARTICULARITIES.

The number of bacterial meningitis cases is estimated at over one million per year worldwide, with more than two thirds of these occurring before 5 years of age. Acute bacterial meningitis (ABM) in children continues to be associated with high morbidity and mortality, despite progress in terms of etiological therapy. In all countries, whether industrialized or developing, the risk of epidemics remains a topical issue. Bacterial meningitis in children is, in all cases, a therapeutic emergency, involving allegations of early clinical diagnosis to be confirmed by CSF examination.

The etiologic agent varies with age, immune system, immunization status and the geographical region. *Haemophilus influenzae* type b (Hib), *Streptococcus pneu-*

moniae, and *Neisseria meningitidis* cause most cases of acute bacterial meningitis (ABM) in children, but epidemiological data are changing rapidly because of immunization practices and changing patterns of bacterial resistance.

Before the introduction of an effective vaccine, *Haemophilus influenzae* (Hi) was the most common etiologic agent before 5 years of age (about 45% of cases). In those countries where Hib vaccine is part of the routine infant immunization, Hib was almost eradicated (1, 2).

Vaccines against pneumococcal and meningococcal diseases have changed significantly the disease profile (3).

Streptococcus pneumoniae (Sp) is involved in about 20% of child MB (about 30% before one year of age, and 40% before 6 months in developed countries). Two features are important to be remembered: a) Sp is common in patients with hemoglobinpathies and splenectomy; b) the increasing rate of penicillin resistance requires rigorous epidemiological surveillance of Sp susceptibility profile. Vaccination at 2 months aims at preventing early infections. In countries where routine pneumococcal immunization was introduced, an increase in invasive pneumococcal disease due to non-vaccine serotypes was not reported (4, 5).

Neisseria meningitidis (Nm) is responsible for severe MB: fulminant meningococcemia and meningitis. The overall incidence of Nm meningitis is approximately 30% of cases (60% over 5 years, 25% before this age) with deadly epidemics reported especially in the "meningitis belt" of sub-Saharan Africa, where Nm serogroup A affects up to 1% of the population. Nm serogroup B predominates in Europe and Nm serogroup C has an increased frequency in France. Effective vaccination against serotypes A and C has reduced the number of secondary cases and epidemics (6).

MATERIAL AND METHODS

The present study aims at evaluating the clinical and biological features and the trends in the course of bacterial meningitis in children in order to optimize the first-line antibiotic therapy for this category of patients. Our study is a retrospective analysis which included 100 patients aged 0-18 years diagnosed with bacterial meningitis at the Iasi Infectious Diseases Hospital Iasi between 2005 and 2010.

The diagnosis of bacterial meningitis was based on clinical and laboratory criteria.

A t-test, Fisher's exact test, or Pearson chi-square test were used where appropriate, and a p-value of < 0.05 was considered significant.

RESULTS

During the 6-year study period, 354 patients with bacterial meningitis, of which 28.24% were children, were admitted to the Iasi Infectious Diseases Hospital. In the interval 2005-2010 the number of pediatric bacterial meningitis cases was relatively constant, peaking in 2008 (24%).

Comparing the incidence of bacterial meningitis in the two genders, a slightly higher value was observed in males (58% vs. 42% in women). Age of patients ranged between 0 and 18 years, with a mean age of 8 years. Bacterial meningitis was more common in the age group 1-5 years (28%), with lower but comparable rates in the age group 6-11 years (27%) and 0-12 months (25%).

Upon admission we noticed that the predisposing factors for bacterial meningitis were ENT infections (20%), height and weight deficit (9%), digestive system infections (5%), trauma (4%), protein-caloric

malnutrition (4%), and ventriculoperitoneal shunt (1%). 71% of patients were admitted within 48 hours after the onset. The most common onset clinical manifestations were fever (84%), vomiting (70%), signs of meningeal irritation (59%), somnolence (23%), loss of appetite (19%), and coma in 5% of patients. CSF examination revealed a turbid macroscopic appearance in 36% of cases, clear CFS in 33%, and in only 13% of cases a typical purulent appearance. 35% of cases had a moderate pleocytosis in CSF, and increased cellularity > 1000/mm³. Glycorahia was found low (0.2 to 0.3 g / l) in almost a half of patients (47%).

Of the 100 children diagnosed with bacterial meningitis, in only 21% the infection was microbiologically documented: in 8% of patients the causative agent was pneumococcus, meningococcus was isolated in 6% of patients, and *Haemophilus influenzae* in 4% of cases. Two (2%) cases of bacterial meningitis with Gram-negative and 1 case (1%) of staphylococcus meningitis were reported. These small positivity rates are most likely due to the fact that more than half of patients received antibiotics prior to admission.

In this study we analyzed the age-related clinical and laboratory features of bacterial meningitis and found significant statistical differences with regard to meningeal irritation syndrome and vomiting at the onset (tab. I, II).

TABLE I

Age-group distribution of onset symptoms

Clinical manifestation	Age group 1-5 Years (n=28)	Age group 6-11 years (n=27)	p
Fever	27 (96.42%)	25 (92.5%)	0.51
Meningeal irritation syndrome	11 (39.2%)	22 (81.48%)	0.001
Vomiting	4 (14.48%)	19 (73.37%)	0.00002
Anorexia	21 (75%)	16 (59.29%)	0.213
Somnolence	20 (71.42%)	19 (73.37%)	0.931
Seizures	14 (50%)	9 (33.33%)	0.210
Coma	2 (7.14%)	3 (11.11%)	0.608

TABLE II

Age group distribution of CSF characteristics

CSF parameters	Age group 1-5 years	Age group 6-11 years	p
Purulent	8 (28.57%)	5 (18.51%)	0.380
Opalescent	11 (39.28%)	12 (44.4%)	0.698
clear	9 (32.14%)	10 (30.07%)	0.702,
100- 500 cel/mm	7 (25%)	8 (29.6%)	0.699
500-1000 cel/mm3	6 (21.4%)	4 (14.81%)	0.524
1000-2000 cel/mm3	10 (35.71%)	8 (29.6%)	0.630
>2000 cel/mm3	5 (17.85%)	7 (25.92%)	0.468
Protein 0,7-1g/l	4 (14.28%)	7 (25.92%)	0.28
1-3 g/l	11 (39.28%)	12 (44.4%)	0.698
3-5 g/l	13 (46.42%)	8 (29.6%)	0.199
Normal CSF glucose	10 (35.71%)	12 (44.44%)	0.508
Low CSG glucose	18 (64.28%)	15 (55.5%)	0.508

To be effective, the etiological treatment must have a rapid microbicidal effect. The majority of patients (60%) received a single beta-lactam antibiotic (ampicillin, ceftriaxone or meronem). 26% of cases were treated with an association of two antibiotics, the most commonly used as first-line being ampicillin + ciprofloxacin, ceftriaxone + ciprofloxacin, or vancomycin + ciprofloxacin (fig. 1). A small number of patients (14%) required a combination of more than two antibiotics, based on CSF culture results, sensitivity testing, and subsequent clinical course.

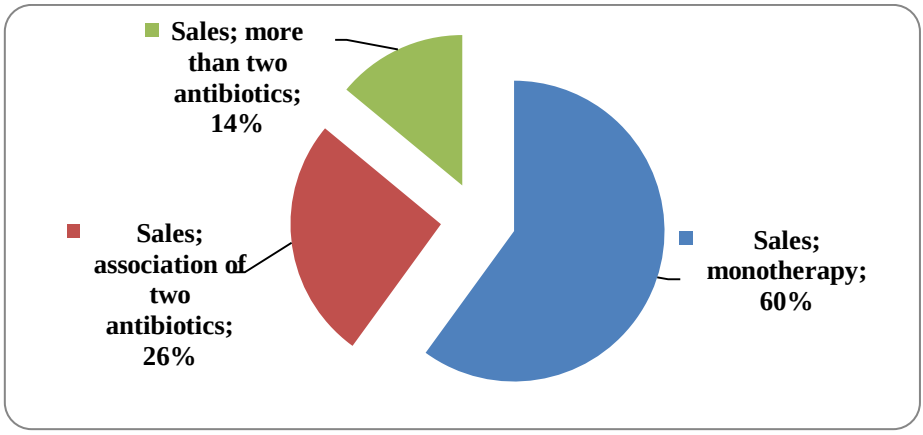

Fig.1. Etiological treatment of bacterial meningitis in children

The course of bacterial meningitis in the studied group was generally favorable; only few cases developed neurological complications (3%) consisting of hydrocephalus and brain abscess. Mortality rate was low - 5%. Unfavorable course was associated with severe general status and age at onset (all children in coma upon admission and those belonging to the age group 1-5 years).

DISCUSSION

Although it includes only 100 cases of bacterial meningitis, compared with multicenter studies our study brings microbiologically documented data sufficient to support the epidemiological features in our geographic area, taking into account that the Iasi Infectious Diseases Hospital serves patients from most districts of North-Eastern Romania. *S. pneumoniae* was the most common cause of bacterial meningitis in children (8%), followed by *N. meningitidis* (6%) and *H. influenzae* (4%).

As in previous studies, the distribution of etiologies was age-dependent. *S. pneumoniae* was the most frequently isolated pathogen in children <1 year, with a peak of incidence in children 4-6 months, while N. meningitidis was the most common pathogen in older children. Mortality rate (5%) was lower than that reported in other industrialized countries (9.2%). Mortality rates associated with *N. meningitidis* and *S. pneumoniae* were 3% and 2%, respectively, lower than those reported in recent studies (7, 8).

The high frequency of *S. pneumoniae* strains with diminished susceptibility to penicillin (53.4% of isolates), supports the use of vancomycin associated with a third

generation cephalosporin (9, 10).

CONCLUSIONS

Bacterial meningitis remains an important public health issue, not just when it occurs in epidemics, but mostly because of its complications and high mortality. The low mortality rate in our hospital was due to an early CSF examination even in children with subtle signs of meningitis.

Bacterial meningitis should be considered in any case of acute fever in infants and children since the clinical picture in young children is less specific.

The high frequency of *S. pneumoniae* strains with diminished susceptibility to penicillin (53.4%) is an alarm signal that recommends the use of a third-generation cephalosporin or vancomycin as first-line therapy.

A good knowledge of the epidemiology of bacterial meningitis in children may serve as a guide for empirical antibiotic treatment, and also justifies the introduction of new conjugate vaccines into national vaccination scheme.

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THE CENTRAL ROLE OF THE NON ALCOHOLIC FATTY LIVER DISEASE IN METABOLIC SYNDROME

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THE CENTRAL ROLE OF THE NON ALCOHOLIC FATTY LIVER DISEASE IN METABOLIC SYNDROME (Abstract): Non-alcoholic fatty liver disease (NAFLD) covers a spectrum of liver disease from steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. Most NAFLD patients are hyperinsulinaemic and more insulin resistant compared with non-steatotic healthy subjects, and there is a near universal association between NAFLD and insulinresistance (IR) irrespective of obesity. The metabolic syndrome (MS) is highly prevalent in the general adult population (approximatively 22%) and it carries an increased cardiovascular morbidity and mortality. Pathophysiologic considerations, clinical associations, and laboratory investigations support that IR and hyperinsulinaemia have a central role in pathogenesis of both MS and NAFLD. The fatty liver is resistant to the action of insulin to suppress hepatic glucose production, which results in hyperglycaemia and, further, in hyperinsulinemia. The MS is associated with maldistribution of body fat, increased free fatty acids (FFAs) and IR, leading to type 2 diabetes, hypertension, dyslipidemia. Visceral fat is an important clinical marker of metabolic cardiovascular risk and a marker of IR in multiple tissues, independent of body mass index (BMI). NAFLD and atherosclerosis share common molecular mediators and NAFLD itself might play an early role in the development and progression of atherosclerosis. These data suggest that NAFLD should be considered part of a multi-organ system derangement in insulin sensitivity, and help explain why NAFLD is so closely linked with diabetes, MS and is an important risk factor for coronary heart disease. NAFLD may be the hepatic manifestation of the MS and raises the possibility that it may play an early role in the etiology of MS. **Key words:** NON-ALCOHOLIC FATTY LIVER DISEASE, INSULIN RESISTANCE, METABOLIC SYNDROME

Non-alcoholic fatty liver disease (NAFLD) has become an important public health problem because of its high prevalence, potential progression to severe liver disease and association with cardiometabolic abnormalities, including diabetes, the metabolic syndrome and coronary heart disease (CHD) (1, 2). Obesity is an important risk factor for NAFLD, and the prevalence of NAFLD is linearly associated with body mass index (BMI) (3). The mechanism responsible for the link between obesity and its metabolic complications involves insulin resistance (IR), which is a common feature of obesity and NAFLD, and is an important risk factor for

cardio-metabolic disease (4). Patients with NAFLD might be at greater risk for CVD than those without NAFLD; however, the putative increased CVD risk in patients with NAFLD might reflect the coexistence of underlying MS risk factors or, alternatively, NAFLD itself might confer a CVD risk above and beyond its association with the individual components of the MS (3, 5).

METABOLIC SYNDROME (MS)

MS is a clustering of risk factors (abdominal obesity, atherogenic dyslipidaemia, hypertension, elevated plasma glucose, a pro-thrombotic state and a pro-inflammatory state) that greatly increases an individual's probability for developing atherosclerotic cardiovascular disease, type 2 diabetes mellitus and chronic kidney disease (3, 5). According to the new definition (IDF and AHA 2009), people with any three of the following five criteria are considered to have MS: increased waist circumference (with ethnicity specific values); high triglycerides (≥150 mg/dl); reduced HDL cholesterol (<40 mg/dl for males and <50 mg/dl for females); elevated blood pressure (systolic ≥130 mm Hg and/or diastolic ≥ 85 mm Hg); increased fasting glucose ≥ 100 mg/dl.

The MS is highly prevalent in the general adult population (approximately 22%), with differences in relation to race, gender, and age (6). It carries an increased cardiovascular morbidity and mortality - they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome, which makes an early and correct assessment mandatory. The prevalence of the metabolic syndrome is very high in type 2 diabetes patients, in whom it influences the risk of chronic complications (1, 6)..

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

NAFLD covers a spectrum of liver disease from steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. According to the American Association for the Study of Liver Diseases, NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight (5, 6). Steatosis attributable to NAFLD is typically macrovesicular rather than microvesicular (4). Alcohol consumption should not exceed 20 g/day and viral (hepatitis B and C), toxic, autoimmune (clearly elevated antinuclear and anti-smooth muscle antibodies), and other causes of steatosis (Wilson disease, hypobetalipoproteinemia) should be excluded (7, 8).

NAFLD affects approximately 15-25% of the general population in various countries, and its prevalence increases steadily to 70–90% in obese or type 2 diabetic patients. NAFLD is currently the most common cause of abnormal liver function tests. Liver enzyme levels do not reliably correlate with liver histology, the full range of disease may be seen in patients who have normal transaminase levels (normal values being present in up to 78% of patients at any time) (4, 8). When elevated, the increase is mild, often restricted to ALAT, with an ASAT/ALAT ratio usually less than 1 (4, 8).

Why Is Liver Fat Related to Components of the Metabolic Syndrome?

Pathophysiologic considerations, clinical associations, and laboratory investigations support that IR and hyperinsulinaemia have a central role in pathogenesis of both MS and NAFLD (6). Even in nondiabetic individuals a fatty liver has been associated with causes of insulin resistance such as obesity and with consequences of insulin

resistance such as hypertriglyceridemia and hyperinsulinemia (9).

Although the epidemic of obesity has been accompanied by an increase in the prevalence of the MS, not all obese develops the syndrome and even lean individuals can be insulin resistant. The reason why some individuals are insulin resistant whereas other equally obese are not has long been unresolved. The development of fatty liver was associated with an increase in BMI even of 1 kg/m2 (around 2-3 kg), suggesting that relatively small changes in body weight may exert significant metabolic effects. Waist circumference is a better predictor of the risk of CVD than obesity and is associated with NAFLD as determined using computed tomography, liver biopsy or ultrasound techniques (10, 11, 12). Although the prevalence of NAFLD is higher in obese population, the liver fat is highly significantly and linearly correlated with all components of the metabolic syndrome independent of obesity (13).

Both lean and obese insulin resistant individuals have an excess of fat in the liver. Most NAFLD patients are hyperinsulinaemic and more insulin resistant compared with non-steatotic healthy subjects, and there is a near universal association between NAFLD and IR irrespective of obesity (3, 5). IR is a recognized risk factor for CVD, and plays a pivotal role in the development of poor clinical outcomes in NAFLD patients (5, 6). IR may be defined as a condition in which higher than normal insulin concentrations are needed to achieve normal metabolic responses or normal insulin concentrations fail to achieve a normal metabolic response (7, 14). Fasting serum insulin correlates closely with liver fat content independent of age, gender and BMI. The fatty liver is resistant to the action of insulin to suppress hepatic glucose production, which results in hyperglycaemia and, further, in hyperinsulinemia (9, 13, 15). ALAT levels correlated significantly with fasting insulin levels, suggesting that hyperinsulinaemia is an important contributor to the development of fatty liver, more crucial to the pathogenesis than anthropomorphic data, blood glucose or serum lipids (13).

The amount of intra-hepatic triglycerides (IHTG) in obese persons without diabetes is directly correlated with impaired insulin action in liver (suppression of glucose production), skeletal muscle (stimulation of glucose uptake) and adipose tissue (suppression of lipolysis), independent of percent body fat and intra-abdominal adipose tissue (IAAT) volume (16). IHTG content is a marker of insulin sensitivity in multiple tissues, independent of BMI. These results suggest that NAFLD should be considered part of a multi-organ system derangement in insulin sensitivity, and help explain why NAFLD is so closely linked with diabetes and the metabolic syndrome and is an important risk factor for coronary heart disease (1, 17).

Thus, NAFLD in its more advanced forms might act as a stimulus for further increased whole-body insulin resistance and dyslipidemia, leading to accelerated atherosclerosis. This hypothesis is also partly validated by few prospective studies demonstrating that raised liver enzymes independently predict the development of the metabolic syndrome (18, 19). So, we can say that NAFLD is an under recognized manifestation of the metabolic syndrome (15).

The role of adipose tissue - ectopic fat. Visceral (intra-abdominal) fat has attracted much interest both as a marker and an inducer of many of the metabolic abnormali-

ties associated with obesity. Abdominal circumference, surrogate marker of visceral fat, is an easily available clinical measurement of both CV risk and type 2 diabetes risks in obesity, being a good predictor of IR, dyslipidemia, low-grade inflammation (7, 8, 11). Excess of intra-abdominal fat in particular may be a key determinant in the pathogenesis of NAFLD, because of its strong association with IR and a source of free fatty acids (FFAs) (7, 20).

The MS is associated with maldistribution of body fat, increased FFAs and IR, leading to type 2 diabetes, hypertension, dyslipidemia and hypercoagulability (11). The role of ectopic fat depots, including the visceral depot, is to "spill-over" the lipids when the subcutaneous sites become insufficient (12, 20). The fact that the portal vein drains the visceral fat makes the liver an important target for the released adipokines. But, visceral adipose cells have an increased lipolytic rate and respond poorly to the anti-lipolytic effect of insulin. Visceral fat is a marker of ectopic fat and an important clinical marker of metabolic CV risk (11). The effect of IR may be mediated by increased portal FFAs, deacreased adiponectin, by cytokines and chemokines, which may exert local effects, as well as contribute to overall systemic release. Obesity with excess visceral fat is associated with reduced adiponectin, whereas obese persons with normal visceral fat have normal adiponectin levels (12).

Role of Adipose Tissue Inflammation. Chronic inflammation is frequently associated with the MS and the main inflammatory mediators are adipocytokines and FFAs. Pro-inflammatory cytokines that have been associated with MS include CRP, TNF-a, IL-6 and others and they result in more insulin resistance and lipolysis of adipose tissue triglyceride stores, in enhanced hepatic glucose and VLDL production. The increased secretion of TNF-α and other pro-inflammatory cytokines by adipocytes and infiltrating macrophages is thought to lead to chronic systemic inflammation as well as obesity-linked insulin resistance (21).

Elevated concentrations of circulating TNF-α have been documented in NAFLD patients and also its implications in the manifestation of fatty liver disease (21); is increased in obese and diabetic individuals, while weight loss decreases its levels. TNF-α inhibit lipoprotein lipase, stimulates hormone-sensitive lipase and induces uncoupling protein expression, down regulates insulin-stimulated glucose uptake via effects on GLUT-4, insulin receptor autophosphorylation and IRS-1 expression. TNF-α decreases the production and secretion of leptin by adipocytes (12, 21).

IL-6 seems particularly abundant in the visceral fat and chronically elevated IL-6 induces IR in both the liver and the adipose cells (11). IL-6 has pro-inflammatory properties and causes activation of Kupffer cells resulting in fibrogenesis; it also enhances hepatic C-reactive protein synthesis. In paracrine fashion, it decreases adiponectin secretion from the surrounding adipocytes, inhibits lipoprotein lipase (LPL) on endothelial cells and activates lipolysis (12).

TNF-α and IL-6 also induce development of hypertension (7, 21). Individuals with MS have significantly higher rate of sodium and water reabsorption at the proximal tubular level (7, 22). Insulin promotes endothelin-1 (ET-1) production, as well as ET-1 action in vascular smooth muscle cells, causing vasoconstriction and increased smooth muscle cells proliferation (7, 23, 24).

LIVER FAT – AN ATHEROGENIC CONDITION ASSOCIATED WITH INCREASED CARDIOVASCULAR RISK

Essential hypertension is considered an insulin resistant state and approximatively 50% of patients with arterial hypertension are reported to be insulin resistant and hyperinsulinemic. These metabolic abnormalities also seem to be involved in the pathogenesis of the complications of hypertension, both in obese and non-obese patients (15, 22). NAFLD and atherosclerosis share common molecular mediators and NAFLD itself might play an early role in the development and progression of atherosclerosis. The biological mechanisms by which NAFLD contributes to higher risk of CVD, independent of other prognostic factors, are not fully understood. It is possible that NAFLD, in its more advanced forms, acts as a stimulus for further increased whole-body IR and dyslipidaemia, leading to accelerated atherosclerosis (10, 24).

Recent studies clearly documented that NAFLD patients have significantly greater carotid artery intima-media thickness (IMT) than control subjects, a reliable index of subclinical atherosclerosis (25, 26). Other small studies showed that NAFLD patients have a significant decrease in endothelium-dependent vasodilatation and this decrease was significantly associated with the histological severity of NAFLD (1, 18). Villanova *et al*. (17-19) reported that brachial artery endothelial flow-mediated vasodilatation was decreased in non-diabetic subjects with NAFLD compared with control subjects. The severity of histopathological features in NAFLD is strongly associated with both early carotid atherosclerosis and the decrease of flow-mediated vasodilatation, independent of classical risk factors, IR and the presence of MS (8, 18). The 10-year probability of coronary events (Framingham equation) was moderately increased in these patients (17).

A possible atherogenic mechanism linking NAFLD and carotid IMT could be represented by increased oxidative stress and subclinical inflammation, which are thought to be causal factors in the progression from simple steatosis to more advanced forms of NAFLD (17, 18). Atherosclerosis is accelerated both directly by effects of cytokines on endothelial cells and indirectly by cytokine effects on the liver, causing increased production of CRP and related factors (27, 28). CRP induces endothelial cell adhesion molecules expression (27), inhibits nitric oxide synthesis, increases plasminogen activator inhibitor (PAI)-1 expression (28), and leads to endothelial dysfunction (21, 28). The concentrations of plasma adiponectin (cytokine of adipose tissue with antiatherogenic properties) are decreased in patients with NAFLD, compared with control subjects. This decrease is associated with the histological severity of NAFLD independent of IR and MS components (4, 10, 29); decreased adiponectin concentrations predict the incidence of CVD and MS in large prospective studies (4, 29) and appear to play a key role in the development and progression of NAFLD.

These strongly support the notion that NAFLD may be the hepatic manifestation of the MS (2). Currently, the importance of NAFLD and its relationship with the MS is increasingly recognized. However, the putative increased CVD risk in patients with NAFLD might reflect the coexistence of underlying MS risk factors or, alternatively, NAFLD itself might confer a CVD risk above and beyond the one associated with the individual components of the MS (11, 12).

Liver Fat Predicts the Metabolic Syndrome and Type 2 Diabetes. The evidences of the fact that NAFLD is a feature of the MS are on growing. Liver markers have been shown to be associated with MS variables in large samples of the general population. ALAT significantly predicts the incidence of MS and type 2 diabetes, associations that were maintained after adjustment for a large number of risk factors (including direct measures of insulin sensitivity and insulin secretion). This finding raises the possibility that NAFLD may play an early role in the etiology of MS (12, 30). First, these markers are known to be significantly correlated with increased hepatic fat content and it has been demonstrated that hepatic fat content is associated with IR independent of body weight (9). In particular, hepatic fat was associated with defects in the suppression of endogenous glucose production by insulin (9, 19); this could explain why ALAT values and ASAT/ALAT ratio predicted the onset of fasting hyperglycaemia (19).

The role of steatosis, estimated using liver enzymes, in the prediction of type 2 diabetes has been examined in 12 prospective studies. Elevated liver enzymes predicted type 2 diabetes independent of obesity. In a Swedish study of 54-year-old men, serum ALAT concentrations predicted a 3.9-fold increase in the risk of type 2 diabetes, which remained significant after adjusting for BMI (13).

CONCLUSIONS

Regardless of the origins of the metabolic syndrome and despite the fact that the pathogenesis of the fatty liver is poorly understood, the liver is responsible for production of many CV risk factors. Increased fat accumulation in the liver is a marker of hepatic IR and closely correlates with all components of the metabolic syndrome, independent of obesity. Adipose tissue inflammation and a fatty liver seem to coexist, but the direction of causality, is unclear. Epidemiological data have shown the fatty liver predict, independent of other factors, the MS, type 2 diabetes, and CVD.

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STUDIU PRIVIND ASOCIEREA METODELOR IMAGISTICE CU DATELE ANATOMO - CLINICE ÎN PREDICȚIA INTUBAȚIEI DIFICILE

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STUDY ON THE ASSOCIATION OF IMAGING AND CLINICAL ANATOMIC DATA IN THE PREDICTION OF DIFFICULT INTUBATION (Abstract). Difficult intubation is a serious challenge for emergency doctors and anesthesiologists. **Aim:** This study was aimed at investigating the effectiveness of imaging methods combined with clinical anatomic data in predicting difficult intubation by developing a score as thorough and easy to use as possible. **Material and methods:** This prospective study was carried out on a series of 121 patients (51 males and 70 females), aged between 18 and 85 years (mean age 45.5 years) in the interval September 2010 0 June 2011. The patients, scheduled for various surgical interventions requiring general anesthesia by oral tracheal intubation, were randomly selected and gave a written consent. Children and pregnant women were excluded. The criteria for clinical anatomic assessment and cerebral and cervical imaging methods were used in all patients. By corroborating these data, a difficult intubation prediction score, named OMLET, was developed. It included 5 parameters: obesity (IMC), Mallampati score (modified), position of the larynx as to the spine, cervicothoracic angle (CTA), and head extension (DTM). Each variable was attributed 0 or 2 points, the highest score being 10 points, the obtained score being directly proportional with the expected difficulty during oral tracheal intubation. **Results:** Of the 121 patients, 9 were difficult cases, the incidence of difficult intubation being 7.43%. The Cormack-Lehane score predicted a difficult intubation in 11 of the 121 cases (sensitivity 77.78%, specificity 96.43%), while the OMLET score predicted 6 of the 9 difficult intubations (sensitivity of 66.67%, specificity of 95.43%. positive predictive value 54.55%, and negative predictive value 97.27%). **Conclusions:** The OMLET score proved effective in predicting difficult intubation expressed in its high specificity and sensitivity, and could be used especially in patients with conditions of the cephalic extremity and neck inevitably requiring before surgery investigation of these areas.

Căile respiratorii care implică intubația dificilă (ID) în situațiile de urgență reprezintă cea mai importantă provocare pentru medicii urgentişti şi anestezişti. Incapacitatea de a controla permeabilitatea căilor respiratorii şi a ventila pacientul în mod eficient (SpO2< 90%) poate duce la complicații grave care au un impact imediat asupra calității vieții sau chiar la decesul acestuia prin hipoxie cerebrală.

Societatea Americană de Anestezie (ASA) defineşte ID ca fiind incapacitatea unui anestezist instruit de a insera sonda de intubație în trahee prin laringoscopie con-

venţională, necesitând mai mult de 3 tentative de intubaţie orotraheală (IOT) şi/sau în mai mult de 10 minute (1). IOT dificilă neanticipată este principala cauză a morbidităţii şi mortalităţii în anestezia pacienţilor (1).

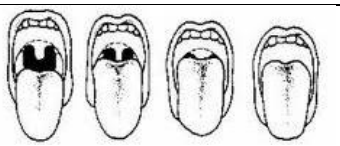
Scopul studiului este investigarea eficienţei metodelor imagistice (IRM) combinate cu datele anatomo-clinice în predicţia ID. Având în vedere coroborarea datelor imagistice cu elementele anatomo-clinice ne-am propus să stabilim un scor care să fie cât mai complet şi cât mai uşor de aplicat la fiecare caz şi care să cuprindă elementele de evaluare în scopul depistării unei posibile situaţii de ID.

MATERIAL ŞI METODE

Studiul prospectiv a fost realizat după obţinerea consimţământului, în perioada septembrie 2010 –iunie 2011. Pacienţii au fost selecţionaţi aleator, fiind programaţi pentru diverse intervenţii chirurgicale sub anestezie generală (AG) cu intubaţie orotraheală (IOT); au fost excluşi copiii, având particularităţi anatomice specifice vârstei şi femeile gravide, datorită modificărilor particulare din timpul sarcinii. Atunci când un pacient a fost exclus din studiu, altul a fost ales aleator în locul lui. Lotul de pacienţi s-a evaluat prin criteriile anatomo-clinice (tab. I).

TABELUL I

Criterii de evaluare anatomo-clinica a pacientilor

Parametru	Metoda de evaluare
Test Mallampati (modificat) (2) (de vizualizare)	Clasa I: palatele dur, moale şi uvula; Clasa II: palatul dur, moale şi partial uvula; Clasa III: palatul dur şi baza uvulei; Clasa IV: numai palatul dur, cel moale mascat  Clasa 1 Clasa 2 Clasa 3 Clasa 4
IMC ($IMC = G/h^2$)	< 18,5 Subponderabilitate 30 – 34,99 Obezitate gr. I 18,5 – 24,99 Normoponderabilitate 35 – 39,99 Obezitate gr. II 25 – 29,99 Supraponderabilitate > 40 Obezitate morbidă
DTM (3)	Distanţa dintre cartilajul tiroidian şi menton pe linia mediană în extensia maximă a capului.
Scorul LEMON (4, 5)	***L***: Examen extern: traumatism facial, incisivi răsfiraţi, excesde par facial (mustăţi, barbă), macroglosie ***E***: Regula "3-3-2": deschiderea gurii (3 lăţimi de deget), distanţa hio-mentonieră (3 lăţimi de deget), distanţa tiro-mentonieră (2 lăţimi de deget). ***M***: Scor Mallampati > 3 ***O***: Obstrucţia căilor aeriene: traumatisme, epiglotită, abcese peritonsilare. ***N***: Mobilitatea gâtului.
Clasificarea Cormack-Lehane (6) (de vizualizare)	Grad I - întreaga apertură laringiană; Grad II - numai comisura posterioară a aperturii laringiene; Grad III - numai epiglota; Grad IV - exclusiv palatul dur. 1 2 3 4

Pacienții incluși în studiu au fost investigati RM, cerebral şi cervical, după protocoale cu secvențe sagitale T1- şi T2 - ponderate fără supresie de grăsime, secvența coronala SPAIR (cu supresie de grăsime) şi secvența T2-ponderată axială fără supresie de grăsime. Pe imagini s-au determinat unghiul cervico-toracal (UCT) şi localizarea laringelui față de vertebrele cervicale. UCT este unghiul dintre dreapta ce unește marginea posterioară a vârfului dintelui axisului cu marginea posterioara a platoului inferior C7 şi dreapta ce unește marginea posterioară a platoului T1 cu marginea posterioara a platoului inferior T4 (fig. 1)

Fig. 1. Unghiul cervico-toracic şi laringe localizat pe secvența IRM sagitală T2-ponderată fără supresie de grăsime.

TABELUL II

Formular scor OMLET

	Variabilă	**Descriere**	**0 pcte**	**2 pcte**
O	**O**bezitate	IMC = G/h^2	< 30	> 30
M	**M**allampati	Scor Mallampati	Clasa 1 Clasa 2	Clasa 3 Clasa 4
L	**L**aringe	Localizarea laringelui	< C_5	> C_5
E	**E**xtensia capului	DTM	> 6 cm	< 6 cm
T	Unghi **T**oraco-cervical	UCT	În intervalul (130°-165^0)	În afara intervalului (130°-165°)
TOTAL scor ”OMLET”				

Coroborând datele obținute din examenul preanestezic cu cele obținute imagistic, am introdus un scor de predicție a ID, pe care l-am numit scor OMLET, care a cuprins 5 parametri: obezitatea (IMC), scorul Mallampati (modificat), laringele localizat față de coloana cervicală, DTM şi UCT. Fiecarei variabile i-a fost atribuit un punctaj de 0 sau 2 puncte (tab. II). Scorul maxim posibil este de 10 puncte, valoarea acestuia fiind direct proporțională cu dificultatea aşteptată în timpul IOT.

Achizițiile de imagine au fost efectuate pe aparat de IRM Siemens Avanto 1.5T cu ajutorul unei antene periferice (Neck Coil), cu poziționarea pacienților în decubit dorsal şi fixarea extremității cefalice în poziție neutră.

Examenul preanestezic şi IOT a fost efectuat folosind laringoscop rigid tip MacIntosh cu lama curbă numărul 4 sau 5, sonda endotraheală adaptată sexului şi particularităților anatomice ale pacientului. În cazul ID s-a folosit laringoscopul cu fibră optică tip MacIntosh şi s-a recurs la algoritmul managementului căilor respira-

torii dificile stabilit de ASA.

Prelucrarea şi interpretarea informaţiilor s-a realizat folosind programul EpiInfo vers. 3.5.1. şi în Microsoft Office Excel 2000. În calculul distribuţiei variabilelor s-a ales un interval de incredere (CI) de 95%; ″p″ < 0.05 a fost considerat semnificativ statistic iar pentru obţinerea sensibilităţii şi specificităţii, valorilor predictive, pozitiv şi negativ, am apelat la tabelul de contingenţă 2x2.

REZULTATE

Lotul de 121 pacienţi a fost alcătuit din 51 (42,1%) bărbaţi şi 70 (57,9%) femei cu vârste între 18 - 85 ani şi media vârstei de aproximativ 45½ ani. S-au evaluat datele clinice, imagistice şi dificultatea întâmpinată în timpul intubaţţiei (tab. III).

TABELUL III

Caracteristici anatomo-clinice şi imagistice evidenţiate pentru lotul de pacienţi incluşi în studiu

Caracteristica ***N = 121***		n*	%	95% CI**
Sex	Masculin	51	42,1	33,2 – 51,5
	Feminin	70	57,9	48,5 – 66,8
IMC	< 18,5	6	5,1	1,9 – 10,8
	18,5 – 24,99	49	41,9	32,8 – 51,4
	25,0 – 29,99	51	43,6	34,4 – 53,1
	30,0 – 34,99	8	6,8	3,0 – 13,0
	35,0 – 39,99	2	1,7	0,2 – 6,0
	> 40	1	0,9	0,0 – 4,7
Mallampati	1	52	43,0	34,0 – 52,3
	2	49	40,5	31,7 – 49,8
	3	18	14,9	9,1 – 22,5
	4	2	1,7	0,2 – 5,8
LEMON	0	46	38,0	29,3 – 47,3
	1	22	18,2	11,8 – 26,2
	2	18	14,9	9,1 – 22,5
	3	13	10,7	5,8 – 17,7
	4	14	11,6	6,5 – 18,7
	5	3	2,5	0,5 – 7,1
	6	5	4,1	1,4 – 9,4
Unghiul cervico-toracal	În afară interval (130°-165°)	15	12,4	7,1 – 19,5
	În intervalul (130°-165°)	106	87,6	78,4 – 94,8
Localizarea laringelui	C_4	5	4,1	0,9 – 8,2
	C_5	70	57,9	48,5 – 66,8
	C_6	44	36,4	28,6 – 46,4
	C_7	2	1,7	0,2 – 5,8
Cormack - Lehane	1	49	40,5	31,7 – 49,8
	2	61	50,4	41,2 – 59,6
	3	9	7,4	3,5 – 13,7
	4	2	1,7	0,2 – 5,8
Intubaţie dificilă	Da	9	7,4	3,5 – 13,7
	Nu	112	92,6	86,3 – 96,5

* n - număr pacienţi
** - pentru valorile mai mici, de 10 proporţiile sunt orientative
*** CI - interval de confidenţialitate

În incercarea de a găsi valoarea de referință a UCT pentru ID s-a efectuat o corelație între acest unghi şi cele mai folosite criterii de evaluare în predicția ID (clasificarea Mallampati, scorul LEMON, Cormack-Lehane) (fig. 2).

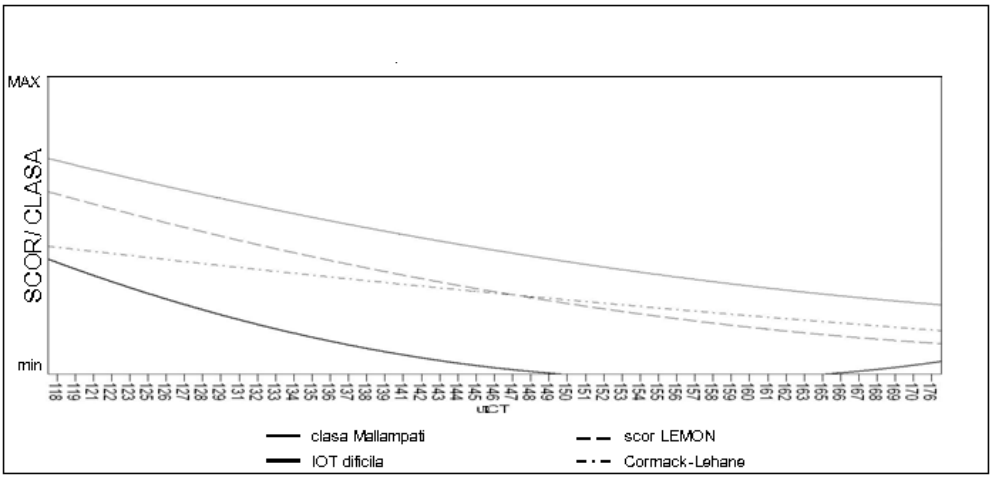


Fig. 2. Corelarea UCT cu cele mai importante scoruri/clasificări folosite în predicția ID.

Coreland UCT cu încadrarea pacienților în clasa Mallampati se observă că cele două variabile sunt în corelație inversă. Cu cât UCT este mai mare, cu atât clasa Mallampati (şi implicit dificultatea intubației) este mai mică. Considerand ca o ID devine posibilă şi probabilă la pacienții care au clasele Mallampati peste 2, vom putea afirma că pacienții cu un UCT mai mic de 140° pot fi candidați pentru o intubație posibil sau probabil dificilă.

De asemenea, a fost corelat UCT cu scorul LEMON. Ca şi în primul caz, corelarea grafică a fost de tip invers. Considerând că o intubație devine probabilă la valori ale scorului LEMON peste 3, putem afirma că pacienții care au un UCT mai mic sau egal cu 128° devin candidați pentru o posibilă sau probabilă ID (fig. 2).

Corelarea UCT cu scorul Cormack-Lehane descoperit în momentul laringoscopiei a fost de asemenea de tip invers, clasa Cormack-Lehane fiind cu atât mai mare cu cât UCT este mai mic (fig. 2).

Considerând că o intubație este posibil/probabil dificilă la un scor Cormack-Lehane peste 2, putem afirma că un pacient care are UCT sub 134° poate fi candidat pentru o intubație dificilă. Corelarea UCT cu dificultatea intubației întâlnită în timpul IOT descrie o curbă cu concavitatea superior şi traseu preponderent descendent, ceea ce evidențiază că pacientii cu valoarea UCT sub 132° pot fi candidați ai unei IOT dificile.

S-a remarcat faptul că, odată ce este depăşită valoarea de 165° a UCT, curba descrie o ascensiune, deci, şi o creştere a dificultății întâlnită în timpul intubației. Astfel, putem presupune că pacienții cu o valoare a UCT sub 130° pot fi încadrați în grupul de risc pentru o eventuală ID.

Datele clinice şi scorurile de evaluare a căilor respiratorii au fost utilizate pentru a obține sensibilitatea, specificitatea, valoarea predictivă pozitivă (VPP) şi negativă (VPN) a testelor (tab. IV).

TABELUL IV

Analiza comparativă a diverşilor factori sau sisteme de evaluare a intubaţiei dificile.

Sistem de evaluare	Se (%)	Sp (%)	VPP (%)	VPN (%)	RR	p
Obezitatea (IMC>30)	66,67	90,18	35,29	97,12	0,36	<0,05
Clasa Mallampati	66,67	85,71	27,27	96,97	0,28	<0,05
Localizarea laringelui (<C_5)	22,22	96,43	33,33	93,91	0,35	<0,05
Distanţa tiro-mentoniera (<6,5m)	66,67	91,96	40,00	97,17	0,41	<0,05
uCT (130-165)	55,56	90,18	31,25	96,19	0,32	<0,05
Clasa LEMON (>4)	66,67	98,21	75,00	97,35	0,77	<0,05
Cormack-Lehane	77,78	96,43	63,64	98,18	0,65	<0,05

Se = sensibilitatea; Sp = specificitatea; VPP = valoare predictivă pozitivă;
VPN = valoare predictivă negativă; RR = risc relativ

Dintre parametrii incluşi în scorul OMLET, DTM mai mică de 6,5 cm şi obezitatea (IMC > 30 kg/m^2) au avut cele mai mari puteri de predicţie a ID.

Scorul OMLET a prezis 6 din cele 9 intubaţii dificile, sensibilitatea testului fiind de 66,67%. Deşi 3 din totalul pacienţilor au avut un scor OMLET < 5, valoare considerată limită pentru IOT dificilă, ei au fost totuşi intubaţi greu (valoare fals-negativă). Din 112 pacienţi intubaţi normal/uşor, scorul OMLET a avut valori corespunzătoare unei IOT dificile (> 5) în 11 cazuri (valoare fals-pozitivă). Astfel, scorul OMLET a avut o specificitate de 95,54%, valoare predictivă pozitivă de 54,55% şi valoare predictivă negativă de 97,27% (tab. V).

TABELUL V

Valorile predictive ale scorului OMLET.

	Se (%)	Sp (%)	VPP (%)	VPN (%)	RR	p
Scor OMLET	66,67	95,54	54,55	97,27	0,56	<0,05

Se = sensibilitatea; Sp = specificitatea; VPP = valoare predictivă pozitivă;
VPN = valoare predictivă negativă; RR = risc relativ

S-a remarcat că valoarea predictivă a scorului OMLET este cel puţin egală cu cea a fiecărei variabile componente, dar riscul relativ (RR) este mai mare decat oricare dintre acestea, acest scor fiind util în practica curentă.

CONCLUZII

În prezent nu există nici un test care să prezică de unul singur probabilitatea unei intubaţii dificile cu o sensibilitate şi o specificitate suficient de mari, de aceea este necesară folosirea unui set complex de teste, diverse algoritme pentru evaluarea preintervenţională cu scopul de a prezice o intubaţie dificilă şi în acelaşi timp medicul urgentist sau anestezist trebuie să fie pregătit şi să stăpânească tehnicile de management a căii respiratorii dificile în cazul apariţiei unei astfel de situaţii neanticipate. Respectarea strategiilor definite şi algoritmilor pot rezolva cele mai multe probleme

în managementul căii respiratorii dificile, permițând intubarea cu succes la majoritatea pacienților cu căi respiratorii dificile. Atunci când gestionarea căilor respiratorii eşuează, s-a inclus un algoritm de gestionare a căilor respiratorii dificile care include dispozitive ce permit intubarea traheală sub control vizual direct, dar şi acestea implică preț de cost ridicat.

Mergând pe principiul că daca anticipăm o ID, aceasta poate să nu mai fie dificilă, fiind mai uşor să previi o anumită situație critică decât să o tratezi, anamneza, examenul clinic şi imagistic al pacientului rămân etape esențiale. În acest sens, prin elaborarea unui scor de predicție a ID folosind şi măsurători imagistice contribuie la identificarea ID la pacienții suspicionați. Comparativ cu factorii predictivi anatomo-clinci, metodele imagistice deşi nu sunt de rutină şi universal aplicabile, utilizarea lor s-a dovedit a fi potrivită pentru predicția de ID. Tehnicile imagistice imagistice – CT, IRM pot face măsuratori cu precizie, pot evidenția patologii asociate ale extremității cefalice şi a regiunii cervicale, dar rămân metode scumpe, disponibile numai în spitalele mari.

Scorul de IOT dificilă propus de noi este suficient de amănunțit, prin datele imagistice oferindu-ne o cantitate mare de informații (unghiuri, localizări, raporturi ale structurilor anatomice, patologii asociate căilor respiratorii superioare şi a zonelor de vecinatate). Acest scor ar putea fi aplicat în special pacientilor cu patologii ale extremității cefalice şi gâtului ce necesită inevitabil investigarea acestor regiuni preintervențional (patologii tumorale, patologii vertebro-medulare).

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NOUTĂȚI

VALOAREA PREDICTIVĂ A *homB* ÎN MANAGEMENTUL BOLILOR GASTROINTESTINALE

În acest studiu au fost investigaţi factorii de virulenţă ai *H. pylori* în special distribuţia genelor *cagA*, *homA* şi *homB* în cazul tulpinilor izolate de la pacienţi cu boli gastrointestinale din Iran. De asemenea, un alt obiectiv a fost stabilirea corelaţiilor dintre stadiul bolii şi fcatorii menţionaţi anterior. A fost extras, amplificat şi secvenţiat ADN-ul genomic de la 138 de tulpini de *H. pylori* izolate de la pacienţi cu tulburări dispeptice (35 pacienţi cu gastrită, 62 pacienţi cu ulcer peptic, 42 pacienţi cu cancer gastric). Prevalenţa pentru *cagA*, *homA* şi *homB* a fost 58%, 54%, şi respectiv 43%. De asemenea s-a remarcat o diferenţă semnificativă a prevalenţei factorilor investigaţi în funcţie de stadiul bolii. Frecvenţa *homB* a fost semnificativ mai crescută în cazul pacienţilor cu cancer gastric (78%) decât în cazul pacienţilor cu ulcer peptic (20%) sau a pacienţilor gastrită (43%). Prezenţa lui *homB* a fost asociată cu prezenţa *cag A*. Aceste date sugerează că evaluarea *homB* poate avea valoare predictivă mai mare pentru prezenţa tulpinilor virulente de *H.pylori* iar prezenţa acesteia influenţează severitatea simptomatologiei clinice (Abadi A.T, Rafiei A, Ajami A, Hosseini A, et all. *Helicobacter pylori homB* but not *cagA* is associated with gastric cancer in Iran. *J ClinMicrobiol*. 2011: 3122-3126).

Ecaterina Enache

PAIN ASSESSMENT EFFECTIVENESS OF PAINKILLERS AT THE PATIENTS ADMITTED IN THE EMERGENCY DEPARTMENT OF THE IASI "SF. SPIRIDON" COUNTY CLINICAL EMERGENCY HOSPITAL

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PAIN ASSESSMENT EFFECTIVENESS OF PAINKILLERS AT THE PATIENTS ADMITTED IN THE DEPARTMENT OF THE IASI "SF. SPIRIDON" COUNTY CLINICAL EMERGENCY HOSPITAL (Abstract): Pain represents a very common symptom and reason for doctor consultation in the patients coming to the emergency rooms. **Aim**: to identify the pathological conditions requiring painkillers in the emergency department and analyze the types of painkillers used in practice and their efficiency. **Material and methods**: patients who received painkillers in the Emergency Room (a total of 121 patients, 47.52% male) with a mean age of 57.57 were included in the study (January-June 2016). To assess pain at the time of admission and after treatment the World Health Organization analgesic scale principles were applied. The data were statistically analyzed by *Microsoft Excel 2010* and *SPSS 20.0* for Windows. **Results**: The most common diagnoses that had pain as the main symptom were physical trauma (30%) in various places, followed by abdominal pain syndrome (15 cases) and renal colic (12.4%). Depending on the diagnosis and experience of the emergency medicine doctor, the patients received analgesics such as paracetamol, fentanyl, and tramadol. Only 10% of the patients who received tramadol as initial treatment needed fentanyl to be added to reduce pain intensity (50-250 mcg). We established a positive correlation of $p< 0.01$ between the patients' degree of pain and their need to receive fentanyl. **Conclusions**: Regardless of etiology, the comparison of pain intensity at emergency department admission and discharge using the numeric pain intensity scale showed lower pain ratings at discharge. **Keywords**: PAIN, ACETAMINOPHEN, OPIOID, EMERGENCY DEPARTMENT.

According to the International Association for Study of Pain (IASP), pain is *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"* (1). Romanian specialists in medical emergencies just like any other emergency room (ER) specialist in the world often face treating patients in pain. That is why the Canadian emergency departments regard the use of painkillers based on studies as a basic skill for the emergency physicians. Thus, protocols were developed based on Grading of Recommendations, Assessment, Development and Evaluation Methodology (GRADE)

seven PICO (Population, Intervention, Comparison, Outcomes) questions were developed, and subsequent scientific research for each question was conducted. Emergency Department analgesia protocols based on GRADE assessment have been suggested *to be used* by emergency physicians as guidelines, being considered as a *"book of analgesia in the emergency department"* (2).

All GRADE methodology has been used to develop an evidence-based approach to analgesia used in pre-hospital trauma. The group of specialists who have set it up recommends that all patients be candidates for analgesia irrespective of the type of transport and opioid medication to be taken in moderate and severe pain (3). Oligoanalgesia would also be caused by changes made by practitioners in the management of pre-hospital pain (4).

In Switzerland, specialists have conducted a study in view of developing and implementing educational interventions and a specific pain management in emergency services and to evaluate their effect on pain management the effect of the implementation of guidelines being evaluated in the same emergency department at admission and during emergency department stay. The study results showed an improvement in the handling and treatment of patients with pain, pain management, analgesia and patient comfort in Emergency Department following education program and guidelines implementation (5, 6).

MATERIAL AND METHODS

The study was conducted on 121 patients who were admitted to the Emergency Department (ED) of the Iaşi "Sf. Spiridon" County Clinical Emergency Hospital, between January-June 2016 and the main presenting symptom being pain of various causes.

The subjects that received one of the studied painkillers (paracetamol, fentanyl and tramadol) were asked to rate the intensity of their pain at admission and at 15, 30, and 60 minutes during treatment on a simple numeric scale (SNS) where 1 = no pain and 10 = high intensity pain (7, 8). The patients received one of the following 3 painkillers: paracetamol, tramadol and fentanyl. Seven work hypotheses were developed and the benefit of treating pain with the three painkillers was statistically assessed.

The data were statistically analyzed using *Microsoft Excel 2010* and *SPSS v.20.0* (9). The benefit of the study painkillers is expressed in a reduction of pain at fixed time intervals following their administration (what percentage of the baseline score is the difference between the baseline score and the final score, or the score measured sometime after administration).

RESULTS

The study group including 121 patients was homogenous. The mean age of the 121 study patients was 57.57 years, and the male patients being the majority (55.37%) with a male/female ratio of 1.2/1. Most patients came from the rural area (60.33%), the rural/urban ratio being 1.5/1.

Hypothesis 1a. Does age influence the benefit of painkillers in the treatment of pain? The benefit of painkillers following pain treatment was analyzed in two age groups: under and over 50 years old (tab. I). The benefit shows a tendency to decrease with age, with the highest benefit recorded in the young patients.

Hypothesis 1b. Does gender influence the benefit of painkillers in the treatment of pain?

The average benefit was around 28%

with insignificant male/female differences.

Hypothesis 1c. Does urban/rural residence influence the benefit of painkillers in the treatment of pain? No significant differences between rural and urban patients were found.

Hypothesis 2a. Do higher than normal ASAT and ALAT levels influence the benefit of painkillers in the treatment of pain? The benefit rate was lower (19.58%) in patients with ALAT and ASAT levels higher than 30 IU (tab. II).

Hypothesis 2b. Do higher than normal ALAT and ASAT levels influence the gender- related benefit of painkillers (tab. III).

TABLE I

Age-related benefit of painkillers at the end of pain treatment

Age group	Cases No. / %	Total score		Benefit (%)	Benefit / case (%)
		before treatment	after treatment		
Under 50	36.74%	162	116	28.40	0.68
Over 50	63.16%	284	204	28.17	0.39
Total	100.00	446	320	28.25	0.25

TABLE II

The benefit of painkillers according to ALAT and ASAT levels

Cases / Score	ASAT and ALAT	
	> 30 IU	< 30 IU
No. of cases	36	58
Total score before treatment	143	224
Total score after treatment	115	146
Treatment benefit (%)	19.58	34.82

TABLE III

The benefit rate of painkillers in women and men with ALAT and ASAT levels higher than 30 IU

Patient gender	No. of cases	Total score		Benefit (%)	Benefit %/case
		before treatment	after treatment		
Women	16	63	50	20.63	1.29
Men	22	87	68	21.84	0.99
Total	38	150	118	21.33	0.56

Hypothesis 3. Does the diagnosis responsible for pain influence the benefit rate of the treatment with painkillers? The main diagnosis was multiple contusions – 31% of cases, followed by renal colic and abdominal pain syndrome - 13% (fig. 1). The highest benefit/case (2.7%) was recorded in acute limb ischemia, followed by renal colic and neuralgia, of approximately 2.5%. The average benefit/case was of 0.25% (tab. IV).

TABLE IV
Benefit according to diagnosis

Diagnosis	% of cases	Total score		Benefit (%)	Benefit case (%)
		before treatment	after treatment		
Multiple contusions	31%	146	104	28.77	0.78
Abdominal pain syndrome	13%	59	45	23.73	1.58
Renal colic	13%	58	36	37.93	2.53
Acute limb ischemia	12%	53	33	37.74	2.70
Neuralgia	11%	52	35	32.69	2.51
Isolated fractures and dislocations	20%	78	67	14.10	0.71
Total	100.00	446	320	28.25	0.25

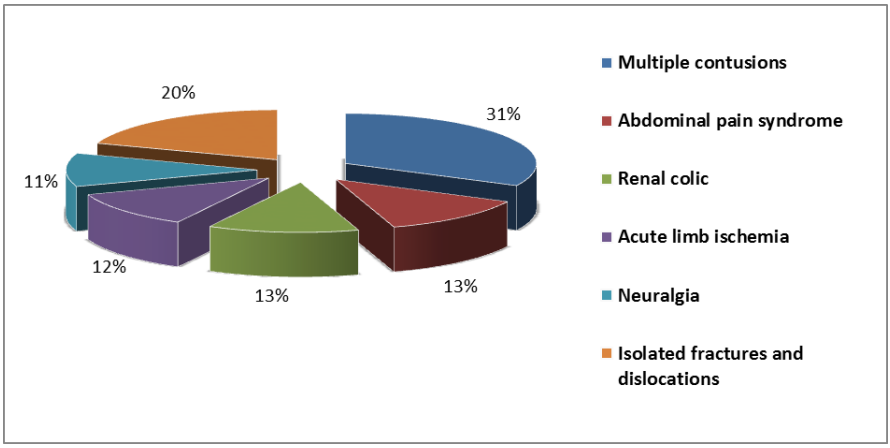

Fig. 1. The percentage of the diagnoses causing pain

Hypothesis 4. Does the heart rate influence the self-assessment of pain? The pain score obtained by the patients on the SNS was not influenced by their heart rate.

Hypothesis 5. Do the cases with concomitant conditions/diseases have a higher benefit rate in the case of pain treatment with painkillers? Half of the studied cases had concomitant conditions. Following pain treatment, the patients received approximately the same benefit rate as those who did not have concomitant conditions.

Hypothesis 6. Are the benefits of painkillers higher in the treatment of pain in patients with associated diseases? Adverse reactions were present in 7.44% of patients and the treatment benefit rates were of about 29% in the patients with or without adverse reactions. The most common adverse reactions were nausea and vomiting.

Hypothesis 7a. How does the concomitant medication use influence the benefit rate of pain treatment with painkillers? The benefit rate of treating pain with painkillers is higher in patients without concomitant medications the difference being of almost 5%.

Hypothesis 7b. Was pain treatment benefit rate influenced by the concomitant medication for preventing the adverse reactions to painkillers? (tab. V).

TABLE V
The influence of concomitant medication against adverse reactions

Medication	No. of cases	Total of pain score		Benefit (%)
		before treatment	after treatment	
Total patients	100	392	283	27.81
With concomitant medication for preventing the adverse reactions to painkillers	62	247	200	19.03
Without concomitant medication for preventing the adverse reactions to painkillers	38	145	83	42.76

DISCUSSION

All patients admitted in the emergency receiving unit are monitored hemodynamically and respiratory by recording the arterial pressure, heart rate and oxygen saturation. Analyzing these values, an increased incidence of those with high heart rate has been noticed (pulse > 100 / min) possibly because the pain causes a physiological stress response that includes the increase of the ventricular rate, respiratory frequency, which implies a more pronounced demand for oxygen and nutrition in the vital organs (10).

Knowing that both paracetamol and opioids are metabolized to the greatest extent in the liver cell, prior to their dosing, liver cytolysis enzymes (ALAT and ASAT aminotransferases) were harvested, but the intensity of pain when the patient was admitted in the emergency care did not allow us to wait for the results of these analyses to initiate appropriate treatment. The mean of ASAT and ALAT values was 30 IU; over 20% of those questioned having elevated transaminases, values that helped directing the subsequent antalgic treatment according to the recommendations of gastroenterology specialists.

A study performed in United States of America, in May 2010, showed that it is recommended to avoid using opioids in patients with impaired liver function because they favor portal systemic encephalopathy as fentanyl and tramadol are mainly metabolized by the liver (11). However, Chadock *et al.* (12) suggest the short-term use of opioids as a solution in such patients with acute pain associated with an underlying disease (other than liver etiology). Being familiar with these recommendations, in our study we administered only fentanyl in patients with liver cirrhosis considering as beneficial both the intravenous hydration and taking antiemetics.

All those diagnosed with acute myocardial ischemia, acute myocardial infarction, ulceration and multiple localization had maximum pain intensity (SNS = 9-10) of all the patients under study and inquired on arrival at our unit. 46.5% of those diagnosed with renal colic, and 50% of the subjects with dislocations and fractures isolated in a member segment had agonizing pain with a score at the top of the scale (SNS = 9-10). In our study, we used paracetamol as a first-line drug for those with moderate and weak pain. If some of those included in the study after treatment with acetaminophen scored the pain significantly lower, others had to add an opioid analgesic (tramadol or fentanyl). Combining the data obtained with the literature shows that acetaminophen has proved its maximum efficacy in moderate and weak pains. But a group of intensive care physicians in Istanbul have concluded that the analgesic

properties of paracetamol are equivalent to fentanyl in suction curettage procedures, concluding that more extensive studies are needed (13). Only 33% of the people with high intensity pain (SNS = 9-10) received tramadol as first-line drug after the medical check-up. Those with unbearable pain (renal colic, neuralgia, multiple fractures) did not benefit from the same treatment. We wonder why not all patients with unbearable pain have received tramadol to relieve pain on primacy? Opioids also have strong side effects (nausea, vomiting, dizziness, orthostatic hypotension) in addition to the powerful analgesic effect, which has made it possible to limit the use of tramadol in some of them. In some patients who received tramadol and paracetamol as an analgesic initiator, it was necessary to add another opioid more than 600 time stronger than morphine, fentanyl. The Pearson correlation was used to evaluate the efficacy of fentanyl treatment. Between the degree of pain of the patients and the need to receive fentanyl, the result obtained showed a strong statistically significant correlation between the most sensitive of 0.01 of the pain scale and the administration of fentanyl. If upon arrival in the emergency department 48% of those interviewed had a score (SNS = 9-10) and 47% respectively had a score (SNS = 7-8), only 9% left our department with a maximum score (SNS = 9). This score may not even reflect reality, with some patients adding an anxiety component in expressing pain (14).

A study performed in France, in 2010 showed that anxiety occurs in trauma patients as a pejorative factor of analgesia, the more anxious patients expressing a higher pain score and independent from other studied factors. This correlation has already been demonstrated in pediatrics, in patients with burns, dental pain or back pain (15).

The benefit of analgesic pain treatment can be noticed and summarizing the statistical data from this study can answer with yes or no to certain assumptions. The patients' age had a slight influence showing that the benefit of the established treatment was superior to those under 50. Only those with renal colic, neuralgia and painful abdominal syndrome benefitted the most of all the diagnoses stated whose main symptom was pain. Even if some subjects had associated comorbidities, there were no differences in the benefit of the treatment used compared to those without comorbidities.

As it is known from medical literature, some opioid drugs also have side effects, but our goal of treating pain has been reached without being influenced by side effects in some subjects.

There is a statistically insignificant difference in patients who have not received concomitantly other medication for the treatment of other associated comorbidities (27.81%) than those who received at least one drug for associated pathologies (24.07%), but this does not prevent us from treating both pain and prior pathologies specific to each patient. Even though our study shows that some patients who received treatment to avoid or treat side effects the benefits to analgesic treatment were lower compared to those who did not receive any medication. In the future, we should not neglect these reactions, which create additional discomfort to the recipient.

CONCLUSIONS

Our studies highlighted that the younger the patients, the higher the benefit rate of pain treatment with painkillers. ASAT and ALAT < 30 IU favor the outcomes of pain treatment. With the acute limb ischemia,

renal colic and neuralgia as pain causes, the benefit rate of pain treatment with painkillers is higher than with other studied causes. Lack of treatment for concomitant conditions does not involve achieving better benefit rates for the treatment of pain with painkillers. Patients without concomitant medication against adverse reactions had improved pain scores after treatment with painkillers. In only 10% of the patients who received tramadol and fentanyl as first-line treatment the pain, intensity scores forced us to use fentanyl dose titration, which proves that the opioids used in the study had a strong analgesic effect according to data in the medical literature. Over 60% of the study subjects required the association of a second analgesic in this case fentanyl or tramadol. Determining the effectiveness of pain management requires other hypotheses, better monitoring during pain treatment, pain assessment at some intervals after treatment, the use of different doses and in different combinations.

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EFECTE ARITMOGENE ALE ETANOLULUI

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ARRHYTHMOGENIC EFFECTS OF ALCOHOL (Abstract): Acute or chronic excessive consumption of alcohol can cause cardiac arrhythmias, particularly supraventricular tachyarrhythmias. Experimental and clinical data show that chronic excessive alcohol ingestion has a destructive role on cardiac tissue, which significantly deteriorates the rhythm and contractile activity, manifested by the appearance of cardiac arrythmias and dilated cardiomyopathy. **Aim:** The aim of this study was to evaluate and analyze the ECG changes - disturbances of cardiac rhythm and conduction in patients after an acute or chronic excessive consumption of alcohol. **Material and methods:** The study included a number of 187 patients (184 males and 3 females). Each patient followed biochemical and hematological analysis as well as paraclinical investigation measurements. **Results:** The most common types of arrhythmia were permanent atrial fibrillation (61%). Paroxysmal atrial fibrillation and supraventricular extrasystoles were found in patients without significant echocardiographic deteriorations. These arrhythmias appeared after an alcohol abuse and paroxysmal atrial fibrillation was converted to normal sinus rhythm after a short period of abstinence or pharmacologic therapy administered. Ventricular arrhythmias (ventricular extrasystoles, non-sustained ventricular tachycardia-4.7%, torsada de point-1.5%) were less frequent. Conduction disturbance (left bundle branch-LBBB 6.3%, right bundle branch-RBBB 10%) were found in patients who had a history of consuming >150 g/day of alcohol for >10 years, with dilated heart chambers and systolic ventricular function much more deteriorated (symptomatic stage of heart failure). **Conclusion:** Excessive alcohol consumption may lead to cardiac arrhythmias in patients with existing heart disease as well as in healthy individuals. **Key words:** ARRHYTHMIA, CONSUMPTION, ETHANOL.

Consumul exagerat acut sau cronic de alcool poate determina tulburări de ritm şi conducere atât la nivel atrial cât şi ventricular.

Efectul aritmogen al etanolului până în prezent nu este pe deplin elucidat, dar se presupune că există mai mulţi factori care contribuie la geneza aritmiilor cardiace condiţionate de etanol. Ettinger şi colab. în 1978 descriu "*Sindromul cardiac de sfârşit de săptămână"* (*Holiday heart syndrom*) constând în aritmii cardiace ce apar după un consum abuziv de alcool la sfârşit de săptămană (după week-end) incluzând atât aritmii supraventriculare cât şi ventriculare.

Se consideră că perturbările cardiovas-

culare întâlnite la consumatorii cronici de alcool şi în special tulburările de ritm şi conducere reprezintă o cauză din ce în ce mai importantă de morbiditate şi mortalitate.

Scopul acestui studiu a constat în evidenţierea şi analizarea modificărilor electrocardiografice - tulburările de ritm şi conducere la pacienţii internaţi după un consum exagerat acut sau cronic de etanol (1,2,3,4).

MATERIAL ŞI METODĂ

Studiul a fost efectuat pe un lot de 187 de pacienţi internaţi în Clinica I Medicală a Spitalului "Sfântul Spiridon" Iaşi în perioda 1996-2002.

În studiu au fost incluşi pacienţii diagnosticaţi cu cardiomiopatie alcoolică ce au solicitat internarea în mod repetat, precum şi cei care au fost internaţi pentru prima dată şi au confirmat prin anamneză că sunt consumatori de etanol. Au fost excluşi din studiu pacienţii care nu au recunoscut consumul de etanol în ciuda faptului că examenul obiectiv şi investigaţiile paraclinice au fost pozitive pentru cardiomiopatie alcoolică. Vârsta medie a pacienţilor cuprinşi în studiu a fost de 52 ± 8,5 ani, variind între 25 şi 79 ani, iar sexul masculin a predominat (184 bărbaţi şi 3 femei).

La fiecare pacient au fost efectuate o serie de teste biochimice şi hematologice precum şi ECG, radiografie toracică, ecocardiografie transtoracică, iar la unii pacienţi s-a făcut şi monitorizare Holter ECG 24 de ore. Pentru urmărirea modificărilor electrocardiografice respectiv tulburările de ritm şi conducere, electrocardiograma s-a înregistrat repetat, zilnic sau de mai multe ori pe zi.

Cu ajutorul ecocardiografiei au fost determinate diametrul cavităţilor cardiace atriale şi ventriculare, cinetica globală a ventriculului stâng, fracţia de scurtare şi fracţia de ejecţie a ventriculului stâng, grosimea pereţilor ventriculari, regurgitările valvulare semnificative hemodinamic.

REZULTATE

Studiul relevă predominanţa sexului masculin - 184 cazuri (98%), dar această diferenţă semnificativă se explică atât prin factorii socio-culturali, tradiţionali, cât şi prin reticenţa femeilor în a declara consumul de etanol. În ceea ce priveşte mediul de proveniență 64% din pacienţi (120) proveneau din mediul rural şi fară activitate profesională, iar restul de 36% (67 pacienţi) din mediul urban şi cu activitate profesională (tab. I).

Aritmiile cardiace întâlnite la lotul de bolnavi au fost destul de numeroase, atât cele supraventriculare cât şi ventriculare. Dintre aritmiile prezente la pacienţii investigaţi, fibrilaţia atrială a fost cea mai frecventă, atât sub formă paroxistică, persistentă şi mai ales permanentă (109 pacienţi - 61%), urmată de extrasistole atriale multifocale la 105 de cazuri (56%). Fibrilaţia atrială paroxistică şi persistentă au apărut în relaţie cu consumul unor cantităţi exagerate de etanol (tab. II).

Datele ecocardiografice obţinute la acest grup de pacienţi au relevat diametre crescute ale cavităţilor cardiace, predominant stângi, scădere marcată a fracţiei de ejecţie şi scurtare (30 ± 2,7%), insuficienţă mitrală semnificativă hemodinamic (67% din cazuri) (tab. III).

Markerii consumlui de etanol reprezentaţi de GGT şi VEM au avut valori medii de 83 ± 4,8 U/l respectiv 96 ± 2,5 γ^3. Nu au existat tulburări electrolitice asociate în procent semnificativ. Fibrilaţia atrială permanentă a fost prezentă la aproape toţi

pacienţii din studiu care au declarat consum peste 100 g alcool/zi, cu durată de > 5 ani. Tulburările de ritm şi conducere la nivel vetricular şi joncţional au fost prezente la un procent mai mic: extrasistole ventriculare monomorfe la 94 pacienţi (50%), tahicardii ventriculare nesusţinute la 9 pacienţi (4%), torsada vârfurilor la 3 pacienţi (1,5%), iar tulburările de conducere interventriculare sub formă de BRD la 19 pacienţi (10%), BRS la 11 pacienţi (6%) şi HBAS la 15 pacienţi (8%).

TABELUL I

Date demografice ale lotului studiat

Numărul pacienţilor		**187**	**%**
Sex	bărbaţi	184	98%
	femei	3	2%
Vârsta	25-79 ani		
Mediul de proveniență	urban	67	36%
	rural	120	64%
Profesia	cu ocupaţie	64	34%
	fără ocupaţie	123	66%
Cantitatea de alcool consumată pe zi	60-100g/zi	9	5%
	100-150g/zi	114	61%
	>150g/zi	64	34%
Durata consumului de alcool/ani	2-5 ani	9	5%
	5-8 ani	61	33%
	> 8 ani	117	62%
Număr de internări	1 internare	71	38%
	2 internări	69	36,9%
	>3 internări	47	25,1%

TABELUL II

Relaţia între tipul aritmiei cardiace şi durata consumului de etanol/ani la pacienţi cu consum >100 g/zi

Tipul aritmiei cardiace	**Durata consumului de etanol > 100 g/zi**			
	> 5 ani	**5-10 ani**	**> 10 ani**	Numărul pacienţilor
Extrasistole atriale multifocale	70 (37%)	9 (4,8%)		
Fibrilaţie atrială - paroxistică →	20 (10%)			
Fibrilaţie atrială - persistentă →	10 (5,3%)	9 (4,8%)		
Fibrilaţie atrială - permanentă →		27 (14%)	43 (23%)	
Extrasistole ventriculare	42 (22,5%)	36 (19%)	23 (12%)	
Tahicardie ventriculară nesusţinută	1 (0,5%)	3 (1,5 %)	5 (2,6%)	
Torsada vârfurilor	3 (1,3%)			
BRD		3 (1,5 %)	16 (8,5%)	
BRS		4 (2,1%)	7 (3,7%)	

TABELUL III

Corelaţia dintre aritmile cardiace şi diametrul telediastolic al ventriculului stâng determinat prin ecocardiografie transtoracică

Tipul aritmiei cardiace	Diametrul telediastolic al ventriculului stăng măsurată prin ecocardiografie transtoracică			
	55- 65 mm	**65-75mm**	**>75mm**	
Extrasistole atriale multifocale	63 (33,6%)	45 (24%)		Numărul pacienţilor
Fibrilaţie atrială - paroxistică →	20 (10%)			
Fibrilaţie atrială - persistentă →	10 (5,3%)	11 (7,4%)		
Fibrilaţie atrială - permanentă →	3 (1,5%)	27 (17 %)	38 (20%)	
Extrasistole ventriculare	32 (17 %)			
Tahicardie ventriculară nesusţinută		3 (1,5 %)	6 (3,2%)	
Torsada vârfurilor	3 (1,5 %)			
BRD		7 (3,7%)	12 (6,4%)	
BRS		3 (1,5 %)	9 (4,8%)	
HBAS	1 (0,5%)	5 (2,6%)	9 (4,8%)	

DISCUŢII

Din datele experimentale şi clinice rezultă că etanolul are rol distructiv asupra ţesuturilor cardiace, ceea ce perturbă semnificativ atât activitatea contractilă cât şi ritmicitatea, manifestate prin apariţia cardimiopatiei dilatative şi aritmiilor cardiace (3,4,5,6).

Koskinen şi colab., în 1987, atestă rolul consumului de alcool în geneza unor tulburări de ritm la nivel atrial la un grup de bolnavi cu vârsta între 35-65 de ani, şi debut al fibrilaţiei atriale în urma unui consum excesiv de alcool. În lotul nostru de studiu am observat că toţi pacienţii au prezentat unul sau mai multe tipuri de aritmii cardiace, fie supraventriculare, fie ventriculare, sub formă de tulburări de ritm sau conducere (6,7).

Fibrilaţia atrială a fost aritmia cea mai frecventă la cazurile investigate, instalată de cele mai multe ori după un consum exagerat de etanol, favorizând agravarea insuficienţei cardiace. Fibrilaţia atrială paroxistică care a fost redusă în ritm sinusal după oprirea alcoolului şi aplicarea terapiei medicamentoase a fost prezentă la pacienţii care au consumat alcool pe o periodă de timp < 5 ani, şi cu parametri ecocardiografici mai puţin alteraţi comparativ cu forma permanentă care a fost întâlnită la marea majoritate a pacienţilor care au consumat cantităţi mari şi timp îndelungat (> 150 g/zi şi > 10 ani). La acest ultim sublot datele ecocardiografice au relevat parametri mult alteraţi: cavităţile cardiace mult dilatate, funcţia contractilă globală mult redusă. Extrasistolele atriale multifocale au fost prezente mai ales la cei care au fost convertiţi în ritm sinusal.

Frecvenţa aritmiilor ventriculare în acest lot a fost mai mică, dar se constată o corelaţie directă între consumul de etanol şi apariţia lor. Trebuie menţionat faptul că instalarea unor aritmii ventriculare: extrasistole ventriculare polimorfe, tahicardia ventriculară nesusţinută, şi nu în ultimul rând torsada vârfurilor, întâlnită în lotul nostru au o semnificaţie severă şi potenţial letală. În ceea ce priveşte tulburările de conducere, acestea au fost întâlnite la cazurile care au consumat cantităţi exagerate de

alcool > 150 g/zi o durată lungă de timp (peste 10 ani).

CONCLUZII

Consumul de alcool poate produce instabilitate electrică a inimii şi se manifestă clinic prin palpitaţii (având drept suport aritmiile) şi agravarea insuficienţei cardiace.

Modificările electrocardiografice întâlnite la pacienţii cuprinşi în studiu au fost destul de variabile sub formă de tulburări de ritm şi de conducere, atât la nivel atrial cât şi ventricular, reversibile la sevraj şi terapie corespunzătoare.

Fibrilaţia atrială şi tulburările de conducere apar îndeosebi la cazurile la care durata consumului de alcool este prelungită şi în cantităti peste 150 g/zi.

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CORELAŢII ÎNTRE INDICII ANTROPOMETRICI ŞI FACTORII DE RISC CARDIOVASCULAR LEGAŢI DE STILUL DE VIAŢĂ ÎNTR-O POPULAŢIE RURALĂ (Nota I)

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CORELATIONS BETWEEN ANTHROPOMETRIC PARAMETERS AND CARDIO-VASCULAR RISK FACTORS RELATED TO LIFESTYLE IN A RURAL POPULATION. (Abstract) **Background and aims.** Prevention and reduction of cardio-vascular mortality are in relationship with understanding and being able to modify risk factors, among which overweight and obesity become the most significant. The aim of this paper is to define cardio-metabolic risk factors which are specific to the studied population, and also to find elements of lifestyle that lead to overweight. We targeted smoking, alcohol consumption, stress, physical exercise, income and educational level. **Material and methods.** We studied a sample of 172 subjects, representative for the population of Deleni, a rural community in Iaşi County; among these subjects, 67 were men, 105 were women, with an average age of 54. For each a chart was filled, containing demographic data, socio-economic status, personal and family medical history. We measured anthropometric parameters: weight (W), height (H), waist circumference (WC) and we calculated body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR). We performed interviews to fill in a food frequency questionnaire, the Perceived Stress Scale and the International Physical Activity Questionnaire. **Results.** In the lot studied, 44.2% are overweight and 18.6% are obese, the majority of these being women. Regarding WC, among normal weight, 52.4% have large WC (≥94cm for men and ≥80cm for women). Obesity markers positively correlate with increase in age and decrease in income (p=0.024), with low educational level, regular consumption of coffee (p=0.093), and negatively correlate with excessive alcohol consumption (p=0.049) and smoking (p=0.003). As expected, there is an inverse relationship between increased physical exercise and BMI (p=0.015) and a direct relationship between increased stress level and WC. **Conclusions.** Anthropometric parameters are sensible markers in evaluating the influence of lifestyle on cardio-metabolic risk. **Key words:** CARDIOVASCULAR RISK, LIFESTYLE RISK FACTORS, ANTHROPOMETRIC PARAMETERS

Stilul de viaţă modern are o mare influenţă asupra energiei utilizate *vs* energia stocată, printr-o evidentă disponibilitate de alimente cu densitate calorică crescută în contrast cu o viaţă tot mai sedentară. Supraponderea şi obezitatea sunt factori de risc majori pentru mortalitatea şi morbiditatea prin boli cardiovasculare, diabet zaharat de tip 2, cancere şi altele care duc la aproximativ 3 milioane de morţi in fiecare an (1).

Indicii antropometrici cum ar fi indicele de masa corporală (IMC) sau circumferinţa taliei (CA), raportul talie/şold (T/S) şi raportul talie/înălţime (T/I) sunt evaluaţi ca predictori ai prezenţei riscului cardiovascular individual sau într-o comunitate, rămânând controverse asupra celui mai bun indicator. În studii populationale, IMC este recomandat de Organizaţia Mondială a Sănătăţii (OMS) pentru a stabili severitatea bolii (2). În 2005, International Diabetes Federation (IDF) propune CA pentru definirea sindromului metabolic (3). IMC este folosit ca indicator al grăsimii totale, în timp ce CA, T/S şi T/I sunt consideraţi markeri surogat ai obezităţii centrale. Numeroase studii vor să ierarhizeze indicii de apreciere ai supraponderii şi obezităţii în relaţie cu creşterea riscului cardiovascular (4). Majoritate a factorilor de risc sunt modificabili prin schimbări ale stilului de viaţă. Nutriţia este principalul factor controlabil în prevenţia şi reducerea obezităţii, diabetului zaharat de tip 2 şi a sindromului metabolic. Evaluarea ei este însă foarte dificilă. Mesele conţin o varietate de alimente cu combinaţii complexe de nutrienţi care sunt interactivi sau sinergici (5). Chestionarele de frecvenţă alimentară sunt un mijloc de apreciere a dietelor uzuale în studii epidemiologice largi, de a clasifica populaţia studiată după consumurile alimentare pe parcursul anului şi de a genera recomandări pentru sănatate (6). Alături de dieta aterogenă şi sedentarism la creşterea riscului cardiovascular contribuie şi adicţia la fumat şi alcool şi stresul (7). Prima aproximare a predicţiei riscului pe evaluările din cabinet sunt esenţiale pentru a iniţia procesul de selecţie a pacienţilor vulnerabili pentru viitoare intervenţii sau teste adiţionale.

MATERIAL ŞI METODE

Suntem în desfăşurarea unui studiu transversal, pe un lot reprezentativ din populaţia comunei Deleni-Iaşi. Scopul studiului este de a defini factorii de risc cardiometabolic specifici acestei populaţii şi cauzele legate de stilul de viaţă care duc la creşterea obezităţii: alimentaţie, fumat, consumul de alcool, stres, efort fizic, starea materială şi nivel de educaţie. Avem un lot de 172 de participanţi, dintre care 67 bărbaţi (39%) şi 105 femei (61%), cu vârste între 21 şi 91 ani, media fiind de 54 ani (53,1 ani la bărbaţi şi 54,7 ani la femei). Toţi participanţii au semnat consimţământul informat înainte de intrarea în studiu. Pentru fiecare persoană s-a completat o fişă cu date demografice şi socio-economice: nivel de educaţie, ocupaţie, venituri, status marital, obiceiul de a fuma, boli antecedente sau prezente, istoric de boli familiale. S-au măsurat parametrii antropometrici: greutatea, înălţimea, circumferinţa abdominală, circumferinţa şoldului şi s-au calculat indicele de masă corporală (IMC), raportul talie/şold (T/S) şi raportul talie/înălţime (T/I). Înălţimea şi greutatea au fost obţinute cu echipamente şi tehnici standardizate. Măsurarea circumferinţei abdominale s-a realizat cu subiectul dezbrăcat, în poziţie verticală, la jumătatea distanţei dintre rebordul costal şi creasta iliacă, la sfârşitul expirului normal, utilizând o bandă gradată, neextensibilă. Indicele T/I se calculează împărţind circumferinţa abdominală la înălţime, considerându-se ca normal orice valoare <0,5 indiferent de sex. IMC se calculează împărţind greutatea la pătratul înălţimii: IMC = $G(kg)/I^2(m^2)$**.** S-a stabilit diagnosticul de supraponderе sau obezitate conform criteriilor OMS: IMC = 25-29,9kg/m^2 – supraponderе, IMC≥30kg/m^2 – obezitate. Obezitatea abdominală a fost diagnosticată la valori ale CA≥94 cm la bărbaţi şi ≥80 cm la femei şi/sau raport T/I ≥0,5.

S-au completat, faţă în faţă cu un intervievator antrenat, chestionare de frecvenţă alimentară, de evaluare a stresului perceput, de activitate fizică. După obiceiul de a fuma s-au structurat 3 grupe: nefumători, foşti fumători (abstinenţă >6 luni) şi fumători.

Pentru evaluarea nivelului de stres s-a folosit *Perceived Stress Scale* (Scala de Stres Perceput, *PSS*), cel mai utilizat instrument psihologic internaţional de măsurare a percepţiei stresului, tradus în limba română (8). Chestionarul cuprinde 10 întrebări (itemi) cu 5 variante de răspuns şi anume: 0 = Niciodată; 1 = Aproape niciodată; 2 = Uneori; 3 = Destul de des; 5 = Foarte des. Scorul la PSS se obţine prin răsturnarea punctajului (adică 0 = 4, 1 = 3, 2 = 2, 3 = 1 şi 4 = 0) la cei patru itemi consideraţi pozitivi (itemii 4, 5, 7 şi 8) şi apoi făcând suma tuturor itemilor. Interpretarea scorului se face în funcţie de sex: la bărbaţi - grad înalt de stres (GIS)=18-24 puncte, iar grad patologic de stres (GPS) >24; la femei – GIS=20,4-26,4 şi GPS >26,4.

Evaluarea activităţii fizice s-a făcut cu Chestionarul Internaţional de Activitate Fizică (*International Physical Activity Questionnaire, IPAQ*) (9) tradus în limba română, care are întrebări legate de intensitatea şi durata activităţii fizice la locul de muncă, în timpul transportului, în activitatea casnică şi pentru recreere şi sport. Nivelul de activitate fizică poate fi scăzut, mediu sau crescut.

Pentru evaluarea nutriţională s-a folosit un chestionar semicantitativ de frecvenţă alimentară adaptat (10) şi validat prin cercetarea anterioară în teritoriu (11). Se analizează alimentele “uzuale” care acoperă diferite aspecte ale vieţii pe parcursul ultimului an. Au fost incluse alimentele care contribuie la variaţia interindividuală a consumurilor, cum ar fi alimentele bogate în grăsimi sau în fibre. Chestionarul are 12 variante de răspuns pentru fiecare aliment sau grup alimentar chestionat (de la "aproape niciodată" la ">6 porţii/zi") şi întrebări despre modul de preparare a alimentelor. Anterior completării chestionarului se discută unităţile de măsură folosite, se estimează porţiile prin descriere şi raportare la elemente culinare cunoscute (lingură, linguriţă, cească, pahar etc). În chestionar se completează ca unitate o porţie standard. S-a chestionat consumul de cafea considerându-se ceaşca standard de 215 ml. S-au pus întrebări despre consumul de alcool. Fiecare tip de alcool este chestionat separat. Se consideră că un consum de < 2 porţii/zi la femei şi < 3 porţii/zi la bărbat este acceptat (1 porţie alcool=50ml tărie=150ml vin=350ml bere).

S-au folosit în analiza statistică SPSS (*Statistical Package for Social Sciences*) versiunea 19.0 pentru Windows în cazul analizei statistice aplicate, respectiv *Microsoft Office Excel* pentru crearea bazei de date (program compatibil cu SPSS în ceea ce priveşte importul datelor).Variabilele continui au fost exprimate ca medie ± DS, iar variabilele discrete ca număr şi proporţii. Determinarea intervalelor de variaţie a indicatorilor statistici şi testarea ipotezelor statistice s-a realizat cu interval de încredere de 95%, rezultând un prag de semnificaţie p = 5% = 0,05. Compararea grupurilor s-a făcut cu testul *t-Student* pentru variabilele continui şi cu testul Pearson Chi-Square (χ^2) pentru datele categoriale. Pentru χ^2 pragul de semnificaţie este p=1, adica p_{calc}<1.

REZULTATE

Structura lotului. În funcţie de IMC, 37,2% sunt normoponderali, o proporţie

mai mare a acestora fiind bărbaţi (50,8% bărbaţi); 44,2% sunt supraponderali, majoritatea femei (65,8% femei); 18,6% sunt obezi, marea majoritate a acestora fiind femei (71,9% femei). În ceea ce priveşte CA, 52,4% dintre normoponderali au CA groasă, marea majoritate a acestora fiind femei (78,8%). În ceea ce priveşte supraponderalii, numai 10,5% dintre aceştia au CA normală (dintre aceştia 75% fiind bărbaţi), restul de 89,5% fiind cu CA groasă (70,6% dintre aceştia fiind femei). În privinţa veniturilor medii/membru de familie/lună, participanţii au declarat: venituri <100 RON/lună în proporţie de 20,3%; 54,1% au declarat venituri între 100-400 RON/lună, 19,8% între 400-800 RON/lună, iar 5,8% au declarat venituri >800 RON/lună. Nivelul de studii al populaţiei : maximum 10 clase au 83,7 %, restul de 16,3% având mai mult de 10 clase cu extreme de la fără nici o clasă (3,5%) la studii universitare (1,2%).

Relaţia dintre statusul socio-economic şi indicii antropometrici:

Venit. S-a constatat diferenţă semnificativ statistică între venitul mediu lunar/membru familie şi IMC, în sensul că cei cu veniturile cele mai mici (<100 RON/lună) au IMC mai mic decât cei cu venituri mai mari (400-800 RON/lună), chi-square=0.024. In sublotul celor cu venit < 100 RON/lună, 60 % sunt normoponderali si doar 11,4% obezi, pe cînd în sublotul persoanelor cu venit > 400 RON/lună doar 23,5 % sunt normoponderali , 52,9% sunt supraponderali şi 23.5% sunt obezi

Ocupaţie. S-a constatat statistic semnificativ că pensionarii au IMC-ul cel mai mare, iar salariaţii IMC-ul cel mai mic (chi-square = 0,004). Totuşi, această constatare este greu de interpretat, odată ce creşterea ponderală este proporţională cu vârsta (11).

Studii. Există un trend către IMC mai mare în sublotul celor cu maximum 10 clase. Astfel, între normoponderali, 76,6% au max.10 clase, în sublotul supraponderalilor 86,8% au maximum 10 clase, iar în cel al obezilor 90,6% au maximum 10 clase.

Studiul factorilor de risc cardiovascular legaţi de stilul de viaţă:

Fumatul. În lotul studiat, 16,9% dintre subiecţi sunt fumători, 7% sunt foşti fumători, iar restul de 76,2% sunt nefumători. Am analizat doar grupele fumători şi nefumători, fără cel al foştilor fumători, care este prea mic. Putem afirma că IMC-ul la fumători este semnificativ mai mic decât IMC-ul la nefumători (p=0.049). Între normoponderali 26,6% sunt fumători, între supraponderali 11,8% sunt fumători, iar dintre obezi doar 9,4% sunt fumători. S-a obţinut semnificaţie statistică şi când s-a comparat CA a fumătorilor, cu a nefumătorilor (chi-square=0,003), fumătorii având CA mai mică decât nefumătorii. Între cei cu CA normală, 33,3% sunt fumători şi 56,4% sunt nefumători, iar între cei cu CA groasă: 12% sunt fumători, 82% fiind nefumători.

Consumul de alcool. În lotul studiat, 10,5% dintre subiecţi au declarat consum excesiv de alcool, restul de 89,5% neîncadrându-se în aceste criterii. IMC-ul celor care consumă excesiv alcool este statistic semnificativ mai mic decât cel al celor care nu consumă alcool excesiv (25,13kg/m^2 *vs.* 26,95kg/m^2, p=0,049). La fel, 19,5% dintre cei care nu consumă excesiv alcool au CA normală, faţă de 50% dintre cei care consumă excesiv alcool, care au CA normală. Astfel, statistic semnificativ (chi-square=0,003), cei care consumă excesiv alcool au CA mai mică.

Stresul. Persoanele cu un grad de stres înalt au în proportie de 80% CA peste valo-

rile admise, iar dintre persoanele cu grad patologic de stres 70,3% au talia groasă.

Relaţia între consumul de cafea zilnic şi indicii antropometrici. S-a constatat, statistic semnificativ, că cei care consumă zilnic de cafea sunt cel mai adesea supraponderali (chi-square=0,093). Astfel, între normoponderali, 56,2% consumă zilnic cafea, între supraponderali 72,4% consumă zilnic cafea, iar între obezi, 56,4% consumă zilnic cafea. Nu s-a constatat nicio relaţie statistic semnificativă între CA şi consumul de cafea, deşi există un trend spre creşterea CA la cei care consumă zilnic cafea. În schimb, dacă se face raportul T/I, se constată o diferenţă statistic semnificativă (chi-square=0,078), 65,6% dintre cei care au T/I≥0,5 consumă zilnic cafea.

Dieta. Am analizat următoarele aspecte ale dietei care cresc riscul cardiovascular: consumul a <5 porţii de fructe şi legume/zi şi consumul >10% din total calorii de grăsimi saturate. Doar 30,8% dintre subiecţi au declarat consumul zilnic de 5 porţii de legume şi fructe. Nu s-a constatat o corelaţie între consumul adecvat de fructe şi legume şi IMC. În ceea ce priveşte consumul de grăsimi saturate >10% din totalul caloriilor, 64,3% dintre subiecţi au declarat consum mai mare decât această proporţie (estimat prin înmulţirea frecvenţei consumului alimentelor care conţin grăsimi saturate cu cantitatea de grăsimi saturate/porţie, iar cantitatea a fost calculată pentru o dietă de 2500 kcal/zi pentru bărbaţi şi 2000 kcal/zi pentru femei). Nu s-a constatat o corelaţie între consumul de grăsimi saturate şi IMC, sau între consumul de grăsimi saturate şi CA, dar aplicând formula T/I, s-a constatat semnificaţie statistică, cei cu T/I<0,5 consumă mai puţine grăsimi saturate decât cei cu T/I ≥0,5 (media de 30,49 g/zi *vs*. 33,40 g/zi, test neparametric). Cele mai frecvent consumate alimente de către populaţia studiată au fost, în ordine: pâinea şi alte produse din cereale, fructe, ulei, lapte, legume şi ouă. S-a putut determina un pattern de tip vestic, predominent cu carne si mai ales cu alimente prăjite în contrast cu o alimentaţie sănătoasă cu lactate şi legume şi fructe, dar loturile comparate nu au dat semnificaţie statistică.

Sedentarismul. Aplicând *IPAQ*, în această populaţie rurală, analizată în anotimpul primăvară-vară, 11,6 % din populaţie este sedentară, 16,7% din populaţie este moderat activă, iar 71,7% din populaţie are activitate fizică mare. Aceste procente se referă la un sublot de 138 de subiecţi la care s-a aplicat chestionarul, care au vârsta ≤69 ani (peste această vârstă, chestionarul nu poate fi aplicat). Statistic semnificativ, IMC-ul persoanelor cu activitate fizică medie este mai mare decât al celor care fac activitate fizică mare (30,13 *vs*. 26,2, p=0,015). Activitatea efectuată de populaţia studiată este eminamente ocupaţională şi casnică.

DISCUŢII

Stilul de viaţă modern este asociat cu creşterea riscului cardiovascular printr-o prevalenţă ridicată a fumatului, sedentarismului şi obezităţii.

Evoluţia supraponderii şi obezităţii în comunitatea studiată este în trendul mondial (12). Deşi am găsit în lotul nostru că fumătorii au IMC şi CA mai mici decât nefumătorii, riscul cardiovascular al fumatului nu se exercită prin intermediul obezităţii şi lupta pentru combaterea lui trebuie continuată. Din literatură rezultă că persoanele cu obezitate viscerală fumătoare au cel mai mare grad de risc (13).

Dacă ne bazăm pe conţinutul energetic, un gram de alcool are 29 kJ (7,1 kcal), consumul de alcool ar induce creşterea în

greutate. Nu sunt însă studii relevante care să arate că odată cu creşterea consumului de alcool, creşte şi IMC (14). Se discută despre cantitatea şi mai ales tipul de alcool consumat (15). În studiul nostru, marii băutori au IMC şi CA mai mică probabil prin tulburări ale apetitului, printr-un grad de denutriţie, relaţia cu factorii de inflamaţie urmând a fi cautată ulterior.

Rezultatele noastre privind relaţia consum de cafea – indici antropometrici s-ar putea să fie în legătură cu consumul de cafea îndulcită cu zahăr, sau cu faptul că adesea consumul de cafea se asociază cu tulburări de somn, care sunt de fapt cele care conduc la suprapondere şi obezitate. Rolul benefic, de antioxidant al cafelei va fi căutat în relatie cu parametrii biochimici revelaţi şi de literatură (16).

Relaţia dietă – indici antropometrici nu a dat rezultante semnificative clinic la această etapă în relatie cu cuantumul caloric sau grupe de alimente. Numeroasele chestionare de frecvenţă alimentară găsite în literatura de specialitate şi-au dovedit valabilitatea raportate la "standarde de aur" cum ar fi o serie de biomarkeri, pe care noi îi vom studia în continuare (17). Nu am putut valida un *pattern* alimentar aterogen deşi multe persoane consumă o dietă cu carne şi prăjeli. Nici o dietă protectivă nu a putut fi clar delimitată, deşi printre principalele alimente consumate sunt lactatele, legumele, fructele şi uleiul. Întrebările legate de gastrotehnie arată însă o rară utilizare a uleiului crud.

CONCLUZII

Stilul de viaţă are influenţă asupra statusului ponderal al indivizilor evaluat prin indicii antropometrici. Obezitatea se corelează pozitiv cu vârsta, stresul şi nivelul redus de educaţie şi negativ cu gradul de efort fizic şi cu consumul excesiv de alcool. Dacă IMC evaluează global populaţia, când sunt de examinat influenţele unor parametri specifici ai stilului de viaţă, CA şi raportul T/I sunt mai sensibili. Continuarea cercetării ne va ajuta să definim şi alti parametri ai stilului de viaţă ca factori de risc independenţi ai riscului cardio-metabolic.

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FACTORI NEUROENDOCRINI IMPLICAȚI ÎN HIPERTENSIUNEA ARTERIALĂ DIN SARCINĂ

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NEUROENDOCRINE FACTORS IN HYPERTENSION DURING PREGNANCY (Abstract): Pregnancy induced hypertension is a condition of high blood pressure during early and mid-pregnancy. This type of hypertension is much like the chronic type, but it occurs only when the woman is pregnant and resolves completely after delivery. **Aim:** to evaluate some stress hormones in both normotensive and hypertensive pregnant women. **Material and methods:** The study investigated the correlation between pregnancy induced hypertension and different immune/inflammatory and other markers. This exploratory investigation was performed on pregnant women diagnosed with pregnancy-induced hypertension, admitted to the Iasi Cuza Voda Hospital. The psychometric assessment was performed by using the daily stress test, daily hassle scale, blood pressure measurements, and determination of anthropometrical parameters. Some parameters, such as the neuroendocrine and immune/inflammatory ones, and specific parameters for pregnancy hypertension were determined. **Results and discussions:** Our study revealed that blood pressure values presented significant differences between systolic, but not diastolic blood pressure values ($p<0.05$). In 75% of subjects blood cortisol levels were not changed. Daily stress level assessment proved that low potential factors and an annoying environment had a high influence on both normotensive and hypertensive pregnant women. Hypertensive women also presented leukocytosis and thrombocytopenia. **Conclusions:** The research results proved that plasmatic cortisol level was higher in hypertensive pregnant women, comparing to the normotensive one. **Key words:** NEUROENDOCRINE, HYPERTENSION, PREGNANCY

Hipertensiunea arterială apărută în timpul sarcinii nu este în mod obişnuit periculoasă, ea fiind denumită "hipertensiune arterială tranzitorie a sarcinii". Totuşi, trebuie monitorizată atent, pentru că poate fi primul semn al unei stări patologice grave, preeclampsia.

Obiectivele cercetării au fost reprezentate de cercetarea nivelurilor unuia dintre hormonii de stres (cortizolul) la paciente care s-au adresat pentru consult in Ambulatoriul Spitalului Cuza Vodă din Iaşi, evaluarea gradului de stres prin teste psihometrice şi a unor parametri biologici, pentru identificarea de noi factori de prognostic în criza de hipertensiune arterială din sarcină.

MATERIAL ŞI METODĂ

Cercetarea în vederea stabilirii unor corelații neuro-imuno-endocrine în sarcină, ca

factori de prognostic în afecțiunile cardiovasculare din timpul sarcinii, a fost realizată printr-un studiu clinic pilot prospectiv unicentric, cu grupuri paralele, randomizat, la paciente diagnosticate cu criză acută de hipertensiune arterială. Comparația s-a realizat cu un lot martor, care a inclus gravide sănătoase din punct de vedere clinic şi al investigațiilor de laborator.

Studiul a fost aprobat de Comisia de Etică Instituțională.

Pentru început, a fost realizat un studiu pilot, care a inclus câte 6 paciente în fiecare lot, pentru a detecta eventualele modificări neuro-imuno-endocrine. Toate participantele incluse au semnat formularele de consimțământ pentru participarea la studiu, după ce au fost informate verbal şi în scris despre scopul şi modul de desfăşurare a studiului.

Criteriile de selecție a pacientelor au fost reprezentate de:

- ***Criterii de includere:*** vârsta: peste 18 ani; consimțământul scris înainte de introducerea în studiu; capacitatea de a înțelege natura şi scopul studiului; capacitatea de cooperare cu medicul şi de a îndeplini în cerințele studiului;
- ***Criterii de excludere:*** paciente cunoscute cu afecțiuni psihiatrice; cu traumatisme multiple; tratate cronic cu antiinflamatoare nesteroidiene sau cu corticosteroizi; refuzul subiecților; orice altă condiție care poate afecta participarea la studiu; persoane fără domiciliul stabil.

Evaluarea psihometrică s-a realizat cu ajutorul unui test de evaluare a stresului zilnic: Daily Hassles Scale. Cu ajutorul acestui test s-a putut evalua în ce măsură stresul a constituit o parte importantă a vieții în ultima lună. Testul evaluează frecvența şi modul de răspuns la factorii zilnici cu potențial de iritare (de exemplu, presiunea timpului, problemele financiare, evenimentele de la locul de muncă sau din familie, problemele din trafic etc). Testul are 51 de afirmații la care persoana care răspunde trebuie să acorde o valoare cuprinsă între 1 şi 4 (este aleasă valoarea 1, în cazul în care situația nu a reprezentat o parte importantă a vieții şi, gradat, se ajunge până la valoarea 4, ce corespunde situației ce a reprezentat o parte importantă a vieții).

Evaluarea clinică a constat în: măsurători ale tensiunii arteriale; determinarea parametrilor antropometrici (greutate, talie); pentru evaluarea indicelui de masă corporală au fost notate valorile dinainte de sarcină (pe baza acestor valori declarative s-a calculat indicele de masă corporală, IMC = G (kg)/T² (m²). Măsurarea greutății s-a realizat cu pacienta dezbrăcată, utilizând un cântar calibrat, standardizat şi etalonat conform normelor internaționale.

Datele anamnestice înregistrate au fost aspectele privind stilul de viață (consum de cafea, alcool, fumatul), afecțiunile cronice asociate, antecedentele heredo-colaterale de HTA asociată sarcinii, tratamentul urmat în mod curent.

Analiza statistică a datelor a fost realizată utilizând testul t-Student. Datele sunt prezentate sub formă de valori medii ± deviația standard. Diferențele au fost considerate semnificative pentru $p < 0,05$.

REZULTATE ŞI DISCUȚII

Caracteristicile demografice ale subiecților

Au fost incluse în studiu un număr de 24 de gravide, repartizate în două loturi: un lot de 12 gravide normotensive, cu vârsta medie de 27,5 ± 4,2 ani, cu limite între 22 şi 29 de ani (lotul martor); un lotul de 12 gravide hipertensive, cu vârsta medie de 26,75 ± 3,3 ani, cu limite între 26 şi 34 de

ani (lotul de studiu). S-a calculat indicele de masă corporală înainte de sarcină, care a fost cuprins între 20.39 şi 27.6, media fiind de 24.16 ± 6.32.

Date privind factorii de risc: stilul de viață al pacientelor cu hipertensiune arterială incluse în studiu

Studiul factorilor de risc cardiovascular la care sunt expuse pacientele incluse în studiu a relevat că aproximativ 80% din gravidele normotensive incluse în studiu declară consumul de cafea (o ceaşcă/zi, declarativ), în timp ce doar 20% dintre gravidele hipertensive asociază acest tip de comportament (fig. 1). Rezultatele studiului nostru sugerează că, deşi consumul de cafea reprezintă un factor de risc pentru dezvoltarea HTA, în cazul gravidelor, acesta nu se corelează permanent cu valori tensionale mari. Rezultatele obținute în cadrul studiului nostru se corelează cu cele din literatură (1,2).

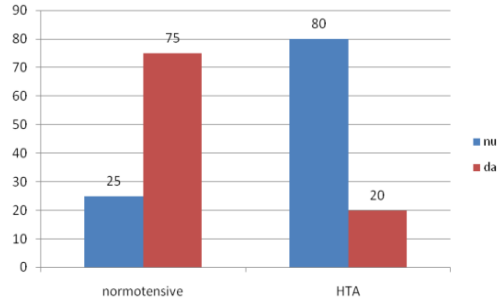

Fig. 1. Procentul consumatoarelor de cafea la loturile studiate

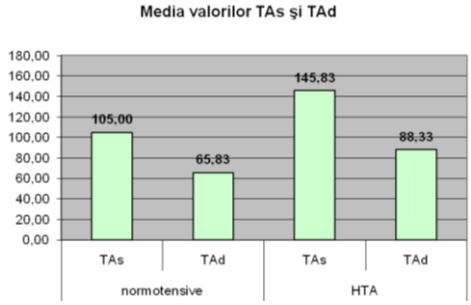

Fig. 2. Mediile valorilor TAs şi TAd la loturile studiate

Rezultate privind factorii de risc cardiovascular: valorile tensionale ale pacientelor incluse

În cadrul lotului martor, valorile TA sistolice (TAs) au fost cuprinse între 90-110 mmHg, iar cele ale TA diastolice (TAd), între 60-70 mmHg. În lotul gravidelor hipertensive, distribuția valorilor TAs s-a situat în intervalul 140-150 mmHg, în timp ce, TAd s-a menținut între 85-100 mmHg (fig. 2). Se observă că valorile TAs la lotul gravidelor hipertensive sunt nesemnificativ statistic mai mari, comparativ cu cele ale pacientelor din lotul normotensiv ($p>0,05$), în timp ce, diferența la nivelul valorilor TAd prezintă semnificație statistică ($p< 0,05$). Creşterea mai ales a valorilor TAd în context de sarcină explică semiologia afecțiunii şi organele afectate preferențial în cadrul crizei eclamptice (creier, ficat, rinichi). Rezultatele obținute se corelează cu datele din literatură care arată creşteri mai importante ale TAd, comparativ cu TAs, ceea ce sugerează că HTA asociată sarcinii determină, prin creşterea postsarcinii, manifestări clinice similare suprasolicitării cordului drept (3).

Rezultatele evaluării psihometrice (testul Daily Hassles)

Evaluarea nivelului stresului zilnic a demonstrat că factorii cu potențial scăzut de iritare au avut cea mai mare pondere, atât la gravidele normotensive, cât şi la cele cu HTA (fig. 3). Factorii stresori de intensitate mare şi extremă au apărut cu frecvență scăzută la ambele loturi, nici una dintre gravidele hipertensive nerelatând prezența unui stres extrem, în perioada evaluării. Pe de altă parte, s-a constatat că percepția tuturor categoriilor de factori

stresori a fost mai accentuată la grupul pacientelor cu HTA. Rezultatele studiului nostru sunt conforme cu datele din literatură, care arată o frecvență mai mare a HTA la persoane supuse stresului sau cu percepție puternică a agenților stresori (4).

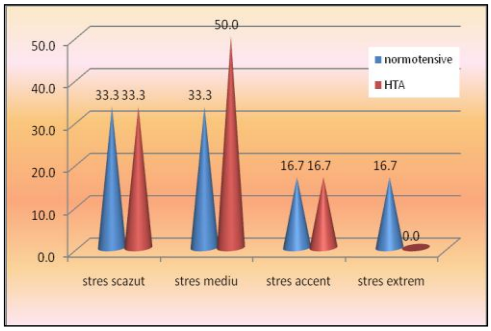

Fig. 3. Rezultatele evaluării psihometrice prin testul Daily Hassles, la pacientele incluse în studiu

Rezultate privind biomarkerii neuroendocrini

Studiul nivelului cortizolului total plasmatic a relevat că 25 % din pacientele incluse în studiu au avut valori normale ale cortizolului plasmatic total, în timp ce restul pacientelor au prezentat valori crescute ale acestui parametru. Valoarea medie a cortizolului plasmatic la pacientele hipertensive luate în studiu a fost de 248,72 ng/ml ± 125,66 (iar mediana a fost de 247,15 ng/ml). Cortizolul are valori normale cuprinse între 60-230 ng/ml între ora 8 şi 10 şi valori normale cuprinse între 30-150 ng/ml la ora 16. La gravide, în al treilea trimestru de sarcină, valorile cortizolului total plasmatic sunt mai mari decât valorile normale. Datele din literatură sugerează că există o influență reciprocă între glucocorticosteroizi şi steroizii sexuali în reglarea funcțiilor imune dependente de timus (5).

Efectele glucocorticosteroizilor pe celulele sistemului imun sunt mediate prin receptori membranari. Există două tipuri de receptori, notate cu I şi II. Tipul I leagă aldosteronul şi corticosteronul cu aceeaşi afinitate. Tipul II (situs de referință al dexametazonei) are afinitate scăzută față de corticosteron, comparativ cu tipul I. În creier şi hipofiza anterioară, una dintre multiplele acțiuni ale acestui receptor este implicată în reglarea feed-back-ului axei hipotalamo-hipofizo-corticosuprarenaliene în timpul răspunsului la stres. Glucocorticosteroizii secretați în timpul stresului acționează pe hipocampus, hipotalamus, hipofiză, pentru a inhiba secreția corticotropinei şi a ACTH şi pentru a termina răspunsul la stres.

Răspunsul imun celular şi umoral este mai activ la femei decât la bărbați, cu excepția perioadei de sarcină. Existența unui dimorfism sexual în răspunsul imun, alterarea marcată a funcției imune induse de gonadectomie, şi alterările imunitare din sarcină, susțin teoretic ipoteza că steroizii gonadali intervin în reglarea funcțiilor sistemului imun. Beneficiul dimorfismului imunologic poate fi parțial legat de capacitatea femeilor de a rezista la stresul reproducerii. Hormonii sexuali sunt principalii factori ai dezvoltării, reglării şi menținerii dimorfismului, deşi intervin şi alți hormoni, cum ar fi, hormonul de creştere, prolactina, glucocorticosteroizii, precum şi o serie de mediatori clasici (5).

Niveluri crescute de corticosteroni, precum şi variații mari ale corticosteronului plasmatic au fost găsite la femei în timpul zilei şi ca răspuns la stres. Datele obținute în acest studiu demonstrează depăşirea posibilităților de intervenție a axei hipotalamo-hipofizo-corticosuprarenaliene în menținerea homeostaziei organismului la gravidele hipertensive (6).

Rezultate privind biomarkerii imuni/ inflamatori

Studiul valorilor formulei leucocitare a demonstrat existența de modificări semnificative, comparativ cu valorile normale la gravidele hipertensive (10200 ± 233 globule albe/ml comparativ cu 8200 ± 361 globule albe/ml în lotul cu gravide normotensive) (fig. 4). Leucocitoza este considerată în limite normale pentru trimestrul al II-lea sau al III-lea de sarcină.

Leucocitoza obținută la gravidele hipertensive evidențiază una din caracteristicile sindromului HELLP (denumire provenită din abrevierea în limba engleză a principalelor modificări de laborator: anemie hemolitică, creşterea nivelului enzimelor hepatice şi trombocitopenie), cu sau fără eclampsie, datele fiind confirmate în literatura de specialitate (6,7).

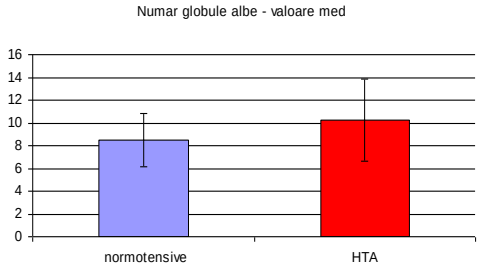

Fig. 4. Valorile medii ale numărului de globule albe

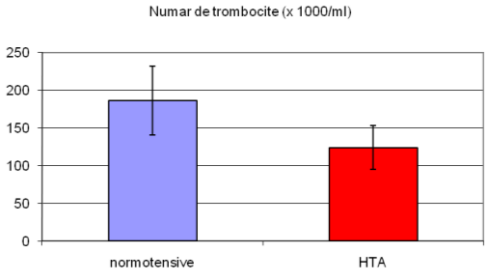

Fig. 5. Valorile medii ale numărului de trombocite.

Rezultate privind alți biomarkeri

Studiul valorilor seriei eritrocitare (număr de eritrocite, hemoglobină şi hematocrit) nu a demonstrat existența de modificări patologice ale acestor parametri. Valorile medii ale trombocitelor la gravidele normotensive au fost 186000 ± 45600 /ml trombocite, comparativ cu 124000 ± 28900 trombocite/ml la gravidele hipertensive. Studiul valorilor trombocitelor a demonstrat existența de trombocitopenie la două dintre gravidele cu hipertensiune arterială incluse în studiu (fig. 5). Datele sunt confirmate de literatura de specialitate, trombocitopenia fiind descrisă la 70 % dintre gravidele cu sindrom HELLP cu eclampsie şi 30 % dintre gravidele cu sindrom HELLP fără eclampsie (7).

Valorile medii ale lactic dehidrogenazei (LDH) la gravidele normotensive au fost 207 ± 54,2 U/L, comparativ cu 263 ± 61,8 U/L la gravidele hipertensive. Studiul valorilor acestui parametru a demonstrat existența de valori uşor peste limita normalului la gravidele cu hipertensiune arterială incluse în studiu (fig. 6).

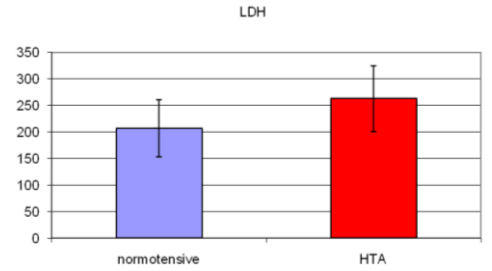

Fig. 6. Valorile medii ale LDH

Studiul altor parametri nu a demonstrat existența de modificări patologice la persoanele incluse în studiu. Astfel, deşi în literatura de specialitate există studii care evidențiază creşteri ale valorilor transami-

nazelor serice (6,7), nici una dintre gravidele hipertensive luate în studiu nu au prezentat modificări semnificative ale acestor parametri.

CONCLUZII

Cauzele şi mecanismele de producere ale HTA de sarcină nu sunt pe deplin elucidate, iar etiologia rămâne necunoscută. Există şi cazuri în care unele femei dezvoltă hipertensiune în timpul sarcinii, preeclampsie, chiar în lipsa acestor condiţii medicale preexistente.

Dintre markerii pentru factorii de risc se pot menţiona:

- sangvini: hematocrit, trombocite, produşii de degradare ai fibrinei, factorii VIII de coagulare, antitrombina III, timpul de protrombină, aspartat aminotransferaza (ASAT), alanin aminotransferaza (ALAT), bilirubina, acidul uric, creatinina, ureea, fibrinogenul, clearance-ul creatininei, trigliceridele, acizii graşi liberi, anticorpii antinucleari, anticorpii antifosfolipidici (8);
- urinari: volumul, densitatea, sedimentul, proteinuria, ionograma, calciuria.

Rezultatele cercetării au evidenţiat că valorile cortizolului plasmatic au fost crescute la gravidele diagnosticate cu valori crescute ale tensiunii arteriale, comparativ cu valorile acestui parametru la gravidele cu valori normale ale tensiunii arteriale, fiind corelate cu factorii de risc aparţinând stilului de viaţă şi cu biomarkerii inflamatori (leucocitoza), precum şi cu o serie de parametri (trombocitopenia) ce caracterizează sindromul HELLP cu şi fără eclampsie.

Persoanele incluse în studiu nu au prezentat modificări semnificative ale altor parametri ce caracterizează sindromul HELLP (de exemplu, hemoliza sau creşteri ale valorilor transaminazelor serice).

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DEPRESSION SCREENING IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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DEPRESSION SCREENING IN PATIENTS WTH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): The **aim** of this study was screening for depression in patients with COPD. **Material and methods:** Depression was diagnosed with the Hamilton 17 items questionnaire. All patients were assessed by history taking, physical exam, blood pressure (BP), spirometry, heart rate (HR), mMRC scale (Modified research Council). **Results:** The study included 312 patients with COPD admitted in the Clinical Hospital of Pneumology Iasi during 1-st March 2015 to 1st March 2017. The subjects were mainly males, with a median age of 65.4±4.1 years, a history of current or former cigarette smoking of 30 packs-year. Items of Hamilton Questionnaire: Item 5 (72%) - Mark 1-2-3 times night awaking, without problems feeling asleep again. Item 7 (50%) - Marks 2,3,4, Decreased performance, considerable loss of interest, frequent abandon of fulfilling tasks, even for those essential, lack of decisions, hesitations (directly or indirectly described). mMRC Dyspnea Grades/patient: Grade 3. **Conclusions:** 70% of COPD patients have low to moderate depression. 100% of subjects are former or current smokers, which is a known risk factor for COPD. 89% of patients were diagnosed with moderate to severe airway obstructive dysfunction. **Keywords:** DEPRESSION, COPD, HAMILTON QUESTIONNAIRE, mMRC SCALE.

Diagnosing depression in patients with chronic obstructive pulmonary disease (COPD) with severe or medium ventilatory deficiency is important for the management of the life quality of these patients.

According to the *Global Diagnostic, Treatment and Prevention Strategy* (GOLD Programme, 2017) COPD is a disease characterized by reduce air inflow, not completely reversible. The limitation of the air influx is usually progressive and associated with an abnormal pulmonary inflammatory response towards noxious particles or gasses (cigarettes smoke or other inhalatory irritants (1).

World Health Organization (WHO) estimates that 210 million people suffer from COPD globally and 3 million died in 2005. COPD is associated with a high morbidity and mortality and a considerable decreased quality of life in the late stage of the disease, representing an important socio-economic problem, through its high direct and indirect medical costs1. Recent research showed that COPD was the third mortality cause worldwide in 2010. This data represents a betted situation in comparison with previous predictions and show that the global mortality through COPD decreased in 2010 reported to 1990, from

6.4% to 2% (2).

However, in Romania COPD frequency increased: in 1990 it was 0,49%; in 20 years, it tripled, to 1.51% in 2010. The number of cases grew with 185% in this period. In 2010 over 1 million persons were diagnosed with COPD.

The clinical picture of COPD includes specific and unspecific respiratory symptoms, such as exertional and rest dyspnea, chronic cough, expectoration, wheezing and chest compression sensation. The cough is chronic, frequently productive, with mucous or purulent sputum, during infectious exacerbation, the so-called morning "bronchial cleansing". By far the most discomfort for the COPD patient is the dyspnea - the central symptom in those seeking medical attention. Its appearance is progressive, first in high exertional effort (running), the patient can no longer sustain the same effort as his age matched healthy subjects (3). Epidemiologic studies that along with the evolution of the respiratory disfunction the dyspnea aggravates and represent a major cause for the deterioration in the life's quality.

The spirometry is a valuable diagnostic and monitoring tool in COPD. It increases the diagnostic accuracy of chronic respiratory diseases and facilitates the treatment's improvement. A good spirometry assessment implies trained staff, good equipment and well standardized measurement procedures (4).

COPD diagnosis includes clinical history, physical examination, thoracic X-Ray and spirometry airways obstruction documentation (5, 6).

Treatment for obstructive dyspnea includes inhaled bronchodilators (anticholinergics, β2-agonists) and corticosteroids, recently added in a single inhaler, improve treatment adherence (4). Inhaled Thiotropium can increase bronchodilation but has little effect on the quality of life in COPD5. Recent research on IL-13 blockade with various monoclonal antibodies suggests that these could also be useful in the treatment of respiratory obstruction (6).

Depression is defined in 2012 by WHO as a common mental condition, including a depressive status, loss of interest and pleasure, decreased energy, guilt feeling or low self-esteem, sleep or eating disorders and reduce concentration (Depression: A Global Crisis - World Health Organization, 2012). Depression is a general emotional state, that involves the whole person (physically and psychic) and manifests through social retire, sorrow, with a global painful impression of hopelessness, fatality despair, longer than a justified reason (Kerneman dictionary, 2010). Depression is defined as a mental illness characterized by a profound change of the timic disposition towards sadness, moral suffering and lower psychomotor activity, associated with anxiety. It can associate delirious ruminations of culpability, indignation, self-appreciation and can progress to suicidal ideation or action. Depression symptoms can be complex and vary considerable from one person to another. As a rule, the person is depressed, sad, hopeless and loose interest in joyful things (7). The symptoms persist for weeks or months and interfere with work, social and family life.

The psychological symptoms include: continuous low mood or sadness, hopelessness, irritability and intolerance towards others, loss of motivation and interest, low decision-making, no happy feeling, anxiety or worry, suicidal or self-harm ideation (8).

Physical signs include: bradylalia and lent movement; appetite changes; low en-

ergy and libido, unexplained pain, constipation, sleep change (difficult sleeping at night and very early awakening)8.

Social symptoms include: fewer social activities and less contact with friends; neglect of hobbies and interests, difficulties in family life and home activities (8).

These component elements of depression are depicted in figure 1.

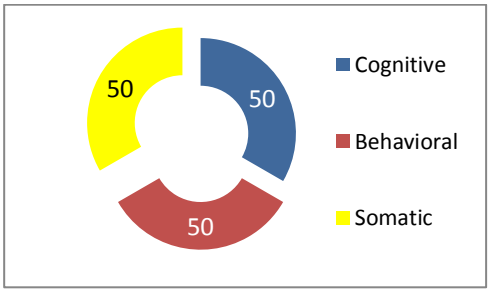

Fig. 1. Depression's components

Depression diagnosis is based on history taking and physical exam. First step is the medical appointment. The examining physician can evaluate the state of mental health of the patient through an interview or indicate a battery of verbal and written tests which diagnose depression. Some clinicians use diagnostic scales, represented in questionnaires by scores that pinpoint the amplitude of patient's sufferance.

A routine specific questionnaire is recommended to assess in due time subjective and affective symptoms and apply a therapeutic intervention to prevent their evolution. WHO and *The American Psychiatric Association* suggest considering symptoms which are present for at least 2 weeks to ease the questionnaire's delivery. The obtained results are diagnostic, especially in elderly (*Depression and anxiety scale; Hamilton's questionnaire 17 items; Beck questionnaire*).

AIM OF THE STUDY

The present study investigated the presence of depression in patients with COPD.

MATERIAL AND METHODS

Inclusion criteria were the following: Caucasian patients, age over 40 years old; with smoking history.

Exclusion criteria: lack of compliance, alcohol intake and drugs in the last 12 months, other major mental health disease, epilepsy, cardiac implanted pacemakers, decompensated heart disease (heart failure, untreated hypertension, angina pectoris, rhythm or conduction disturbances), rheumatic or neuromuscular active disease, low testosterone levels disease such as Klinefelter syndrome, chemo- and radiotherapy for cancer, excessive alcohol intake, Kallman syndrome, pineal gland or hypothalamic tumor, severe acute pathologies that needed hospital admission in the last 12 weeks, other respiratory diseases (idiopathic pulmonary fibrosis, bronchiectasis, pulmonary tuberculosis, bronchial asthma, diagnosed malignant tumors with radio or chemotherapy).

Depression was diagnosed with the Hamilton 17 items questionnaire.

All patients were assessed by history taking, physical exam, blood pressure (BP), spirometry, heart rate (HR), peripheral oxygen saturation in ambient air, mMRM scale (Modified research Council).

RESULTS

The study included 312 patients with COPD admitted in the Clinical Hospital of Pneumology Iasi between the 1st of March 2015 and 1st of March 2017. The subjects were mainly males, with a median age of 65.4±4.1 years, a history of current or former cigarette smoking of 30 packs per year.

TABLE I

Items of Hamilton Questionnaire

Item 1	61.1%	Mark 1	The symptoms are due to hidden sadness in every person. It can be discovered only if the subject is asked and is manifested as a feeling of lack of wellness, indisposed, discouraged, pessimist, sad; higher than an occasional feeling. Can be eased by company, work, activity.
Item 2	55%	Mark 1	Poor self-esteem, with a persistent regret for past actions, self-reproaches, feeling of having caused a prejudice to other people
Item 3	61.1%	Mark 1	Lack of symptoms. In some subjects there is a feeling that life can't be lived (7%)
Item 4	67.1 %	Mark 1	Discrete difficulty in falling asleep (duration of 30-60 minutes). The duration up to 30 minutes is considered normal
Item 5	72%	Mark 1	time night awaking, without problems feeling asleep again
Item 6	68%	Mark 1	The subjects awake one hour prior to the usual awaking time, but goes back to sleep without difficulties
Item 7	50%	Marks 2,3,4,	Decreased performance, considerable loss of interest, frequent abandon of fulfilling tasks, even for those essential, lack of decisions, hesitations (directly or indirectly described)
Item 8	40%	Mark 1	Discrete decrease in conversation, communication, fixed expression, or fluctuation of the emotional response
Item 9	63%	Mark 1	Fidgeting of hands, hair, or feet (restless persons)
Item 10	68%	Mark 1	Subjective tension and irritability – not fundamented worries, intermittent tensions
Item 11	72%	Mark 1	Increased intensity and severity of symptoms, that appear more frequent than twice a week
Item 12	64%	Mark 0	Normal appetite
Item 13	80%	Mark 1	Tiredness disproportionate to the made effort, back pain, headache or member pain, low energy, fatigability
Item 14	60%	Mark 1	A small decrease in interest or a decrease in activity
Item 15	70%	Mark 1	Fixed attention on own body, evidenced when asked, but not apparent spontaneously in the conversation
Item 16	80%	Mark 1	Loss of weight related to the actual disease
Item 17	70%	Mark 1	Mark 1– Partial loss. The patients acknowledge his/her disease, but attributes it to food, climate, workload, and silence need

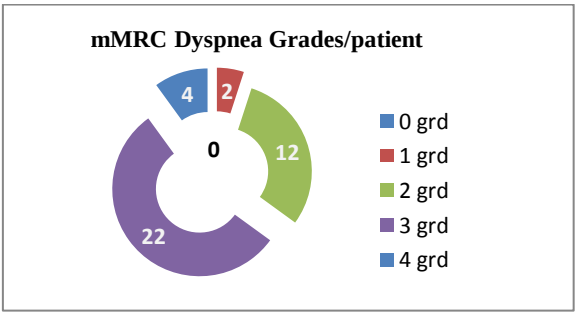

Fig. 2. Mmrc Dyspnea Grades

TABLE II
Blood pressure, heart rate, peripheral oxygen saturation in ambient air before and after spirometry

Spirometry before and after	Minimum	Media	Maximum
BP rest	130/80mmHg	145/85 mmHg	170/90 mmHg
BP during spirometry	145/85 mmHg	165/85 mmHg	160/80 mmHg
BP after spirometry	140/85 mmHg	160/80 mmHg	170/90 mmHg
HR rest	82 b/min	87 b/min	105 b/min
HT during spirometry	90 b/min	95 b/min	170 b/min
HT after spirometry	88 b/min.	90 b/min	130 b/min
SpO_2 rest	83% a.a.	86% a.a.	92 % a.a.
SpO_2 during spirometry	80% a.a.	85% a.a.	90% a.a.
SpO_2 after spirometry	82% a.a.	88% a.a.	94% a.a.
FR rest	17 c.	20 c.	22 c.
FR during spirometry	20 c.	25 c.	35 c.
FR after spirometry	18 c.	23 c.	28 c.

DISCUSSION

The aim of this study was screening for depression in patients with COPD. Most of enrolled patients were over 65 years old, current or former smokers, with 30 pack cigarettes / year history.

This study is similar with Thomas George Willgoss work, who searched pneumology journals from 1966 to 31st of January 2012 for articles focused on clinical interviews to assess a psychologic diagnostic in patients with COPD. The study found a frequency of 55% for current depression in COPD patients, consistent with our study. This is a moderate to increased value, but with over symptomatology – clinic, psychologic and psychiatric (9).

One of the most severe symptoms is the decreased work capacity, over the accepted minimum, with decreased work performance and lack of efficiency, impossible to hide, inducing commentaries, making chores impossible, complete disorganized work, lack of hygiene and self-neglect, friends' abandon.

These patients become unable to handle themselves in life, society and seek the hospital for protection (not for investigations or observation). They abandon work due to their disease and need consistent help of friends and neighbors for living.

The limitations of this study consist in its low number of patients, the rate of COPD patients understanding of the task of 80%, 20% of subjects not responding due to a cluster of mental problems, which make impossible for them to understand the questions or the possible answers in the questionnaire.

CONCLUSIONS

70% of COPD patients have low to moderate depression.

All the subjects are former or current smokers, which is a known risk factor for

COPD.

89% of patients were diagnosed with moderate to severe airway obstructive dysfunction.

COPD is of the most severe public health problems, and the fourth global mortality cause. Being a chronic, progressive and not curable disease, the medical treatment can reduce symptoms and reduce the airway obstructive dysfunction.

Recent studies started to recognize the importance of the psychological factors in COPD progression and the need to consider their management. High prevalence of depression in COPD is consistent with a poor evolution.

More inclusive evaluation of anxiety and depression through specific tests (psychologic, psychiatric, antismoking and antialcohol counselling) associated with pulmonary rehabilitation in COPD can result in life's quality improvement.

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MARKERI NON-INVAZIVI PENTRU VARICE ESOFAGIENE ÎN CIROZA HEPATICĂ COMPENSATĂ

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NON-INVASIVE MARKERS TO ESOPHAGEAL VARICES IN COMPENSATED LIVER CIRRHOSIS (Abstract): **Material and methods**: There were 91 patients in this study, with liver cirrhosis classified as Child A, diagnosed using clinical, biological and ultrasound methods, and a control group. We used a special questionnaire and biostatistical methods in order to assess the presence of esophageal varices (EV), followed by performing upper gastrointestinal endoscopy (UGE). **Results**: Models made with biostatistical methods differentiated the cases with or without EV. ROC curve showed sensitivity and specificity values of various parameters. We obtained an overall rate of correct classification of 84.62% for the presence of EV and 71.30% scale assessment. **Conclusions**: Using non-invasive tests may be useful as therapeutic and prognostic considerations, to reduce the number of upper gastrointestinal endoscopy in the disease surveillance also. **Key words**: EPIDEMIOLOGY, LIVER CIRRHOSIS, ESOPHAGEAL VARICES

În România, patologia hepatică cunoaşte o incidenţăcrescută ca urmare a evoluţiei endemice a infecţiilor cu virusul hepatitei B (VHB) şi C (VHC) şi a consumului de alcool (1). Studiile efectuate au evidenţiat valoarea de prognoză asupra prezenţei cirozei hepatice şi a varicelor esofagiene (VE), a unui număr mare de parametri individuali accesibili, alături de metodele reprezentate de puncţia biopsie hepatică şi elastografia Fibroscan sau Fibromax (2).

Numeroase încercări au reuşit să îmbunătăţească valoarea predictivă şi a testelor sangvine simple sau în combinaţii, între care sunt scorurile APRI, ASPRI, AAR, ARI, Pohl, Forne, care folosesc date biochimice şi hematologice (3,4,5). Cu scopul de a valida rezultatele obţinute anterior, au fost analizate acurateţea, reproductibilitatea şi funcţia de prognoză a datelor estimate în modelul precedent, într-un lot nou de pacienţi recrutaţi după criterii similare.

MATERIAL ŞI METODĂ

Studiul de tip prospectiv s-a desfăşurat în perioada 1.01.2009-1.11.2010, în compartimentul de gastroenterologie al Spitalului Judeţean "Mavromati", Botoşani, pe un lot de 91 pacienţi diagnosticaţi cu ciroză hepatică, clasa Child A, prin metode clinice, biologice şi ultrasonografice. S-a utilizat o fişă de evaluare complexă, alături de calculul matematic propus pentru estimarea prezenţei VE, urmat de efectuarea endoscopiei digestive superioare. S-a folosit clasificarea VE în absenţa şi prezenţa, respectiv gradul 1 (VE care dispar la insuflarea endoscopică), gradul 2 (VE care nu confluează, dar protruzează în

TABELUL I
Calcularea indicatorilor SPRI, ASPRI, AAR, APRI, API

Indicator	Calcul
SPRI	Diametrul longitudinal al splinei (cm) / nr. trombocite (10^6 / ml) * 100
ASPRI	Vârsta (ani) : <30 = 0 ; 30-39=1 ; 40-49=2 ; 50-59=3 ; 60-69=4 ; =70=5
	ASPRI = vârsta + SPRI
AAR	TGP/TGO
APRI	TGP/42/ nr. trombocite*100
API	API=vârsta + nr. trombocite
	Vârsta (ani) : <30 = 0 ; 30-39=1 ; 40-49=2 ; 50-59=3 ; 60-69=4 ; =70=5
	Nr. trombocitex1000, >225=0,200-224=1,175-199=2,150-174=3,125-149=4, <125=5

lumen, sub insuflaţie endoscopică) şi gradul 3 (VE care confluează şi proemină în lumen). Pentru prelucrarea statistică, s-a mai utilizat clasificarea în VE mici, corespunzător gradului 1 şi VE mari, care cuprind gradele 2 şi 3 (6, 7).

Criteriile de excludere s-au referit la pacienţii cu carcinom hepatocelular primar, cu sângerare variceală în antecedente, cu tromboză portală la examinarea ecografică şi alte cauze pentru splenomegalie, precum şi cei aflaţi în tratament cu antagonişti ai receptorilor beta-adrenergici.

Parametrii urmăriţi au fost serologici (nivelul trombocitelor, valoarea TGP, TGO) şi ecografici (valoarea diametrului maxim al splinei, în combinaţii sub forma scorurilor SPRI, ASPRI, AAR, APRI, API) (tab. I).

Analiza statistică s-a realizat utilizând programele Microsoft Excel şi SPSS 16.0., testul t-Student şi chi^2, analiza univariată şi multivariată prin regresie logistică. Curba ROC (Receiver Operating Characteristic Curve) a folosit la calculul celei mai reprezentative valori, prin sensibilitate si specificitate, putând considera, astfel, valorile de cutoff ale diverşilor parametri. De asemenea, acurateţea modelului s-a apreciat prin măsurarea ariei de sub curba ROC, astfel, o arie $< 0,7$ a fost considerată bună, iar o valoare $> 0,8$ a indicat o acurateţe excelentă. Datele au fost exprimate ca medie a valorilor, cu un interval de încredere de 95%. S-a utilizat şi analiza dispersională univariată, obţinând indicatorii statistici ai variabilei dependente, total şi pe grupuri (testul Levene), pentru analiza omogenităţii dispersiilor (testul ANOVA), iar, în cazurile când dispersiile au fost diferite, s-a calculat semnificaţia diferenţei dintre medii prin testele robuste Welch sau Brown-Forsythe. Aprecierea semnificaţiei globale a modelului s-a realizat prin aplicarea testului Hosmer-Lemeshow (8,9). S-a propus un model logistic de prognoză în urma calculului matematic al lotului 1, cu verificarea datelor estimate in lotul 2.

REZULTATE

Lotul 2 (experimental) a cuprins 91 pacienţi, 47 femei şi 44 bărbaţi, în vârstă de 19-80 ani, cu o medie de 51, faţă de lotul 1 (martor), cu vârsta de 19-85 şi o medie de 52 ani. Intervalul de vârstă 40-59 ani a fost dominant. Analizând caracteristicile etiologice ale lotului 1 si 2, s-a observat o distribuţie relativ omogenă a principalelor etiologii reprezentate de consumul de alcool singur (28% lot 1, 30% lot 2) sau în asociere (18% lot 1, 15% lot 2) şi implicarea VHC (29%) (fig. 1).

Rezultatele obţinute după examinarea prin endoscopie digestivă superioară s-au exprimat, comparativ, prin clasificarea în grade (absente, 1, 2, 3). Am constatat diferenţe mici în repartiţia pe grade a VE, totuşi, semnificative, mai ales la categoria absente, 29% în lotul 1 faţă de 36% în lotul 2 şi pe palierul VE de gradul 2, unde datele arată o incidenţă de 24% în lotul 1 şi 18% în lotul 2 (fig. 2).

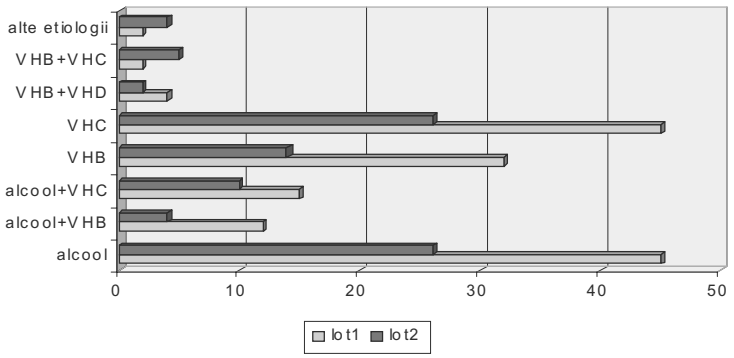


Fig. 1. Reprezentarea spectrului etiologic, comparativ în lotul 1 şi 2

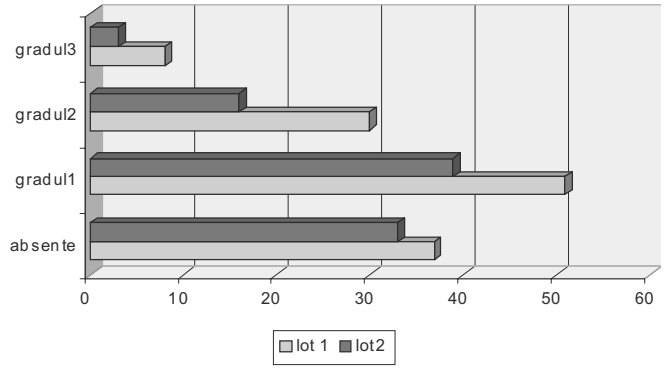


Fig. 2. Repartiţia varicelor esofagiene pe grade, comparativ în lotul 1 şi 2

A fost calculată valoarea modelelor propuse în cadrul lotului experimental şi anume, corelaţia scorurilor ASPRI, SPRI, AAR, API, APRI cu prezenţa sau absenţa VE, respectiv cu gradul acestora, determinat endoscopic. S-a constatat că singurii parametri cu semnificaţie statistică sunt SPRI şi ASPRI, valoroşi pentru ambele evaluări de detecţie a prezenţei VE, cât şi de corelare cu mărimea VE. Pentru restul indicatorilor, AAR, API, APRI, care sunt alcătuiţi din combinaţii de parametri biochimici, nu s-a demonstrat o asociere semnificativă (tab. II, III).

Utilizând datele care caracterizează lotul 2, s-a estimat un model logistic pentru prezenţa/absenţa VE. Rezultatele obţinute în urma estimării modelului arată că acesta este semnificativ statistic cu o semnificaţie globală a modelului indicată de testul Hosmer-Lemeshow de 0,172 (chi-square 11,55 şi grade de libertate 8). De asemenea, analiza valorilor rezumative denotă o bună concordanţă a modelului cu datele introduse astfel circa 56,9% (Nagelkerke R Square) din varianta iniţială este regăsită în model.

În privinţa valorilor prognozate de model, s-a obţinut un procentaj global al clasificărilor corecte de 84,6%, indicând o bună adecvanţă a modelului. Se poate observa că are loc o clasificare mai bună pentru cazurile de absenţă a VE (87,8%) decât pentru cele de prezenţă (82,7%).

Un alt model logistic matematic s-a obţinut după analiza coeficienţilor modelului 1, care va fi verificat pentru prognoza în lotul 2. Această funcţie a fost calculată pentru valorile SPRI şi ASPRI ale pacienţilor din lotul 2, utilizând curba ROC pentru a găsi valoarea de cut-off, care permite o prognoză acceptabilă. Utilizând funcţia logistică cu pragul de clasificare de 0,5, pentru lotul 2,

TABELUL II
Compararea grupurilor determinate de prezenţa/absenţa VE

Testul	Egalitatea dispersiilor - testul Levene		Egalitatea mediilor - testul t	Diferența mediilor				
scor	F	p	T	p	diferența	er. std.	IC 95% al diferenței	
							inf.	sup.
SPRI	8,91	0,004	7,08	0,000	7,13	1,01	5,13	9,14
ASPRI	7,87	0,006	6,42	0,000	6,69	1,04	4,62	8,76
AAR	0,42	0,517	-0,76	0,447	-0,05	0,07	-0,19	0,08
API	0,43	0,513	0,54	0,592	0,17	0,32	-0,46	0,81
APRI	0,81	0,371	0,90	0,372	0,29	0,32	-0,35	0,92

TABELUL III
Compararea grupurilor determinate de VE mari / mici

Testul	Egalitatea dispersiilor - testul Levene		Egalitatea mediilor - testul t		Diferența mediilor			
scor	F	p	t	p	Diferența	Er. std.	IC 95% al diferenței	
							Inf.	Sup.
SPRI	9,32	0,003	3,91	0,001	7,95	2,03	3,72	12,18
ASPRI	8,69	0,004	3,94	0,001	7,75	1,97	3,66	11,84
AAR	1,59	0,211	-1,32	0,190	-0,10	0,08	-0,26	0,05
API	0,86	0,356	0,28	0,777	0,11	0,38	-0,65	0,86
APRI	1,99	0,162	1,01	0,314	0,38	0,38	-0,37	1,13

s-a obţinut un procentaj de 68,03% de clasări corecte, cu precizarea că modelul prognostichează mai bine cazurile de prezenţă a VE (73,68%) decât cazurile de absenţă a VE (48,15%).

Analiza ROC pe cele două loturi, experimental şi martor, ne-a permis o apreciere a acurateţei modelelor şi a asigurat stabilirea unor valori cutoff. Deoarece semnificaţia statistică a fost $< 0,001$, a rezultat că modelul face o distincţie semnificativă între cazurile cu VE prezente, respectiv absente. Din analiza datelor, rezultă că VE prezente au fost prognosticate corect (82,7%) şi 12,1%, cazuri fals pozitive. Analiza similară în cadrul unui nou model privind clasificarea VE în mici şi mari a evidenţiat rezultate semnificative (tab. IV).

Utilizând funcţia logistică cu pragul de clasificare de 0,5 s-au obţinut date care atestă că în 71,3% cazuri, clasările au fost corecte, cu precizarea că modelul prognos-

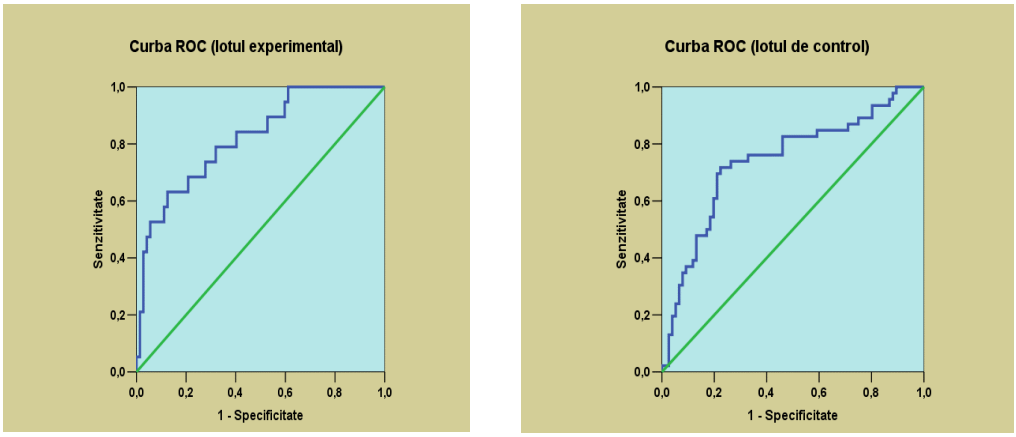
TABELUL IV
Estimarea prognozată de model privind prezenţa VE mari /mici

Valori observate		Valori prognozate de model		
		varice esofagiene		Clasare corectă (%)
		mici	*mari*	
varice esofagiene	***mici***	70	2	97,2%
	mari	11	8	42,1%
Clasare corectă (%)				85,7%

TABELUL V

Estimarea pe grade a VE prognozată în lotul 2 prin analiza lotului 1

Valori observate		Valori prognozate de model		
		varice esofagiene		Clasare corectă (%)
		mici	*mari*	
varice esofagiene	***mici***	65	11	**85,5%**
	mari	24	22	**47,8%**
Clasare corectă (%)				**71,3%**



a. **b.**

Fig. 3. Curba ROC pentru modelul prezentei VE mari/mici: **a.** în lotul 2; **b.** prognozate de analiza lotului 1

tichează mai bine cazurile de VE mici (85,5%) decât cazurile de VE mari (47,8%) (tab. V).

Pentru a realiza o clasare mai bună, s-a utilizat analiza ROC în care, pentru lotul experimental, discriminarea VE mari/mici obţine o valoare de 0,819, iar pentru lotul martor, de 0,741 (fig. 3 a, b).

Semnificaţia statistică fiind $<0,001$, rezultă că modelul face o distincţie foarte bună între cazurile VE mari şi VE mici. Astfel, de exemplu, alegând cutoff =-1,13748, va rezulta un prognostic corect, pentru cazurile cu VE mari (63,2%) şi cazuri fără VE, prognosticate fals pozitiv (12,5 %).

DISCUŢII ŞI CONCLUZII

Utilizarea testelor non-invazive propuse pentru diagnosticarea prezenţei şi dimensiunilor VE în cadrul evoluţiei cirozei hepatice poate fi utilă, atât din considerente terapeutice şi prognostice, cât şi pentru reducerea numărului de endoscopii digestive superioare şi pot fi repetate în cadrul monitorizării evoluţiei bolii.

Patogenia infecţiilor cu VHB şi VHC şi alcoolismul au fiecare particularităţi care se reflectă, în final, în producerea fibrozei hepatice. Mărimea splinei s-a dovedit a fi un parametru important în boala hepatică cronică, fiind corelată cu transformările fibrotice hepatice şi poate susţine singură diagnosticul de ciroză hepatică forma compensată în 82-88% din cazuri. În studiul nostru anterior (10), splenomegalia s-a dovedit alături de trombocitopenie parametru independent semnificativ corelat cu prezenţa VE, dar cu o acurateţe mai mică la măsurarea ariei ROC decât raportul lor calculat sub forma scorurilor SPRI şi ASPRI. Trombocitopenia are o corelaţie inversă cu mărimea VE şi corelaţie directă cu progresia fibrozei hepatice.

Analiza univariată şi multivariată a confirmat puterea de predicţie a combinaţiilor ce folosesc dimensiunile splinei, nivelul trombocitelor, vârsta şi acurateţea redusă a scorurilor ce folosesc nivelul transaminazelor TGP, TGO. Astfel, este de menţionat că doar ASPRI şi SPRI şi-au menţinut constantă acurateţea în toate analizele, aceste două scoruri fiind cele mai promiţătoare. Nu s-a putut stabili o valoare universală de cutoff, reproductibilă pentru ASPRI şi SPRI prin analiza matematică pe cele două loturi. În schimb analiza pe subloturi grupate pe etiologiile dominante arată ca o valoare prag >12 pentru ASPRI în hepatopatiile cu VHB şi >16 cu VHC are o valoare predictivă pozitivă pentru prezenţa VE (92%).

Analizând modelul experimental am obţinut un procentaj global de 84,62% pentru clasificarea corectă a cazurilor cu prezenţa VE. Exprimată în valori practice 10 pacienţi care aveau prezente la examenul endoscopic VE au fost diagnosticaţi fals negativ în model. Valoarea diagnostică a scăzut considerabil când s-a aplicat funcţia de prognoză la lotul martor când cu un procentaj global de clasificări corecte de 68,03%, 25 cazuri au fost greşit diagnosticate. În ceea ce priveşte modelarea după mărimea VE am obţinut două loturi care au dovedit semnificative la analiza prin testul Hosmer. Se menţine însă procentajul optim pentru prognoza mărimii VE (mari/mici), cu un total al clasificărilor corecte de 85,7% cu 11 cazuri prognozate ca având VE mari fals negative, unde practic s-ar fi pierdut momentul introducerii prevenţiei. Datele obţinute după aplicarea funcţiei de prognoza din analiza realizată lotului 1 şi aplicată lotului 2, în privinţa diagnosticării mărimii VE a determinat un procentaj global de 71,3% de clasificări corecte, cu o prognoză mai bună pentru cazurile cu VE mici de 85,5%, dar 24 cazuri au fost subevaluate în privinţa nivelului VE mari.

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WHAT CAN HIDE BEHIND A DEEP VENOUS THROMBOSIS? CASE REPORT

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WHAT CAN HIDE BEHIND A DEEP VENOUS THROMBOSIS? CASE REPORT (Abstract): Deep venous thrombosis is frequently associated with various malignancies and, in particular, with pulmonary ones and is two times more common in patients with malignancies than those without. Recognition or diagnosis of a DVT as a paraneoplastic syndrome is important because it could represent the early manifestations of an occult cancer, allowing diagnosis at an initial stage. We are reporting the case of a 62-year-old patient who was hospitalized in the Cardiology Clinic with clinical symptoms of deep venous thrombosis at the right lower limb. The diagnosis was confirmed by the imaging methods: venous echo doppler and phlebography. Catheter directed thrombolysis was performed, followed by conventional anticoagulant treatment with favorable response. The patient was also investigated etiologically but without a clear result. After one month, chest radiography revealed a suggestive image for lung cancer, which required thoracic CT and bronchoscopy with biopsy. These investigations confirmed the diagnosis of left pulmonary cancer with carinal invasion, of the scumamocellular type, with multiorganic secondary disseminations. The patient was directed to the Oncology Clinic. This case highlights the difficulty of making an etiologic diagnosis of deep venous thrombosis and a particular form of clinical onset of occult cancer. Since in the present case the occurrence of DVT preceded the diagnosis of the pulmonary cancer, the need to perform a screening of neoplasia is evident in case of any thromboembolic event occurring without an apparent cause, especially in the case of male patients. **Keywords**: PULMONARY CANCER, PARANEOPLASTIC SYNDROMS, DEEP VENOUS THROMBOSIS.

Paraneoplastic syndromes (PNS) represent a heterogeneous group of signs and symptoms associated with the progression of malignancy but not due to the direct

effects of primary tumor, adenopathy or metastasis (1). Recognition or diagnosis of a PNS is important because it could represent the early manifestations of an occult cancer, allowing diagnosis at an initial stage (2), can mimic metastatic disease and thus discourage the application of curative treatment for localized cancer, may also contribute to delaying treatment since complications of a cancer can be confused with a PNS (3). Cardiovascular paraneoplastic syndromes also include thrombophlebitis. Paraneoplastic thrombophlebitis (PNT) is one of the most suggestive manifestations under certain circumstances for cancer. They may be superficial and profound, occurring after the age of 50, more frequent in smokers and chronic alcohol users (4, 5, 6).

Venous thrombosis in patients diagnosed with malignant pathology is twice as high as patients without malignant pathology. Within this particular patient population, pulmonary cancer is the second in frequency regarding the relation with venous thrombosis second to hematological cancers (7, 8).

CASE REPORT

We are presenting the case of a 62-year-old patient, with no medical history of venous pathologies, who was a smoker until the age of 30 and an alcohol consumer, hospitalized in the Cardiology Clinic with clinical signs of edema in the lower right limb with hot tegument, erythema, pain in mole and functional impotence, suggestive for the diagnosis of deep venous thrombosis. The onset of the symptoms was 4 days prior. High-dose D-dimers (1,000-2,000 ng/mL) sustained the diagnosis.

The diagnosis was confirmed by imaging methods: venous echo doppler that revealed an almost complete thrombosis of the right femoral vein and almost complete venous thrombosis of the right popliteal vein. Phlebography of the right lower limb shows the opacity of the internal saphenous vein which communicates with the deep popliteal veins through the perforating veins. The femoral vein appears segmentally thrombosed, with dilated collateral muscular veins, with stasis and permeable anastomoses with the internal saphenous vein (fig. 1). The other veins were permeable.

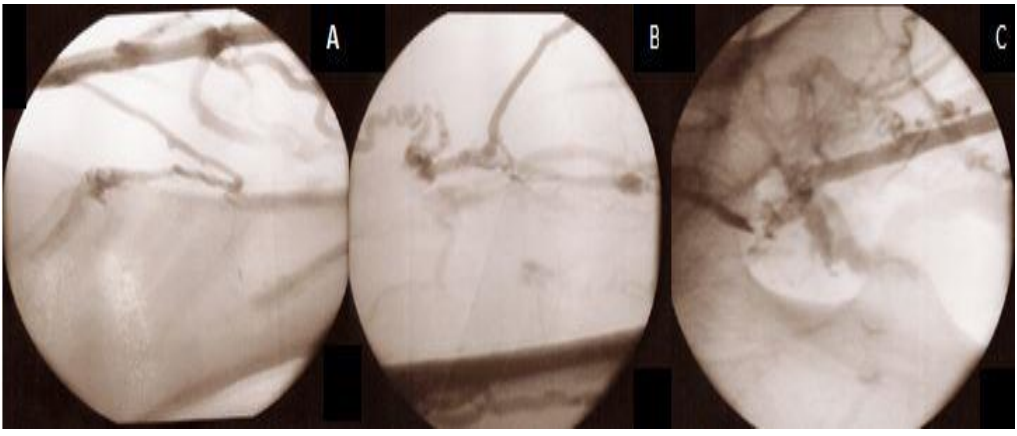

Fig. 1. A: Internal saphenous vein developing popliteal collateral circulation; B: Segmental thrombosis of femoral vein; C: The internal saphenous vein is still permeable throughout its entire length

Thrombolysis was subsequently conducted on the catheter with Rapilysin 1U/hr. followed by administration of Heparin 500 UI/hour. After 48 hours, phlebography showed reperfusion of calf veins in the upper 1/3 and femoral veins in the upper 1/3 of the thigh. The therapy was continued with conventional anticoagulant therapy (first heparin therapy, then oral anticoagulation) with favorable evolution.

At the same time, etiological investigations aimed at detecting a possible cause of deep venous thrombosis (DVT) were made, with the patient apparently not presenting prothrombotic risk factors (lack of varicose veins). Antiphospholipid antibodies were within normal range (Ig M = 10.3 U/mL, Ig G = 11.4 U/mL), and the thoracic radiography showed calcification of aortic knob; the suspicion of a possible type of alveolar filling syndrome arose although the patient was afebrile and did not have an inflammatory syndrome. Prostate ultrasound revealed a 21 mm hypoechogenic median node, but with negative prostate antigen, and upper digestive endoscopy revealed the presence of a hiatal hernia, corroborated with grade I esophagitis, atrophic gastritis in the corpus and fornix, and four inflammatory polyps with the diameter of 5- 10 mm.

Electrocardiogram showed the presence of a major left bundle branch block, echocardiography revealed dilated cardiomyopathy with moderate dilated ventricle with diffuse hypokinesia (FEVS 40%), normal valves, minimal valvular regurgitation.

The patient was discharged from hospital with oral anticoagulant medication and recalled a month later to resume etiological investigations.

After a month, the patient presented with influenced general state, effort dyspnea, cough, dysphonia and dysphagia. The right lower limb exhibited (up to the lower third of the thigh) petechial lesions, confluent in the lower half of the calf and which did not disappear in vitropress.

The blood pressure was 155/80 mmHg and the blood count showed an increased GGT (207 U/L), moderate leukocytosis (GA = 12,500/mm^3) associated with a fibrinogen of 4,64 g/L.

Thoracic radiography showed a larger predominantly enlarged mediastinum on the right; suprahilar adenopathy and a 2.7 cm round opacity in the middle left lung lobe, narrow tracheal lumen, which raised the suspicion of left bronchopulmonary neoplasia, requiring further investigations (fig. 2).

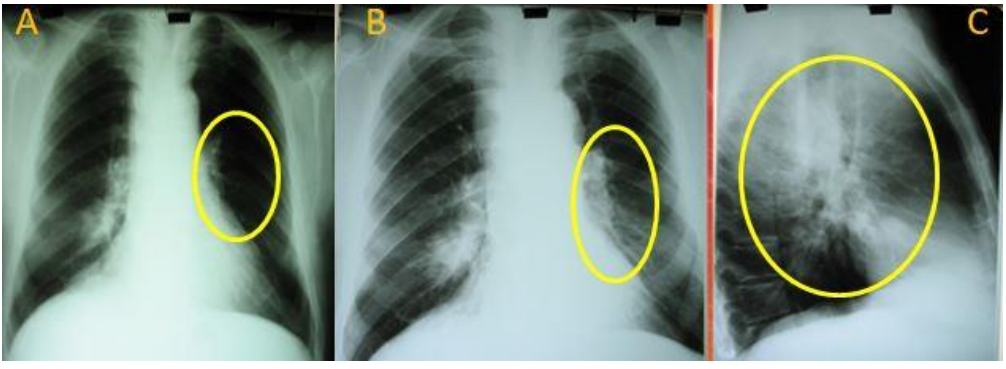

Fig. 2 A: initial posterior-anterior thoracic Rx, without changes in lung areas; B and C: postero-anterior thoracic and profile thoracic Rx after 30 days and presence of an opacity in the middle left lung lobe

The bronchoscopic examinations confirmed the diagnosis of pulmonary cancer into the left lung with the invasion of the tracheal carina. At the level of the left upper main bronchus a congestive infiltration process was observed, which interested the left primary bronchus from the origin and which then completely obstructed the inferior and superior left lobar bronchia. The bronchial biopsy revealed the mucosa with chronic inflammation and squamous metaplasia.

Computed topographies have been performed, confirming the presence of a 5.2/5 and 2/6 cm adenopathic tumor block, imprecisely delineated, located on the left primary bronchial emergence with posterior-lateral extension. The tumor was related anteriorly to the trachea, posteriorly to the esophagus, the vertebral body, the descending aorta and the azygos vein; medially is related to the mediastinal pleura, the medial wall of the main lower right bronchus and lateral to the right branch of the pulmonary artery (fig. 3). The interazigoaortic esophagus was almost occupied by a soft, non-homogeneous tissue mass with a cranial-caudal extension of about 6 cm, with the upper limit about 1 cm above the bifurcation of the trachea. The mediastinum was pushed to the left. There was another solid expanding formation of 2.5/3.0/1.0 cm in the topography of the right adrenal gland, hypodense after administration of the contrast substance. Another formation with the same characteristics was located on the left adrenal gland topography (1.5 cm).

Oncological consultation was required, and symptomatic cytostatic treatment was initiated. After the treatment, an improvement in dyspnea, coughing and dysphagia was noted. However, the progress of the diseases was rapidly progressive and ended with the patient's death 14 months after oncological treatment began.

The particularities of this case are the appearance of deep vein thrombosis as a first sign of a bronchopulmonary cancer in a paraneoplastic syndrome associated with the appearance of a scumamocellular carcinoma with a relatively rapid evolution of the tumor and of the adenopathies within one month to a current non-smoker. The association between the cardiovascular pathology, smoking and alcohol consumption - dilatory ethanolic cardio-myopathy, with such a brief clinical expression is also very rare.

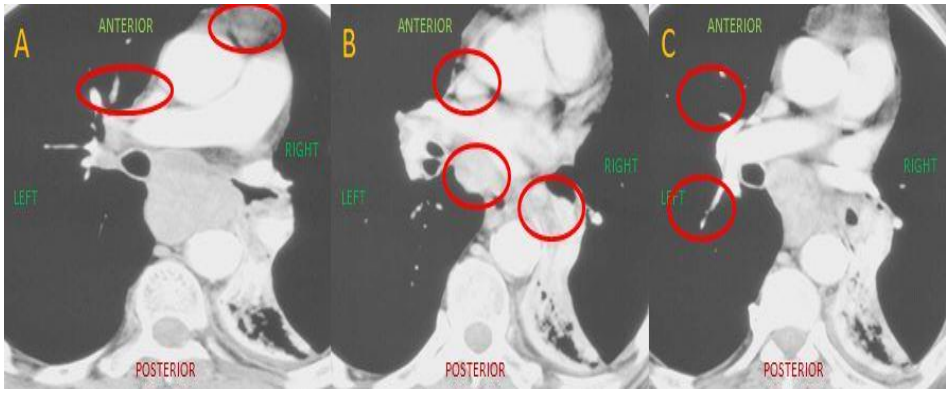

Fig. 3. High mediastinal adenopathies: A = on the sides of the trachea, B = posterior to the trachea, C = between pulmonary lobes; CT evaluation

DISCUSSION

Regarding thromboembolic events occurring in the neoplastic context, their incidence is 4 times higher than in the general population (9, 10, 11).

Nearly half of cases with migratory thrombophlebitis associate a neoplasia, and almost all of these are adenocarcinomas. Metastatic stages of cancers are associated with increased incidence of venous thromboembolism (12, 13, 14). Furthermore, chemotherapy increases the risk of venous thromboembolism 6 times than in the healthy population. Researchers (15) developed and validated a predictive model for the risk of venous thromboembolism (VTE) in patients undergoing chemotherapy (tab. I). The 0 score signifies a VTE risk below 1%, the intermediate score between 1-2% is associated with a 2% risk, and the score ≥ 3 signifies increased thrombotic risk (7%).

TABLE I

The risk of VTE associated with chemotherapy

Localization of neoplasia - Very high risk (stomach, pancreas) - High risk (pulmonary, lymphoma, urogenital)	 2 1
Pre-chemotherapy platelets ≥350 x 10^9/L	1
Hemoglobin <10 g/dL or the use of erythropoiesis growth factors	1
Pre-chemotherapy leukocytes > 11 x 10^9/L	1
Body Mass Index ≥ 35 kg/m^2	1

A percentage of 10-20% of VTE patients hide a malignancy and it is a predictive independent marker of death in these patients. Studies have also shown that approximately 10% of patients with apparently no cause of THP will develop a neoplasm over the next 5-10 years, most of which are detected within the first 1-2 years (16).

The combination of neoplasia - VTE decreases survival, thrombotic events being reported as the second cause of mortality in this category of patients. Neoplastic patients with VTE have a much higher risk of bleeding events under anticoagulant treatment but also recurrence of thrombotic events with the same or another location (17).

To evoke a paraneoplastic mechanism in a thrombophlebitis, a direct tumor mechanism (extrinsic compression, venous invasion) and other circumstances such as radiotherapy must be eliminated. Differential diagnosis should eliminate a disseminated intravascular coagulation syndrome, frequent in neoplastic pathology. Dysphonia in this patient is based on the damage of the infraglotic filter of the vocal tract (18) and the dysphagia is determined by the compression exerted by the tumor on the thoracic esophagus.

Causes may be related to increased expression of tissue factor, activator of extrinsic coagulation mechanism, indirect induction of tumor-induced cytokines on tissue factor expression, tumor excretion of cysteine proteinase as direct procoagulant trigger of X factor, independent of factor VII, inducing a state of relative resistance to protein C or direct action of the platelets by tumor membrane fragments.

Along with a thorough clinical examination and a careful history, the following are necessary: hematological balance, venous echo doppler examination, phlebography, pletismography, thoracic radiography, superior digestive endoscopy, computed tomography, tumor markers.

The main treatment is intravenous therapy with heparin or low molecular weight heparins, an important feature of paraneoplastic deep venous thrombosis being the lack of response to oral anticoagulants. In the case of anticoagulant medication contraindications, it is possible to use a filter on the inferior vena cava.

The rapid unfavorable progression represents a characteristic of neoplastic patients who suffer also from deep vein thrombosis (19).

CONCLUSIONS

Since in the present case the occurrence of DVT preceded the diagnosis of the pulmonary cancer, the need to perform a screening of neoplasia is evident in case of any thromboembolic event occurring without an apparent cause, especially in the case of male patients.

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FACTORI DE RISC PENTRU INFECŢIILE CU *PSEUDOMONAS AERUGINOSA* REZISTENT LA CARBAPENEME

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RISK FACTORS FOR CARBAPENEMS-RESISTANT PSEUDOMONAS AERUGINOSA INFECTIONS(Abstract) : Since their introduction in clinical practice,carbapenems have been among the most powerfull antibiotics for treating serious infections cased by Gram-negative nosocomial pathogens,including *Pseudomonas aeruginosa*. The emergence of betalactamases with carbapenem-hydrolyzing activity is of major clinical concern.*Pseudomonas aeruginosa* is a leading cause of nosocomial infection. **Results** : Risk factors for colonization with carbapenems–resistant Pseudomonas in hospital are : history of *P. aeruginosa* infection or colonization within the previous year, (length of hospital stay, being bedridden or in the ICU, mechanical ventilation, malignant disease, and history of chronic obstructive pulmonary disease have all been identified as independent risk factors for MDR *P. aeruginosa* infection. Long -term -care facilities are also reservoirs of resistant bacteria. Risk factors for colonization of LTCF residents with resistant bacteria included age >86 years, antibiotic treatment in the previous 3 months, indwelling devices, chronic obstructive pulmonary disease, physical disability, and the particular LTCF unit. **Key words** : CARBAPENEMS –RESISTANT PSEUDOMONAS AERUGINOSA, RISK FACTORS, NOSOCOMIAL INFECTION, LONG -TERM CARE FACILITIES

Studiul de faţă, privind factorii de risc pentru infecţia cu *Pseudomonas aeruginosa* a fost efectuat pe 9 pacienţi diagnosticaţi cu acest tip de infecţie, internaţi în Clinica de Boli Infecţioase Iaşi în perioada ianuarie 2008 - septembrie 2010.

MATERIAL ŞI METODE

Pacienţii, 5 bărbaţi şi 4 femei s-au internat, cu o singură excepţie, prin transfer din alte servicii medicale şi au fost diagnosticaţi cu sepsis conform criteriilor ACCP/SCCM, şi cu disfunctii organice (tab. I).

REZULTATE

Toţi pacienţii prezentau comorbidităţi care au impus spitalizări prelungite în alte servicii medicale, chiar de terapie intensivă postoperator. Pacienţii cu vârsta peste 75 de ani erau diagnosticaţi cu demenţă senilă sau AVC vechi, soldate cu deficite neurologice. În cazul celorlalţi pacienţi notăm stenoză pulmonară infundibulară, tetraplegie spastică postfractură C5-C6, boală pulmonară cronică obstructivă şi peritonită biliară operată. În 4 cazuri erau purtători de sondă urinară a demeure (tab. II). Toţi bolnavii primiseră diferite asocieri de antibiotice in ultimele 3 luni, inclusiv cefalosporine de generaţia a III-a şi fluoroquinolone.

Pseudomonas aeruginosa rezistent la carbapeneme a fost izolat din 5 uroculturi (pacienţii 1,2,5,6,7), 2 hemoculturi (pacienţii 3,4), din escare (pacientul 8) şi din lichidul pleural (9). În toate cazurile s-a observat sensibilitatea la colistin şi rezistenţa la cefalosporine de generaţia a III-a, asocieri cu inhibitori de betalactamază, aminoglicozide,

TABELUL I
Caracteristic demografice si diagnostic

Nr.		Modalitate de internare	Diagnostic
1	86 ani/M	domiciliu	Sepsis Infecţie urinară cu *Pseudomonas aeruginosa* Pneumonie bazală dreaptă
2	27 ani/M	transfer medicina interna	Sepsis cu stafilococ auriu meti-R Bronhopneumonie Endocardită bacteriană acută VP Infecţie urinară cu *Pseudomonas aeruginosa*
3	75 ani/F	transfer spital psihiatrie cronici	Sepsis cu *Pseudomonas aeruginosa* Escare multiple
4	75 ani/F	transfer spital îngrijiri paliative	Sepsis cu *Pseudomonas aeruginosa* Escare multiple Şoc septic
5	32ani/M	transfer recuperare neuro-motorie	Sepsis Infecţie urinară cu *Pseudomonas aeruginosa*
6	45 ani/F	transfer boli infecţioase	Sepsis Infecţie urinară cu *Pseudomonas aeruginosa*
7	14 ani/M	transfer chirurgie	Sepsis cu stafilococ auriu meti-R Infecţie urinară cu *Pseudomonas aeruginosa* Bronhopneumonie
8	31ani/F	transfer chirurgie	Sepsis Escare suprainfectate cu *Pseudomonas aeruginosa*
9	49 ani/M	transfer pneumologie	Sepsis Empiem cu *Pseudomonas aeruginosa*

TABELUL II
Comorbidităţi

Nr	Comorbidităţi
1	AVC vechi, sondă urinară a demeure
2	Stenoză pulmonară infundibulară, sonda urinară a demeure
3	Demenţă senilă, obezitate morbidă, escare
4	AVC vechi, paraplegie flască, sondă urinară a demeure
5	Tetrapareza spastică post-traumatică, sondă urinară a demeure
6	AVC vechi, hemiplegie, obezitate, diabet zaharat tip2 IN
7	Peritonită bilioasă operată
8	Tetrapareza spastică post-traumatică, sondă urinară a demeure
9	BPOC, etilism cronic

fluoroquinolone şi carbapeneme. S-a iniţiat tratament cu colistin (în cazurile în care funcţia renală a permis acest lucru) în asociere cu vancomicină, clindamicină, carbapeneme, fluoroquinolone în funcţie de particularităţile clinico- paraclinice ale fiecărui caz. Evoluţia a fost favorabilă în primele două cazuri, bolnavul cu empiem a fost transferat în Clinica de Chirurgie Toracică pentru tratament de specialitate. Au decedat chiar în primele 24 ore pacientele cu hemoculturi pozitive cu *Pseudomonas aeruginosa* prin fenomene de şoc septic şi fibrilaţie ventriculară, acestea având şi cel mai mare scor APACHE II la internare (32, respectiv 38). Pacientul în vârstă de 14 ani, cu peritonită bilioasă operată, a fost diagnosticat cu sepsis cu stafilococ auriu Meti–R, bronhopneumonie, infecţie urinară cu *Pseudomonas aeruginosa*, asociind disfuncţie organică multiplă (insuficienţă respiratorie, renală, hepatică) şi decedează în a 14-a zi

de la internare. In cazul sau functia renala nu a permis utilizarea colistinului, singurul antibiotic la care tulpina de Pseudomonas era sensibila.

DISCUŢII

Rezistenta bacteriana este o problema in continua agravare in spitalele de pretutindeni. Desi in ultimii 20 ani s-a observat o incidenta crescuta a infectiilor asociate ingrijirilor medicala cu germeni gram-pozitivi, agentii patogeni gram-negativi continua sa se coreleze cu o rata crescuta a mortalitatii la acesti pacienti. Pentru ca posibilitatile terapeutice nu evolueaza de multe ori in acelasi ritm cu rezistenta bacteriana, masurile de preventie costituie constituie prima linie de aparare. Pseudomonas aeruginosa este unul dintre germenii cel mai frecvent incriminati in infectiile nozocomiale, fiind pe primul loc in etiologia pneumoniei in serviciile de terapie intensiva. Utilizarea excesiva a antibioticelor in spitale, a dus la emergenta tulpinilor de *Pseudomonas* cu rezistenta extinsa la antibiotice,inclusiv la noile generatii de betalactamine cu spectru extins, aminoglicozide si fluoroquinolone. (1) Carbapenemele au fost, pana recent, antibioticele de electie pentru tratarea infectiilor determinate de tulpini de *Pseudomonas aeruginosa* multidrog–rezistente. Totusi, *Pseudomonas* poate dezvolta rezistenta la carbapeneme prin variate mecanisme : scaderea permeabilitatii membranare, supraexpresia pompelor de eflux si productia de carbapenemaze. In rezistenta la betalactaminele cu spectru larg atribuita betalactamazelor sunt implicate mai ales metallo--betalactamazele (MBLs) : IMP, VIM, SPM and GIM. Metallo-betalactamazele hidrolizeaza carbapenemele si cefalosporinele antipseudomonas si nu sunt inhibate de inhibitorii de betalactamaze disponibili in acest moment.(2)

In Romania, conform studiului MAR-T derulat in patru centre universitare (Iasi, Constanta, Timisoara si Institutul „Matei Bals" Bucuresti) in perioada iulie 2008-decembrie 2009 sensibilitatea tulpinilor de Pseudomonas aeruginosa fata de ceftazidim s-a mentinut in proportie de 55,14%, nivelul activitatii piperacilina –tazobactam a fost de 57,88%, apropiata de cea a altor betalactamine, cu activitate anti-pseudomonas" iar pentru imipenem rata sensibilitatii a fost apropiata de cea a celorlalte betalactamine analizate : 57,12%, ceea ce infirma ideea activitatii sale superioare corelata cu utilizarea excesiva.Conform aceluiasi studiu, in perioada ianuarie- decembrie 2009 nivelul activitatii imipenemului si meropenemului a scazut sub 50%.(3)

Pe masura ce prevalenta P.aeruginosa multidrog –rezistent(MDR) a crescut de-a lungul ultimilor 20 ani, multe studii s-au concentrat asupra factorilor de risc specific asociati izolarii acestui agent etiologic. Desi datele variaza in literatura pornind de la insasi definitia multidrog-rezistentei se identifica : tratament anterior cu antibiotice, istoric de infectie sau colonizare cu P. aeruginosa in ultimul an, durata crescuta de spitalizare, internare in servicii de terapie intensiva, ventilatia mecanica, neoplaziile, istoric de boala pulmonara cronica obstructiva. (4) Factori de risc independenti pentru infectia cu Pseudomonas MDR au fost tratamentele anterioare cu cefalosporine, aminoglicozide si fluoroquinolone (prin alterarea ADN gyrazei,reducerea sau alterarea proteinelor membranei externe si a efluxului) si vancomicina (prin scaderea populatiei endogene gram-pozitive permite proliferarea P. aeruginosa). (5) Pacientii diagnosticati cu infectii cu Pseudomonas MDR sunt de obicei pacienti critici, cu spitalizari prelungite, tratati anterior cu multiple antibiotice cu activitate anti-pseudomonas. Toti cei 9 pacienti inclusi in studiul nostru aveau comorbiditati severe (deficite neurologice cu imobilizare la pat,escare multiple,diabet zaharat dezechilibrat sau interventii chirur-

gicale recente) implicand spitalizari prelungite in diferite servicii medicale, chiar in terapie intensiva post-operator. Istoricul tuturor a relevat date despre tratamenta anterioare cu antibiotice cu spectru larg, inclusiv cefalosporine de generatia a III-a si quinolone.Cel mai tanar pacient din lot a fost diagnosticat la aproximativ 10 zile post-operator cu Sepsis cu Stafilococ auriu meti –R si infectie urinara cu Psedomonas rezistent la carbapeneme cu evolutie fatala.

Serviciile de ingrijire a pacientilor pe termen lung (case de batrani, sanatorii, spitalele pentru bolnavii cronici) sunt de asemenea rezervoare de germeni rezistenti. Studii efectuate in Italia in aceste servicii medicale au indicat faptul ca 74,8% dintre rezidenti erau contaminati cu cel putin un germene rezistent, 64% cu germeni producatori de betalactamaze, 38,7% cu stafilococ auriu meticilino-rezistent (MRSA), 6,3% cu producatori de metallo-beta-lactamaze si 2,7% cu enterococ rezistent la vancomicina (frecventa colonizarii pacientilor din casele de batrani a fost mai redusa). Factorii de risc pentru colonizarea rezidentilor din serviciile de ingrijire pe termen lung cu bacterii rezistente au fost: varsta peste 86 ani, tratament antibiotic in ultimele 3 luni, dispozitive invazive, boala pulmonara cronica obstructiva, dizabilitatile fizice si specificul ingrijirilor iar pentru cei din casele de batrani au fost varsta si dementa. (6) Cele doua paciente diagnosticate cu sepsis cu P. aeruginosa,cu evolutie fatala in primele 24 ore erau internate pe termen lung intr-un serviciu de ingrijiri paliative respectiv in unul pentru boli psihice cronice indeplinindu-se conditiile de colonizare si infectie cu germeni rezistenti.

Rezidentii acestor unitati sunt vectori de transfer pentru bacterii rezistente in spitalele obisnuite, bacterii ce pot determina infectii atat in cazul gazdelor cat si al altor pacienti vulnerabili. P.aeruginosa poate supravietui timp indelungat in mediul de spital contaminand suprafete si surse de apa. Transmiterea de la un pacient la altul este posibila in conditiile nerespectarii riguroase a normelor de igiena. Tulpinile de Pseudomonas aeruginosa imipenem –sensibile pot fi transmise de la un pacient la altul si pot deveni rezistente la carbapeneme prin presiune antibiotica selectiva.(7) Colistinul a fost considerat terapia de salvare in infectiile cu BGN non-fermentativi, dar,conform studiului MAR-T, are o rata de eficienta in scadere ingrijoratoare.

CONCLUZII

1. Pseudomonas aeruginosa este unul din germenii cei mei frecvent incciminati in etiologia infectiilor nozocomiale, utilizarea pe scara larga a antibioticelor ducand la aparitia tulpinilor cu rezistenta extinsa la antibiotice, inclusiv la noile generatii de betalactamine cu spectru extins, aminoglicozide si fluoroquinolone.
2. Factorii de risc pentru infectiile cu Pseudomonas aeruginosa rezistent la carbapeneme sunt: spitalizarile prelungite, mai ales in servicii de terapie intensiva, ventilatia mecanica, dispozitivele invazive, tratamentele cu cefalosporine, quinolone, vancomicina.
3. Serviciile de ingrijire pe termen lung (de ingrijire paliativa, pentru pacientii cu afectiuni psihice cronice si chiar casele de batrani) sunt surse de bacterii cu rezistenta multipla la antibiotice.Intre cele doua tipuri de unitati spitalicesti exista un schimb continuu si un cerc vicios pentru transmiterea bacteriilor rezistente.
4. In studiu s-a observat ca singurul antibiotic la care au fost sensibile aceste tulpini de Pseudomonas a fost colistinul care, datorita nefotoxicitatii nu a putut fi folosit in toate cazurile.

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NOUTĂŢI

Sindroamele confuzionale acute (delirium) din serviciile de terapie intensivă au o evoluţie defavorabilă sub tratament cu Rivastigmină

Un studiu multicentric randomizat dublu-orb contra placebo, efectuat în Noua Zeelandă, a inclus 440 de pacienţi, internaţi în servicii de terapie intensivă, care prezentau un sindrom confuzional acut (delirium), la care s-a administrat pe lângă tratamentul clasic cu haloperidol, şi rivastigmină, un inhibitor de colinesterază utilizat mai ales în boala Alzheimer, considerându-se că aceşti bolnavi pot prezenta o tulburare la nivelul neurotransmiterii colinergice. Studiul a fost întrerupt prematur de comitetul de securitate ca urmare a mortalităţii crescute observate în grupul celor trataţi cu rivastigmină (12 decese - 22%, faţă de 4 - 8% în grupul placebo ; $p=0,07$) În plus, rivastigmina a prezentat şi alte efecte nedorite : o durată mediană a sindromului confuzional de 5 zile, faţă de 3 zile în cazul administrării de placebo ($p=0,06$), cât şi o evoluţie mai severă a stărilor confuzionale sub rivastigmină, faţă de placebo, ceea ce a necesitat prescrierea unor doze crescute de neuroleptice şi de propofol pentru calmarea pacienţilor. A rezultat clar că rivastigmina nu numai că nu reduce durata sindroamelor confuzionale acute în terapia intensivă, dar este posibil să le prelungească durata şi chiar intensitatea. Schema de de tratament încercată a fost considerată inutilă şi periculoasă, rămânând a fi explicate bazele fiziopatologice ale acestui eşec terapeutic (Van Ejik M et al. Effect of rivastigmine as an adjunct to usual care with haloperidol on duration of delirium and mortality in critically ill patients : a multicentre, double-blind, placebo-controlled randomised trial. *Lancet* 2010 DOI : 10.1016/ S0140-6736(10)61855-7).

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NEW CONCEPTS IN THE DIAGNOSIS AND TREATMENT OF IDIOPATHIC PULMONARY FIBROSIS

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NEW CONCEPTS IN THE DIAGNOSIS AND TREATMENT OF IDIOPATHIC PULMONARY FIBROSIS (Abstract): Idiopathic pulmonary fibrosis is a chronic, progressive form of diffuse parenchymal lung disease, leading to respiratory failure. It has unknown causes, affects older adults, with a predilection for men, cigarette smokers, and it has a poor prognosis with a median survival time of approximately 2-3 years from diagnosis. Considering the rapid decline in lung function, this review brings into attention the importance of a multidisciplinary team for an early diagnosis. The methods for the assessment include spirometry which identifies restricted lung volume and impaired gas exchange, and imaging procedures. High-resolution computed tomography is the gold standard for the early diagnosis of idiopathic pulmonary fibrosis, allowing treatment initiation on mild-to-moderate form. Surgical lung biopsy is required in about a third of cases, in the lack of definite usual interstitial pattern on high-resolution computed tomography. The procedure is limited by its complications and increase mortality rate. At present, there are two antifibrotic therapies available for a mild-to-moderate form of idiopathic pulmonary fibrosis with positive effects on lung function but are not a cure. Other treatment options are lung transplant, pulmonary rehabilitation, stem cell therapy, and biotechnology. Mesenchymal stem cells could have protective effects, reducing inflammation and slowing fibrotic process. The review aims to illustrate the management of this rare disease and future directions for treatment research. **Keywords:** IDIOPATHIC PULMONARY FIBROSIS, MULTIDISCIPLINARY TEAM, ANTIFIBROTIC THERAPY, BIOTECHNOLOGY.

Idiopathic pulmonary fibrosis (IPF) is defined as a progressive diffuse parenchymal lung disease, leading to an irreversible decline in lung function of unknown etiology (1). IPF has a poor prognosis with a median survival time of approximately 2-3 years from diagnosis (2, 3). It is limited to the lungs, affects people in the later decades of life (IPF is rare before age 50) and is often initially misdiagnosed (4). IPF is a disease of the elderly and death rate increases with increasing age (5). Studies

have reported 3 million patients diagnosed with FPI, with a higher prevalence among men compared to women.

Etiopathogenesis

With the lung volumes reduced, patients are experiencing a decline in lung function, characterized by progressively worsening dyspnea (3, 6). Although IPF is still considered to be an idiopathic disease, some factors increase the risk of getting IPF. More than 70% of patients have a history of cigarette smoking (5). Studies reported that nonsmokers had a higher survival rate than former and current smokers. Other factors associated with lung injury are microbial agents (fungal, bacterial), gastroesophageal reflux, and having certain genetic predispositions (7, 8). Viral infections, such as hepatitis C virus, human herpesvirus-8, cytomegalovirus (9) and Epstein-Barr-Virus, were considered to be risk factors but this was not demonstrated (9). Many patients with IPF have comorbid conditions like sleep apnea (10, 11), diabetes mellitus, depression and pulmonary hypertension (10).

Histologically, the American Thoracic Society/European Respiratory Society (ATS/ERS) classification defines IPF as an entity of usual interstitial pneumonia (UIP) findings (12). IPF lungs have regions of normal lung parenchyma alternating with interstitial inflammation, fibrosis, and honeycombing (13). The IPF pathophysiology is not fully understood, but a repeated injury of alveolar epithelial type II cells (AECII) is considered a key factor (14). This leads to apoptosis, epithelial homeostasis is disrupted and finally, fibrosis occurs. Normal lung (rich in elastin) is replaced with an abnormal matrix rich in fibrillar collagen (15). Structural changes lead to multiple alterations of the alveolar-capillary membrane limiting the pulmonary gas exchange (16).

Diagnosis

The signs and symptoms lack specificity and resemble common lung diseases such as shortness of breath (exercise dyspnea), dry chronic cough, fatigue, unexplained weight loss, tiredness, aching joints and muscles, chest pain, finger clubbing. Dry, inspiratory bibasilar "Velcro-like" crackles can be heard when listening to the patient's lungs in over 80% of patients (17). This signs and symptoms of IPF can be mild or even absent early in the disease process but worsen over time.

The chest X-ray of a patient with IPF is initially normal but, after a while, it shows diffuse interstitial fibrosis associated with bronchiectasis and bullous emphysema described as honeycomb lung. Spirometry is one of the most commonly used tests of pulmonary function; it can be used to identify restricted lung volume and impaired gas exchange (18). Another lung function test is the 6-minute walk test (6MWT) which measures how far patients with IPF can walk in six minutes, how fast they exhibit exercise-induced oxygen desaturation and if they need supplemental oxygen therapy (19).

At present, high-resolution computed tomography (HRCT) is the gold standard for the diagnosis of UIP using standard criteria (20). Typical UIP pattern on HRCT is characterized by subpleural, basal predominance (21), reticular abnormality (22), honeycombing with or without traction bronchiectasis, and absence of non-UIP characteristics (23). Lester S. King (24) mentioned that "The history of medicine is the history of distinguishing one condition

from another." The difference between IPF ("idiopathic UIP") and "secondary UIP'' is very important because multiple diseases may be associated with UIP (25): collagen vascular disease (26), chronic hypersensitivity pneumonitis (27), drug-induced pulmonary fibrosis (28), desquamative interstitial pneumonia (29), respiratory bronchiolitis (30), asbestosis (31), sarcoidosis (32). Another problematic question is: How can we differentiate honeycombing between traction bronchiectasis and emphysema? A honeycomb lung in UIP can be regarded as being subpleural, predominant in the posterior and lower lobes, and a variable combination of thick-walled cysts (33). Emphysema appears with thinner walls and cystic airspaces located away from the chest wall (centrilobular/paraseptal emphysema) (34, 35). More extensive fibrosis and traction bronchiectasis on HRCT are often associated with increased risk of death (36). Presently, a certain UIP pattern on HRCT in a patient with typical clinical findings can define IPF without another investigations (22), like surgical lung biopsy or bronchoalveolar lavage (37). HRCT scan could help in the early diagnosis and treatment leading to a decreased decline lung function and mortality rate.

Surgical lung biopsy is required only in one-third of cases (in the lack of definite usual interstitial pattern on high-resolution computed tomography) due to the high risk of mortality and complications within the first 30 days after this procedure (38). The most common cause is an acute exacerbation of IPF, defined as a sudden acceleration of this disease and an increased mortality rate (39). Bronchoalveolar lavage is used to exclude other inflammatory disorders such as chronic hypersensitivity pneumonitis or eosinophilic pneumonia, but it is a nonspecific and insensitive method (40, 41). The current guidelines for the diagnosis and management of IPF do not recommend bronchoalveolar lavage in suspected IPF patients (42).

The diagnostic criteria for IPF include:

- exclusion of three other important causes of interstitial lung disease, like connective tissue disease, drug toxicity and long-term environmental exposures;
- a certain UIP pattern on HRCT;
- pulmonary biopsy for the lack of definite UIP pattern on HRCT (21).

Treatment

The ATS/ERS guideline for the pharmacological treatment of IPF includes two antifibrotic agents, nintedanib and pirfenidone. In the past, glucocorticosteroids and immunosuppressants were treatment options. Then, drug combinations were used: prednisone and azathioprine (43) or prednisone, azathioprine and N-acetyl-cysteine (NAC), but the latter combination proved to be harmful (44, 45). Triple therapy (prednisone, azathioprine and NAC) was excluded because PANTHER study (46) demonstrated an increased risk of death compared with placebo (45). Other molecules for the treatment of IPF that have been proposed and tested were interferon-ɤ, etanercept, vasodilators like bosentan, macitentan and ambrisentan, imatinib mesylate (a tyrosine kinase inhibitor), but no benefits were found (47). Bosentan, ambrisentan and macitentan are mentioned in the current guidelines for the treatment of pulmonary hypertension. Bosentan has proven its efficiency by increasing exercise capacity, right ventricular myocardial function and improving the symptoms but it can be hepatotoxic. It was

the first drug in the endothelin-receptor antagonist class. More ongoing studies have mentioned macitentan, another endothelin-receptor antagonist, as the last therapeutic option for pulmonary hypertension (48).

At present, nintedanib, an intracellular inhibitor of tyrosine kinases, is used for the treatment of IPF in US, Europe and Asia, slowing disease progression (49, 50). Pirfenidone, an antifibrotic agent, was approved in Europe in 2011. Pirfenidone and nintedanib were tested and approved in our country, both antifibrotic drugs showing benefits, with some differences between them. The adverse effects are dose depended and include gastrointestinal events, skin rash and photosensitivity. It has proven to reduce the decline in lung function during exercise, demonstrated by the 6-minute-walk distance (51). Nintedanib and pirfenidone can both reduce lung function decline but cannot cure the disease (52, 53). These therapies are indicated in a mild-to-moderate form of IPF (54). According to the TOMORROW (55) and INPULSIS (56) trials, nintedanib is superior to pirfenidone in terms of time to the first acute exacerbation of the disease (50).

Millan-Billi P and collaborators (57) mentioned the recombinant human soluble thrombomodulin as another treatment option. It has anti-inflammatory, antifibrotic and anticoagulant effects, and some studies have shown a decreased mortality rate. Presently, a phase three clinical study is underway (58). Other future treatments, like hemoperfusion with polymyxin B-immobilized fiber column and therapies against autoantibodies, are in the study (57). For IPF exacerbation cases, some theories argue that statins (59) and antacid treatment (60) have beneficial effects, but studies are needed to prove that. Follow-up and improving socio-economic factors may have a long-term risk reduction role, as with other chronic conditions (61).

We currently need alternative therapeutic options to reduce mortality and morbidity from IPF. The database of US National Institutes of Health includes 493 clinical trials of mesenchymal stem cells (MSCs), as a potential treatment for IPF. Different types of tissues, like bone marrow, adipose tissue, and umbilical cord blood can help us to obtain MSCs. Stem cells have been used in phase I clinical trials as a therapy for respiratory disease. Recent studies demonstrated that MSCs have protective effects, reducing inflammation and slowing the fibrotic process (62). The only ongoing study (a phase 1/2 trial) conducted in China, aims to isolate lung stem cells from patients' own bronchi. The stem cells will be processed in the laboratory and then the patients will receive the treatment in a single injection directly into the fibrotic lung zone. The first results of this trial are expected in December 2018, but the approval of such therapies will probably take several years (63).

IPF guidelines mention pulmonary rehabilitation as a therapeutic option for improving the quality of life in these patients (64, 65). Patients with severe disease may receive palliative care. Bajwah S and collaborators (66) suggest that patients appreciate conversations with the care team about IPF diagnosis and severity. Patients with depression symptoms can receive antidepressant drugs (62). The treatment option for end-stage IPF is lung transplantation which offers a long-term survival. Bajwah S and collaborators (66) mentioned that approximately 50% of patients with this rare disease are alive five years after

surgery for transplantation. Another problematic fact is the lung donor shortage compared to the high demand.

New treatment options were reported. Bioengineers from the State University of New York at Buffalo and the University of Toronto in Canada proposed a solution: lung-on-a-chip. This method is similar with microlithography, and it could make the drug testing process more efficient. Organ-on-chip technology is not only a more rapid method of drugs testing. The electronic chips can mimic the changes that occurs in the lung with IPF, reducing the number of tests performed on animals and lowering the costs of possible antifibrotic therapies. The researchers used pulmonary tissue produced in the laboratory and placed on chips, instead of semiconductor materials. This new method allows the lung-on-a-chip to expand and contract, like the real organ. Fibrosis has been induced by a protein that affects the lung cells (67).

CONCLUSIONS

Our research emphasized the importance of idiopathic pulmonary fibrosis as a progressive disease, with an increasing incidence. Two important aspects are essential for patients with this irreversible disease: an early and correct diagnosis, and the initiation of antifibrotic treatment as early as possible. IPF is a little-known disease, underdiagnosed, with a higher mortality rate than many forms of cancer. Equally important is teaching the population about the symptoms of this disease. Chronic dry cough and progressive dyspnea on exertion are the main clinical features. Over time, the disease limits the patient's ability to perform current activities. Patients often require supplemental oxygen. Diagnosis of IPF is difficult and thus we need a multidisciplinary team: pneumologists, radiologists and anatomo-pathologists.

We have mentioned that only two antifibrotic treatments are available for IPF. These drugs can reduce the decline in lung function, not decreasing the degree of fibrosis. We need more therapeutic options for IPF, but *in vitro* models are necessary to reproduce the development and progression mechanisms of lung scarring over time. Biotechnology represents a good method for testing new drugs, reducing the additional costs. Researchers from Buffalo University have found the solution: lung-on-a-chip.

Finally, patients with IPF need psychological support and care in palliative centers. The only treatment option left for end-stage IPF patients is a lung transplant.

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PROFILUL METABOLIC AL PACIENTULUI CU LITIAZĂ BILIARĂ COLESTEROLICĂ

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METABOLIC PROFILE OF PATIENTS WITH CHOLESTEROL GALLSTONE DISEASE (Abstract): Metabolic syndrome is a clustering of cardiovascular risk factors as well as associated co morbidities, including cholesterol gallstones disease.The aim of our study is to prove the association between metabolic syndrome and cholesterol gallstones, to identify the main risk factors for this association and to trace the particularities of the metabolic syndrome in such patients. **Material and method:** Our cohort study enrolled 449 subjects, 312 patients diagnosed with metabolic syndrome and gallstones or cholecystectomy (group 1) and 137 patients diagnosed only with metabolic syndrome (group 2), hospitalized in the Respiratory Rehabilitation Medical Clinic between 2007-2009. **Results:** 85.25% of the patients with metabolic syndrome and cholesterol gallstones and 79.92% of the patients with metabolic syndrome alone had a body mass index (BMI) ≥ 25 Kg/m^2. Univariate and multivariate logistic regression were used to calculate the risk of gallstone disease associated with different metabolic syndrome variables. Waist circumference, BMI, fasting glycemia, insulinemia and insulin resistance index (HOMA-IR) registered statistical significant differences between the two study groups and were significantly associated with a higher risk of cholesterol gallstone as well as were blood pressure values ≥130/85 mmHg. The presence of 4 or 5 components of the metabolic syndrome increased the risk of gallstone disease by 3 times (OR = 3.3, $p<0.001$) compared to a 2 times higher risk (OR=2.1, $p=0.02$) in case of increased insulin resistance; there was no statistical significance for any of the lipid parameters. **Conclusion:** In our study, cholesterol gallstones appeared strongly associated with anthropometric measurements, fasting glycemia and insulin resistance index, as well as with the presence of metabolic syndrome as defined by the new clinical definition, despite the negative results for the lipidic components. These findings are consistent with the hypothesis that insulin resistance plays an important role in the pathogenesis and that cholesterol gallstone disease belong to metabolic syndrome.
Key words: INSULIN RESISTANCE, METABOLIC SYNDROME, CHOLESTEROL GALLSTONES

Sindromul metabolic a fost adus în prim-plan de Reaven (1988), sub denumirea de sindrom X, odată cu relevarea agregării, la o aceeaşi persoană, a unei constelaţii metabolice centrată de insulinorezistenţă: hiperinsulinism, hiperglicemie (scăderea toleranţei la glucoză sau diabet zaharat tip 2), dislipidemie aterogenă (hipertrigliceridemie, HDL-colesterol scăzut) şi hipertensiune arterială (1). Ulterior au fost adăugate o multitudine de alte componente, care alcătuiesc un tablou polimorf, ce include factori de risc cardiovascular tradiţionali şi nontradiţionali, precum şi condiţii patologice asociate diverse, cum ar fi litiaza biliară colesterolică (LBC), steatohepatita nonalcoolică

TABELUL I
Criteriile de diagnostic ale sindromului metabolic

Criterii	Valoare de referinţă
Perimetru abdominal crescut	>94 cm la bărbaţi şi >80 cm la femei
Hipertrigliceridemie (sau tratament medicamentos pentru scăderea trigliceridelor, ca indicator alternativ)	>150 mg/dL
HDL-colesterol scăzut (sau tratament medicamentos pentru creşterea acestuia)	<40 mg/dL pentru bărbaţi, <50 mg/dL pentru femei
Tensiune arterială crescută (sau medicaţie antihipertensivă)	Tensiunea arterială sistolică ≥130 mm Hg şi/sau diastolică ≥85 mm Hg
Glicemie bazală (à jeun) crescută (sau tratament medicamentos hipoglicemiant)	≥100 mg/dL

şi steatoza hepatică, sindromul ovarelor polichistice etc. (2,3).

De-a lungul timpului, au fost elaborate mai multe definiţii ale sindromului metabolic (SM), recent fiind publicate rezultatele unui consens al mai multor organizaţii, care recomandă pentru diagnosticul clinic prezenţa a minimum trei dintre criteriile prezentate mai jos (tab. I) (4).

Studiile epidemiologice au relevat ca factori de risc modificabili pentru LBC dislipidemia, obezitatea, în special cea abdominală şi diabetul zaharat. Se pare că hiperinsulinismul şi insulinorezistenţa reprezintă veriga de legătură între LBC, SM şi obezitatea propriu-zisă (5,6,7). Cercetări recente au indicat rolul modulator al unor receptori nucleari– receptorul farnesoid X (FXR), în conexiunile metabolice dintre acizii biliari, trigliceride, carbohidraţi şi rezistenţa la insulină; prin această mediere, se pare că se realizează o legătură la nivel molecular între litiaza biliară şi sindromul metabolic (8,9).

Pornind de la argumentele prezentate, obiectivele studiului de faţă sunt reprezentate de identificarea factorilor de risc pentru LBC în cazul pacienţilor cu SM şi evidenţierea celor cu cea mai înaltă semnificaţie statistică, prin utilizarea parametrilor clinici şi de laborator definitorii pentru SM (insulinorezistenţa, perturbarea metabolismului glucidic şi lipidic, hipertensiunea arterială) precum şi stabilirea particularităţilor sindromului metabolic în cazul pacienţilor cu LBC.

MATERIAL ŞI METODĂ

Profilul pacienţilor. S-a realizat un studiu de cohortă pe un număr de 449 pacienţi, internaţi în Secţia Clinică Medicală – Recuperare Respiratorie în perioada ianuarie 2007 – decembrie 2009. În funcţie de diagnosticul stabilit în urma evaluării clinico-paraclinice, pacienţii au fost împărţiţi în 2 loturi: lotul 1, format din 312 pacienţi cu sindrom metabolic şi LBC şi lotul 2, format din 137 pacienţi cu sindrom metabolic dar fără LBC.

Criteriile de includere a pacienţilor în cele 2 loturi au fost reprezentate de vârsta peste 18 ani, obiectivarea imagistică (ecografică sau prin tomografie computerizată) a litiazei biliare veziculare sau prezenţa istoricului de colecistectomie pentru litiază biliară, date sugestive de laborator.

Criteriile de excludere. Din studiu au fost excluşi pacienţii cu afecţiuni cunoscute că asociază litiază biliară prin mecanism hemolitic sau infecţios (ciroză hepatică alcoolică, infecţie virală B, D sau C, siclemie, proteze valvulare metalice, litiază coledociană sau intrahepatică); de asemenea, au fost excluşi din studiu pacienţii diagnosticaţi cu diabet zaharat franc, luând în considerare doar pe aceia care au prezentat glicemie < 126 mg/dL à jeun şi/sau < 200 mg/dL la 2 ore după administrarea pe cale

orală a 75 g de glucoză.

Parametrii analizaţi în prezentul studiu au fost reprezentaţi de indexul de masă corporală (IMC), circumferinţa abdominală, greutatea corporală slabă (*lean body weight*--LBW), suprafaţa corporală (*body surface area*-BSA), tensiunea arterială sistolică (TAS) şi diastolică (TAD), glicemia à jeun, colesterolul total, HDL-colesterolul, LDL-colesterolul, trigliceridele (TG), indexul de acumulare lipidică (*lipid accumulation product*-LAP), insulinemia bazală şi indexul de insulinorezistenţă (*Homeostasis Model Assessment of Insulin Resistance* - HOMA-IR). Nivelul insulinei bazale a fost determinat în 65 de cazuri prin metoda imunochimică de detecţie prin electrochemiluminiscenţă (ECLIA), iar indexul de insulinorezistenţă HOMA-IR a fost calculat după formula: HOMA-IR = insulinemia bazală (μU/mL) $\times$ glicemia à jeun (mmol/L) / 22,5. Indexul de acumulare lipidică (LAP), un nou parametru de evaluare a riscului cardiovascular, a fost calculat pe baza trigliceridemiei serice şi a perimetrului abdominal, diferenţiat pe sexe: (P abdominal 65) x TG la bărbaţi şi (P abdominal 58) x TG la femei.

Materialele de lucru au fost reprezentate de foile de observaţie clinică şi biletele de ieşire din alte servicii medicale, pe baza cărora s-au completat fişe de evaluare a pacienţilor, după obţinerea în prealabil a consimţământului lor informat.

Analiza statistică. Datele au fost analizate şi exprimate statistic descriptiv. Riscul de a dezvolta LBC în funcţie de prezenţa anumitor factori a fost evaluat cu ajutorul raportului cotelor şi regresiei logistice, după o prealabilă codare în formă binară a variabilelor continue. Prelucrarea statistică a fost realizată prin intermediul programului *Statistica 8.0*.

REZULTATE ŞI DISCUŢII

Lotul 1 a fost alcătuit din 312 pacienţi (248 femei şi 64 bărbaţi) diagnosticaţi cu SM şi LBC, iar lotul 2 din 137 pacienţi (91 femei şi 46 bărbaţi) diagnosticaţi cu SM dar fără LBC. Vârsta medie a pacienţilor nu a înregistrat diferenţe semnificative între cele 2 loturi ($64,21 \pm 11,67$ ani în cazul lotului 1 şi $62,25 \pm 10,45$ ani în cazul lotului 2). Repartiţia pe grupe de vârstă a pacienţilor cuprinşi în cele 2 loturi este redată în fig. 1.

Obezitatea şi statusul supraponderal au înregistrat o prevalenţă ridicată în loturile studiate, 85,25% dintre cazurile cuprinse în primul lot şi 79,92% dintre cele din al doilea lot având IMC $\geq$ 25 Kg/m^2.

Între cele 2 loturi au existat unele diferenţe statistic semnificative din punct de vedere al valorilor medii ale parametrilor

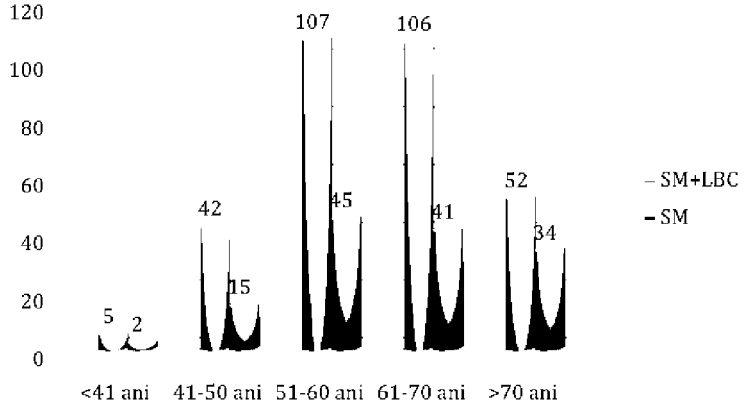

Fig. 1. Repartiţia pe grupe de vârstă a pacienţilor din cele două loturi

TABELUL II
Compararea valorilor medii ale parametrilor evaluaţi

	Medie lot 1	Medie lot 2	p
IMC	31,40	28,31	<0,001
LBW	48,33	49,11	NS
BSA	1,84	1,82	NS
Circumferinţa abd.	106,50	98,2	<0,001
Glicemie	109,62	103,47	0,02
Col total	201,17	197,53	NS
HDL	47,40	48,46	NS
LDL	124,18	125,52	NS
COL/HDL	4,36	4,28	NS
TG	142,65	137,85	NS
LAP	6694,49	6657,36	NS
Insulinemie bazală	9,56	8,67	0,05
HOMA-IR	1,30	1,20	0,03
TAS	131,7	128,5	<0,001
TAD	72,8	71,5	0,004

evaluaţi, după cum este redat în tab. II. Pacienţii cu SM şi LBC au prezentat valori mai mari ale IMC, circumferinţei taliei şi valorilor tensionale ; de asemenea, valorile glicemiei, insulinemiei bazale şi HOMA-IR au fost crescute, comparativ cu pacienţii diagnosticaţi doar cu SM, diferenţele fiind statistic semnificative.

Obezitatea şi statusul supraponderal (IMC ≥25 Kg/m^2), circumferinţa taliei, hiperglicemia, rezistenţa crescută la insulină (HOMA-IR >2,5) şi numărul de criterii din definiţia SM s-au asociat semnificativ cu un risc crescut de LBC.

Niciuna dintre variabilele ce evaluează metabolismul lipidic (colesterol total, HDL--colesterol, colesterol total/HDL, LDL-colesterol, trigliceride, LAP) precum şi unii indici antropometrici (greutatea corporală „slabă" şi suprafaţa corporală) nu s-au asociat cu riscul de LBC (tab. III).

Întrunirea a 4 sau 5 dintre criteriile SM

TABELUL III
Asocierea parametrilor evaluaţi cu probabilitatea de LBC

	Raportul cotelor	Interval de încredere	p
Sex	0,58	0,29-1,08	NS
Vârsta (F>55, M>45)	2,17	1,32-3,95	0,008
Circumferinţa abd. (F>80, M>94)	3,65	1,98-6,85	<0,001
IMC (?25)	3,02	1,57-6,12	<0,001
Glicemie (>100)	2,21	1,15-4,02	0,02
Trigliceride (>150)	1,12	0,57-2,15	NS
Col total (>190)	1,31	0,62-2,07	NS
LDL (>100)	1,15	0,71-2,24	NS
HDL (M<40, F<50)	1,18	0,55-2,42	NS
Col total/HDL	1,09	0,37-2,52	NS
3 criterii SM	8,57	2,56-29,91	<0,01
4 criterii SM	6,43	1,55-18,72	0,02
5 criterii SM	9,46	1,75-102,31	0,02
HOMA-IR (>2,5)	1,82	1,14-4,74	0,01
TAS >130 mmHg, TAD >80 mmHg	3,26	1,65-6,43	0,002

a determinat o creştere de peste 3 ori a riscului de LBC (OR = 3,3, p<0,001), iar rezistenţa crescută la insulină, o creştere de peste 2 ori (OR=2,1, p=0,02). Deoarece pacienţii de sex feminin şi cei vârstnici au înregistrat o pondere ridicată în loturile studiate, am ajustat riscul funcţie de cele două variabile, în cadrul regresiei logistice multivariate. Consecutiv acestei ajustări, SM a fost asociat în continuare cu o creştere de 2,9 ori a riscului de LBC iar rezistenţa crescută la insulină, cu un risc de 1,8 ori mai mare.

Am încercat analizarea unor parametri antropometrici suplimentari, cum ar fi compararea suprafeţei corporale şi a greutăţii corporale „slabe" între loturile luate în studiu, fără a se înregistra diferenţe statistic semnificative ; în schimb, IMC şi perimetrul abdominal au fost puternic asociaţi cu riscul de LBC (p<0,001), aşa cum arată şi datele din literatură (7,11).

În studiul nostru, încercarea de a găsi corelaţii între parametrii metabolismului lipidic şi litiaza biliară a eşuat, chiar şi pentru cei incluşi în definiţia sindromului metabolic : HDL-colesterolul şi trigliceridele serice, contrar datelor din literatură (12).

Insulinemia bazală şi glicemia bazală au avut valori semnificativ mai mari la pacienţii cu LBC şi SM iar indexul de insulinorezistenţă peste 2,5 a fost asociat cu un risc crescut de LBC (OR=1,82 ; p=0,01).

CONCLUZII

Studiul de faţă a avut rezultate similare cu cele din literatură în ceea ce priveşte asocierea litiazei biliare cu unii parametri antropometrici şi cu indicii de insulinorezistenţă, fiind discordant în ceea ce priveşte indicatorii dislipidemiei. În plus, au fost puternic asociate cu riscul de LBC valorile tensionale mai mari de 130/80 mm Hg, posibil şi în prezenţa unei multitudini de factori de risc comuni pentru boala cardiovasculară şi litiaza biliară.

Rezultatele obţinute capătă o relevanţă în plus dacă amintim că datele marilor studii epidemiologice privind litiaza biliară (NHANES III, MICOL) au obţinut asocieri mai puternice între parametri glucidici, insulinorezistenţă şi perimetrul abdominal mai ales la sexul masculin, în timp ce în lotul nostru de studiu au predominat populaţia feminină şi vârstnicii (5,13).

În concluzie, litiaza biliară colesterolică poate fi integrată în contextul sindromului metabolic, considerat de Grundy un factor de risc cardiovascular „multiplex", putând avea multiple semnificaţii : semnal, factor de agravare a prognosticului prin numeroase complicaţii şi implicaţii terapeutice, ţinând cont de faptul că medicaţia hipolipemiantă, indispensabilă pacientului cu sindrom metabolic, amplifică riscul litogen.

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NOUTĂŢI

TELOCITELE DIN EPICARDUL UMAN

Studii de imunohistochimie şi imunofluorescenţă au demonstrat existenţa telocitelor în epicardul uman. S-a evidenţiat faptul că telocitele apar în epicard, acţionând drept celule doică pentru progenitorii cardiomiocitelor. Sunt caracterizate de prezenţa telopodelor-prelungiri celulare foarte lungi (zecimi de micrometri), foarte subţiri (0,1-0,2 micrometri), cu o configuraţie moniliformă. Telocitele epicardice interconectate creează reţele celulare tridimensionale, conectate cu reţeaua tridimensională a telocitelor miocardice ; în acelaşi timp, stabilesc legături spaţiale strânse cu capilarele sanguine miocardice şi cu terminaţii nervoase. Telocitele eliberează microvezicule sau corpi multiveziculari exocitotici în spaţiul extracelular. Fenotipul telocitelor umane epicardice este similar celui al telocitelor localizate printre cardiomiocitele umane. Rămane de stabilit rolul telocitelor în procesele de regenerare şi de reparare la nivel cardiac, precum şi în procesele patologice induse de inhibiţia lor funcţională sau de variaţia lor numerică. Telocitele, prin proprietăţile lor, ar putea asigura baza terapiei celulare în diverse afecţiuni celulare (Popescu LM, Manole CG, Gherghiceanu M, Ardelean A, Telocytes in human epicardium. *J Cell Mol Med* 2010 ; 14 (8) : 2085-2093).

Carmen Zamfir

DIABETUL ZAHARAT TIP II INDUCE MODIFICĂRI STRUCTURALE ŞI FUNCŢIONALE ALE MEMBRANEI ERITROCITARE

Un studiu prin spectroscopie FTIR demonstrează că valorile mari ale glicemiei pacienţilor cu diabet zaharat tip II afectează membrane eritrocitară. Atât proteinele cât şi lipidele membranei eritrocitare sunt afectate, apărând modificări ale structurii secundare tip b a proteinelor şi restricţii vibraţionale la nivelul interfeţei fosfolipidice. Modificările au apărut doar în cazul grupului de studiu cu valoarea medie a glicemiei de 285mg/dL, comparativ cu al doilea grup de studiu cu o valoare medie a glicemiei de 185mg/dL (Mahmoud SS. The impact of elevated blood glycemic level of patients with type 2 diabetes mellitus on the erythrocyte membrane : FTIR study. *Cell Biochem Biophys* 2010 ; 58 (1) : 45-51).

M. Guţu

SECONDARY HYPERTENSION DUE TO PHEOCHROMOCYTOMA. CASE REPORT

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SECONDARY HYPERTENSION DUE TO PHEOCHROMOCYTOMA. CASE REPORT (Abstract): Pheochromocytoma, a rare endocrine tumor characterized by massive catecholamine secretion is found in less than 1% of patients with hypertension. We report the case of a 40-year-old patient who presented to the VIth Medical Clinic of the Iaşi Rehabilitation Hospital with discrete digestive and anxiety symptoms, normal blood pressure, without orthostatic hypotension and sudden paroxysms. The diagnosis was slightly late, with the random detection of clinical and biological changes. Laboratory tests detected a right adrenal tumor and twice the normal plasma and urinary metanephrine and normetanephrine levels confirmed the imaging diagnosis. Surgery and careful postoperative monitoring yearly, possibly for life, are key elements in the management of pheochromocytoma. Prior to surgery, the patient required treatment with alpha-blockers and nitrates to maintain normal blood pressure levels. **Keywords**: SECONDARY HYPERTENSION, PHEOCHROMOCYTOMA, ALPHA-BLOCKERS, SURGICAL TREATMENT.

Secondary hypertension (SH) is a relatively uncommon condition, accounting for about 5% of all potentially reversible hypertensive patients. The most common causes of SH are renovascular disease, aortic coarctation, pheochromocytoma, primary hyperaldosteronism, thyroid dysfunction and Cushing's syndrome. Of the causes of SH, more than half have endocrine causes (1, 2). Hyperaldosteronism is the major endocrine disorder that causes high blood pressure, followed by pheochromocytoma and Cushing's syndrome. When a patient is found to have high blood pressure, a comprehensive history and a complete physical examination are essential for detecting a possible cause of hypertension. Depending on the findings, additional screening and diagnostic studies may be required. Hypertension is a major cardiovascular risk factor with a continuously increasing incidence (3).

Pheochromocytoma is a rare endocrine tumor (less than 1% of patients with hypertension) of neuroectodermal origin, located in the medulla of the adrenal gland, uni- or bilateral, characterized by massive catecholamine secretion: adrenaline / epinephrine, noradrenaline / norepinephrine, dopamine, especially in people aged 40 to 50 years (4).

Despite technical progress in modern diagnostic methods and treatments for pheochromocytoma, early examination for the presence of this tumor remains the key for a best possible outcome in these patients. Timely diagnosis and appropriate treatment can prevent a wide variety of potentially

catastrophic cardiovascular complications (5). The effect on the cardiovascular system is mediated by membrane type α and β receptors, expressed by sustained elevated blood pressure or hypertensive crisis, accompanied by nonspecific symptoms such as pain, nausea, abdominal pain, coronary pain, dyspnea, visual disturbances, dizziness, tremor, anxiety. Differential diagnosis with migraine, panic attack, acute hypoglycemia, acute coronary artery disease, use of sympathomimetics, cocaine is essential (6). Modern biochemical tests should include tests that offer the best diagnostic performance available. In order to minimize the false positive test results, particular attention should be paid to pre-analytical and sampling conditions (7). Postoperatively, patients should be followed-up yearly for at least 10 years by measurements of plasma or urinary metanephrine for the timely detection of recurrent or metastatic disease. Patients at high risk for recurrence or metastatic disease (young age, multiple or large tumors, genetic background) should be followed-up lifelong (8).

CASE REPORT

A 40-year-old man residing in an urban area, approximately one-month smoke-free presented to our clinic complaining of epigastralgia, profuse sweating, nervousness, dizziness, headache, weight loss (7 kg in one year), symptoms that occurred 6 months earlier. Objective physical assessment revealed moderately influenced general health status, afebrile, no changes in respiratory system, tachycardia (100/minute), rhythmic heart sounds, no heart murmurs, normal blood pressure (120/80 mmHg), slight sensitivity to palpation in the right hypochondrium, bilateral nystagmus. Neurological consultation did not reveal any focal signs, nystagmus being considered congenital. From the biological point of view, hematologic profile, renal function, liver function and blood electrolytes were within the normal range. Of the investigated tumor markers increased levels (8.58 ng/ml) were found only for the carcinoembryonic antigen. Endocrine assessment revealed normal thyroid hormone levels and thyroid ultrasound normal plasma ACTH and cortisol levels. Abdominal ultrasound revealed enlarged right adrenal gland (78/73/73 mm) with a non-homogeneous structure transonic area inside and Doppler signal present, mass compressing the vena cava and coming in contact with the portal vein (fig. 1).

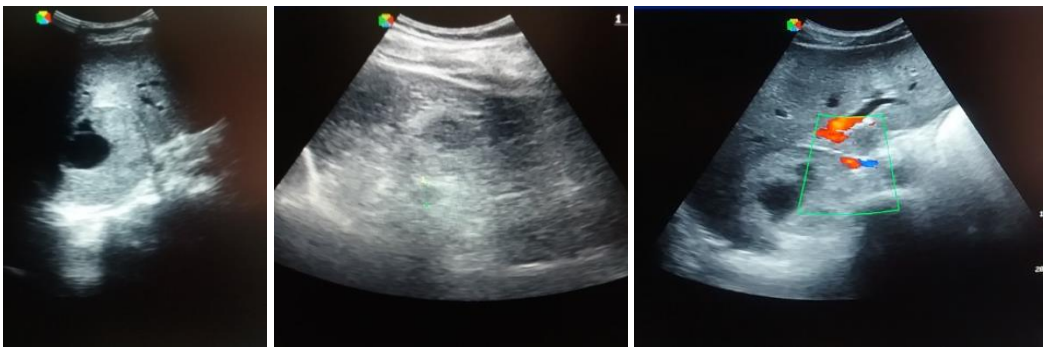

Fig. 1. Abdominal-pelvic ultrasound - enlarged adrenal gland with a non-homogeneous structure with transonic areas inside and present Doppler signal

As to the biochemical diagnosis of the adrenal gland tumor, the measurement of plasma and urinary metanephrines and normetanephrines (catecholamine metabolites)

is the most sensitive method (9). A firm diagnosis was made based on levels exceeding twice the upper limit of normal. Determination of urinary vanillylmandelic acid has currently a rather historical value.

Electrocardiogram on admission showed sinus rhythm, 78 beats/minute, without significant morphological changes. Echocardiography did not reveal appearance of catecholamine cardiomyopathy. The patient was monitored with ABPM, the following being recorded: systolic blood pressure between 106 and 149 mmHg and diastolic pressure between 54 and 99 mmHg, with a maximum heart rate of 124 beats/minute at noon; heart rate ranged from 71 to 124/minute.

In making a correct diagnosis it was necessary to complete the radiological investigations with an abdominal computed tomography. In the upper abdomen, a bulky right adrenal tumor was described, with no clear-cut cleavage with the posteroinferior surface of the liver, with vascular elements of the liver and right renal hilum and inferior vena cava, respectively.

DISCUSSION

By corroborating clinical and laboratory data, the final diagnosis was pheochromocytoma (based both on ultrasound and computed tomography findings and high levels of catecholamine metabolites). Useful for this purpose would have been serum chromogranin A measurement and 1 mg dexamethasone suppression test with cortisol sampling, considering the normal fasting ACTH and cortisol levels, but they could not be performed.

In the presented case differential diagnosis was necessary given the unusual onset and course. Of the included differential diagnoses we mention the following:

- Hyperthyroidism: thyrotoxicosis with intense sweating, weight loss, tachycardia, irritability, nervousness; counter-arguments - euthyroid status, thyroid ultrasound without pathological changes, a number of symptoms of thyrotoxicosis overlap with those caused by pheochromocytoma;
- Angina pectoris was included due to epigastralgia, taking into account the risk factors: smoker with a slight tendency to hypercholesterolemia (total cholesterol 220 mg/dL), presenting sinus tachycardia; counter-argument – electrocardiogram without terminal phase changes (broad T wave but <1/2 R wave) in crisis, normal thoracic radiography, echocardiography without changes in left ventricular kinetics;
- Insulinoma: adrenergic symptoms - weakness, excessive sweating, tachycardia; counterargument: normal blood glucose (82-87 mg%);
- Anxiety disorders (panic attacks) (10).

When diagnosing a patient with pheochromocytoma, the only curative treatment is laparoscopic surgical removal. Surgical intervention is much riskier than in the case of any other non-catecholamine-secreting tumor of similar size. The goal is to destroy the source of catecholamines and to neutralize the effects of their excess.

Extremely important is preoperative antihypertensive medication, initiated about 1-2 weeks before surgery. The treatment of choice in our case was alpha-blockers (prazosin, phentolamine). Calcium channel blockers and nitrates can also be used, preventing coronary spasm and catecholamine-induced myocarditis. Note that the isolated use of β-blockers may induce tensional paroxysm because of the loss of the vasodilatory compensatory effect induced by the stimulation of vascular β-receptors. Thus, β blockers are not used in case of SH onset if an endocrine etiology is suspected (11).

In our case, the patient received both nonpharmacological (volemic repletion) and

pharmacological treatment with alpha-blocker (Cardura (Doxazosin) 1 mg/day), monitoring blood pressure and heart rate to prevent possible incidents that might have occurred intraoperatively. The evolution during hospital stay was good, constant, with normal, repeatedly measured blood pressure levels, including the ones on the ABPM monitoring.

CONCLUSIONS

The particularity of the case is given, first of all, by the unusual onset symptoms in a young individual with constantly normal blood pressure, without orthostatic hypotension and sudden paroxysms. The diagnosis was slightly late, with the random detection of clinical and biological changes. The associated symptoms were headache, profuse sweating, tachycardia, anxiety, hyperexcitability, abdominal pain, weight loss. Also, an important aspect of this case was the absence of complications, namely hypertensive retinopathy, hypertensive nephropathy, stroke, hypertensive encephalopathy, acute pulmonary edema, catecholamine cardiomyopathy, hypotension and shock, intratumorally hemorrhage or aortic dissection aneurysm.

Surgical treatment and careful yearly, possibly lifetime postoperative follow-up are key elements in the management of pheochromocytoma. In their absence, the prognosis is very poor. It is important to note that the endocrine etiology of SH should be considered in case of treatment resistance.

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THERAPEUTIC ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY FOR CHOLEDOCHOLITHIASIS IN PATIENTS 80 YEARS OF AGE AND OLDER: EFFICACY AND SAFETY

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THERAPEUTIC ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY FOR CHOLEDOCHOLITHIASIS IN PATIENTS 80 YEARS OF AGE AND OLDER: EFFICACY AND SAFETY (Abstract): Choledocholithiasis is common in octogenarians and open surgery for its treatment has high operative risks. Thus, therapeutic endoscopic retrograde cholangiopancreatography (ERCP) has become the method of choice for management of such patients. The aim of this study is to evaluate the efficacy and safety of therapeutic ERCP for choledocholithiasis in octogenarians. **Patients and methods:** Data of 2325 patients who underwent therapeutic ERCP for choledocholithiasis from January 2014 to December 2018 was reviewed. Out of these, 222 patients were 80 years or older (study group) and the assessment of efficacy and safety of therapeutic ERCP was compared to that of 260 patients aged 51-60 (control group) randomly selected during the same period. Patient demographics, laboratory parameters, complications of the procedure, as well as post ERCP outcome were carefully reviewed in both groups. **Results:** The elderly group had a significantly higher incidence of comorbidities that the younger group (85.6% *vs.* 68.5%). Common bile duct cannulation was successful in approximately 84% of patients in both groups. The rate of ERCP-related complications was different in the two groups. The rates of pancreatitis particularly were significantly lower in the elderly compared to the younger patients (2.7% *vs.* 9.2% p=0.037). Procedure related mortality was zero in both age groups. Furthermore, successful stone extraction was not significantly different between the two age groups. **Conclusions:** Therapeutic ERCP for choledocholithiasis is an effective and safe procedure in patients 80 years of age and older, comparable to its use in those younger than 60 years of age.
Keywords: ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATO-GRAPHY, CHOLEDOCHOLITHIASIS, EFFICACY, SAFETY.

Life expectation has increased over the last 2-3 decades worldwide, and consequently, the number of elderly population is growing steadily (1,2). With advancing age, the risk of choledocholithiasis is increased, and complications of this disorder have led to a high mortality rate (3, 4). Moreover, surgery for its treatment is characterized by high surgical morbidity and mortality rates (5, 6).

Endoscopic retrograde cholangiopancreatography (ERCP) is one of the most important advances in the treatment of choledocholithiasis and it is now recognized as the first choice and gold standard procedure for the therapy of choledocholithiasis (7, 8). It is therefore understandable why therapeutic ERCP procedures are increasingly required in elderly patients with choledocholithiasis. However, therapeutic ERCP remains the riskiest routinely used endoscopic procedure, associated with several complications such as pancreatitis, bleeding, perforation and overall post-procedural mortality which are expected to have a higher incidence in elderly patients because of multiple and severe comorbidities (1, 8). Nevertheless, several studies reported the safety and efficacy of therapeutic ERCP in patients age 80 years and older diagnosed with choledocholithiasis, with a success rate and adverse events comparable to those found in younger patients (2, 4, 9-11).

The aim of this study was to evaluate the efficacy and safety of therapeutic ERCP in patients aged 80 years and older with choledocholithiasis.

PATIENTS AND METHODS

Patients

Between January 2012 and December 2018, a total of 2,325 patients underwent therapeutic ERCP for choledocholithiasis at the Institute of Gastroenterology and Hepatology, "Sf. Spiridon" County Clinical Emergency Hospital of Iasi, Romania. Patients were divided in two groups: octogenarians – the study group (defined as all patients aged 80 years or older) consisting in 222 patients, and a population of younger patients – the control group (a randomly selected cohort of patients aged between 51 and 60 years old, who underwent therapeutic ERCP during the same period) consisting in 260 patients.

Entry criteria included adult patients undergoing therapeutic ERCP for choledocholithiasis. Exclusion criteria were presence of ongoing or past acute pancreatitis, inability to perform proper follow-up, presence of additional risk factors for pancreatitis, and bleeding, history of previous ERCP, the need for reintervention or surgery during the same admission.

All past medical records together with periprocedural clinical and laboratory parameters, imagistic findings (ultrasonography and/or magnetic resonance cholangiopancreatography (MRCP)) and endoscopy reports were analyzed retrospectively. All patients were hospitalized and underwent before therapeutic ERCP routine hematologic and biochemistry testing, abdominal ultrasound, abdominal computed tomography or MRCP evaluation. The diagnosis of choledocholithiasis, with or without the presence of concomitant gallstones was confirmed by ultrasonography, MRCP or computed tomography, and all patients were hospitalized for at least 48 hours after therapeutic ERCP to assess the post-procedure complications. All patients signed an informed consent prior to therapeutic ERCP.

Methods

Endoscopic procedures were performed by 3 experienced endoscopists in therapeutic ERCP, using a standard duodenoscopes (TJF 160 Series Olympus, Japan). All patients were given topical pharyngeal anesthesia with lidocaine 10% spray, and endoscopic procedures were performed under sedation with intravenous midazolam and/or propofol (either alone or in combination) administered by a specialist in anesthesiology. Blood pressure, heart rate, ECG, and peripheral oxygen saturation (pulse oxime-

try) were continuously monitored. All patients were administered oxygen through a nasal cannula during the endoscopic procedure. Most frequently the procedures included endoscopic sphincterotomy alone, balloon dilatation was used only in few cases. Stone extraction was attempted by means of a balloon catheter, and/or Dormia basket, and only in difficult cases by endoscopic mechanical lithotripsy. At the end of each procedure a NSAID suppository (diclofenac 100 mg) has been administered to all patients.

Data collected included age, gender, significant comorbidities (classified as cardiovascular (e.g., hypertension, ischemic heart disease), metabolic (e.g., diabetes mellitus), pulmonary (e.g., chronic obstructive pulmonary), renal or cerebrovascular diseases), complete laboratory panels, endoscopic findings (anatomy of the papilla, periampullary diverticulum, cannulation success rate, stone clearance rate), ERCP-related complications such as post-ERCP pancreatitis, bleeding, cholangitis and perforation, all defined according to published criteria by Cotton *et al*., according to the lexicon for endoscopic adverse evolutions published by Cotton *et al*.; early mortality was defined within 30 days after the procedure.

Statistical analysis was performed with the *SPSS 16.0* software. *Chi-Square* (X^2) and p values were calculated and subsequently Odds Ratios (OR) were issued and compared for specific events.

TABLE I

Baseline therapeutic outcomes and ERCP – related complications

Parameter	Age >= 80 years		Age between 51–60 years		X^2 / p	OR		
	n	%	n	%		Value	95% confidence level	
							lower	upper
Concomitant gallbladder stones	40	36.0	59	45.4	2.162 / 0.141	.678	.403	1.139
Previous cholecystectomy	33	29.7	56	43.1	4.580 / .032*	.559	.327	.955
Concomitant chronic diseases	95	85.6	89	68.5	9.723 / .002*	2.735	1.434	5.219
Periampullary diverticule	28	25.2	19	14.6	4.293 / .038*	1.971	1.031	3.769
Failed cannulation	18	16.2	21	16.2	.000 /.990	1.005	.505	1.998
Stone clearance	79	71.2	86	66.2	.698 /.403	1.263	.730	2.186
Acute pancreatitis	3	2.7	12	9.2	4.372 /.037*	.273	.075	.994
Bleeding	0	0.0	8	6.2	7.065 /.008			
Acute cholangitis	0	0.0	4	3.1	3.473 /.062			

*Statistically significant data

RESULTS

The mean age of the study group was 84 (range: 80-98 years old) and the number of female patients was significantly higher in both groups. Baseline therapeutic outcomes and ERCP-related complication rates of the studied groups are shown in first table. Presence of concomitant gallbladder stones

was non-significantly higher in the control group, but history of previous cholecystectomy was significantly less frequent in octogenarians, compared to younger patients (33% *vs.* 56%, p = .032). More than two thirds of elderly patients had concomitant chronic diseases (mainly cardiovascular), significantly more compared to only one third in control group (85.6% *vs.* 68.5%, p = .002). Periampullary diverticula were significantly more frequent in the elderly group (25.2% *vs.* 14.6%, p = .038). There was no significant difference between the two age groups regarding rate of cannulation success (83.8% in both groups). Although not statistically significant, complete stone clearance rates were higher in the study group (71.2% *vs.* 66.2%).

Overall, the ERCP-related complications were less frequent in control group than in study group (2.7% *vs.* 18.5%, NS), as shown in first table. The most common complication was post-ERCP pancreatitis. Interestingly, there were only three cases of post-ERCP pancreatitis in elderly patients. Post-sphincterotomy bleeding occurred in no patient in the study group but in 5.4% of the control patients. In all cases it was easily managed endoscopically by injection therapy. There was also no perforation in both groups. Post-ERCP cholangitis occurred in no patient in the study group and in 2.7% of the control patients. There was no death related to the procedure in both age groups.

DISCUSSION

Life expectation has increased over the last 2-3 decades worldwide and subsequently, the number of elderly population is growing steadily (1-4). With advancing age, the risk of choledocholithiasis is also increased (1, 2, 4, 9). Clearly, the need for ERCP in the management of choledocholithiasis will become age dependent. However, therapeutic ERCP is an invasive method, associated with complications, which may have a higher incidence in elderly patient because of the presence of multiple comorbidities (1, 8, 13, 14). Nevertheless, several studies have reported that therapeutic ERCP for choledocholithiasis is comparable in terms of efficacy and safety between elderly (≥ 65 years of age) patients and younger ones (< 65 years of age) (1, 3). It should be underlined that "elderly" patients have been defined in most studies as those patients aged 65 years or older and only few studies were conducted on patients over 80 years old (4, 9, 10, 12).

The results of our study show that therapeutic ERCP is a successful and safe therapy method for choledocholithiasis in octogenarians, even safer than that performed in younger patients. Similar results were obtained in other previous studies that included patients aged 80 years or older (1, 4, 9, 10, 12, 13). Most studies indicate that despite the significantly greater number of comorbidities in octogenarians, the procedure-related complications rates were not significantly different compared to the younger group. Moreover, in the current study procedure-related complication rates were lower in the octogenarian group than in the younger group. Not significantly different complication rates between the age groups were reported in several other studies (2, 4, 9, 10, 12, 13, 15).

Comparable cannulation rates between the age groups are reported in many of the similar studies to date (2, 15, 16, 17). Despite an important incidence of juxtapapillary diverticula, not only cannulation rates but also complete bile duct clearance are

similar and consistent with widely reported rates in both groups.

Complication rates in our study are comparable to those reported in the literature both in octogenarians (2, 16-19) or in younger patients. Post-ERCP pancreatitis (PEP) is the most common complication, with reported prevalence ranging from 1.3% to 8% (1,2,19,20). Interestingly, Finkelmeier *et al*. (21) found PEP rate is significantly lower in patients above 80 years old. Recently, Han *et al*. (1) reported that PEP occurred in only 1.3% of octogenarians compared to 2.9% of youngers, probably due to changes in pancreatic histology leading to pancreatic atrophy with age that has been traditionally cited (22, 23). As stated, our study shows that the rate of PEP was lower in octogenarians (4.1%) than in youngers (12.3%) and all were mild disease, a finding reported in other studies (2) and explained by a decline in pancreatic exocrine function with aging (24). Therefore, age is considered by some authors to be a protective factor for the development of PEP (9). To be stressed is that in our study all PEP, independently on the age group were mild cases that did not prolong the hospitalization period more than 48 hours. Bleeding incidence was also in line with those reported in literature from 0.6% to over 2% (1-3), with a smaller prevalence in the elderly group. Similar results were obtained also regarding the post-ERCP cholangitis rates which were identified in isolated cases, the same as in current studies (1, 25). Consequently, as part of the literature suggests, perforation rates and procedure related mortality are of a scarce incidence, reported only in isolated cases (15). Some other previously published studies reported procedure-related mortality in the elderly from 0.6% to 1.6% (19).

CONCLUSIONS

Our study has several limitations: the single center distribution, it's retrospective methodology and the fact that the efficacy and safety of therapeutic ERCP for choledocholithiasis were compared only between two age groups (80 years or older and 51-60 years old), while patients ≤ 50 years and those between 61-79 years were not included. Despite these potential down sides, this study shows clearly that therapeutic ERCP for choledocholithiasis can be successfully and safely performed on patients aged 80 years or older.

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THERAPEUTIC RESPONSE TO PEG-INTERFERON PLUS RIBAVIRIN IN CHRONIC HEPATITIS C

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THERAPEUTIC RESPONSE TO PEG-INTERFERON PLUS RIBAVIRIN IN CHRONIC HEPATITIS C (Abstract): **Aim** To evaluate the epidemiological, clinical-biological, therapeutic and evolutive characteristics of HCV-infected patients admitted in Hospital of Infectious Disease "Sf. Parascheva" Iaşi between 2012-2017. **Material and methods**: 408 patients received peginterferon alfa-2a/alfa-2b plus ribavirin for 48 weeks. Clinical and laboratory data were assessed at baseline, week 4, 12, 24, 48 (end of treatment, EOT), and 24 weeks after therapy (sustained virological response, SVR). **Results**: The mean age of patients was 48.78±11.30 years old (range 18-75 years), most patients being female (63.5%). Ten (2.5%) patients had co-infection with hepatitis B virus (HBV), and in 14 (2.1%) IL28B polymorphism was determined. Of the 408 patients receiving PEG-IFN α and ribavirin, 35% had SVR, but 22.8% had relapses and 36.3% were non-responders. The most common side effects were leukopenia (26.2%), followed by thrombocytopenia (21.4%), anemia (17.9%) and neutropenia (12.6%). **Conclusions**: The SVR rate in the studied group was only 35%, this fact demonstrates the need for new antiviral therapies, especially in patients with advanced fibrosis. **Keywords:** HCV, PEG-INTERFERON, RIBAVIRIN.

Viral hepatitis is important challenge in infectious pathology due to high incidence of the acute infections, risk to develop chronic infections and severity of these infections and also costs associated to the disease.

Chronic hepatitis C viral (HCV) infection is the main cause of hepatic cirrhosis, hepatocellular carcinoma and liver-related mortality in developed countries. Even if the disease has a major importance on morbidity and mortality, its natural history of the disease is not completely known. Chronic hepatitis C is a slowly progressive infection characterized by persistent hepatic inflammation which develops cirrhosis in 10-20% cases in 20-30 years (1).

HCV is an important cause of chronic liver disease both in Romania and worldwide. There has long been evidence that hepatitis C can lead to persistent infection in a large proportion of infected individuals, and may develop toward chronicization, cirrhosis, and hepatocellular carcinoma (2).

The most efficient initial therapy in HCV infected patients was interferon alpha-2b plus ribavirin for 24 or 48 weeks. Addition of one molecule of polyethylene glycol to interferon lead to a new biologically active molecule with longer half -life which allowed one weekly administration (3).

Peginterferon alpha plus ribavirin were the gold standard of HCV infection treatment for more than one decade, and its effectiveness was carefully monitored using accurate and sensitive techniques of molecular biology.

MATERIAL AND METHODS

The aims of our study were to evaluate the epidemiological, clinical-biological, therapeutic and evolutive characteristics of HCV-infected patients admitted in Hospital of Infectious Disease "Sf. Parascheva" from Iaşi between 2012-2017.

Ethical approval. The study was carried out according to Declaration of Helsinki 2008, and the International Ethical Guidelines. The study was approved by the Ethics Committee of the Infectious Diseases Hospital "Sf. Parascheva" from Iasi.

Patients and treatment. Eligible patients were included according to the criteria established by Romanian National protocol approved for HCV infection treatment, as follows: treatment naïve or experienced patients aged over 18 years with HCV genotype 1 infection, liver enzymes increased or normal, detectable RNA hepatitis C virus, liver biopsy, Fibromax test results: A ≥1, F ≥1 and /or S≥1 and Fibroscan results > 1.

Exclusion criteria were decompensated liver cirrhosis, mental illness, decompensated diabetes, neurological disease, autoimmune disease, coronary ischemic disease or uncontrolled severe heart failure, severe, uncontrolled respiratory disease.

Patients with HCV genotype 1 infection received 48 weeks of treatment with one of the following regimens: peginterferon alfa-2a at a dose of 180 μg per week plus ribavirin at a dose of 1000 to 1200 mg per day, or peginterferon alfa-2b at a standard dose of 1.5 μg per kilogram of body weight per week plus ribavirin at a dose of 1000 to 1200 mg per day. Treatment administration was in accordance to the approved Summary of Product Characteristics.

Patients evaluation. Clinical data related to gender, age, treatment history and comorbidities were collected at baseline. According to protocol, laboratory data included HCV RNA level, determination of genotype and sub genotype, alanine aminotransferases, HBsAg, anti-HIV antibodies, hemoglobin, platelets, leukocytes, neutrophils and IL28B polymorph/ism. Serum HCV RNA levels were measured with the Cepheid GeneXpert system (Cepheid, Sunnyvale, CA) with a lower limit of quantification and detection of 15 IU/mL. The degree of liver fibrosis was evaluated based on Fibromax / Fibroscan test, or liver biopsy.

HCV RNA was measured before treatment initiation, after 4 weeks of therapy and was repeated after 12 weeks in case of detectable viremia at 4 weeks. Measurement was repeated at 24 - weeks after treatment in cases when negativity test was not obtained, but a decrease of ≥ 2 log10 HCV RNA was found after 12 weeks of treatment, at end - of treatment (EOT) and 24 weeks after EOT. Biological measurements were performed at baseline, at week 4, 12 and 24 of treatment.

Outcomes. Effectiveness of therapy was assessed by the percentage of patients achieving sustained virologic response (SVR, defined as HCV RNA below the limit of detection 24 weeks after the EOT). According to the protocol, therapeutic failure was defined as detectable HCV RNA at

EOT. Relapse was considered detectable HCV RNA at SVR in patients with undetectable viremia at EOT.

Statistical analysis. The data were centralized in a *SPSS 18.0* database and processed with the statistical functions to which they are expected, at the significance level of 95%. Continuous variables were reported as a mean ± standard deviation, while categorical variables are reported as frequencies and percentages.

RESULTS

Patient characteristics

Between 2012 and 2017, 408 patients with chronic HCV infection were treated with peginterferon alfa-2a/alfa-2b plus ribavirin in our clinic. Most cases were observed in 2012 (243 cases), subsequently, a downward trend was observed, with only 22 cases in 2017 (fig. 1). The mean age of patients was 48.78±11.30 years (range 18-75 years), most patients being female (63.5%).

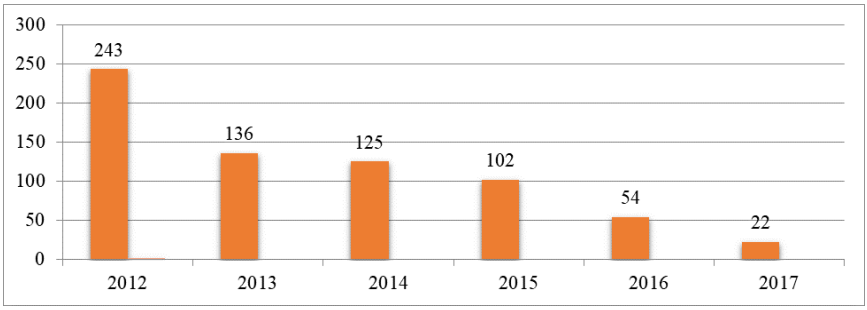


Fig. 1. Distribution of cases of chronic HCV between 2012-2017

Ten (2.5%) patients had co-infection with hepatitis B virus (HBV), and in 14 (2.1%) IL28B polymorphism was determined, of which 2 (14.3%) patients had CC genotype, 11 (78.6%) CT genotype and one patient (7.1%) had TT genotype.

Most of the patients had a mild or moderate liver fibrosis (F1 - 15.8%, F2 - 35.2%, F3 - 23.6%), and 20.7% had advanced fibrosis (F4) (fig. 2).

Of the 408 patients receiving PEG-IFN α and ribavirin, 35% had SVR, but 22.8% had relapses and 36.3% were non-responders (fig. 3).

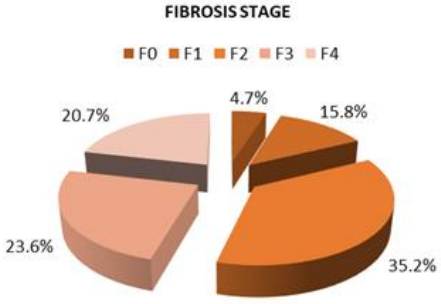


Fig. 2. Group structure according to the fibrosis stage

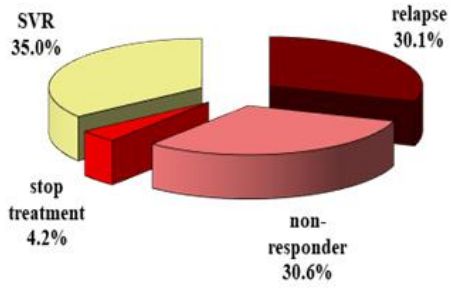


Fig. 3. Distribution of patients according to the post-treatment evolution

More than half of the patients (53%) with hepatic cirrhosis were non-responders, 13.6% had relapses and only 30.3% had an SVR (p = 0.019) (fig. 4).

Of the 408 patients who received treatment, 10 (2.5%) had co-infection with HBV, and 70% of them were non-responders and 20% had relapses (p = 0.05).

Longitudinal evaluation of biological markers

Analyzing the mean level of **HCV RNA** according to the post-treatment evolution, an initially significantly higher mean level was found in the non-responders and those with relapse, compared to the one registered in the patients with SVR (2,345,520 vs 1,632,242 ; p = 0.05) (fig. 5).

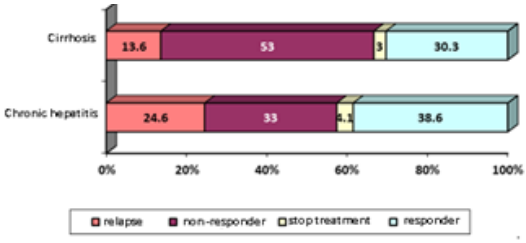


Fig. 3. Distribution of patients according to the diagnosis and evolution

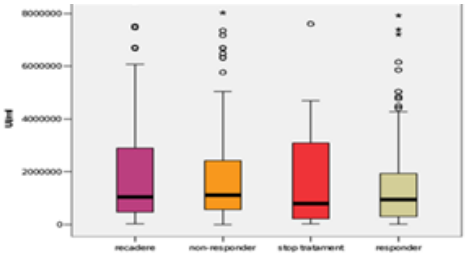
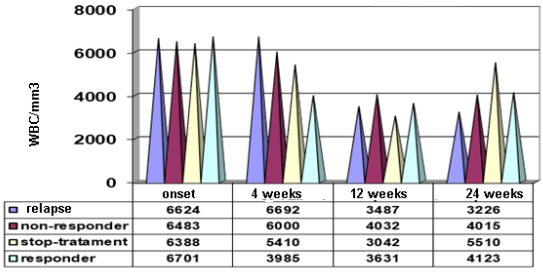


Fig. 5. Mean HCV-RNA values at baseline depending on the post-treatment evolution

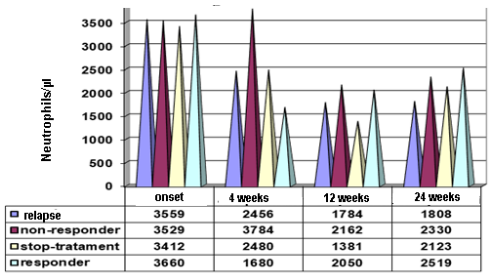
At the 12-week evaluation, the mean **leukocytes** values remained in a decreasing trend in all the analyzed groups (p = 0.001) (fig. 6). At week 4, 12 and 24 after the initiation of the treatment, we observed a significant decrease of the mean values of **neutrophils** compared to baseline (fig. 7).

At 4, 12 and 24 weeks after initiation of treatment, mean **hemoglobin** levels decreased significantly compared to baseline (fig. 8). At 24 weeks after treatment initiation, mean **platelet** counts decreased significantly from baseline (p = 0.001), but no significant differences were observed depending on treatment response (p = 0.159) (fig. 9).



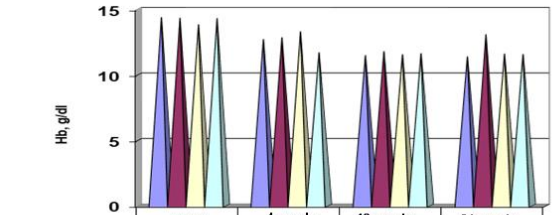
	onset	4 weeks	12 weeks	24 weeks
relapse	6624	6692	3487	3226
non-responder	6483	6000	4032	4015
stop-tratament	6388	5410	3042	5510
responder	6701	3985	3631	4123

Fig. 6. Evolution of mean leukocytes values during the study period depending on the treatment response



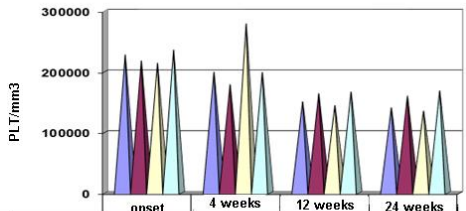
	onset	4 weeks	12 weeks	24 weeks
relapse	3559	2456	1784	1808
non-responder	3529	3784	2162	2330
stop-tratament	3412	2480	1381	2123
responder	3660	1680	2050	2519

Fig. 7. Evolution of mean neutrophils values during the study period depending on the treatment response



	onset	4 weeks	12 weeks	24 weeks
relapse	14.39	12.71	11.48	11.39
non-responder	14.34	12.85	11.79	13.08
stop-tratament	13.84	13.3	11.55	11.6
responder	14.32	11.7	11.63	11.57

Fig. 8. Evolution of mean hemoglobin values during the study period depending on the treatment response



	onset	4 weeks	12 weeks	24 weeks
relapse	227965	199429	150795	140737
non-responder	218029	179000	164101	159884
stop-tratament	214188	279000	144500	135333
responder	235862	199000	166729	168652

Fig. 8. Evolution of mean platelet count values during the study period depending on the treatment response

Side effects

Of the total 408 patients receiving treatment, 17 (4.16%) discontinued the treatment, most due to severe adverse effects but also to poor adherence to the triple therapy (4 of the patients). Of those with severe adverse effects, 1 patient had pulmonary thromboembolism, 2 patients discontinued treatment due to depression, 8 had severe hematological disorders, 1 patient had hepatic decompensation, and one of them developed autoimmune thyroiditis.

Anemia was noted in 17.9% of patients, statistically significant increase in relapses (39.3%) and patients who did not respond to treatment (18.0% cases) ($p = 0.002$). Leukopenia was found in 26.2% of patients, of whom 34.7% had relapses and 20.1% did not respond to treatment ($p = 0.001$). Only 12.6% of the total study group had neutropenia, of which SVR had 37.2% ($p = 0.012$). Thrombocytopenia was observed in 21.4% of patients, of whom 34.9% had relapses and 20.5% did not respond to treatment ($p = 0.009$).

DISCUSSION

In this retrospective study, carried out between 2012-2017 within the Clinical Hospital of Infectious Diseases "Sf. Parascheva" from Iasi, we noticed a decreasing tendency of the number of patients with chronic HCV infection.

According to the National Institute of Public Health of Romania (CNSCBT), the incidence of chronic HCV infection has a downward trend, from 32 new cases diagnosed in 2012 to only 3 cases reported in 2017 (1, 4, 5, 6, 7, 8).

Of the total patients included in the study, 10 (2.5%) of them had co-infection with HBV. The estimated prevalence of HBV-HCV coinfection is 5-20% in AgHBs positive patients and 2-10% in HCV positive patients, but with a significant geographical variation (2).

We have noticed in our study a higher prevalence of chronic HCV infection in women, with a sex ratio of 1.7 women per 1 man. In an epidemiological study of 2851 adult patients regarding the prevalence of viral hepatitis in Romania, Voiculescu *et al.* demonstrated a significantly higher prevalence among women than in male (9).

Histological examination of the liver is an integral part of the evaluation of patients with chronic hepatitis C. Knowing the stage of hepatic fibrosis is essential for the prognosis and decisions regarding antiviral treatment (10). Recent data have shown

that the development of hepatic fibrosis in hepatitis C is multifactorial and many co-factors have been identified to be involved in increasing the individual risk of developing significant fibrosis or cirrhosis. These include age at the time of infection, male sex, alcohol consumption, obesity, insulin resistance, type 2 diabetes, co-infection with HBV or HIV, immunosuppressive therapy and genetic factors (2).

The combination of PEG-IFN α-2a or α-2b and ribavirin has been the standard of care for the treatment of chronic HCV infection and, in many countries, is still the only therapeutic option available, other than conventional IFN. It is still the standard of therapy in Egypt, a country with an extremely high prevalence of HCV (11).

Until 2011, the only standard treatment regimen for HCV genotype 1 was PEG-IFN and ribavirin for 48 weeks, resulting in SVR rates of 40-50% according to randomized clinical trials (12). SVR rates obtained with PEG-IFN and ribavirin are affected by a number of different variables: HCV genotype (non-1 genotypes are associated with higher RVS rates), serum viremia level before initiation of treatment, degrees of fibrosis and inflammatory activity , as well as the host genotype IL28B (CC genotype is associated with higher SVR) (11).

In our study we observed that the difference between the RVS rate in the patients treated with PEG-IFN α-2a and the patients treated with PEG-IFN α-2b was not statistically significant. PEG-IFN α-2a and PEG-IFN α-2b, the two types of IFN available for the treatment of chronic hepatitis C, differ in their pharmacokinetics and chemical properties (13). In the published articles, there are some observations regarding these two drugs; some argue that PEG-IFN α-2a has a better outcome and many say there are no significant differences (14).

In this study we also set out to identify the biochemical and hematological parameters of the patients receiving PEG-IFN plus ribavirin treatment in order to evaluate the effect of the therapy on the number of leukocytes, neutrophils, thrombocytes and serum hemoglobin level. Various changes were observed during 24 weeks of monitoring, and also these changes were very significant.

Assessing the number of leukocytes during 24 weeks of treatment, a significant decrease is observed in the first 12 weeks in all the studied groups. According to the data published by Iliescu *et al*, the number of leukocytes decreases by approximately 20-30% during treatment (15). Neutrophil values also decreased over the 24 weeks, with a more significant decrease after the first 12 weeks of treatment. Neutropenia is one of the most common reasons for changing the dose of IFN. A granulocyte stimulation factor could be used to stabilize the number of neutrophils during PEG-IFN therapy. However, various studies suggest that IFN-induced neutropenia is not generally associated with an increased risk of bacterial infections (16).

There was also a significant decrease in hemoglobin level after 24 weeks of treatment. Treatment with PEG-IFN and ribavirin is associated with a decrease in hemoglobin concentration and anemia. Anemia is the main adverse effect associated with dose reduction or discontinuation of ribavirin, which can significantly affect SVR, especially in patients with genotype 1. Erythropoietin treatment may increase hemoglobin concentration and allow complete adherence to treatment (16).

In our study, there was a significant decrease in platelet counts, especially at 24

weeks of treatment, but no significant differences were observed depending on the response to treatment (p = 0.159). The effect of IFN on bone marrow results in reduction of granulocytes and platelets during treatment. These are usually moderate if the initial number is normal. However, changes in the dose of IFN are required, especially in patients with a low initial number. This limits the use of IFN in patients with advanced cirrhosis who often have low platelets and are also more vulnerable to infection (16).

In the event of adverse reactions during this therapy, timely dose modification or stopping treatment may be life-saving (17). A study of 257 patients with HCV-induced liver cirrhosis concluded that the adverse effects of PEG-IFN and ribavirin therapy, namely, anemia, neutropenia and thrombocytopenia affect 75%, 19.5% and 65% of patients respectively (18).

CONCLUSIONS

In our retrospective study, we noticed a decrease in the incidence of chronic hepatitis with HCV, probably due to the routine screening of blood products. About a quarter of the patients included in the study had hepatic cirrhosis, most of whom were found in those over 50 years of age, so knowledge of the stage of hepatic fibrosis is essential for prognosis and decisions regarding antiviral treatment. The SVR rate in the studied group was only 35%, this fact demonstrates the need for new antiviral therapies, especially in patients with advanced fibrosis.

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NEWS

SGLT-2 INHIBITORS AND CARDIORENAL EFFECTS

Sodium-glucose co-transporter 2 inhibitors (SGLT2i) represents the latest class of hypoglycemic agents used in antidiabetic therapy. These compounds work through a novel mechanism of reducing renal tubular glucose uptake, lowering blood glucose without stimulating insulin release. Since 2008, the Food and Drug Administration has approved the introduction of 4 SGLT2i into therapy, based on studies demonstrating their safety and efficacy: canagliflozin (Invokana®), dapagliflozin (Farxiga®), empagliflozin (Jardiance®) and ertugliflozin (Steglatro®). A fifth SGLT2i, sotagloflozin (Zynquista®) is currently in the latest clinical trials. The cardiovascular outcomes trials have confirmed the cardiovascular and renal clinical benefits of SGLT2i: decreased risk of developing or worsening cardiovascular and renal pathologies in diabetic patients (myocardial infarction, stroke, cardiovascular death or chronic kidney disease). The SGLT2i represents the newest treatment option for type 2 diabetes mellitus with high therapeutic potential (Kluger AY, Tescon KM, Lee AY, *et al.* Class effects of SGLT2 inhibitors on cardiorenal outcomes. *Cardiovasc Diabetol* 2019; 1-13).

ASPECTE CLINICE ŞI EPIDEMIOLOGICE PRIVIND BOLNAVII CU HEPATITE VIRALE ACUTE INTERNAŢI ÎN SECŢIA DE BOLI INFECŢIOASE A SPITALULUI JUDEŢEAN BACĂU, ÎN PERIOADA 1996-2005

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CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF PATIENTS WITH ACUTE VIRAL HEPATITIS IN BACĂU COUNTY, FROM 1996 TO 2005 (Abstract): The purpose of the study is to emphasize the clinical and epidemiological peculiarities of acute viral hepatitis in the last decade. **Material and method**: We studied retrospectively the files of 2014 patients, hospitalized with the diagnosis of acute viral hepatitis in the Bacău County Hospital, from 1996 to 2005. **Results**: We saw that the only type the incidence of which lowered in time was the B-type hepatitis. The A acute hepatitis witnessed two epidemic peaks. The clinical cases of the male young patients prevailed. In 91.6% of cases, the infection source could not be established. The average incubation period was 21 days for viral hepatitis A, 65 for B and 80 for C. Hospitalized anicteric forms represented 2.8% of total. Extended forms were recorded with 3.6% patients (50.9% of them with B virus) and severe forms with 10.3% (among these, 66.7% with B virus). A fulminate evolution was noticed with 0.65% of cases. The global mortality was 0.4% and 0.95% for B type. **Conclusions**: We noticed several peculiarities and changes regarding the clinical and epidemiological aspects of acute viral hepatitis in the last decade. **Key words**: ACUTE VIRAL HEPATITIS, VIRAL HEPATITIS B, CLINICAL MANIFESTATIONS, EPIDEMIOLOGY

ACTUAL STATUS OF RADIOTHERAPY IN LOW-GRADE GLIOMA

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ACTUAL STATUS OF RADIOTHERAPY IN LOW-GRADE GLIOMA (Abstract): Radiotherapy is commonly used in the treatment of brain tumors but using it for low-grade gliomas is not yet standardized. The purpose of this study is to evaluate the survival benefit of radiotherapy for patients with low-grade gliomas. We also tried to find out how radiotherapy influences the functional status of these patients. **Materials and methods:** We performed a retrospective study on 57 patients admitted in the Neurosurgery department of Bucharest Emergency Hospital in a period of 20 years (between January 1st, 1996 and January 1st, 2016)**.** Obtained data was centralized using *Microsoft Excel* and analyzed with *Stata 13*. **Results:** We found that increasing age ($p = 0.02$) and tumors of astrocytical origin ($p = 0.036$) have a higher risk of relapse, whereas radiotherapy increases time to relapse especially when it is administered without delay ($p = 0.0608$). We also found early radiotherapy to be associated with better functional prognosis ($p = 0.0266$). Increasing Karnofsky Performance Status (KPS) at diagnosis was associated with an increased likelihood of malignant transformation. **Conclusions:** Radiotherapy is proven to significantly increase time to progression for low-grade glioma patients, especially when administered early on. Radiotherapy does not seem to negatively influence the functional status of the patients, but we did not analyze the neurocognitive status of the patients and do not have data regarding short or long-term evolution of this aspect. **Keywords:** LOW-GRADE GLIOMA, RADIOTHERAPY, OUTCOME, SURVIVAL

Low-grade gliomas are defined by the World Health Organization (WHO) as being grade I or II primary brain tumors. They are slow-growing and affect mainly the young adult population in the fourth decade of life. Because patients with these tumors have a long survival rate, a great controversy has risen over the years regarding the best treatment option. Management considered for these tumors range from surgery, radiotherapy, chemotherapy and even "wait and see" approach. Because of the long survival of these patients, one must carefully weigh the impact of treatment on the quality of life of different therapeutic options (1, 2, 3, 4).

OBJECTIVES

In this article we assess the radiotherapeutic option in the treatment of low-grade glioma, aiming to see the impact of radiotherapy on survival of patients, progression of tumor, optimal timing of radiotherapy (early *versus* late) and other variables that

influence survival: tumor site (eloquent versus non-eloquent), histology type (oligodendroglioma / mixed oligo-dominant *versus* astrocytoma), age, estimated tumor volume and Karnofsky performance score (KPS) at diagnosis.

MATERIAL AND METHODS

We performed a retrospective study of 57 patients who were admitted in the Neurosurgical Department of Bucharest Emergency University Hospital between 1996 and 2016. The inclusion criteria were supratentorial and confirmed histopathological diagnosis of low-grade glioma (grade II WHO comprising astrocytoma, mixed oligoastrocytoma, oligodendroglioma), age between 18-70 years old, with no other significant clinical conditions. Mean age was 40 years with a slight male predominance (32 male patients *versus* 25 female patients).

We did not admit in this study patients with prior radiation therapy, chemotherapy or brain surgery for other pathologies. Of the 57 patients, 27 had postoperative radiotherapy either early (18) or delayed (9) by fractioning 54 Gy during a time-period of 6 weeks. Early radiotherapy was defined as intervention as soon as possible after surgery, delayed radiation therapy being deferred until tumor progression was observed. Patients were diagnosed either by biopsy, subtotal resection or gross total resection (as observed by intraoperative inspection and immediate postoperative imaging).

Our primary endpoints were death and tumor progression/recurrence. Secondary endpoints for analysis of functional autonomy, survival after progression and time to malignant transformation were used: Karnofsky performance score (KPS) < 70, death after relapse and malignant transformation, respectively.

The patient database was obtained from the Neurosurgical Department of Bucharest Emergency University Hospital. Patients' records were studied and collected data was analyzed using *Microsoft Excel* and *Stata 13*. We used Kaplan-Meier survival curves and tests of equality (Log rank) for categorical variables (radiotherapy treatment status, tumor site, histology) and the Chi-squared test for continuous variables (age, Karnofsky performance score, tumor volume) to explore whether or not to include the co-variate in the final model. Co-variates who had a *p* value of more than 0.2 after univariate analysis were not included in final Cox models for proportional hazards, except for radiotherapy which we included in our final models no matter the result on univariate analysis. We compared patients who received radiotherapy with those who haven't, but also early radiotherapy with late radiotherapy patients.

RESULTS

We did not find any significant difference when analyzing overall survival by radiotherapy status (p=0,6911). The results for the CPHM based on radiotherapy and KPS were also not significant.

When analyzing overall survival for the radiotherapy group we found that histology type had a significant influence (p=0.0219). Radiotherapy (p=0.0706) and tumor site were added to the final CPHM, but no other significant results were obtained.

On univariate analysis only histology (p=0.0472) proved significant influence on progression-free survival (PFS) for the entire group. The final model including site, age and radiotherapy status showed that the three significantly influence PFS (tab. I).

TABLE I

Progression-free survival predictors analyzed using Cox proportional hazards models. Hazard ratios show us that increasing age and astrocytoma histology have higher risk of relapse while eloquent area and radiotherapy treatment are associated with prolonged PFS.

t	Hazard Ratio	Std. Err.	p > z	[95% Conf. Interval]	
AGE AT DIAGNOSIS	1.043723	.0191919	**0.020**	1.006777	1.082024
SITE	.227095	.1494608	**0.024**	.0625167	.8249337
RADIOTHERAPY	.700632	.3552927	**0.483**	.2593241	1.892941
HISTOLOGY	4.181093	2.855501	**0.036**	1.096369	15.94493

When observing only the radiotherapy group, there was no significant predictor on univariate analysis. Radiotherapy timing (fig. 1), tumor site and KPS were selected for the multiple regression where again no significant result appeared.

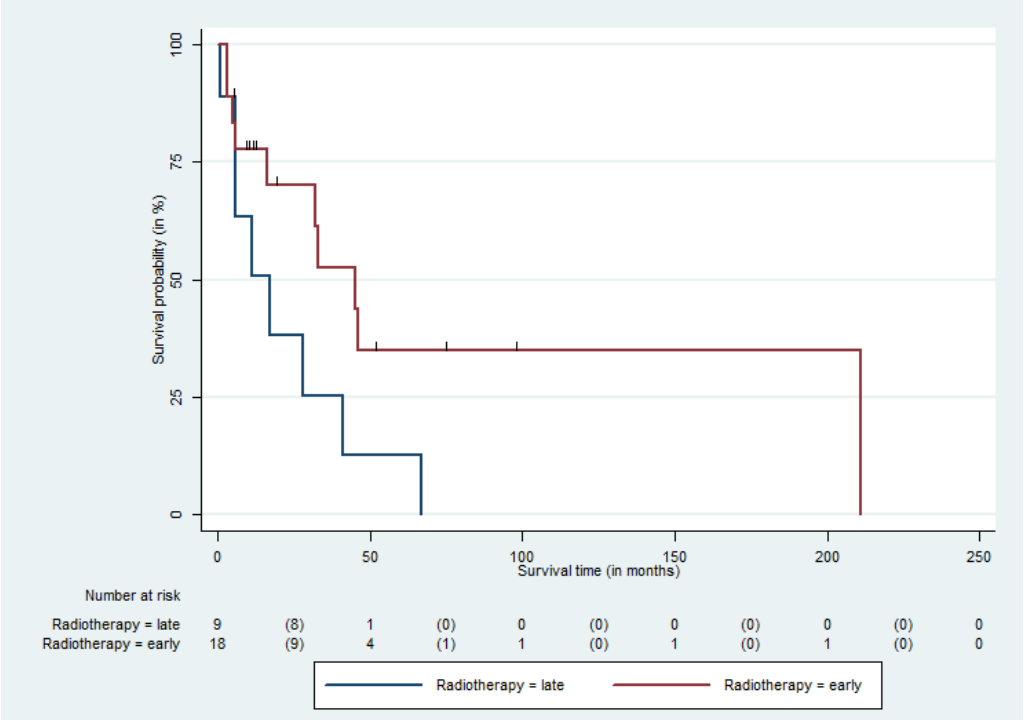

Fig. 1. Kaplan-Meier survival curves showing a longer progression free survival for patients treated with early radiotherapy (in red); numbers marked at the bottom of the table are patients at risk with failed events between parentheses. Vertical axis is the probability of survival; horizontal axis is the survival time in months. Log-rank = 0.0608.

No predictor for functional independent survival was identified when performing univariate analysis or multiple regression for the whole group. In the radiotherapy group, we found early radiotherapy to be significantly associated with greater functional independent survival (fig. 2). Multiple regression based on site and radiotherapy didn't show any significant co-variates.

There were no registered deaths of patients with relapses, so no survival analysis could be performed regarding survival after progression.

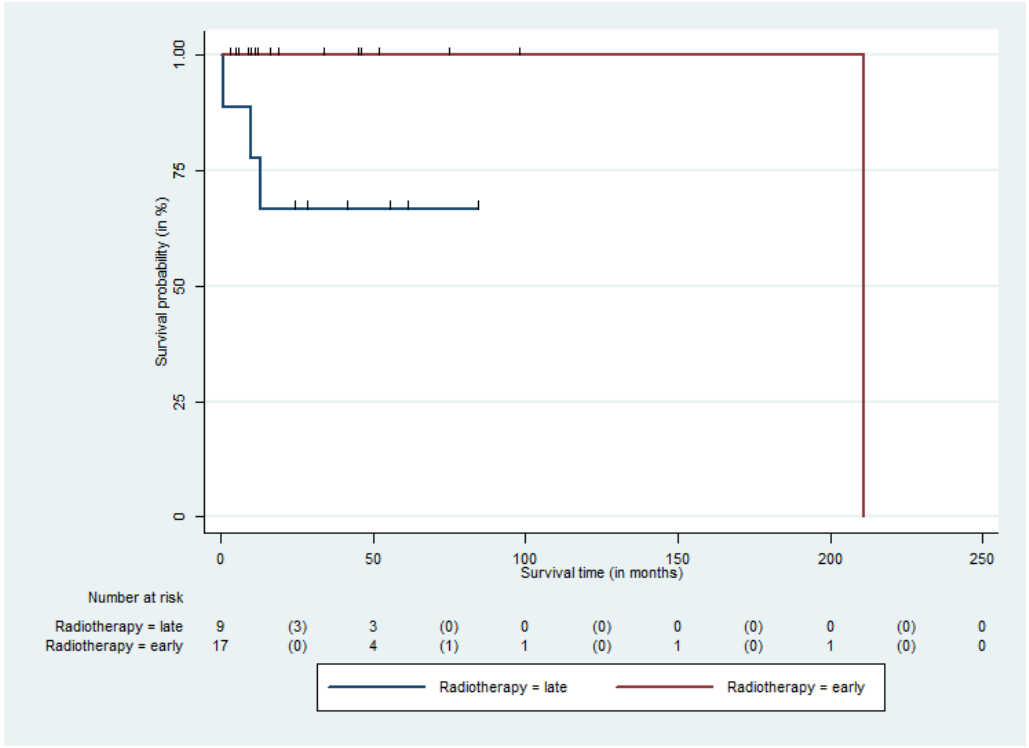

Fig. 2. Kaplan-Meier functional independent survival curves showing better prognosis for early radiotherapy treated patients (in red); numbers marked at the bottom of the table are patients at risk with failed events between parentheses. Vertical axis is the probability of survival; horizontal axis is the survival time in months. Log-rank = 0.0266

Oligodendroglioma / mixed histology was found to suffer malignant transformation less frequently than astrocytoma. KPS was also found to be a predictor for malignant transformation on univariate analysis. For the multiple regression we also added radiotherapy (p = 0.8785) and tumor volume, but only KPS remained a significant co-variate in this setting. When analyzing only the patients who received radiotherapy, timing of the treatment, site of the tumor, histology, age and tumor volume were found non-significant. KPS was the only variable with significance in the univariate analysis. In the final model of multiple regression that included: tumor volume, KPS and radiotherapy status, none of them showed any significance.

DISCUSSION

We could not prove any influence of our studied variables on global overall survival. However, we were able to prove that oligodendroglial subtype has a better prognosis on overall survival analysis in the radiotherapy treatment group.

Tumors of astrocytical origin were 4 times more likely to relapse than tumors of oligodendroglial origin. Older patients were more prone to relapse, along with tumors located in non-eloquent areas. Radiotherapy decreased relapse likelihood with 30%. Eloquent located tumors had a better prognosis which could be explained by the increased likelihood of radiotherapy treatment for this group.

Patients who benefitted of early radiotherapy had a significantly better functional independent prognosis. If radiotherapy increases progression-free survival, we can see why it makes sense that patients treated earlier (when symptomatology is not that important) keep their status for a longer time than those for whom we wait for neurological degradation in order to initiate treatment.

Oligodendroglioma histology was proven to be less aggressive in regard to malignant transformation. Higher KPS at diagnosis was also associated with a higher rate of malignant transformation which may be explained by the fact that patients with a good clinical condition were less likely to be treated aggressively thus more likely to be victims of the natural course of the disease. Also, if the patient lives longer because of a good clinical condition there is more time for the tumor to progress. KPS was important in the radiotherapy treatment group probably because a low KPS urged the physician to initiate treatment as opposed to patients in good condition which are prone to be kept under surveillance.

Although radiotherapy was proven to be effective especially concerning progression-free survival (5, 6), only up to 5% of patients with low-risk low-grade gliomas and up to 30% of patients with high-risk LGG receive this treatment (7). Almost half of the patients in our study were subjected to radiotherapy. Often it is recommended that patients who present with high-risk low-grade gliomas should undergo early radiotherapy treatment, even though this may associate side effects. Usually the decision of initiating early radiotherapy is argued by a few principles: the responsiveness of low-grade glioma to radiotherapy, the diseases' aggressive pathobiological behavior, characterization as a high-risk LGG, the apparent reduction of adverse effects with modern techniques of radiotherapy and possible increased efficacy of commencing radiotherapy at diagnosis (6, 8, 9). In our study we decided not to use classical high-risk/low-risk criteria, like Pignatti risk factors (10). Instead we tried a different approach choosing uncategorized age and a volumetric estimation of the tumor despite other studies not finding volume measurement clinically useful (11). What caught our attention was the fact that radiotherapy determines a decrease in tumor volume over several years (12).

Regarding safety of the treatment for high-risk patients and early complications, recent data shows that tumor progression is responsible for the poorer quality of life of radiotherapy-treated patients and not irradiation itself (13).

Concerning the fact that low-risk LGG patients are less likely to benefit from radiotherapy treatment (7), we do not know for sure if this is a completely justified action

at this time, because after all we are depriving a patient from a verified efficient treatment, that is growing safer and safer with new technologies. If our findings that early radiotherapy is associated with a better functional prognosis are confirmed by other studies, then we have a path for an alternate approach.

Literature data suggests superior brain functions following radiotherapy for diffuse gliomas are stable no matter the timing chosen for treatment administration (14). This fact aids in decision making both for patients and physicians who have doubts about taking this step.

Our study was limited by the small sample size and retrospective design, but nonetheless we managed to get some interesting results not only concerning the behavior of LGG patients, but also the attitude of physicians regarding this pathology.

CONCLUSIONS

Radiotherapy is proven to be effective in the treatment of low-grade glioma, especially regarding progression-free survival with early treatment being further associated with a longer time to progression.

Questions to be answered in the future must direct research towards analyzing clinical status, symptoms control, adverse effects (are they acceptable even for patients where there is no primary intention for radiotherapy) and functional status. An interesting morphological parameter to be investigated is tumor volume and its evolution under the effect of radiotherapy.

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NEWS

THE ENDOMETRIOSIS EFFECT ON OVARIAN MALIGNANCY AND ITS IMPLICATION IN GRANULOSA CELL TUMORS

Even if endometriosis is considered a benign pathology, it may play an essential role in ovarian cancer developing. There are data about the endometriosis effect on ovarian cancer, but it is a lack regarding its involvement in granulosa cell tumor. Ashari *et al*. (2019) applied steroid-depleted serum or whole serum from endometriosis patients to the granulosa cells obtained from tubal or infertile women due to a male factor and used flow cytometry and quantitative real-time polymerase chain reaction in order to establish the expression level of inflammatory and apoptotic genes and also the apoptosis level of granulosa cells. The study shown that the granulosa cells which went under treatment with steroid depleted serum from patients with endometriosis presented a considerable increase expression of NFkB/RelA and AKT compared with control group. On the other hand, the genes related to apoptosis had a lower expression in treatment group compared with the control but also the synthesis of saturated fatty acids whereas the levels of unsaturated fatty acids were increased. The implication of steroids on endometriosis is contradictory and the sex hormones and cortisol levels may be modified due to this pathology, leading to alteration of the condition progression. Thus, decreased level of sex hormones in endometriosis females may be considered as a decisive risk factor for granulosa cell tumor (Asghari S, Dizajeykan AV, Ahmadi M *et al*. Evaluation of ovarian cancer risk in granulosa cell treated with steroid-depleted endometriosis serum: role of NF-kB/RelA and AKT. *J Cell Physiol* 2019; 234(7): 12011-12018).

BODY COMPOSITION AND METABOLIC PARAMETERS COMPARISON IN YOUNG WOMEN WITH ANOREXIA NERVOSA AND CONSTITUTIONAL THINNESS

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BODY COMPOSITION AND METABOLIC PARAMETERS COMPARISON IN YOUNG WOMEN WITH ANOREXIA NERVOSA AND CONSTITUTIONAL THINNESS (Abstract)**:** Anorexia nervosa and constitutional thinness are two separate medical entities resembling in terms of age and body weight. Even though the former is defined by unique psychological traits, its diagnosis is based solely on clinical judgment, which can sometimes lead to confusion in terms of diagnosis accuracy and clinical management. **Aim:** The aim of this study is to search for discriminative metabolic and body composition traits for each of the aforementioned pathologies. **Material and methods:** In this transversal study we compared two cohorts of female patients aged 11 to 37 years: 11 subjects with on-going anorexia nervosa restrictive type and 12 subjects with constitutional thinness, respectively. The evaluation was comprised of clinical, biochemical and hormonal assessment, as well as of body composition measurements during total body-scan on dual X-ray absorptiometry. **Results:** In anorexia nervosa group versus constitutional thinness, there is a tendency towards lower values of TSH, fT4, IGF1 and insulin (1.6±0.8 *vs.* 2.2±0.8, $p<0.05$, 0.94±0.1 *vs.* 1.07±0.1, $p<0.05$, 105±11 *vs.* 217±59, $p<0.05$ and 3.8±2.2 *vs.* 15.5±1.4, $p<0.05$, respectively), as well as for total fat percentage (22.8±4 *vs.* 26.7±4.6, $p<0.05$). **Conclusions:** Patients with anorexia nervosa differ from subjects with constitutional thinness not only from psychiatric perspective, but also with regard to several metabolic and body composition parameters. **Keywords:** ANOREXIA NERVOSA, CONSTITUTIONAL THINNESS, INSULIN, LIPID PROFILE, BODY COMPOSITION, THYROID, IGF1.

Anorexia nervosa (AN) is a psychiatric condition characterized by food phobia and severe caloric deprivation achieved by extreme starvation and intense physical exercise (1). It predominantly affects young women, being one of the most debilitating psychiatric diseases as it is characterized by multiple somatic injuries due to malnutrition (2), as well as by high mortality rates (3, 4).The current diagnostic criteria as defined by the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in 2013 consist of low body weight, distorted perception of

body image and fear of gaining weight (1). This new definition of AN is more lenient, because, on the one hand, it leaves to the clinician's appreciation to define "low weight" and, on the other, it no longer imposes amenorrhea as mandatory.

Consequently, due to the more permissive diagnostic criteria, it is sometimes more difficult for untrained clinicians to differentiate AN from a less-known, yet fundamentally different entity, called constitutional thinness (CT). CT patients are in the same age range and have low, but stable body weight, accompanied by normal caloric intake and hormonal, metabolic and reproductive profile (5). They do not exhibit any psychological traits of AN and only resemble it by leanness (6). The condition is considered rare especially in obesogenic societies, but exact prevalence is not known (7). Moreover, there is only scarce evidence in the literature regarding CT state and only a limited number of studies focusing on AN and CT comparison have been published.

The aim of this study is therefore to compare two groups of female patients representative for AN and CT, respectively, in terms of anthropometric, metabolic and body composition parameters in search for significant differences between them in the DSM-5 era.

MATERIAL AND METHODS

The study was conducted between October 2017 and January 2020 in the Department of Endocrinology, Diabetes and Metabolic Diseases of "Elias" Hospital and it originally included 27 female patients 11 to 37 years old, referred to us for malnutrition, anorexia nervosa or thinness management. The cohort was split in two, resulting 15 patients suffering from AN and 12 CT subjects. In the AN cohort, 4 individuals were weight-recovered anorexic patients on ongoing psychiatric medication and were excluded from the analysis for better bias control. All of the remaining subjects had ongoing typical AN restrictive type or have had a lifetime diagnosis of AN restrictive type, without complete remission (they continued to engage in food depriving behavior, were low weight and had attenuated misperception of body image). Six patients from the AN group had secondary amenorrhea at the time of the evaluation. In the CT group, one patient presented with oligomenorrhea and 2 pubertal subjects did not experience menarche by the time of evaluation. Clinical examination assessed that all pubertal girls were Tanner stage 4. All of the remaining patients reported normal menses and no subject was under substitutive hormonal therapy or psychiatric medication. One of the 23 study subjects did not either complete blood tests or body composition due to personal convictions, but anthropometric evaluation is available.

To avoid biased classification of the two medical entities, we performed a careful selection of study subjects and we closely collaborated with pedopsychiatry specialists from the Child and Adolescent Psychiatry Clinic within "Obregia" Hospital, who have large expertise in eating disorders management and gave us guidance during entire study period. As stated above, all of the 11 patients in the AN group expressed intense fear of gaining weight, had misperception of body image and were low weight, and therefore fulfilled the DSM-5 diagnostic criteria. Moreover, eight of them were either already psychiatrically or psychologically evaluated by the time of study visit, or underwent psychiatric evaluation

soon after, which confirmed the above-mentioned diagnosis. To our knowledge, the remaining 3 subjects refused any direct psychiatric or psychologic assessment near the time of our metabolic evaluation. However, there is no doubt on the accuracy of the diagnosis, as they fulfilled the DSM-5 algorithm diagnosis of AN, being on an extremely restrictive diet, remarkably calorie-conscious and very unsatisfied with their body image as they were considering themselves overweight or obese despite their striking leanness. All of the 3 subjects also suffered from secondary amenorrhea. In the group of CT, all 12 subjects stated a frustration in failure to reach a weight that is socially accepted as normal and expressed normal feelings towards food. Also, when asked to rate themselves on a virtual scale from 0 to 10, 0 meaning "extremely lean" and 10 meaning "extremely fat", they evaluated themselves at a maximum 5, as opposed to anorexia nervosa subjects who generally situated above 7.

The study was approved by the Local Ethics Committee of "Elias" Hospital and was conducted in accordance with Helsinki Declaration. Written informed consent was obtained from each patient above 18 years old or from the legal representative for patients under 18, respectively.

Anthropometric evaluation

Physical data such as height, weight, waist and hip circumference were gathered during subjects visit. Height was measured to the nearest 0.1cm and weight was assessed during body composition evaluation on dual X-ray absorptiometry (DXA). Waist and hip circumference were measured in standing position, directly on skin, at the level of umbilicus and greater trochanter, respectively. Body Mass Index (BMI) was calculated as weight/height^2 (kg/m^2). We also assessed the standard deviation of relative weight for child age and sex (BMI-z score)by using external references provided by the Center of Disease Control (CDC) growth charts. For patients 20 years of age or older we used the 20-age BMI-z score reference, in order to have consistent metric of relative weight across study population (8).

Biochemical evaluation

Blood samples were collected in the morning after a minimum eight-hour fasting and were analyzed for plasma glucose, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), total proteins, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), insulin, thyroid stimulating hormone (TSH), insulin-like growth hormone 1 (IGF1), free thyroxin (fT4) and 25-hydroxy vitamin D (25 OH vitamin D). The derived-parameter calculated was the Homeostatic model assessment for insulin resistance (HOMA-IR) as fasting plasma glucose (mg/dL) x fasting insulin (μIU/mL)/405(9).

Body composition evaluation

Body composition was assessed during total body scan on DXA evaluation using a GE Healthcare- Lunar iDXA software. Several parameters were included in the statistical evaluation, such as resting metabolic rate (RMR) for non-pediatric population, total fat percentage (TFP) and total fat mass (TFM). We also calculated predicted resting metabolic rate (RMRp) derived from the equation of Harris and Benedict modified by Roza and Shigal (10), RMRp=667.051+ 9.74×(weight)+1.729×(height)−4.737×(age).

Statistical analysis

All data were analyzed using *SPSS version 21.0* and a p value < 0.05 was considered statistically significant. Parametric data were presented as means ± standard deviations (SD) and categorical variables as percentages (%). The Shapiro-Wilk test, visual inspection of the histograms, Q-Q plots and box plots as well as z value calculation for skewness and kurtosis were performed in order to assess the distribution of variables within each group. Group differences in means were compared using a two-sample independent group t-test for normally distributed data and the Mann-Whitney test for non-Gaussian distributions. The Spearman's rank correlation method was calculated to assess the relationship between the variables.

RESULTS

General characteristics

The age range of the 23 subjects included in the study was between 11.3 and 36.7 years, with a mean age of 24.1±7.3 and a median of 25.8. Mean BMI was 16±1.7 kg/m^2 (range 12.24 to 18.27), corresponding to a mean BMI-z score of -2.72±1.3 SD (range -6.23 to -1.39).The cohort was split in two groups depending on the existence of AN diagnosis. Group 1 consisted of 11 AN individuals and group 2 of 12 CT subjects. The six subjects with secondary amenorrhea represented 54.5% from the whole group of AN.

Group comparison

The AN group was characterized by a younger age and a tendency towards smaller abdominal circumference. Both BMI and BMI z-score were significantly lower in AN group. Hip circumference was also slightly lower in patients with AN. Results for anthropometrical parameters are shown in table I.

TABLE I

Anthropometrical characteristics in AN and CT groups

	AN (mean ± SD) n=11	CT (mean ± SD) n=12	Significance value
Age	22.1±7	26±7.5	NS*
BMI (kg/m^2)	15.1±1.7	16.8±1.2	p<0.05
BMI z-score (SD)	-3.4±1.4	-2±0.6	p<0.05
Waist circumference (cm)	60.1±3.7	62.7±2.6	p=0.06
Hip circumference (cm)	86±6.7	88.9±4.3	p<0.05

*NS=non-significant

The biochemical markers considered for statistical analyses (tab. II) showed that the AN group displayed significantly lower values (p<0.05) for fT4, TSH, IGF1, insulin and HOMA-IR, but also a slight increase in total proteins when comparing to CT group. The AN group also had a tendency of increased TG, TC and LDL compared to CT group, but the p value failed to reach the 0.05 threshold. All of the other parameters examined did not reach statistical significance (results not shown).

Regarding body composition analyses, the AN group differed from the CT group in terms of TFP (22.8±4 *vs.* 26.7±4.6, p<0.05, fig. 1) and RMRp values (1090 k±81 *vs.* 1122±46 kcal/day, p<0.05).

In addition, we performed correlation analyses to determine the relationship between glucose homeostasis (assessed by

HOMA-IR) and body composition parameters (TFP, RMR and RMRp) within each group. We found a positive strong association in the AN group between HOMA-IR and TFP (r=0.7, p=0.03). This relationship was not valid for the CT group (r=-0.1, p=0.6). All of the other correlations tested did not reach statistical significance.

TABLE II

Biochemical parameters in AN and CT subjects

	AN (mean ± SD) n=10	CT (mean ± SD) n=12	Significance value
TC (mg/dL)	193.4±43	163±34	p=0.08
LDL (mg/dL)	116.4±36	91.2±27	p=0.07
HDL (mg/dL)	63.6±11.9	61.5±13.6	NS*
TG (mg/dL)	66.9±30	49±11	p=0.08
TSH (μIU/mL)	1.6±0.8	2.2±0.8	p<0.05
fT4 (ng/dL)	0.94±0.1	1.07±0.1	p<0.05
IGF1 (ng/mL)	105±11	217±59	p<0.05
Insulin (μIU/mL)	3.8±2.2	15.5±1.4	p<0.05
HOMA-IR	0.8±0.5	1.17±0.3	p<0.05

*NS=non-significant

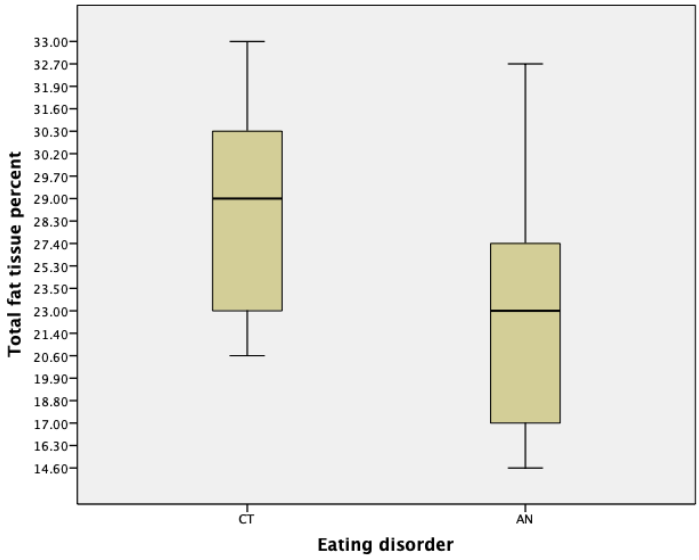

Fig. 1. Total fat percent in CT and AN groups (p<0.05)

DISCUSSION

In this study investigating metabolic parameters and body composition in AN and CT patients, we searched for particularities that can help clinicians better understand the differences between these two rare

entities. The distinction between the two is highly important because it prevents us from unnecessarily stigmatizing lean patients as "anorectic" and also lowers the risk of overlooking AN in a patient without menstrual disturbances(7).

Our study had 3 major findings. First, an impaired lipid profile seems to be representative for AN population, despite their lower weight and total fat percentage.

Probably due to the small number of patients included in the study, our results did not reach statistical significance, but they are interesting to look at, especially because the p value was very close to 0.05 with respect to TC, LDL and TG. This finding highlights a first difference between the metabolic profile of patients with AN versus CT. The increase in cholesterol and TG of AN compared to normal weight subjects has been studied before and, while there is evidence that invalidate this hypothesis (11), most studies plead for hypercholesterolemia and hypertriglyceridemia, as reported in a recent meta-analyses (12). In addition, alterations in the lipid profile seem to be more prevalent for AN restrictive type when compared to any other form of AN or eating disorder (13), or to other causes of malnutrition (14). Moreover, longitudinal studies focused on the dynamics of lipid profile in weight restored AN patients revealed a delay of lipids correction when compared to other metabolic parameters (15). So far, there is no widely accepted explanation for this phenomenon, given that the diet of these patients is typically low in lipids, but some of the hypotheses worth considering are: dehydration, liver dysfunction, increased absorption of exogenous cholesterol due to alterations in gut microbiota, elevated LDL secondary to rapid and severe loss of body fat and to low thyroid hormones or decrease in cholesterol turnover (12, 13, 16).

Second, endocrine disturbances related to metabolic balance were also more pronounced in AN group. This finding is not surprising, as it is widely accepted that AN patients, especially restrictive type, confront themselves with several hormonal misbalances. In relation with our theme, we only focused on thyroid hormones and IGF1 adaptations and found that in AN, contrary to CT, there is a significant drop in IGF1 levels, as well as in TSH and fT4 hormones, pleading for a diagnosis of euthyroid sick syndrome.

There is evidence that in acute forms of AN, free T3 (fT3) levels drop significantly in order to preserve energetic resources, while TSH and fT4 have a slight decrease or remain normal (17). These adaptations are secondary to weight changes and seem to be caused by low levels of leptin that alter the hypothalamic secretion of TRH(18).It is worth mentioning that in 2017, Estour *et al.* proposed fT3 and leptin as metabolic markers for differentiating CT from AN (7).

Similar reasoning explains the drop in IGF1 levels, required in order to minimize expenditure on growth. While AN is a state of malnutrition, low insulin seems to downregulate the hepatic growth hormone (GH) receptor expression (17), leading to acquired GH resistance.GH is a hyperglycemic hormone, and a beneficial effect of high GH-low IGF1 dynamic is preserving euglycemia(19). Both thyroid function and GH-IGF1 axis fully recover after weight restoration (20).

Third, for subjects with AN, there seems to be a strong association between HOMA-IR and TFP. As expected, when comparing these variables separately, insu-

lin, HOMA-IR and TFP were all significantly higher in the CT group, yet the positive correlation between the last two was not valid for lean healthy subjects.

The lower fat mass in AN *versus* normal weight individuals is confirmed by numerous studies (21-24), and, in concordance with our results, there is evidence to be lower than in CT subjects, also (7). Furthermore, we also noticed the RMRp to be lower in AN. This finding is also enforced by existing data (25). Moreover, a group of researchers focused on AN in adolescent girls described positive correlations of RMRp and weight, BMI, fat mass, glycaemia, HOMA-IR, leptin and IGF1 (26). Regarding HOMA-IR results in AN, they suggest a state of insulin sensitivity. This positive association has been demonstrated by earlier researches that consider BMI to be a confounder (27) or describe the association as independent from fat mass (21). Based on these observations, there is a speculation in literature regarding the role of insulin in influencing the reward circuit through its central receptors situated on dopaminergic neurons (27).

In the end, we also want to shed some light on our potentially surprising result on total proteins level, that display higher values in AN group, probably secondary to food supplements administration confessed by some of the AN subjects. This finding has been previously documented by Bossu *et al.* that also described a higher percentage of protein intake in their cohort of AN girls (28).

Study strengths

The present study addresses a less-explored subject and has several strengths. First, we assessed body composition by DXA measurement, which is the gold standard technique and is highly accurate especially in the evaluation of body fat in non-obese premenopausal females(29). Next, we assessed fasting insulin and IGF1 in two categories of patients suffering from neither diabetes nor short stature. Lastly, despite of the lack of an integrative medical center for eating disorders in Romania, we collected data from a group of patients with AN, who are in denial of their condition, being highly reluctant to seek for medical help or to have any scientific exposure.

Study limitations

Overall, we are aware that our study has several limitations. First, the groups were not matched for age and BMI, therefore there are several significant anthropometric differences between them. However, it has the advantage of evaluating representative patients for each category of disease and, in search for a tool for differentiating CT from AN in clinical practice, these differences in BMI and age might come at help. Moreover, in order to increase the homogeneity of the study, we only included female subjects, but both entities, though rare, can also occur in men. Second, several biological parameters with potential relevance to the subject, such as leptin, adiponectin, free T3 or cortisol, were not available. Moreover, we assessed insulin sensitivity by measuring HOMA-IR index, which, opposed to hyper insulinemic-euglycemic clamp, is an indirect, but still efficient indicator of insulin-resistance(30). Another limitation worth mentioning is that, because of its dependency on adiposity and age, there is scarce evidence in the literature regarding accuracy of HOMA-IR index in assessing insulin sensitivity in lean individuals, but available data is rather encouraging (31, 32). Third, due to the poor addressability of AN and CT patients to the doctor, the

study was conducted on a relatively small sample size that may weaken the statistical power of the analyses.

In conclusion, our study postulates that patients suffering from ongoing AN develop several metabolic disturbances that are likely due toa negative energy balance and to an impaired hormonal profile. On the contrary, CT individuals are a unique group of people, who, despite a very low BMI, differ from AN in terms of metabolic, hormonal or body composition parameters.

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EPIDEMIOLOGICAL, CLINICAL AND HISTOLOGICAL CONSIDERATIONS REGARDING BASAL CELL CARCINOMA OF THE FACE

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EPIDEMIOLOGICAL, CLINICAL AND HISTOLOGICAL CONSIDERATIONS REGRADING BASAL CELL CARCINOMA OF THE FACE **(Abstract):** Skin cancer has become one of the most common forms of cancer in recent years. Two major categories of skin cancer have been identified: malignant melanoma (MM) and non-malignant skin cancer (NMSC). Among non-melanoma skin cancer lesions, basal cell carcinoma (BCC) is the most common, with a frequent localization in the face. **Aim:** The current study aims to demonstrate and confirm the frequency of BCC on the face. The increased frequency of these lesions on certain aesthetic units more exposed to the sun proves once again that ultraviolet radiation is the most important risk factor for the appearance of this skin lesion type. **Material and methods:** The study was performed on a group of 95 patients diagnosed with various histological forms of BCC, located in the face. The data has been collected from the observation files, the hospital's reports and eventually processed with the help of the statistical programs *MS Excel 2010* and *SPSS ver. 18.0*. **Results:** The results of the study showed the most frequent affectation of females (86.3%). The elderly are most often affected (average age 71 years old). The anatomical region that BCC develops most often was the nasal pyramid (32.6%). The nodular form of BCC was the most common. **Conclusions:** The increased frequency of BCC on the more preeminent anatomical areas of the face, so more exposed to solar radiation shows once again the importance of avoiding exposure to ultraviolet radiation. Our study confirms the importance of epidemiological studies in detecting risk factors for various pathologies. **Keywords:** SKIN CANCER, BASAL CELL CARCINOMA, MALIGNANT MELANOMA, NON-MALIGNANT SKIN CANCER, FACE.

Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) represent almost 99% of diagnosed non-melanoma skin cancer, BCC being more frequently reported (1).

BCC is originated from keratinocytes from basal layers of the epidermis, from pluripotent cells of the interfollicular epidermis and from cells localized at the outer root of hair follicles (2). Although BCC has

been described on different sites of the body, face is the most frequently affected skin area, due to direct exposure to ultraviolet (UV) radiation, especially sun (3). It is well known that BCC is locally very aggressive and recurrent, invading adjacent skin and very often, goes profoundly into close structures. Based on clinical and histopathological features different types of BCC have been described, but on the face, superficial and ulcerative types are reported as predominantly (4). Present study analyzed 95 cases of BCCs diagnosed and treated at Plastic Surgery Unit of Iasi, Romania, analyzing correlations with age, gender, anatomical site, clinic-histological type.

MATERIAL AND METHODS

The study lot consisted of 95 participants diagnosed with basal cell carcinoma, admitted in the Plastic Surgery Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital Iasi. All of the patients elected to participate in the study signed the terms and conditions concerning the treatment methods and the processing of data with scientific purpose. The data has been collected from the observation files, the hospital's reports and eventually processed with the help of the statistical programs *MS Excel 2010* and *SPSS ver. 18.0.*

Statistical analysis. The *Skewness, Kurtosis tests* ($-2 < p < 2$) were used, these representing normality tests, available for when the distribution platform for the examination of a variable continues, is in use; the ANOVA test for the analysis of dependent variables inside a study lot and in between lots. The threshold with a significance of 95 percent (confidence interval 95%). I have applied the *t-student test* for quantitative variables, parametrical test that compares the average registered values in two groups with normal distributions and F_{ANOVA} test used in the situation of medium degree comparison for more estimations of the same variable in normal distribution groups.

As nonparametric test, we have used the χ^2 *test* in order to compare two or more same population frequencies distributions. When we compared the ordinary variables from three or more groups, we have used the Kruskall-Wallis correlation. The correlation *quotient* "*Pearson" (r)* represents the (direct / indirect) correlation between two variables belonging to the same group.

RESULTS

Age distribution was homogenous, varying from 31 to 98 years old, median value was 70.67 ± 10.67 years, medium value 71 years old and the result of *Skewness test* p = -1,104. Age wise distribution of cases was homogenous, varying from 31 to 98 years old, the group's value was 70.67 ±10.67 years, average value 71 years and the result *Skewness test* p = -1.104 (tab. I) (fig. 1).

TABLE I

Statistical indicators of age (years)

N		95
Mean		70.67
Median		71.00
Standard deviation		10.67
Variant		15.10
Skewness Test		-1,104
Skewness Standard error		0.490
Minimum		31
Maximum		98
Percentiles	25	67
	50	71
	75	78

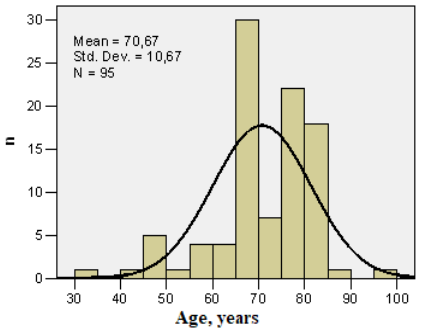

Fig. 1. Distribution of patients by age

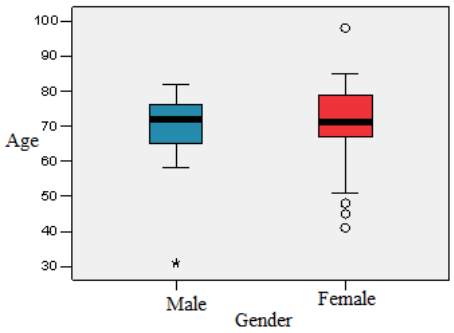

Fig. 2. Average age by sex

Out of all cases 86.3% were female patients, with an average age insignificantly higher in comparison with the cases registered in men (71,01 vs 68,54 years, p=0,440) (fig. 2).

As far as the anatomical region involved, most identified carcinoma cases were localized in the nasal region (32.6%), followed by the facial area (22.1%) and the ocular one (21.1%) (fig. 3, fig. 4) (tab. II).

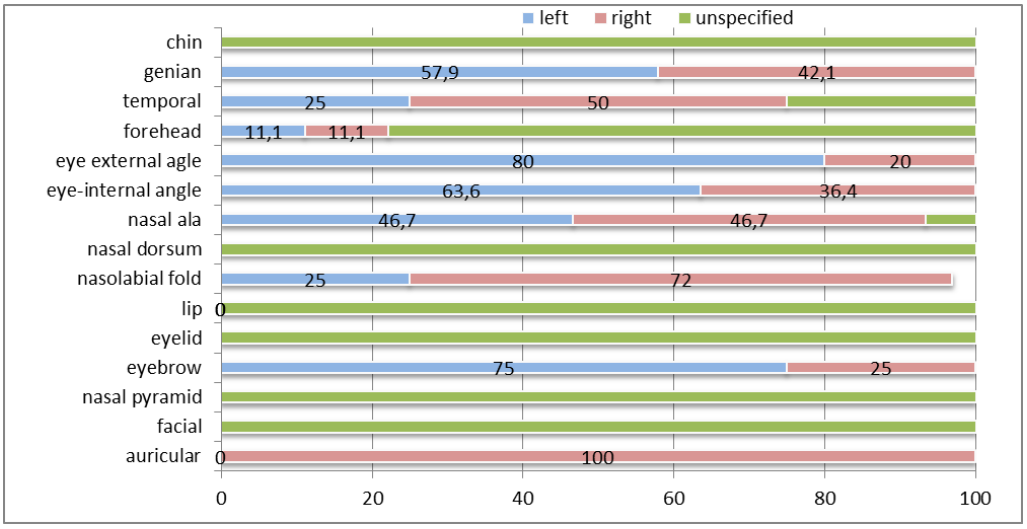

Fig. 3. Distribution by carcinoma location

TABLE II

Distribution by carcinoma location

Anatomical region	Location (%)		
	left	right	Unspecified
Auricular		100	
Facial			100
Nasal pyramid			100
Eyebrow	75	25	

Anatomical region	Location (%)		
	left	right	Unspecified
Eyelid			100
Lip			100
Nasolabial fold	25	72	
Dorsum nasal		100	100
Nasal ala	46.7	46.7	6.7
Eye - internal angle	63.6	36.4	
Eye - external angle	80	20	
Forehead	11.1	11.1	77.8
Temporal region	25	50	25
Genian region	57.9	42.1	
Chin region			100

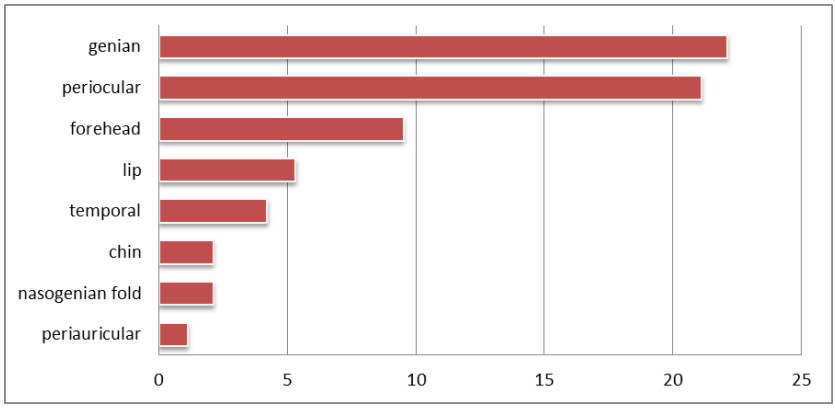

Fig. 4. Distribution by anatomical region

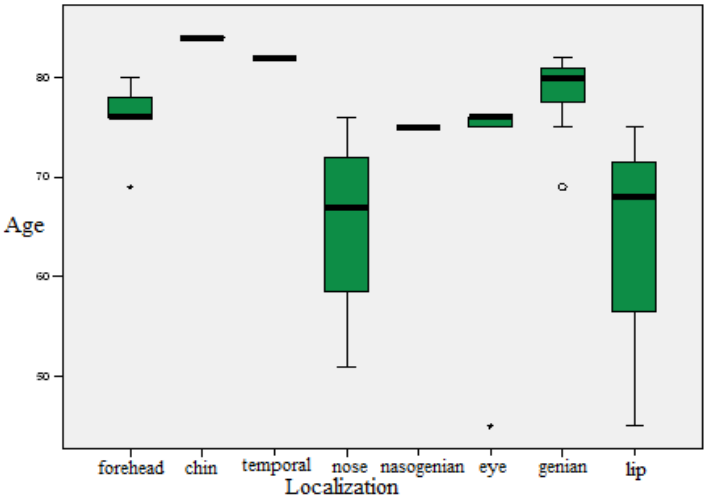

Fig. 5. Average age correlated with the affected anatomical part

Correlation age-anatomical site: younger patients were diagnosed with BCC placed around the lips (62.67±9.06) and older patients presented BCC on the chin (84±0) (p=0.022).

No direct correlation was found between the size of the tumor, regardless the type, and the patient age (r=0.153; p=0.140) (Figure 5). The smallest carcinoma size was associated with the nasolabial region (0.68±0.02), and the largest with the temporal region, the results having intermediate statistical significance (2.26±1.37) (p=0.047) (fig. 7).

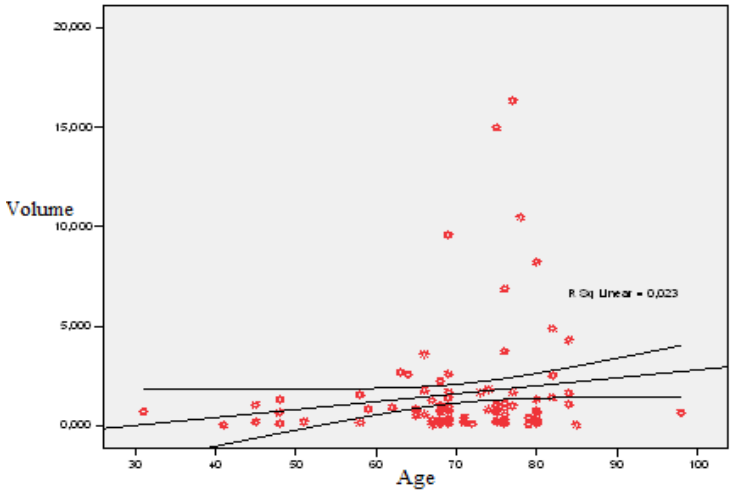

Fig. 6. Correlation of carcinoma size with age

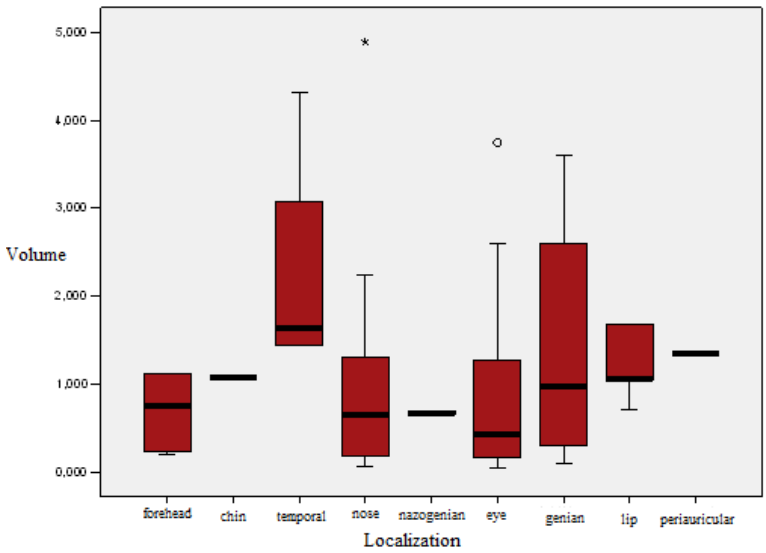

Fig. 7. Average volume depending on the anatomical region

The average size did not register significant differences depending on the anatomo-pathological shape, although the largest volume was associated with the ulcerative form, and the smallest one with the solid form (1.52±0.40) (p=0.929). Among the patients presenting nodular forms, the average size of the carcinoma did not significantly differ between the sexes (fig. 8).

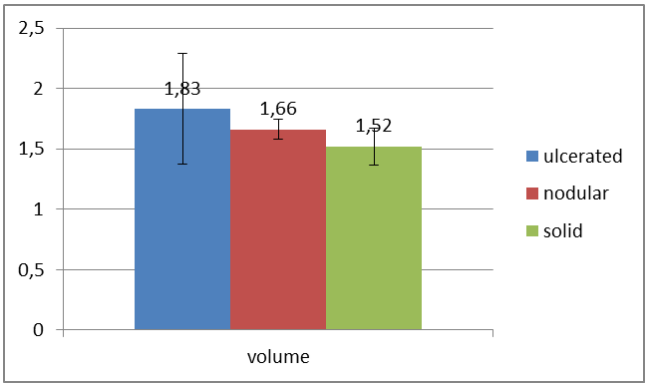

Fig. 8. Average volume depending on the anatomopathological form

In patients with the ulcerated form, the mean size of the carcinoma was significantly higher in females (1.59 ± 0.98 *vs.* 0.41 ± 0.39; p = 0.048), while in the solid form, the mean size of the carcinoma was significantly higher in males (1.58 ± 1.23 *vs.* 0.70 ± 0.43; p = 0.045). In patients with nodular forms, the mean size of the carcinoma did not differ significantly between the sexes (1.00 ± 0.33 *vs.* 1.26 ± 0.48; p = 0.569) (fig. 9).

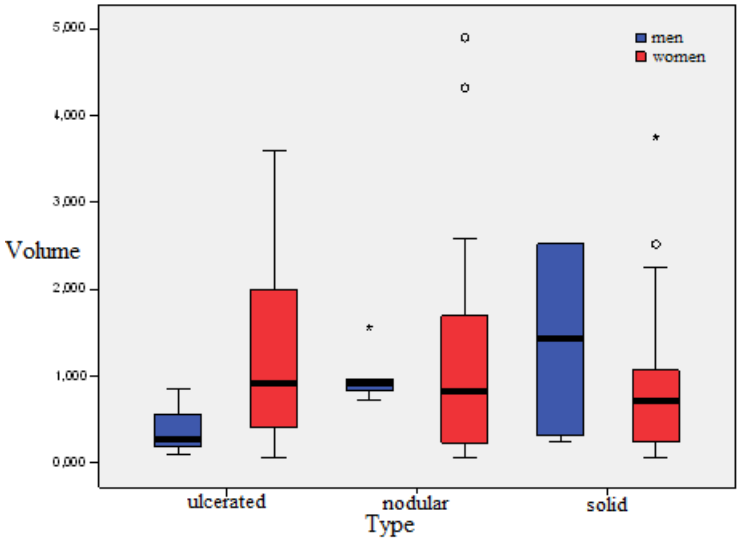

Fig. 9. Average volume by sex depending on the anatomopathological form

DISCUSSION

It is known that BCC frequently occurs on sun-exposed areas, such as the face and neck, but predominantly across the cheeks, nose and periorbital regions (4). Many clinical variants of the BCC (more than 26), have been described, however the most acknowledged classifications tend to presenting superficial, nodular and morphea-like BCC; in daily practice the existence of ulceration is of outmost importance and supports the notion of both ulcerative and non-ulcerative BCC. 81,6% out of BCC had a facial localization (5, 6).

According to more recent studies, BCCs mostly occurred across the nasal area, regardless of the age or gender or the patient. Although BCC was mostly diagnosed among the elderly, younger patients in their thirties, may also develop BCC, especially around the lip area. Women were at higher risk of developing ulcerative BCC in comparison with men, probably due to increased sun exposure, lack of protective measures (men tend to use hats and caps more often than women do), and exposure to artificial light (in tanning salons). No direct correlation between the age of the patient and the size of the tumor was noticed, although the majority of the patients participating in the study were 60-70 years old.

Recent studies by the American Cancer Society show that in 2012, 5.4 million cases of non-melanoma skin cancer (NMSC) were diagnosed. In 8 out of 10 cases, a form of BCC was identified. It is reported that in 40% of patients diagnosed with a form of BCC, a new BCC lesion was diagnosed in the next 5 years of life (5).

The localization of malignant skin lesions on the face, especially in women, will always pose treatment problems, because the aesthetic aspect of the scars is particularly important. Skin lesions, even small, malignant or non-malignant, can cause problems regarding the surgical treatment (7). Although BCC is not an aggressive form of cancer, its ability to cause extensive tissue damage determines that this type of skin lesion to benefit from surgical treatment (8, 9).

When choosing the surgical technique, the surgeon should consider the dimensions of the tumor, the edges of the excision that must be respected, the aesthetic unit of the face at the level of which the tumor formation is found. This will determine the type of anesthesia used. Because facial BCCs are often small, it will not be necessary to use complex surgical techniques (flaps) or other types of therapies (NPWT) (10, 11).

The risk of developing BCC after surgical treatment is reported to be between 5% and 15% (12). The most frequent recurrences were recorded when the initial lesion was located in the lower lip or medial canthus.

Histopathological analysis of excised tumors shows a high frequency of the nodular form of BCC (5, 13). The results obtained by the present study can be compared with those in the literature (14, 15).

CONCLUSIONS

The frequent localization of BCC on the face confirms, once again, that ultraviolet radiation is one of the most important risk factors for such a skin lesion.

Most cases of BCCs were diagnosed in women, during 6-7th decade of life, larger tumor on the forehead, while smallest on perinasal area and a high risk of ulceration

in women, independent of type of BCC or age of the patient. Reducing sun exposure and adequate protection can significantly reduce the risk of BCC on the face.

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FATIGABILITY – FROM CONCEPT TO CLINICAL PROFILE. CASE REPORT

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FATIGABILITY–FROM CONCEPT TO CLINICAL PROFILE. CASE REPORT (Abstract): We present the case of a female patient, aged 57, who undergoes her first cardiovascular evaluation and gets admitted to the emergency room complaining about palpitations, deep physical asthenia, dyspnea on average effort and discrete edema at the lower limbs level. The examination shows a severe anemic syndrome and serositis, with the development, thus, of a systemic immunological affectation at the visceral level. Immunology tests confirmed the diagnosis supposition of systemic lupus erythematosus, the patient meeting 6 of the 11 American College of Rheumatology (ACR) criteria: malar rash, photosensitivity, serositis, hematological affectation, immunological affectation, antinuclear antibodies. The activity of the disease was assessed against the SLEDAI questionnaire (Systemic Lupus Erythematosus Disease Activity Index), which delivered a 12-point scoring, therefore showing a high activity level disease. **Keywords**: FATIGABILITY, SYSTEMIC LUPUS ERYTHEMATOSUS, ANEMIC SYNDROME, SEROSITIS.

Systemic Lupus Erythematosus (SLE) is an autoimmune condition, of complex and incompletely elucidated etiology, characterized by an exaggerated activity of the immune system which triggers the formation of specific autoantibodies (antinuclear antibodies) with the occurrence of inflammation and the destruction of multisystemic tissues (1).

The disease has an increased incidence among female patients (aged 15 to 45, although the onset can be found at any age) suggesting the involvement of hormonal factors in the development of the actual disease; however, men can suffer from more aggressive forms of the disease. Recent studies have shown that there is also an increased rate of disease occurrence in people of color (2, 3).

SLE etiology is unknown, yet it involves a series of genetic, immune, hormonal, and environmental factors, while the main clinical manifestations appear at the cutaneous level (malar rash), muscle–skeletal level (myalgia, arthritis), hematological level (cytopenia) renal level (glo-

merulonephritis)(3).

CASE REPORT

We present the case of a female patient aged 57, from the rural environment, who undergoes her first cardiovascular evaluation, she gets admitted to the emergency room complaining about palpitations, deep physical asthenia, dyspnea in average effort and discrete edema at the lower limbs level.

Upon resuming the anamnesis, the patient appears to suffer from a possible anemic syndrome accidentally diagnosed approximately 5 years ago, in a medical care unit abroad yet without showing any medical documents to support such statement, and without being able to provide any other relevant anamnesis related details.

The clinical examination upon commitment shows malar rash (Fig.1), pale teguments, slightly icteric, discrete myalgia, varices at the level of lower limbs on both sides, dorsal kyphosis with average curve radius, static and dynamic lumbar vertebral syndrome, showing pain upon palpation of the metacarpophalangeal joint I both hands – bilateral level; Blood pressure =100/70 mmHg, ventricular allure=80 beats/minute- heart rate = 80 bpm, rhythmic cardiac murmurs, systolic murmur in the aortic focus 3/6 degree, systolic murmur in the mitral focus – 3/6 degree.

Rest Electrocardiogram shows a sinus rhythm, ventricular rate =59/min, QRS intermediary axis, standard morphology (Fig. 2).

Fig. 1. Malar rash

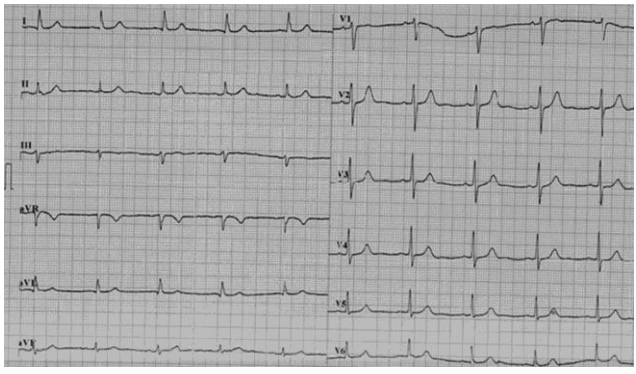

Fig. 2. Electrocardiogram upon commitment

The abdominal ultrasound shows a homogenous liver, lower vena cava = 22 mm, present pericardial fluid; the rest of the abdominal ultrasound test shows no other modifications whatsoever.

At the biological level, we found a microcytic Hypochromic anemia (hemoglobin upon commitment=4,4g/dl), Syderemy =26 µg/dl, Ferritin=10 ng/ml, hyperglycemia, and discreet inflammatory syndrome. The examination of the peripheral blood smear showed an intense erythrocyte anisopoikilocytosis, microcytosis and erythrocyte hypochromia. We have excluded any source of active bleeding at the gastrointestinal tract level or in the gynecological area.

The chest X-ray shows a heart with enlarged transverse diameter, a rectilinear left middle arch, the lung with no pleural –

pulmonary evolutive lesions, calcareous contour aortic knob, moderate intensification of the pulmonary interstitium of the reticular – micronodular display, at the basal level on both sides (fig. 3).

The echocardiographic imaging has showed serositis with a predominant pericardiac determination (the display was that of fluid chronic pericarditis with a thin layer of pericardium fluid displayed at 12 mm circumferential level), with an ejection fraction of 60%. The immunological parameters have endorsed the diagnosis supposition of systemic lupus erythematosus (fig. 4). The patient met 6 of the 11 ACR criteria (malar rash, photosensitivity, serositis, hematological affectation, immunological affectation, ANA – antinuclear antibodies). Moreover, 6 of the Criteria of the Group of Experts in Systemic Lupus International Collaborating Clinics (SLICC) were also met: acute cutaneous lesions, serositis, hematological affectation, increased ANA titer and anti – DNA double – catena antibodies (Ac anti-ADNdc), low C_3 complement level.

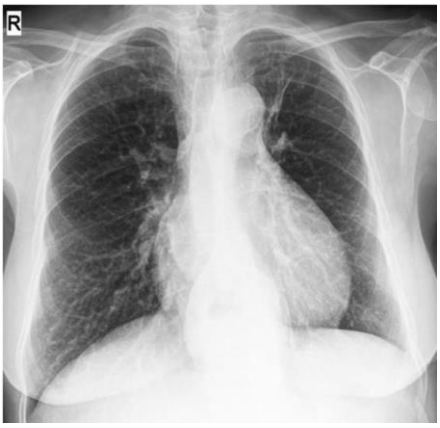

Fig. 3. Chest X-ray

Antinuclear antibodies	338 ↑
Double – catena Anti DNA antibodies	42 ↑
anti SS – A antibodies	475 ↑
anti SS – B antibodies	335 ↑
Cryoglobulins	absent
C3	74 mg/dL
C4	16,4 mg/dL

Fig. 4. Biochemical plasma changes suggestive for SLE

IV with erythrocytes were used, followed by protons pump inhibitors, with a positive clinical evolution, the patient being directed towards the Rheumatology section for registration and therapeutic conduct. The treatment in case of systemic lupus erythematosus aims at diminishing the systemic inflammation with a positive impact, the activity of the disease slowed down leading to a remission of the disease and lowering the occurrence of prophylaxis relapses and therefore the risks of other complications.

DISCUSSION

Fatigability is one of the major symptoms that affects the quality of life, often being described as a *"feeling of physical fatigue and lack of energy deemed to be pathological, excessive, chronic, persistent and problematic"* and it is generally felt when energy reserves are exhausted (4). Moreover, fatigue as common symptom affects more than 90% of the patients suffering from systemic lupus erythematosus (5, 6), with a negative impact on labor performance, family responsibilities and/or

social activity (7, 8) which usually / ultimately triggers a polymorph clinical picture (9).

Cardiovascular pathology varies from subclinical to clinical, with the occasional manifestation of life–threatening complications. The cause of any anemic syndrome should always be identified and specifically treated (10).

Fatigue can be defined as an excessive feeling of exhaustion that does not resolve with sleep or adequate rest (11). More than 50% of patients describe fatigue as their most disabling symptom, furthermore it can cause employment disability and it can have a major impact on the patient's quality of life. Evidence from literature review show an important relationship between fatigue and disease activity which researchers strongly associate with anxiety and depression (12).

Fatigue is a multidimensional concept in rheumatic diseases and is a common symptom in SLE. The treatment and understanding of fatigue remain challenging despite the evolution of alternative therapies (12).

Recent studies have also revealed that cardiovascular disease (CVD) is the main cause of death in patients with SLE. Cardiac diseases in lupus patients may involve the pericardium, myocardium, and endocardium. Compared to the risk in the general population, patients with SLE have a higher risk of stroke, myocardial infarction (MI), congestive heart failure (CHF) and mortality (13). Furthermore, accelerated atherosclerosis is one of the most important comorbidities of SLE and causes of death in relatively young patients (14).

In the course of SLE the most frequent heart manifestation is pericarditis, other causes are rarely observed. It is usually associated with fever and positive antinuclear antibodies (ANA) and treatment should be prompt. In this case, the patient was admitted to the emergency room complaining about palpitations, deep physical asthenia, and dyspnea on average effort, which demonstrates that SLE is characterized by various clinical manifestations.

The complex clinical presentation of SLE often involves multisystemic damage including renal, dermatological, neurological with undulatory, chronic or remission evolution, often with risk of relapses, complications, and comorbidities.

The research of literature shows evidence to support that SLE is an independent risk factor for cardiovascular disease (CVD) development in patients without previous cardiovascular disease. The management, therapies, and cardiac evaluation in patients with SLE are important procedural steps and require a multidisciplinary approach (15).

In the absence of treatment, the prognostic is reserved given the development of systemic complications: cardiovascular, renal, pulmonary, neuropsychiatric, and thromboembolic (16).

Despite current therapeutic advances, the mortality associated with SLE remains high, the probability of survival at 5 years after diagnosis is about 95% and after 20 years is about 78% (17.18).

According to studies, mortality in the first years after diagnosis is associated with active disease and intercurrent infections. Subsequently, the causes of death were reported due to complications of corticosteroid therapy, associated pathologies and last but not least cardiovascular disease.

Lupus nephritis affects about 60% of patients with SLE is one of the most common and severe manifestations of SLE often with poor evolution and prognosis.

In the absence of identification of patients with lupus and lupus nephritis, treatment and rigorous monitoring, the main risk of lupus nephritis is progression to chronic kidney disease (10-30%) (19, 20) with a significant impact on survival, mortality, morbidity and decrease. quality of life (21).

These new data highlight the importance of histopathological diagnosis in lupus nephritis and the usefulness of serial renal biopsy protocols in assessing response to treatment, and the most accurate biomarker in assessing lupus nephritis is renal biopsy (22, 23).

The goal of early treatment is to maintain normal kidney function, prevent skin damage, improve quality of life and longevity among these patients. Current immunosuppressive therapies achieve partial or complete remission in only 50-70% of cases, and complete remission according to the current definition is installed in only 30% of cases (24, 25).

Under immunosuppressive therapy, the diagnostic criteria for remission are under constant debate, especially when its onset should be evaluated, especially due to the fact that many patients achieve remission at least 12-24 months after initiation of immunosuppressive therapy (26).

Current research has also shown that the onset of clinical remission does not necessarily involve immunological remission, just as the lack of clinical response does not necessarily imply persistent lupus nephritis (27, 28).

Relapses due to lupus nephritis cause irreversible lesions and loss of nephrons (29), accelerating the evolution to BCR in the final stage and the need to replace renal function. Periodic nephrological evaluation is essential among these patients.

Currently, approximately 30% of patients diagnosed with lupus nephritis are refractory to current immunosuppression regimens, requiring the identification of new molecules and effective immunosuppressive regimens. According to literature data, although the current evidence is limited, one of the new therapeutic methods being evaluated in severe autoimmune diseases, including SLE, is stem cell transplantation (30, 31).

The occurrence of pericarditis is usually related to the evolution of the disease. According to current guidelines, the appearance of cardiovascular problems in systemic diseases requires a dual treatment: the treatment for the main disease and the treatment of the actual heart disease.

CONCLUSIONS

The report describes the case of a female patient who got admitted into a medical care unit on grounds of the limitation of daily activities as a result of fatigability, further examination showed a severe anemic syndrome and the presence of serositis, with the development of a systemic immunological affectation at the visceral level.

The systemic lupus erythematosus is a complex autoimmune condition, quite often with severe outcomes and a nonlinear evolution, with the risk of relapse in the absence of an accurately established treatment, as well as of complications and comorbidities.

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NEWS

THE RELATIONSHIP BETWEEN NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND SELENIUM-DEPENDENT ANTIOXIDANT SYSTEMS

A review conducted by Polyzos S *et al.* (2020) aims to synthesize the existing information on the link between NAFLD, selenium and selenoprotein P, starting from the conflicting literature data link of nonalcoholic fatty liver disease (NAFLD), a disease with no currently approved treatment, with selenium (Se) and selenoprotein P (SELENOP), a glycoprotein synthesized and primarily secreted by the hepatocytes, functioning as a Se transporter from the liver to other tissues. The experimental studies included in the review investigated the association of Selenium with antioxidant defense, which may help modulate hepatic inflammation and fibrosis. Animal studies using Se-deficient diets showed a decrease in glutathione, an increase in lipid peroxidation and a decline in hepatic GPx implying increased oxidative stress and impaired antioxidant function. A number of studies showed a favorable effect when using selenium supplementation. Other studies displayed concern for high exposure to selenium (high doses and/or longer duration) reporting possible adverse effect (formation of nodular regenerative hyperplasia with sinusoidal damage around nodules of the liver). One of the relevant findings is that lower selenium and selenoprotein P levels are found in patients with advanced liver disease. The results of this review pave the way for future research. (Polyzos SA, Kountouras J, Goulas A, Duntas L. Selenium and selenoprotein P in nonalcoholic fatty liver disease. *Hormones* (*Athens*) 2020; 19(1): 61-72).

CLINICAL EVOLUTION OF RARE VARIANTS OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN ROMANIA

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CLINICAL EVOLUTION OF RARE VARIANTS OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN ROMANIA (Abstract): Alpha-1 Antitrypsin deficiency (AATD) is a genetic inherited autosomal dominant condition, characterized by decreased serum and pulmonary level of alpha-1 antitrypsin, affecting the lung, liver, rarely skin, associated with an increased risk of emphysema and chronic obstructive pulmonary disease (COPD) early in life, under 40. Under the minimum protection value of Alpha-1 Antitrypsin (AAT) (11 μmol/L or 80 mg /dL), neutrophil elastase may cause the alveolar septa destruction with the early appearance of pulmonary emphysema. Although, AAT is a highly pleomorphic glycoprotein, due by mutations Z and S of approximately 120 alleles. At least 30 AAT alleles other than PI*Z or PI*S were described in literature, some of them as single cases. The prevalence of rare genotypes, other than PI*ZZ or PI*SZ, is between 11% and 21.3%. These rare genotypes were recorded in areas with low prevalence of PI*Z. Rare genotypes of AATD with low plasma levels are Mprocida, Mpalermo, Q0isola di procida, Q0trastevere, Mmalton, Harleen, etc. These evolve clinically with severe emphysema with early onset between 30-40 years. The use in screening programs of gene sequencing for exons 2-5 of SERPINA-1, when genotyping demonstrates the absence of the Z and S alleles, will allow in the coming years to identify several cases of rare variants. Rare variants of alpha1 antitrypsin should be considered on the same risk for emphysema as PI*ZZ. **Keywords**: ALPHA-1 ANTITRYPSIN DEFICIENCY, EMPHYSEMA, RARE VARIANTS.

Alpha-1 Antitrypsin deficiency (AATD) is an autosomal inherited disease, characterized by decreased serum and pulmonary level of alpha-1 antitrypsin (member of the serpin family of protease inhibitors), associated with an increased risk of emphysema and chronic obstructive pulmonary disease (COPD) early in life (1, 2, 3). Alpha-1

Antitrypsin (AAT) is a 52-kDa glycoprotein that functions as the major inhibitor of human neutrophil elastase, a potent serine protease capable of destroying most connective tissue matrices (2, 4, 5). The most common "normal" AAT alleles are M1 (Va1213) (allelic frequency 44-49%), M1 (Ala213) (20-23%), M2 (14-19%), and M3 (10-11%). Individuals inheriting any homozygous or heterozygous combination of these alleles have normal serum AAT levels (20-53 μmol /L or 120-220 mg/dL), enough to provide anti-elastase protection. In normal individuals, the presence of AAT at normal plasma levels keeps the action of neutrophil elastase under control. Under the minimum protection value of AAT (11 μmol/L or 80 mg/dL), neutrophil elastase may cause the alveolar septa destruction with the early appearance of pulmonary emphysema. A reasonable threshold for differentiating normal PI*MM from other genotypes with one or more deficient alleles is 20 μmol/L (100 mg/dL).

Although, AAT is a highly pleomorphic glycoprotein, with approximately 120 alleles being identified, the two major variant mutations, Z and S, represent most cases of the AATD (3, 5, 6). The vast majority of Alpha patients carry the PI*ZZ or PI*SZ genotype, coding for approximately 15% and 25% of cases, respectively, of normal AAT plasma levels (3, 5, 6).

Nevertheless, there are at least 30 AAT alleles other than PI*Z or PI*S associated with absent or reduced levels of AAT. There are limited data about the epidemiology, natural history, clinical manifestations of these rare Genotypes, which are often cited in literature as single cases. The recent study on AAT Deficiency in Romania suggest that rare variants of AAT are more frequent than PI*Z and PI*S in Romania (5).

Lung manifestations and Alpha-1 Antitrypsin Deficiency

Lung manifestations in AATD are emphysema, chronic obstructive pulmonary disease (COPD) and bronchiectasis. Typically, emphysema is pan acinar with basal predominance, and is associated with lower values of alpha-1 antitrypsin < 11 μmol/L; as severe is reduction in plasmatic alpha1antitrypsin value, emphysema develops early and extensively (1, 2). The mean age of emphysema onset in AATD is between 32 and 45 years old, while in typical COPD patients onset of symptoms occur later, after 45 years old.

The early onset of emphysema is related to the level of plasma value, the closer to zero, the earlier is the onset of emphysema. It was observed that individuals with near-zero values of alpha-1 antitrypsin, with the homozygous Null/Null variant, develop emphysema at younger ages than subjects with the AATD homozygous PI*ZZ form (7, 8). The 15 to 20% of the normal plasma concentration of alpha-1 antitrypsin associated with the PI*ZZ allele seems to provide some protection to the lung in comparison with a complete deficiency, like in Null/Null variant (7, 8).

Progression of emphysema in alpha-1 antitrypsin deficiency is known to be at an accelerated rate when compared to common COPD. Frequency of exacerbations appears to be correlated with FEV1 decline and the presence of chronic bronchitis were independent predictors of FEV1 decline (9, 10). Currently, there are uncertainties about patients with severe AATD and rare variants experiencing the highest rate of progression of emphysema, thus reflecting a lack of knowledge of the natural history of this disease (10).

In Italian Registry of AATD, Luisseti

and Ferrarotti found a prevalence of 21.3% cases with rare variants, highest prevalence of rare variants identified overwide, in a country with medium-low prevalence of ZZ (4, 8). Romania seems also to have high prevalence of rare variants and low prevalence of ZZ (5).

Rare mutations and early onset of emphysema

The Italian study demonstrate that COPD and emphysema occur early in life in severe deficiency in rare variants of AATD, like Mmalton, Mprocida, Plowell, Q0procida, I, Mharleen (8). Plasmatic values of alpha-1 antitrypsin in rare variants were severe decreased, compared with ZZ individuals, concluding that severe AAT deficiency in rare variants, especially in smoking exposure, have the same risk and the same natural evolution as ZZ, which is much more studding (2, 3, 4, 8).

Risk factors for early development of emphysema are lower plasmatic values of alpha-1 antitrypsin, smoking exposure, professional exposure to noxes, exacerbations (8, 9, 10). Also, risk factors associated with accelerated decline of lung function in AATD were demonstrated to be smoking exposure, frequent exacerbations > 3/year, chronic bronchitis, extensive emphysema, particularly in the upper zones (11).

Mprocida - rare variant of severe AAT deficiency

Procida allele is an allele based M1 (Val 213) by a T → C mutation at codon 41, exon II; Mprocida is associated with AAT serum levels ≤ 10 mg/dL (n.v. 120-220 mg/dL), codes for an AAT molecule that focuses on immobilized pH gradient isoelectric gels slightly cathodal to the common normal M1 (12, 13). Mprocida it was first detected in the late period of the 80's, in an Italian American family, by Takahashi (12, 13).

The homozygous variant Mprocida/Mprocida evolves with very close to zero plasma levels. The clinical phenotype is not described in the literature as well as the natural history of AATD by Mprocida/Mprocida. Existing data thus far suggest a clinical evolution similar to Null/Null, with an early and severe onset of emphysema < 35 years, rapid decline in pulmonary function in the context of smoking exposure and respiratory noxes (13).

Plowell - rare variant of severe AAT deficiency

Plowell allele (also referred to asQO*Cardiff) is an allele-based is characterized by an A→T transversion at codon 256 exon III, leading to a valine for asparagine substitution (8). This substitution causes a severe AAT deficiency when it occurs in homozygosis form: Plowell/Plowell or in association with other deficiency variants such as PI*Z (14). Plowell evolved with near zero values of alpha1 antitripsina and seems to have an increased risk for early emphysema.

Clinical case-Mprocida/Mprocida

We present the case of a 35-year-old male patient, from Romanian National Registry of AAT Deficiency, with Mprocida/Mprocida, with early onset of severe emphysema at the age of 35 years old and symptoms mimicking asthma. A 35-year-old male, active smoker, with a tabacum consumption of 15 pack/year, construction worker for 10 years, is addressed by the family doctor to the pneumologist for repeated episodes of dyspnea at moderate

efforts with suspicion of asthma. Chest X-ray revealed hyper-inflated lung fields with attenuation of peripheral vasculature, flattened diaphragms, and increased retrosternal clear space. Pulmonary function test revealed moderate obstructive dysfunction (FEV1 = 51%, FVC = 74%); post bronchodilation FEV1 increased with 14% and 300 mL. High-resolution computed tomography showed centrilobular emphysema, predominantly in both lower lobes.

Serum levels of AAT, was determined by nephelometry, using an IMMAGE 800 analyzer (Beckman Coulter, USA); plasmatic value was near zero mg/dL, respectively 0.12 mg/dL. Dried bloodspots (DBS) were used for collecting, transporting blood samples, for analysis and diagnosis, to NITBLD in Warsaw, Poland, the main laboratory in the Central Eastern Europe Alpha-1 Antitrypsin Network. DBS were used for extraction of genomicdezoxyribonucleic acid (DNA) and detecting the presence of Z or S allele, by a real time polymerase chain reaction (RT-PCR), method in the Light Cycler 480 II instrument (Roche Diagnostics Ltd., Switzerland). No Z, no S were found, and we continue with sequence analysis of AAT exons 2-4 was determined by a 16-capillary 3130 x l Genetic Analyzer (Applied Biosystems, USA) (Genomed, Poland) (5). Genetic sequencing determined Mprocida (5). Family testing was following, we diagnosed two daughters with AAT value 91.4 mg/dL, respectively 92.5 mg/dL, which were heterozygous MMprocida. The brother had 0.16 mg/dL AAT plasmatic value, and genetic sequencing detected Mprocida Plowell.

Clinical case- Mprocida/Plowell

The second case we reported was the brother of index case, male, 37 years old, e-cigarettes user for 10 years, but with no professional exposure. Lung function test showed an FVC = 97%, FEV1 = 80%. High-resolution computed tomography showed centrolobular emphysema predominant in inferior lobes. Plasmatic value of alpha-1 antitrypsin was near zero, 0.16 mg/dL, genetic sequencing evidentiate Mprocida Plowell. From our knowledge, there is no case reported with Plowell and Mprocida.

DISCUSSION

AATD is inherited by autosomal co-dominant transmission, characterized by decreased serum and pulmonary level of alpha-1 antitrypsin. Alpha-1 antitrypsin deficiency is still an unrecognized disease, medium years necessary for diagnosis been 9.0 ± 12.1 years (16). Common lung manifestations included emphysema, COPD, bronchiectasis. AAT is a protease inhibitor of the proteolytic enzyme elastase and also of the proteases trypsin, chymotrypsin, and thrombin. The imbalance between neutrophil elastase in the lung (which destroys elastin) and the elastase inhibitor AAT (which protects against proteolytic degradation of elastin), the increasing of elastase secondary to cigarette smoking and infection contribute to local inflammation and tissue destruction in the lung (17). Alpha-1 antitrypsin was encoded by SERPINA-1 gene (MIM +107400) located on the long arm of chromosome 14. At least 120 alleles of AAT (SERPINA-1) have been identified. The normal allele is referred to as "M", AAT is a protease inhibitor, the designation "PI" denotes "protease inhibitor" and the letters denotes the alleles that are present ("PI*MM" refers to homozygosity for the normal gene, while PI*ZZ denotes homozygosity for the Z allele. Some indi-

viduals have compound heterozygosity, meaning that they carry two different mutations of the same gene. Genotyping of the protease inhibitor (PI) using polymerase chain reaction (PCR), can detect the normal M allele and the most commonly known pathogenic variants (e.g., F, I, S, Z).

Variants of AAT can be categorized into four basic groups:

- **Normal** – Normal alleles are associated with normal levels of AAT and normal function.
- **Deficient** – Deficient alleles are associated with plasma AAT levels less than 35 % of the average normal level.
- **Null** – Null alleles lead to no detectable AAT protein in the plasma.
- **Dysfunctional** – Dysfunctional alleles produce a normal quantity of AAT protein, but the protein does not function properly (17).

Data are conflicting regarding the risk of emphysema among PI*MZ heterozygotes so called heterozygous variants (17). For patients who have a low level of AAT, with none I, S, or Z variants on genotyping or phenotyping, sequencing of DNA may be needed to assess for null or other rare deficient alleles.

Regarding the extensive educational efforts and the publication of evidence-based guidelines for diagnosis and management of AATD the recognition of AAT deficiency has be found to be under-recognized, with a 5-8-year delay between the first symptom and diagnosis (18). European Respiratory Society statement described the additional feature that should lead clinicians to test for AATD: Emphysema in a young individual (e.g., age ≤ 45 years).

- Emphysema in a nonsmoker or minimal smoker.
- Emphysema characterized by predominant basilar changes on the chest radiograph.
- A family history of emphysema and/or liver disease.
- Adult-onset asthma (when airflow obstruction fails to normalize after bronchodilators).
- Clinical findings or history of panniculitis.
- Clinical findings or history of unexplained chronic liver disease (18).

The natural history most studied is PI*ZZ, but from natural history of rare variants, a little is known. Rare variants of alpha-1 antitrypsin are published as unique or few cases in literature. A number of rare variants of alpha-1 antitrypsin evaluated with near zero plasmatic values, like Mprocida, have a risk of developing emphysema early in life < 35 years old. Few studies about the evolution of Null variants that demonstrated variants of alpha-1 with near zero plasmatic values of alpha-1 antitrypsin will evolute with severe emphysema and accentuated lung function decline. Risk factors are also important in the evolution of AAT deficiency with rare variants (7). Cigarette smoking, dusty occupational exposure, parenteral history of COPD and personal history of chronic bronchitis, asthma or pneumonia can be important risk factor for lung disease in AATD patients (18). Our examples also demonstrated the role of smoking exposure and construction dusts exposure in the onset of emphysema.

CONCLUSIONS

Rare variants of alpfa-1 antitrypsin could evolve with near zero plasmatic values, and clinically with early onset of emphysema < 35 years old, with a rapid decline in pulmonary function, especially in the context of smoking exposure and res-

piratory noxes and dust. From Romanian screening programme preliminary results, rare variant seems to be more prevalent than PI*ZZ, especially in Nord-West region of the country (5).

The use in screening programs of gene sequencing for exons 2-5 of SERPINA-1, when genotyping demonstrates the absence of the Z and S alleles, will allow in the coming years to identify several cases of rare variants. Rare variants of alpha-1 antitrypsin should be considered on the same

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NEWS

THE 4S-AF SCHEME FOR STRUCTURED CHARACTERIZATION OF ATRIAL FIBRILLATION

Atrial fibrillation (AF) is a complex condition requiring holistic management with multiple treatment decisions about optimal thromboprophylaxis, symptom control (and prevention of AF progression), and identification and management of concomitant cardiovascular risk factors and comorbidity. Sometimes the information needed for treatment decisions is incomplete, as available classifications of AF mostly address a single domain of AF (or patient)-related characteristics. The most widely used classification of AF based on AF episode duration and temporal patterns (that is, the classification to first-diagnosed, paroxysmal, persistent/long-standing persistent, and permanent AF) has contributed to a better understanding of AF prevention and treatment but its limitations and the need for a multidimensional AF classification have been recognized as more complex treatment options became available. The authors proposed a paradigm shift from classification toward a structured characterization of AF, addressing specific domains that have treatment and prognostic implications to become a standard in clinical practice, thus aiming to streamline the assessment of AF patients at all health care levels facilitating communication among physicians, treatment decision-making, and optimal risk evaluation and management of AF patients. Specifically, they proposed the 4S-AF structured pathophysiology-based characterization (rather than classification) scheme that includes four AF- and patient-related domains-Stroke risk, Symptoms, Severity of AF burden, and Substrate severity-and provide a hypothetical model for the use of 4S-AF characterization scheme to aid treatment decision making concerning the management of patients with AF in clinical practice (Potpora TS, Lip G, Boriani G, *et al.* The 4S-AF Scheme for Structured Characterization of AF. *Thromb Haemost* 2020; 18(8): 1-9).

THE SURGICAL MANAGEMENT OF LARGE BOWEL OBSTRUCTION IN PATIENTS WITH LEFT COLON AND RECTAL CANCER – A RETROSPECTIVE STUDY

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THE SURGICAL MANAGEMENT OF LARGE BOWEL OBSTRUCTION IN PATIENTS WITH LEFT COLON AND RECTAL CANCER - A RETROSPECTIVE STUDY (Abstract): The optimal surgical attitude towards obstructed left colon and upper rectal cancer remains elusive, one of the main postoperative endpoints being to avoid the most dreaded complication of this type of procedures - the anastomotic leakage. **Material and methods:** We conducted a retrospective analysis of all patients with left colon and rectal cancer admitted and having emergency surgery performed in the surgical departments of the University County Hospital of Craiova, Romania, between 2001 and 2018. The timeline analysis showed an increasing interest for single-stage resection and anastomosis in the second decade (2011-2018) compared to the first decade of the study (28% *vs.* 11.2%). **Results:** The incidence of anastomotic leak for these types of procedures remained higher than for the two-stage procedure (15% *vs.* 4.8%), similar to the overall postoperative morbidity (30.3% *vs.* 20%) and mortality (3% *vs.* 1.1%). Postoperative morbidity and anastomotic leak rate were statistically higher in the single stage procedure group when compared with the two-stage procedure ($p < 0.05$). **Conclusions**: Deciding between single-stage resection with anastomosis and a two-stage procedure requires a careful patient selection due to the relatively high risk of anastomotic leak in this specific category of patients. **Keywords:** COLORECTAL SURGERY, ANASTOMOTIC LEAKAGE, COMPLICATION.

Despite the presence of screening programs, colorectal cancer remains the most frequent cause of large bowel obstruction in adult patients (1, 2, 3). An increasing incidence was observed in patients less than 50 years of age with approximately 15%-25% of them presenting with symptoms of obstruction which usually requires emergency surgical procedure. Surgery is however associated with an increased risk of mortality and morbidity, probably because most of these patients have comorbidities, fluids and electrolytes imbalance, and an advanced-stage cancer (4, 5, 6, 7).

Nonetheless, most tumors leading to large bowel obstruction are located on the

sigmoid colon, and around 75% of obstructing malignancies occur distal to the splenic flexure. The optimal management of left-sided large bowel obstruction is less clear and represents a real surgical challenge (2, 8). Treatment of obstructing colorectal cancer, especially in left-sided locations (oLCRC), often involves 2-stage or even 3-stage surgery while primary resection and anastomosis – the so-called one-stage surgery - has been gradually applied in certain patients such as oLCRC to reduce hospitalization due to multiple stage surgery) (9). Due to smaller diameter of the bowel in this location, left colon tumors and rectal tumors will lead more frequently and in a shorter amount of time to bowel obstruction than those localized on the right colon.

MATERIAL AND METHODS

We performed a retrospective study on patients with left colon and rectal cancer which were admitted and underwent emergency surgical treatment in all three surgical wards of the Emergency County Hospital of Craiova, Romania, between 2001 and 2018.

This study was conducted in accordance with the ethical principles stated by the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all subjects involved in the study. The study protocol was revised and approved by the local Ethics Committee of the University of Medicine and Pharmacy of Craiova with code number 56/23.04.2021.

The inclusion criteria were patients admitted in the emergency setting with the diagnosis of bowel obstruction and the site of obstruction either at the level of the rectum or the left colon. The exclusion criteria referred to benign colorectal pathology, prior chemotherapy or radiation therapy for colorectal cancer, and elective surgical procedures.

The aim of the current study was to assess the current management of these patients and compare it with the international literature data. As the possibility of endoscopic stenting was unavailable in our institution, the main option in case of bowel obstruction was open surgery.

Along with descriptive statistics (presented as mean and percentage), a timeline analysis was performed, comparing relevant surgical data such as the type of procedure and the immediate postoperative outcomes, such as postoperative complications, the overall morbidity, and mortality rate. Categorical variables were displayed as absolute and percentage values. Research data were uploaded to the electronic database and the statistical analysis was performed using *Microsoft Excel* (Microsoft, Redmond, USA) and *GraphPad Prism version 8.0* (GraphPad Software, San Diego, USA). For normally distributed values, the t-Student's test was used to assess potential statistically significant differences between the two groups. The statistical significance level was considered for $p < 0.05$.

RESULTS

Between 2011 and 2018, a total number of 442 cases of colorectal cancer were admitted in our surgical department, out of which 157 cases (35.5%) displayed clinical signs of bowel obstruction. Regarding the site of obstruction, most cases (119 cases) had left colon or upper rectum obstruction (group A). Between 2001 and 2010, 377 colorectal cases were admitted in our department, of which 98 patients underwent surgery for oLCRC (group B).

In group A, single step surgery was performed in 33 cases (27.7%), while in 86

cases (72.3%) a two-stage surgery was preferred, with 71% of cases (62 patients) undergoing the anastomosis as a second stage procedure. The operative procedures performed were left hemicolectomy (18 cases), rectosigmoidectomy (10 cases), subtotal colectomy (5 cases), Hartmann procedure (53% -63 cases) followed by anastomosis (45 cases), and loop colostomy (23 cases – 19%). Single stage restoration of digestive continuity after primary resection was performed in 27.7% of patients (33 cases).

In group B, resection with primary anastomosis was performed in 11.2% of cases (11 patients), while Hartmann procedure was performed in 28.5% of cases (28 patients). Only 60% of those patients undergoing Hartmann procedure had a restorative procedure performed (17 cases). Proximal colostomy was performed in 40 patients (40.8%) followed by second stage left or segmental colectomy in 22 patients (tab. I).

TABLE I

Type of emergency procedures performed between 2001-2010 and 2011-2018 for oLCRC

Procedure – stage I	No.	%	Procedure – stage II	No.	%
2001-2010					
Hartmann	28	28.5%	Anastomosis	17	60.7%
Proximal colostomy	40	40.8%	Segmental colectomy	11	55%
			Left colectomy	11	
Cecostomy	7	7.1%	Left colectomy	2	28.5%
Internal diversion	12	12.2%	Left colectomy	7	58.3%
Colectomy	11	11.2%	-	-	-
Total	**98**	**100%**		**48**	**55%**
2011-2018					
Hartmann	63	53%	Anastomosis	45	71%
Proximal colostomy	23	19%	Left colectomy	12	74%
			Rectosigmoidectomy	5	
Colectomy with anastomosis:	33	28%	-	-	-
- left colectomy	18				
- rectosigmoidectomy	10				
- subtotal colectomy	5				
Total	**119**	**100%**		**62**	**72.1 %**

Between 2011 and 2018, the postoperative course was marked by a significantly higher incidence of anastomotic leaks in the single stage restoration group - 15% of cases (5 out of 33 patients), *vs.* 4.8% of cases (3 out of 62 patients) in the two-stage procedure group (tab. II).

Out of the 86 patients in which no anastomosis was initially performed, the global morbidity was 10.4% (9 cases) and 1.1% mortality rate (one case). Most of the patients with postoperative adverse effects had only mild postoperative complications (classified as Clavien-Dindo grade I-II), such as urinary infections (5.8% - 5 cases) and wound infections (9.3% - 8 cases). During the restorative procedure (62 cases), the overall postoperative morbidity was 9.6% (6

cases) with 3 cases (4.8%) of anastomotic leak which were managed by conservative treatment. No postoperative mortality was registered in this group (tab. II).

In the single stage restoration group, the postoperative mortality was 3% (one out of 33 cases), while the postoperative morbidity accounted for 30.3% (10 out of 33 cases). Anastomotic leaks were diagnosed in 5 patients (15%) and in 2 cases (6%) emergency surgery for postoperative peritonitis was required. Death occurred due to severe sepsis in one of these 2 patients.

Postoperative morbidity and anastomotic leak rate were statistically higher in the single stage procedure group when compared with the two stage procedures ($p < 0.05$).

TABLE II

Postoperative outcomes for single-stage *vs.* two-stage procedures in patients with oLCRC operated between 2011 and 2018

	Single stage procedures (33 cases)	Two-stage		p
		Stage I (86 cases)	**Stage II** (62 cases)	
Wound infection	6 (18.1%)	8 (9.3%)	5 (8%)	NS
Anastomotic leak	5 (15%)	-	3 (4.8%)	<0.05
Peritonitis	2 (6%)	-	-	<0.05
Urinary infection	4 (12%)	5 (5.8%)	2 (3.2%)	NS
Sepsis (MODS)	1 (3%)	-	-	NS
Global morbidity	10 (30.3%)	9 (10.4%)	6 (9.6%)	NS
Mortality	1 (3%)	1 (1.1%)	-	<0.05

oLCRC - obstructed left colon and rectal cancer

DISCUSSION

Despite the new additions in the therapeutic armamentarium of colorectal surgery, such as total mesorectal excision, fast-track perioperative management, laparoscopic, robotic and other minimal invasive surgical approaches, many controversies persist concerning the emergency surgical treatment in obstructive cases (10, 11, 12). This problem appears more stringent as the incidence of complicated colorectal cancer remains as high as 40%, with obstructive colorectal cancer accounting for up to a third of the cases admitted for this pathology. To add more complexity to the problem, almost 60% of obstructive cases occur in elderly patients. Given the fact that the location of the tumor in most of these patients (up to 75%) is distal to the splenic flexure, special attention should be paid to the surgical management and the outcome of these specific cases (13, 14).

So far, there is no consensus for the best management of oLCRC, and the quality of evidence for current guidelines is poor (15, 16). In addition to the very survival of the patient, the main issue in the assessment of the optimal treatment protocol for this pathology is the occurrence of anastomotic disruption and leakage, which remains surgeon's most feared complication, and one of the leading causes for postoperative mortality (17). Other aspects to be considered for the optimal management of oLCRC are the quality of the oncologic surgery and of the resected specimen, decrease of definitive colostomies, and on the whole the improvement in the quality of

life (18, 19).

In view of these aspects, the management options for oLCRC diversified during the last 3 decades and were reconsidered, to some extent, in order to include endoscopic procedures such as self-expandable metallic stents placement or to offer a larger role to single stage resection and anastomosis to the expense of two-step procedures. Other measures that contributed to the wider adoption of single stage resection and anastomosis procedures included the optimization of the perioperative diagnostic and therapeutic protocol, with more emphasis on aspects such as complex preoperative clinical assessment, parenteral nutrition, pharmacologic and non-pharmacologic management, and more importantly, improved selection of patients for this type of procedure (20, 21, 22).

In our study, Hartman procedure was preferred for elderly patients, for patients with severe anemia, or for patients with severe comorbidities, in which potential anastomotic leak could have proven fatal. Mortality rate for patients with primary resection and anastomosis was 3%, compared to 1.1% in case of Hartman procedure. Hartman resection remains a good solution for elderly patients, patients with severe anemia, or for those with multiple comorbidities, but primary resection with anastomosis could offer superior results in selected cases, both from a mortality and quality of life point of view (10).

CONCLUSIONS

The optimal therapeutic algorithm of patients presenting in the emergency setting with left colon or rectal obstructive cancer is still difficult, especially in cases where the tumor is resectable, as the choice between single stage resection and anastomosis and two stage procedure requires a careful patient selection due to the risk of anastomotic leak.

AUTHORS CONTRIBUTIONS

All authors have read and agreed to the published version of the manuscript. Marian Diaconescu, Andrei Nicolaescu and Marius Bica equally contributed to this study.

CONFLICT OF INTEREST AND FUNDING

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WIDE AWAKE LOCAL ANESTHESIA NO TOURNIQUET TECHNIQUE IN HAND SURGERY DURING THE COVID-19 PANDEMIC - PERSONAL EXPERIENCE

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WIDE AWAKE LOCAL ANESTHESIA NO TOURNIQUET TECHNIQUE IN HAND SURGERY DURING THE COVID-19 PANDEMIC - PERSONAL EXPERIENCE (Abstract) : Short-time exposure of the patient to the hospital environment and the significantly lower costs make WALANT technique a "gold-standard" anesthesia in hand surgery during the COVID-19 pandemic. **Material and methods**: We studied a group of 180 patients treated for acute and chronic conditions during the first 10 months of the COVID-19 pandemic. A statistical analysis was conducted in order to demonstrate the efficacy and usefulness of the WALANT technique. **Results**: The average length of hospital stay was 118.05 minutes, the mean time of onset of local anesthesia was 12.79 minutes, the average patient waiting time from admission to surgery was 15.72 minutes. We used ANOVA regression, establishing CI 95% and significance F of 95%. A p-value <0.05 was considered statistically significant. **Conclusions**: Using WALANT technique the length of hospital stays and implicitly the risk of contracting SARS-CoV-2 decreases and also the costs are lower, surgical care is good, and patient satisfaction is high. **Keywords**: HAND SURGERY, LOCAL ANESTHESIA, WALANT, COVID-19 PANDEMICS.

The Wide-Awake Local Anesthesia No Tourniquet (WALANT) technique of local anesthesia, initially proposed for small surgeries and wrist surgeries, in time has proven its efficacy and safety, currently being used for extensive surgeries in the field of plastic surgery and orthopedics (1, 2). Obtaining an exsanguinated surgical field by using a solution of a local anesthetic (usually 1% lidocaine) with adrenaline at 1:100,000 without using the tourniquet, which is one of the main causes of intraoperative pain experienced by the patient, is one of the reasons why this anesthesia technique has become very widespread in recent years (3,4). As patient sedation is no longer necessary, there is a better patient and surgeon comfort, the degree of patient satisfaction is high, and the patient's shorter operating time and

length of hospital stay, as well as the substantial decrease in costs have made the WALANT technique to be used for a wide range of surgeries. The myth according to which adrenaline injection would result in ischemia of the anatomical segment at which it is injected has been overcome (5, 6, 7). Given the efficacy and safety of this local anesthesia technique, it is used with confidence and with very good results in hand surgery (8).

From the onset of the COVID-19 pandemic, rules and restrictions were imposed in the epidemiological context, making the admission and surgical treatment of patients with surgical emergencies and especially chronic diseases more difficult (9). A shorter exposure time of the patient to the medical staff and vice versa, the shortest possible duration of hospitalization, reduction of surgical costs without compromising the quality of patient and surgeon comfort, were some of the aspects imposed by the COVID-19 pandemic.

To comply with these rules, a common denominator has been found in hand surgery, namely the use of the WALANT anesthetic technique, with the help of which all these epidemiological criteria can be observed, this being one of the multiple advantages of this local anesthesia technique (10, 11).

The ***study aim*** was to evaluate a group of patients admitted as emergencies or by appointment during the first year of COVID-19 pandemic. Surgical emergencies included in the study were represented by traumas of various etiologies resulting in lesions of the superficial and deep flexor tendons or extensor tendons, with sections of digital collateral nerves, median and ulnar nerves without concomitant vascular damage, foreign body retention, fractures of the metacarpals and phalanges. As to non-emergency surgeries, these were for carpal tunnel syndrome (CTS), Dupuytren's disease (DD), trigger finger (TF), soft tissue tumors of the fingers.

MATERIAL AND METHODS

We studied a group of 180 patients, of which 117 male patients and 63 female patients. Of these, 140 were surgical emergencies, the remaining 40 being chronic conditions. Of the 140 patients with surgical emergencies, 94 patients were male and 46 females. Of the 40 patients with chronic diseases 23 were male and 17 females. The study patients had been admitted to the Clinic of Plastic Surgery and Reconstructive Microsurgery between March 15 and December 15, 2020. The study was approved by the Hospital Ethics Committee and all participants signed a written informed consent.

Of the 180 study patients, 86 were living in urban areas, 62 of them being surgical emergencies and the remaining 24 were patients with chronic condition; of the 94 patients living in rural areas, 78 were surgical emergencies and 16 had chronic diseases. Admission diagnoses for the study patients with chronic conditions were DD - 20 patients, 19 male and one female, CTS - 12 patients, 1 male and 11 female, TF - 3 cases all female, and 5 cases of soft tissue tumors (2 case of glomus tumor of the nail bed and three cases of lipomas of the hand) of which 3 male and 2 female patients. In this group, the right hand was affected in 18 cases and the left hand in 22 cases.

The group of patients presenting to the emergence department had traumas of different etiologies which resulted in wounds with flexor tendons lesions in 15 cases (11 male and 4 female patients), trauma of

finger extensor apparatus or extensors on the back hands in 28 cases (19 men and 9 women), nerve sections (ulnar or median nerves without vascular damage, or digital collateral nerves) in 15 cases (10 male and 5 female).

The surgical indication was established in all cases, consisting of primary tenorrhaphy of superficial and/or deep flexor tendons, extensor tendons, and primary neurorrhaphy in the case of nerve injuries. Of the study group, 54 male patients and 28 female patients had fractures. There were 34 metacarpal fractures (23 male and 11 female patients) and 48 phalangeal fractures (31 male and 17 female patients). All of these patients had indication for open reduction and internal fixation (ORIF) surgery. In this emergency group, the right hand was affected in 63 cases and the left hand in 77 cases. All these patients underwent day-surgery procedures performed under local anesthesia with 1% lidocaine and 1:100,000 adrenaline. 8.4% sodium bicarbonate buffering was not used in any case. In all 180 cases, a 27 G needle was used for the injection, which was inserted perpendicular to the skin into a skin fold kept by the surgeon between the thumb and index finger of his/her non-dominant hand. In all cases the protective equipment was used in accordance with the specific rules recommended during the COVID-19 pandemic and the rules of asepsis and antisepsis.

All patients were notified that they were about to receive the local anesthetic. In none of the cases was ice used to reduce injection site pain. In all cases the anesthetic was injected according to the recommendations: initially 0.2-0.4 mL sub dermally, followed by deep injection. The injection points recommended in the literature for each type of lesion were observed, as well as the recommended amounts to be injected for each anatomical region (e.g. TF cases, no more than 1 mL anesthetic solution was injected into a digital phalange, 15 mL for each digital ray affected by Dupuytren's disease (DD), the amount of anesthetic solution used did not exceed 20 mL for CTS). In patients with chronic conditions requiring surgery for carpal tunnel syndrome (CTS), Dupuytren's disease, soft tissue tumors, or trigger finger the amount of 20 mL was not exceeded. In emergency interventions, the amount of 7 mL/kg was not exceeded.

Intraoperative pain was assessed using the Visual Analogue Scale (VAS) score for pain, bleeding based on the need of using a tourniquet, efficacy of anesthesia depending on the need for intraoperative use of intravenous sedatives, time from admission to initiation of surgery, actual operation time, total length of hospital stays, the number of people the patient had come in contact with during hospital stay, considering the existing epidemiological context.

Statistical analysis

The statistical analysis included demographic, anatomical and surgical variables. The *ANOVA* regression was applied, establishing a 95% CI and significance F of 95%. The *Pearson's* r was calculated, its positive values showing a direct correlation between the two variables, and the negative values exemplifying an inverse, indirect correlation. The further away the coefficient r is from zero, the stronger the correlation, directly or indirectly. A p-value <0.05 was considered statistically significant.

RESULTS

Demographical data. Gender distribution showed a predominance of male patients (64.44%), with an M:F ratio of 1.81.

TABLE I

Descriptive statistics of study group demographical and clinical characteristics

Variables / descriptive statistics	Average	Minimum	Maximum
Demographical			
Age	Average = 41.09	19	73
Gender	M = 116 cases (64.44%) F = 64 cases (35.56%) M: F= 1.81	N/A	N/A
Residence area	U = 117 cases (65.00%) R = 63 cases (35.00%) U: R= 1.80	N/A	N/A
Anatomical			
RH / LH	LH = 96 (53.33) RH = 84 (46.67%)	N/A	N/A
Clinical			
Emergency / chronic	Emergency = 140 (77.78%) Chronic = 40 (22.22%)	N/A	N/A
Diagnosis	P fracture = 48 (26.67%) MC fracture = 34 (18.89%) ET = 28 (15.55%) DD = 20 (11.11%) FT = 18 (10.00%) NERVE TRAUMA = 15 (8.33%) CTS = 12 (6.67%) TUMOR = 5 (2.78%)	N/A	N/A
Amount of anesthetic	5.46	2	23
Time from admission to surgery	15.72	5	23
Anesthesia installation time	12.79	10	15
Total time	118.05	65	200
VAS test	1- 86 (47.77%) 2- 76 (42.22%) 3- 18 (9.10%)	N/A	N/A

Legend: M=male, F=female, U=urban, R=rural, RH=Right hand, LH=left hand, P=phalanx, MC=metacarpal, ET=extensor tendon, DD=Dupuytren's disease, FT= flexor tendon, CTS=carpal tunnel syndrome

The distribution by area of residence showed a predominance of urban patients (65.00%), with a U: R ratio of 1.80. We considered other demographic characteristics of the participants as well as anatomical, clinical and diagnosis characteristics, the amount of anesthetic, time from admission to surgery, anesthesia installation time, total time and VAS test scores (tab. I).

The age histogram showed that the most affected age groups were 31-40 years and 41-50 years (fig. 1).

The distribution by type of diagnosis revealed that phalanx fractures were the most common diagnosis (48 cases - 26.67% of total cases), followed by metacarpal fractures (34 cases - 18.89%) (fig. 2).

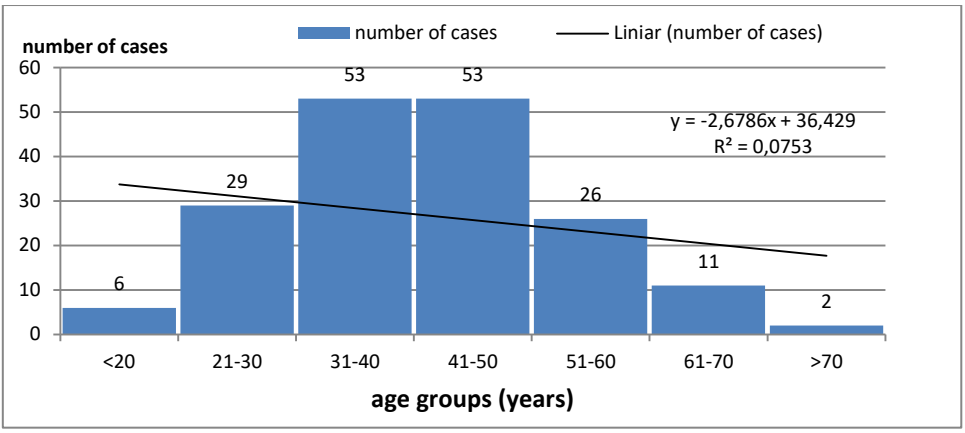

Fig. 1. Age histogram

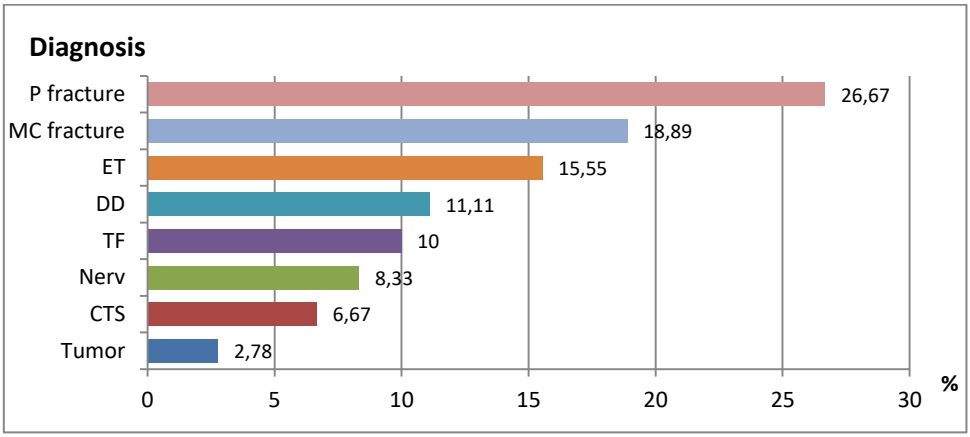

Fig. 2. Distribution of cases by diagnosis

Correlations. Regarding the correlations between different variables, the following results were recorded: the correlation coefficient between patients' age and the amount of anesthetic was r= 0.1121, highlighting a positive, direct, but weak correlation (p= 0.133). The correlation between the amount of anesthetic used and patient's age was statistically positive but weak, meaning that the amount of anesthetic was established based on patients' condition and not their age**.** Correlating patient age with length of hospital stay - surgical start time, a positive correlation coefficient r= 0.1179 was obtained, suggestive of a direct but weak relationship between the two variables (p= 0.114).

The correlation coefficient between patient age and time needed for induction of anesthesia was r= 0.0925, suggesting a positive, direct but weak relationship (p= 0.216). The correlation coefficient between patient age and the amount of anesthetic was r= 0.1121, highlighting a positive, direct but weak relationship (p= 0.465). These two correlations show that the anesthetic solution used in the WALANT technique has maximum efficacy regardless of

patient age when used in the amounts established and indicated in the literature.

The correlation coefficient between patient age VAS test was r= 0.0113, highlighting a positive, direct but weak relationship (p= 0.880). The correlation coefficient between the amount of anesthetic and the results of the VAS test was r= 0.1489, highlighting a positive, direct, weak and statistically significant relationship (p= 0.046).

DISCUSSION

Patient satisfaction was high in all cases, the intensity of pain felt during surgery not being that high as to influence patients' general condition. The VAS test results are not influenced by the amount of anesthetic and patient age, this type of local anesthesia providing full patient comfort during surgery. Regarding intraoperative bleeding, the 4 cases with metacarpal fractures who presented bleeding (2.22%) required the placement of the tourniquet on the arm. These patients had associated cardiac conditions. In none of the study cases was intraoperative administration of intravenous sedatives necessary. No intraoperative accidents or incidents were recorded. There were no cases of ischemia or digital necrosis. From hospital admission, during surgery, until discharge each study patient came in contact with a maximum of 4-5 people, figures modified by the presence or absence of a surgical assistant in the surgical team. If surgery was performed using a different type of anesthesia, a minimum of 2 people forming the anesthesia team (physician anesthesiologist and anesthesiologist assistant) were added to the 4-5 people the patient came in contact with, and the time spent in the operating room would have been extended by a minimum of 30 minutes required to perform loco-regional anesthesia and a longer hospital stay after the surgery for patient monitoring. In these circumstances, the length of hospital stays and the number of contacts during hospital stay and implicitly the risk of contracting SARS-CoV-2 decrease and also the costs are lower, surgical care is of good quality, and patient satisfaction is high.

The advantages and safety of using the WALANT technique have been described and demonstrated over time by multiple studies performed on large groups of patients, WALANT being used for a multitude of pathologies in several surgical specialties (6, 12). The WALANT technique avoids the use of tourniquet, a source of intraoperative pain for the patient, as well as intravenous sedation, associated with the risk of neurological damage, a fact demonstrated by this study in which the use of a tourniquet was required in only 2.2% of cases.

In 2013, Lalonde establishes and demonstrates the safety of the anesthetic solution of 1% lidocaine with 1:100,000 adrenaline, in the amount of 7 mg/kg in hand surgery (13). Lalonde reported in his studies that for optimal visualization in the surgical field, the time needed to achieve the adrenaline vasoconstrictor effect of lidocaine/adrenaline solution is 25 minutes after injection (14). In our study the maximum anesthesia induction time was 15 minutes, with an average of 12.79 minutes. Buffering the anesthetic solution with 8.4% sodium bicarbonate in a 10:1 ratio was not used in any of the cases. The technique of injection and the heating of the anesthetic solution before injection are described in the literature, and in the current study they were performed accordingly (15).

The broad spectrum of hand surgeries in which WALANT can be used as a local

anesthesia technique, as well as its multiple advantages in the context of the COVID-19 pandemic, increase the indications for its use (16).

The short hospital stay and exposure of the patient to the hospital environment, the decrease in the number of people the patient comes in contact with in the operating room, and lower surgical costs tend to make WALANT a gold standard anesthesia in hand surgery during the COVID-19 pandemic and not only (2).The absence of ischemic or necrotic complications in the fingers and hands as a result of using the WALANT technique in the current study are consistent with those described in the literature, thus demonstrating the safety of using adrenaline in the anesthetic solution (17, 18).

The possibility of intraoperative collaboration with the patient allows both the assessment of his/her general condition but specially to perform active intraoperative movement examination of the injured and reconstructed anatomical segment (19). The good locally provided exsanguination obtained by the addition of adrenaline in the anesthetic solution, thus avoiding the use of the tourniquet and the administration of intravenous sedatives is only one of the many advantages of the WALANT technique (20, 21, 22). Both in the literature and in our study, patient satisfaction in terms of intraoperative comfort in relation to the presence of pain, short hospital stays, and lower cost are other benefits associated with WALANT technique (21).

The advantages of this technique represent a way to comply with the hospital protocols and guidelines during the COVID-19 pandemic (9). In our study group the average length of hospital stay was 118.05 minutes. The WALANT technique makes it possible that during the COVID-19 pandemic to treat surgically patients with chronic diseases that should be operated as soon as possible because a delay in surgical treatment may result in worsening disease like soft tissue tumors, finger tumors, carpal tunnel syndrome, or Dupuytren's disease. In the current epidemiological context, these patients would be delayed because of change in the hospitalization rules and decrease of local financial resources (23).

Surgical treatment of metacarpal and phalangeal fractures has good results using WALANT as an anesthetic technique (24, 25). Also, in the current study the outcomes of the surgical treatment of metacarpal and phalangeal fractures were good using the solution of lidocaine 1% with 1:100,000 adrenaline, solution used the vast majority of study cases. The fields in which the WALANT technique can be used have expanded, being frequently used both in hand surgery and in orthopedic surgery (heel) for the surgical treatment of clavicle fracture, distal radius fracture, ankle fracture and other sites (24, 26, 27, 28).

CONCLUSIONS

The WALANT anesthesia technique must be considered by any hand surgeon especially during this pandemic period so that patients to undergo surgical treatment in good conditions, in a short period of time and safely both in terms of surgery outcomes and measures to prevent the spread of coronavirus infection.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

THE MINDMAP PROJECT: PROCESS AND RESULTS

The MINDMAP (promoting mental well-being and healthy ageing in cities) project implemented a multinational data infrastructure to investigate the direct and interactive effects of urban environments and individual determinants of mental well-being and cognitive function in ageing populations. Using a rigorous process involving multiple teams of experts, longitudinal data from six cohort studies were harmonized to serve MINDMAP objectives. This article documents the retrospective data harmonization process achieved based on the Maelstrom Research approach and provides a descriptive analysis of the harmonized data generated. As methods, a list of core variables (the Data Schema) to be generated across cohorts was first defined, and the potential for cohort-specific data sets to generate the Data Schema variables was assessed. Where relevant, algorithms were developed to process cohort-specific data into Data Schema format, and information to be provided to data users was documented. Procedures and harmonization decisions were thoroughly documented. The MINDMAP Data Schema (v2.0, April 2020) comprised a total of 2841 variables (993 on individual determinants and outcomes, 1848 on environmental exposures) distributed across up to seven data collection events. The harmonized data set included 220 621 participants from six cohorts (10 subpopulations). Harmonisation potential, participant distributions and missing values varied across data sets and variable domains. In conclusion, the MINDMAP project implemented a collaborative and transparent process to generate a rich integrated data set for research in ageing, mental well-being and the urban environment. The harmonized data set supports a range of research activities and will continue to be updated to serve ongoing and future MINDMAP research needs. (Wey TW, Doiron D, Wissa R, *et al.* Overview of retrospective data harmonization in the MINDMAP project: process and results. *J Epidemiol Community Health* 2021; 75: 433-441).

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ENDOSCOPIC MANAGEMENT OF POSTERIOR THIRD VENTRICLE LESIONS

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ENDOSCOPIC MANAGEMENT OF POSTERIOR THIRD VENTRICLE LESIONS (Abstract): The pathology of the posterior 3rd ventricle are often complex lesions involving high functional structures and often present in emergency with symptoms caused by the secondary obstructive hydrocephalus. The **aim** of the study was to evaluate the use of ventricular endoscopy in the setting of posterior 3rd ventricle lesions. **Material and methods**: the criteria for inclusion in the study was represented by the patients with posterior 3rd ventricle lesions and hydrocephalus treated endoscopically with ventriculocisternostomy with or without endoscopic biopsy. **Results**: a number of 20 patients were included in the study, most frequent lesions were located at the level of pineal gland 14 cases, followed by quadrigeminal plate 3 case, 19 patients had endoscopic ventriculocisternostomy was effective in 18 patients, one patient needed a redo procedure, and one had an endoscopic cyst fenestration, accurate histological exam was obtained in 13 cases of 14 biopsies. **Conclusions:** Neuroendoscopy is a safe and effective technique for the management of the posterior 3rd ventricle lesions allowing the treatment of associated hydrocephalus and endoscopic tumor sampling during the same surgical procedure. **Keywords**: HYDROCEPHALUS, ENDOSCOPIC THIRD VENTRICULOSTOMY, 3rd VENTRICLE.

Posterior 3rd ventricle lesions often present with hydrocephalus requiring treatment (1) and endoscopic third ventriculostomy (ETV) has been accepted as the procedure of choice for the treatment of obstructive hydrocephalus (2). Endoscopic biopsy of the pineal region lesions is useful in providing tissue for histopathological diagnosis and represent the 1st option in the management of pineal lesions in many centers (3).

Simultaneous ETV and biopsy, using one or the burr holes (4), is nowadays a common strategy for tumors of the posterior 3rd ventricle region (3). Common complications are represented by lesion of the fornix, as the endoscope shaft has to pass the foramen of Monro during the procedure (4) and intraventricular hemorrhage from the biopsy site, which may occlude the stoma from the 3rd ventricle floor (5), sometime requiring an external ventricular derivation (EVD).

MATERIAL AND METHODS

We retrospectively reviewed consecutive patients surgical treated for pineal region lesions admitted to the Department of Neurosurgery, "Professor Dr. N. Oblu" Clinical Emergency Hospital Iasi. Inclusion criteria were as follows: patients with lesions in the pineal region confirmed by MRI at our center between January 2015 and July 2021, enlarged ventricular system based on the preoperative MRI, depictable lesion on the MRI, preoperative MRI evaluation.

The categories consisted of solid and cystic lesions at the level of posterior 3rd ventricle including pineal gland, aqueduct or superior mesencephalon or posterior thalamus.

All patients had undergone preoperative and postoperative neurological and radiological evaluation. Patient data including, age, sex, symptoms, lesion location, surgical procedure, histopathologic diagnostic and outcome were recorded.

Exclusion criteria: patients with no preoperative MRI evaluation, negative MRI for expanding mass, patients in whom no postoperative imagistic evaluation was available.

The outcome following endoscopic procedures was determined by chart review of hospital stay and postoperative clinic and imagistic revisit records at 3 months. Successful endoscopic treatment was defined as decrease of the ventricular system volume and/or decrease of the 3rd ventricle dimension alone requiring no additional treatment for hydrocephalus.

Preoperative evaluation was done based on preoperative MRI images, for the diagnostic of hydrocephalus we used T2 and FLAIR images on axial and coronal plane. Hydrocephalus was defined as enlargement of the ventricular system with an Evans index (6) greater than 0.3, and the presence of the trans ependymal edema, defined as hyperintensity around the frontal horns on T2 and FLAIR sequences. Surgical lesions were evaluated using the T1 sequences before and after contrast injection.

Surgical planning for ETV and biopsy was performed using the T2 sequences in sagittal and coronal plane which allow the setting of entry point, targets (3rd ventricular floor- for ETV/lesion for biopsy) and surgical trajectory.

Surgical procedures were performed using a 30^0 rigid lens endoscope (Karl Starz, Tuttlingen, Germany). After the canulation of right ventricular horn, anatomical landmarks were identified (fornix, septal vein, thalamostriate vein and foramen of Monro), the endoscope is then advanced at the level of the 3rd ventricle and the entry point for ETV is set between the mammillary bodies and infundibulum, on the midline. The stoma is created using a blunt perforator and is enlarged using a 3F Fogarty balloon. When simultaneous ETV and biopsy are planned, the ETV is perform prior to tumor biopsy, this order is advocated since the most pressing clinical condition (that is, noncommunicating hydrocephalus) should be definitively addressed prior to any potential visual obscuration by hemorrhage from tumor biopsy. After successful ETV the endoscope is rotated to obtain posterior view and direction. Once the tumor is identified cupped biopsy forceps are used to sample the tumor, without prior use of cautery in order to reduce artefacts, multiple samples being collected. After biopsy hemostasis is obtain by using saline irrigation, bug bee wire compression or coagulation. After we obtain the hemostasis a final inspection of the stoma and ventricular system is performed. Surgical wound is performed in standard fashion.

RESULTS

This retrospective study includes 20 patients operated at "Professor Dr. N. Oblu" Clinical Emergency Hospital Iasi for symptomatic hydrocephalus caused by lesions located at the level of the posterior 3rd ventricle. Patient age ranged from 8 to 73 years (34.4 mean years) at the time of surgery; there were 8 males and 12 females. All patients presented with symptomatic hydrocephalus, the most common clinical manifestation was represented by headache in 18 patients (90%), Parinaud syndrome in 4 patients (20%), altered level of consciousness in 2 patients (10%) cranial nerve palsy in 4 patients (20%), amblyopia in 2 patients (2%) and one case presented with left hemiparesis (tab. I). The rise intracranial pressure resolved in 19 patients (improved in 1 patient with a cyst of the septum pellucidum in which a unilateral fenestration was performed and resolved after bilateral cyst wall fenestration), patients presenting with Parinaud syndrome and hemiparesis showed no improvement after ETV (tab. I).

TABLE I

Clinical presentation and post-operative course

Clinical presentation	Frequency	%	Resolved	Improved	Unchanged
Headache	18	90	17	1	0
Parinaud syndrome	4	20	0	0	4
Cranial nerve palsy	4	20	1	1	2
Amblyopia	2	10	0	2	0
Altered level of consciousness	2	10	2	0	0
Hemiparesis	1	5	0	0	1

Preoperative MRI identified the primary lesion at the level of the pineal gland in 14 cases (70%), at the level of the quadrigeminal plate in 3 cases (15%), at the level of posterior thalamus 2 cases (10%) and one at the level of the septum pellucidum. There were 2 cystic lesions (pineal and septum pellucidum) with no contrast enhancement and 18 solid lesions, and all showed contrast enhancement except 3 cases (2 located at the level of quadrigeminal plate and one at the level of thalamus).

All patients had endoscopic 3rd ventriculostomy, as primary treatment of hydrocephalus except one (case 20 - in which a cyst fenestration was performed), endoscopic biopsy was performed in 14 cases, in 4 cases was considered unnecessary (cases 8 and 20 - had cystic lesions, and cases 3 (fig. 1) and 11 - had non enhancing quadrigeminal plate lesions) 2 cases (16 and 18) had a profound location within highly functional structures and a stereotactic biopsy was planned, one patient (case 12) had inconclusive biopsy (tab. II).

Histological exam revealed 9 germ cell tumors, 2 pineoblastomas, 1 metastasis and 3 glial tumors (1 grade II astrocytoma and 2 grade III astrocytoma), in one patient the samples were inconclusive.

As complications we had one case of ETV failure (case 7) - the patient presented with a pineal tumor, but intraoperative seeding at the level of the 3rd ventricle floor was identified (fig. 2) ETV and biopsy from both 3rd ventricle floor and pineal

tumor where obtained, and the histopathological diagnosis returned pineoblastoma. After 3 months the patient presented with signs on raised ICP and MRI confirmed the presence of hydrocephalus and progression of the pineal lesion, a redo ETV was performed, intraoperative findings consisted of stoma closure, after ETV the inspection of the prepontine cistern reveled tumoral spreading at the level of the prepontine arachnoid membrane, resection and samplings being undertaken. After 3 days the patient had open surgery with subtotal resection of the lesion.

Another complication was represented by CSF seeding after biopsy of a grade 3 astrocytoma of the quadrigeminal plate (case 4), that developed carcinomatous meningitis after 3 months (fig. 3).

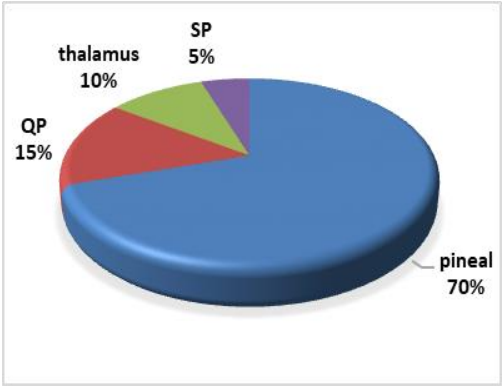

Fig. 1 Lesion location at the level of posterior 3rd ventricle (QP- quadrigeminal plate, SP-septum pellucidum)

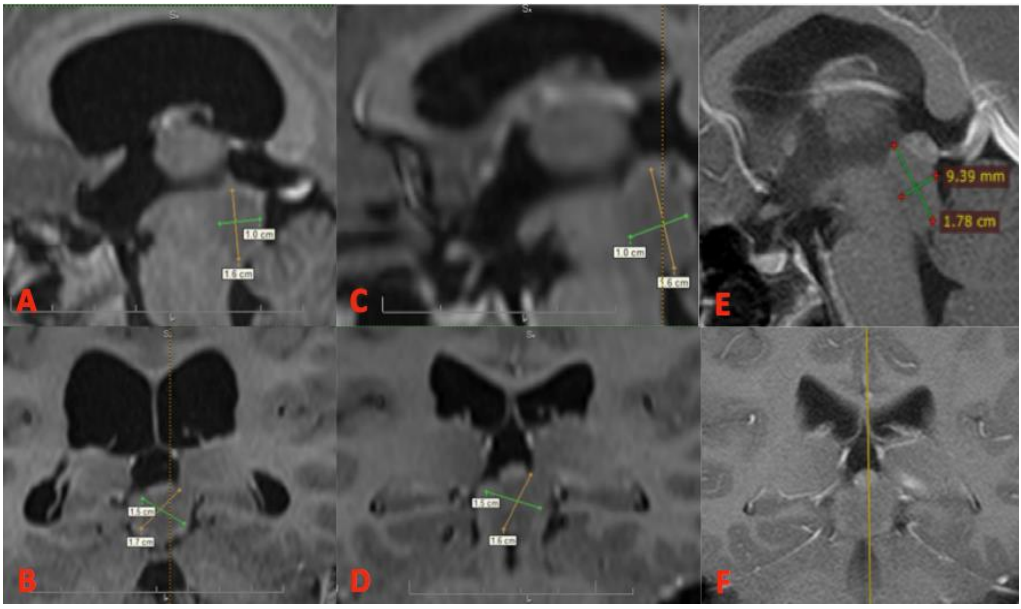

Fig. 2. Quadrigeminal plate glioma treated with ETV and follow-up (case 3)
A and B sagittal and coronal MRI images with contrast showing a non-enhancing lesion of the quadrigeminal plate compressing the aqueduct and causing hydrocephalus, C and D MRI images at 1 year after ETV showing the decrease of the ventricular system and stationary aspect of the posterior lesion, E and F MRI images 3 years after the diagnostic – stable lesion, no hydrocephalus.

TABLE II

Demographics of patients with posterior 3rd ventricle lesions treated endoscopically

Case No.	Age/sex	Location	MRI aspect	Diagnosis	Treatment
1	59,M	Pineal	Solid/enhance	Metastasis	Gamma-knife
2	11,M	Pineal	Solid/enhance	Teratoma	Surgery
3	8,F	Q plate	Solid/no enhance	Indolent glioma	Follow-up
4	55,F	Q plate	Solid/enhance	Anaplastic astrocyto-ma	CHT+ RxT
5	74,M	Pineal	Solid/enhance	Germinoma	CHT+ RxT
6	67,F	Pineal	Solid/enhance	Germinoma	CHT+ RxT
7	31,F	Pineal	Solid/enhance	Pineoblastoma	Surgery + CHT+ RxT + Redo ETV
8	57,M	Pineal	Cyst/no enhance	Pineal cyst	Follow-up
9	52,F	Pineal	Solid/enhance	Germinoma	CHT+ RxT
10	25,F	Pineal	Solid/enhance	Germinoma	CHT+ RxT
11	21,F	Q plate	Solid/no enhance	Indolent glioma	Follow-up
12	67,F	Pineal	Solid/enhance	Inconclusive	Stereotactic biopsy
13	12,M	Pineal	Solid/enhance	Germinoma	CHT+ RxT
14	17,F	Pineal	Solid/enhance	Germinoma	CHT+ RxT
15	17,M	Pineal	Solid/enhance	Germinoma	CHT+ RxT
16	13,F	Thalamus	Solid/no enhance	Grade II astrocytoma (stereotactic)	Follow-up
17	9,M	Pineal	Solid/enhance	Germinoma	CHT+ RxT
18	14,M	Thalamus	Solid/enhance	Grade III astrocytoma (stereotactic)	CHT+ RxT
19	17,F	Pineal	Solid/enhance	Pineoblastoma	Surgery + CHT+ RxT
20	73,F	Septum pellucidum	Cyst/no enhance	Cyst of the septum pellucidum	Follow-up

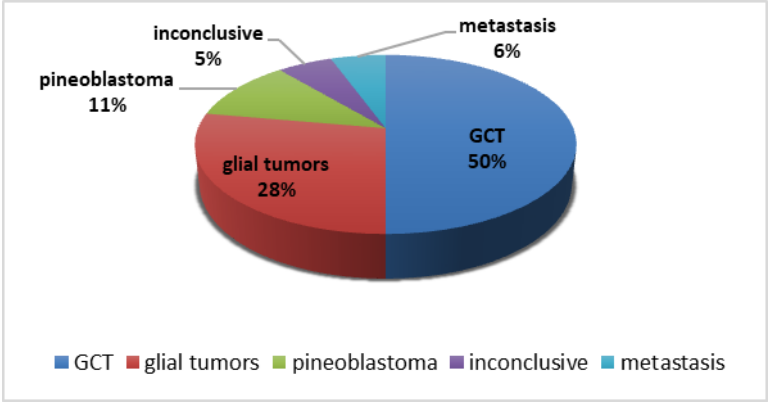

Fig. 3. Lesion distribution according to histologic examination (GCT-germ cell tumor, * results from stereotactic biopsies were included)

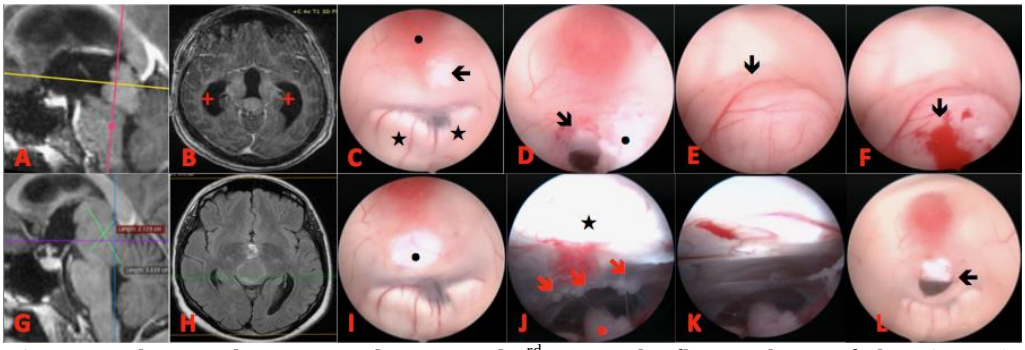

Fig. 4. Synchronous lesions pineal region and 3rd ventricular floor with ETV failure (case 7). A and B contrast enhanced MRI sagittal and axial images showing enhancing pineal tumor with hydrocephalus (+), C intraoperative findings from the level of the 3rd ventricle floor with identification of the infundibular recess (point), mamillary bodies (stars) and synchronous lesion at the level of 3rd ventricular floor (arrow), D intraoperative image showing the created stoma (arrow) and biopsied lesion (point), E and F intraoperative images showing the tumor (arrow) from the posterior 3rd ventricle before and after biopsy, G and H MRI images showing the progression of the posterior lesion and hydrocephalus, I intraoperative image from 2nd surgery showing the closure of the stoma (point), J exploration of the prepontine cistern after 2nd ETV with identification of dorsum sellae (star), basilar artery (point), and dissemination at the level of the arachnoid membrane (stars), K image after resection of the infiltrated arachnoid, L aspect of the newly created stoma (arrow).

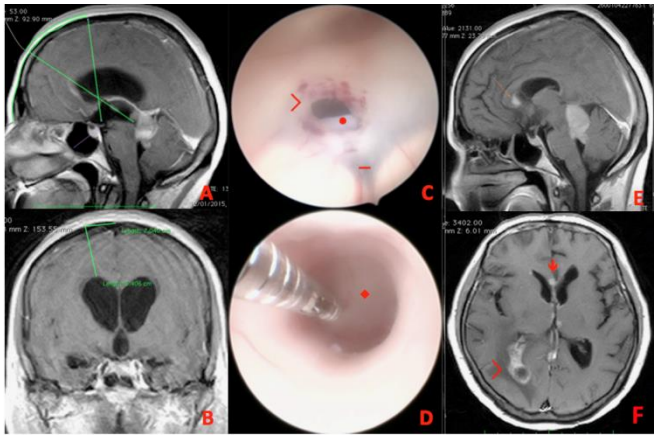

Fig. 5. CSF seeding after endoscopic biopsy (case 4). A and B contrast enhanced preoperative MRI images (sagittal and coronal) showing contrast enhancing lesion at the level of the quadrigeminal plate and aqueduct and hydrocephalus and preoperative planning (green lines) - planned trajectories using 2 burr hole technique. C and D intraoperative images C newly created stoma on the 3rd ventricle floor (arrowhead), basilary artery (point) and mamillary bodies (line), D biopsy of the tumor (diamond) E and F postoperative MRI images at 3 months showing CSF dissemination at the level of the genu of the corpus callosum (arrow) and at the level of the right occipital horn of the lateral ventricle and a slight progression of the primary tumor.

From the patients with germ cell tumors those harboring germinomas were treated successfully with chemotherapy and radiation therapy except the patient with pineal teratoma (case 2) that suffered a rapid degradation and needed emergency surgery for tumoral resection, the patients with anaplastic astrocytoma had radiation therapy and Temozolomide and the patient metastasis was sent to gamma-knife radiotherapy while the patients with pineoblastoma (cases 7 and 19) had surgical resection and adjuvant radiotherapy.

DISCUSSION

The posterior 3rd ventricle comprises the pineal region with the pineal gland inside the quadrigeminal cistern and surrounded by several important structures i.e. third ventricle, tectum, the complex of the great cerebral vein of Galen, thalamus and splenium of corpus callosum (7) thus being one of the most difficult and challenging regions for surgery in terms of surgical approach and neurological outcome.

Tumors of the posterior 3rd ventricle are mainly represented by pineal parenchymal tumors, germinal tumors, gliomas and miscellaneous tumors (meningiomas, metastases, ependymomas, dermoid and epidermoid tumors, pineal cyst) (8, 9). Most of the tumors in the pineal region are germ cell tumors, divided in germinomas and non-germinomatous germ cell tumors (choriocarcinoma, embryonal carcinomas and teratomas) (10). In our series we had a heterogeneous mix of the histopathologic spectrum, but most frequent lesion were germ cell tumors (50%) followed by glial tumors 28% and pineoblastomas 11%, which are in accordance with the data from the literature for germinal tumors and pineoblastoma (11-13). In previously published series gliomas account for 10% (12) but in our study they represent 28% of the cases, probably due to the inclusion of cases located at the level of the posterior thalamus.

The main feature of posterior 3rd ventricle lesions is the fact that cause hydrocephalus, which determine symptoms and require treatment.

90% of patients in our series presented with signs and symptoms of rides ICP along with secondary amblyopy and/or VI nerve palsy leading to visual deterioration.

Clinical status is completed by Parinaud syndrome (20%) due to superior collicular compression, resulting in up-gaze palsy, near-light reflex dissociation, pupillary asymmetry and nystagmus (14), and hemiparesis (1 case) due to cerebral peduncle involvement or by compression of the posterior limb of the internal capsule.

Other clinical manifestations that are cited in the literature such as cerebellar ataxia due to compression of the superior cerebellar peduncle/vermis, endocrine dysfunction due to invasion of the 3rd ventricle floor were not observed in our series (14). There are data in the literature sustaining the synchronous presence of germinomas, both at the level of posterior 3rd ventricle and suprasellar region, some centers considering this situation as pathognomonic for germinomas and considering the biopsy unnecessary (15). In our series we had a patient with synchronous lesions (case7-figure 3) where the histopathologic exam returned pineoblastoma, thus confirming that no imagistic or intraoperative finding could elude the histological examination, but the particularity of the case is that the lesion was identified intraoperative and was not visible on preoperative MRI.

The management of posterior 3rd ventricle lesions is based on two pillars, the

treatment of hydrocephalus and the treatment of the primary lesion depending on the histopathological type(16), which vary from follow-up, chemotherapy, radiation therapy and surgery, alone or in various combinations. In order to have histopathologic diagnosis tissue samples should be obtained, either by stereotactic guidance or by endoscopic biopsy.

Stereotactic biopsy pose some challenges given the proximity of important neural and vascular structures such as internal cerebral veins, straight sinus and does not address the hydrocephalus, and it has a high rate of complications, mostly postoperative bleeding (17). On the other hand, the endoscopic approach is capable in resolving the most urgent problem, the hydrocephalus, which is often life threatening and gives the possibility to obtain tissue samples for pathological exam in the same surgery. There is still debate in the literature weather to use a single burr hole for both procedures or two separate burr holes with two different trajectories (3, 4), although it is recommend the use of single burr approach for lesions that pass anteriorly the interthalamic adhesion (3). In our series we used single burr hole in 93% of the cases and two burr holes in 1 case in which we used a 0° endoscope, of mention the single burr technique cannot be performed using the 0° degree endoscope without damaging the brain. By accurate image study and the use of 30° degree scopes and slightly flexible instruments, we were able to calculate the optimal trajectory in order to perform ETV and sample lesions that are posterior to interthalamic adhesion without damaging the fornix.

In the setting of this approach the sequence of surgical procedures is to do first the ETV in order to resolve the hydrocephalus (18), the most urgent problem, and then to perform the biopsy which usually produce some bleeding thus compromising the realization of ETV. It is known that in ventricular endoscopy a quantity of 5ml of blood in the ventricular system is enough to obscure the scope vision (2).

Sometimes endoscopic biopsy is not suitable, if the tumor has no exophytic component and is surrounded by a layer of functional neural tissue such as mesencephalon, quadrigeminal plate or thalamus.

Reports from the literature are reveal a success rate of histological diagnosis trough endoscopic biopsy ranges from 88% to 95% (1, 19), we had a success rate of obtaining histological diagnosis after endoscopic biopsy 93% of the cases, more precisely we had one case (case 12) in which the samples were very small and thus not sufficient for diagnosis. Stereotactic biopsy on the other hand has a comparable success of diagnosis (94%) but a higher rate of complication with reported morbidity rate as high as 8.1% (20).

Germ cell tumors have a good response to chemotherapy and radiation therapy, surgery seldom being necessary(16), hence the importance of obtaining bioptic material for diagnosis and thus avoiding complex and laborious surgery and ventricular endoscopy is a suitable procedure to obtain tissue samples with low complications rate (19).

Second most common lesions are represented by glial tumors that include pure pineal astrocytomas and astrocytomas that invade the posterior 3rd ventricle from the surrounding structures such as brainstem and thalamus (21). Usually they are low grade lesions (grade 1 pilocytic and grade II fibrillary astrocytomas) as in our series cases 3 and 11, that have all the characteristic features of LGG: localization at the

level of quadrigeminal plate and the absence of contrast enhancement. They are benign, indolent lesions and the treatment of choice is treatment of hydrocephalus and close imagistic follow-up (21), biopsy seldom being necessary as in case 3 where the imagistic appearance, contrast enhancing lesions suggested a more aggressive lesion.

The ETV has a high rate of success in treating obstructive hydrocephalus caused by lesions in the posterior 3rd ventricle with an estimated rate of 87% (22), being the most physiological method of CSF derivation and preventing the installation of a ventriculoperitoneal drainage system with its known complications (infection, obstruction, malfunction, over drainage, hardware failure) (23).

In our series, 19 patients had a successful ETV with a success rate of 95% at 6 months one case had a closure of the stoma at 3 months and needed a new surgical procedure to redo the ETV, interestingly in this case the closure of the stoma was due to tumoral carcinomatosis at the level of prepontine cistern, observed intraoperative but not on preoperative MRI images case 7 (fig. 2).

CONCLUSIONS

Ventricular endoscopy is a safe and reliable technique for the management of the posterior 3rd ventricle lesions. Trough ETV allows the treatment of hydrocephalus with high rates of success in a physiologic fashion and with no need for additional shunting.

The endoscopic biopsy allows safe sampling of the lesion, in the same surgical procedure with ETV, for accurate pathologic exam, thus avoiding further surgical intervention.

CONFLICT OF INTEREST AND FUNDING

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of the paper. There are no financial interests or funding to declare.

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NEWS

BARIATRIC AND METABOLIC SURGERY IN TIME OF COVID-19 PANDEMIC

The coronavirus pandemic is a source of unprecedented pressure on the health-care systems, including disrupting bariatric and metabolic surgery. Like most elective surgery, bariatric and metabolic procedures are being postponed worldwide during the pandemic. With the outbreak, numerous of people seeking these operations will exist. Many candidates for bariatric and metabolic surgery are at high risk of morbidity and mortality from comorbid conditions. For these patients, access to surgical treatment should be prioritized based on disease-focused clinical needs, rather than weight-centered approach, to reduce harm from delaying surgery. Disease-oriented, medically meaningful strategies to triage patients seeking metabolic surgery after the COVID-19 crisis should help prioritize patients in more urgent need, both now and long into the future. (Rubino F, Cohen R, Mingrone G, *et al*. Bariatric and metabolic surgery during and after the COVID-19 pandemic: DSS recommendations for management of surgical candidates and postoperative patients and prioritization of access to surgery. *Lancet Diabetes Endocrinol* 2020; 8: 640-648).

MULTIPLE CEREBRAL VASCULAR MALFORMATIONS IN SAH – WHAT IS THE ROLE OF ANGIOGENESIS?

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MULTIPLE CEREBRAL VASCULAR MALFORMATIONS IN SUBARACHNOID HEMORRHAGE – WHAT IS THE ROLE OF ANGIOGENESIS? (Abstract): Subarachnoid hemorrhage (SAH) is a very serious form of cerebral hemorrhagic stroke, with devastating potential. The multiplicity of cerebral aneurysms in patients that suffer from SAH is a known fact, but all scientific date available today puts it on the same risk factors as for having one single ruptured aneurysm: gender, age, smoking status, ethnicity. We noticed that certain vascular complications tend to occur more frequently in patients with multiple intracranial vascular lesions. **Material and methods:** Data were collected from the Department of Neurosurgery I of the University Emergency Hospital of Bucharest archive from 2017 to 2020. We selected for this study a group of 147 patients hospitalized for SAH and treated in this service during the said period of time. **Results** included strong positive correlations with *Pearson* R coefficient higher than 0.4 between pertaining to the group with multiple brain aneurysms/cerebral vascular malformations and developing re-permeabilization of the previously secured aneurysm, ocular complications (bleeding), DVT/PE, symptomatic vasospasm or hematoma at arterial puncture site during hospitalization for this pathology. We investigated the pattern of those complications and their possible causes and a link between angiogenesis, vascular endothelium and the studied complications seems to be present. Functional outcome scores depending on the multiplicity of the lesions and their differences were also investigated. **Conclusions:** The nature of multiple intracranial vascular lesions in the subjects of the current study cannot be attributed to connective tissue disease. Given these aspects, it can be stated that the profile of these complications suggests, rather than a defective structure of the vascular wall, a defect in the intimal endothelial layer of the vascular wall. **Keywords:** SAH, MULTIPLE ANEURYSMS, VASCULAR LESIONS, VASCULAR COMPLICATIONS, ANGIOGENESIS, VASCULAR ENDOTHELIUM.

In this present study, the starting idea came from an empirical clinical observation resulting from daily activity in the neurosurgery clinic, following careful revision of the parameters of hospitalized patients with subarachnoid hemorrhage (SAH). We noticed that, in patients with similar metabolic profile and similar ethnographic characteris-

tics, with SAH of fairly similar etiology (hypertensive, idiopathic, aneurysmal or by cerebral AVM) and belonging to similar Hunt and Hess (1968) (1) and modified Fischer scale (2006) groups (2), some of them developed vascular complications with a major impact, while for the group with unique vascular intracranial malformation the clinical evolution remains unmarked by such events.

This observation has led to the need to identify other factors, independent of the above, which may be incriminated for the development of these complications.

Currently there is data in the literature that attributes the multiple character of cerebral vascular malformations (mainly aneurysms) to female patients who smoke (3), but there are very few studies on the evolving complications of this type of patients and there are also studies that refute association of smoking with increased risk of complications or poorer prognosis in SAH (4). Out of a desire to distance ourselves from this association, which could sometimes pass as a stigma capable of distancing the researcher from the depth of the problem, we chose to investigate whether there is an association with statistical significance between belonging to the group with multiple cerebral vascular malformations and the development of certain vascular complications in the presence of SAH and whether we can find new risk factors to explain the association.

MATERIAL AND METHODS

The present study is a descriptive clinical study with a retrospective and prospective approach, conducted in the Neurosurgery Clinic, Department of Neurosurgery I of the University Emergency Hospital of Bucharest. We selected the group of patients hospitalized for SAH and treated in this service for a period of four years (01.01.2017-31.12.2020) and we followed the patients from discharge for a period of three years (01.10.2018-30.09.2021). Selection criteria were rigorously applied. The prospective approach includes was designed for the monitoring of patients through a series of parameters that are the subject of personal study.

We centralized the following data in this chapter: the single or multiple character of the intracranial vascular malformations, the type of complications suffered during hospitalization, GOS score at discharge and follow-up visits.

Out of the 147 patients with aneurysmal SAH, 36 were diagnosed angiographically or by computerized tomography angiography (CTA) scan with more than one aneurysm or multiple vascular malformations. The vascular complications that will be studied are the ones with the highest occurrence rates among the group of subjects with multiple intracranial vascular malformations. We summarized them in table I. For systemic complications such as metabolic imbalances, fever, infections or hospital acquired pneumonia, the rate of occurrence was similar in the two groups.

We made use of *Pearson's* Chi-square test as a statistical test applied to categorical data sets. It offers high values of Pearson's coefficient and probability rate, in a proportional manner to the strength of the association (< 0.2 – positive but weak correlation, > 0.2 – strong correlation, >04.- very strong correlation). The *McNemar* test was used to evaluate the association between paired variables (4). The *ANOVA* one-way test was used to compare the aver-

age values of two groups and the statistical significance of the difference.

RESULTS

In the group of 147 patients hospitalized for aneurysmal SAH, 36 (24.48%) were diagnosed angiographically or by angio-CT with aneurysms or multiple vascular malformations. This result is consistent with recently published data, which estimates that in one-fifth of patients with cerebral aneurysms this malformation is multiple (3). The summary of these data is exemplified in the first figure.

Regarding the ethnographic data, the average age in the group of those with multiple cerebral vascular malformations was 51 years old (min = 30, max = 67), with a sex ratio F: M 6.2: 1.

The following aspects were observed directly based on descriptive statistical data: complication rate, type of complications (vascular or non-vascular) and the association between belonging to the group of subjects with simultaneous intracranial vascular malformations and the development of vascular complications (tab. I)

The *Pearson* R coefficient of 0.455867 and p value for *McNemar*'s association test of 0.001 indicates a very strong positive correlation between belonging to the group with multiple vascular aneurysms/ malformations and the development of vascular complications if the subject suffers a subarachnoid hemorrhage (fig. 2).

We thus obtained strong positive correlations, with a *Pearson* R coefficient greater than 0.4 for the following associations: belonging to the group of subjects with multiple intracranial vascular malformations and developing symptomatic cerebral vasospasm, hematoma at the site of arterial puncture for angiography, development of thrombosis of cerebral veins or arteries, ocular complications. This risk of aneurysmal re-permeabilization could be underestimated because patients are still being followed and some of the re-permeabilizations may have not yet been diagnosed (tab. II).

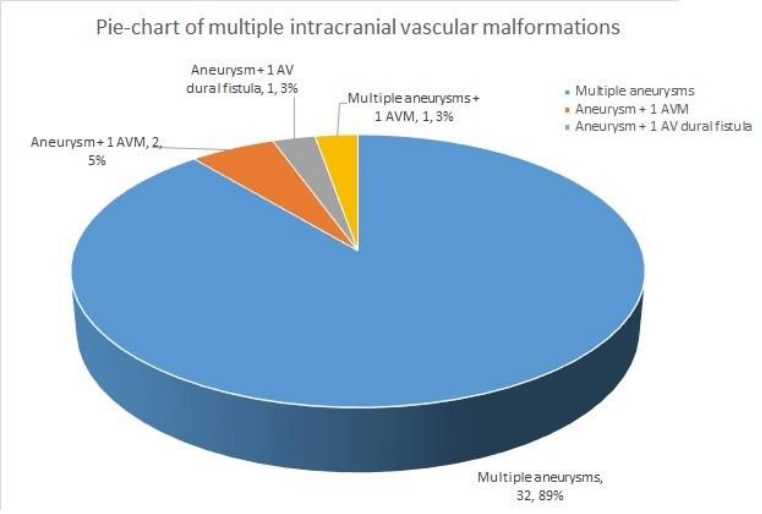

Fig. 1. Subjects with multiple intracranial vascular malformations. Designed based on personal study's results using *MicrosoftExcel*

TABLE I.

Summary of vascular and non-vascular complications suffered by subjects in our study. Results based on personal study using *Minitab software*

	Complication	Multiple aneurysms / cerebral vascular malformations group (36)	Unique aneurysm (111)
Vascular	Rebleeding	3 (8.33%)	1 (0.90%)
	Symptomatic vasospasms	16 (44.44%)	27 (23.42%)
	Ocular complications	5 (13.88%)	1 (2.80%)
	Intracerebral hematoma	4 (11.11%)	4 (3.60%)
	Hematoma at puncture site	3 (8.33%)	0 (0%)
	Re-permeabilization of the aneurism	3 (8.33%)	1 (0.90%)
	Transient ischemic stroke	2 (5.55%)	1 (0.90%)
	Thrombosis of cerebral arteries or veins	4 (11.11%)	3 (2.70%)
	DVT/PE	4 (11.11%)	2 (1.80%)
Nonvascular	Infections	1 (2.77%)	3 (2.70%)
	Significant metabolic imbalances	2 (5.55%)	7 (6.30%)
	Others (including non-coronary cardiac complications)	6 (16.66%)	28 (25.22%)

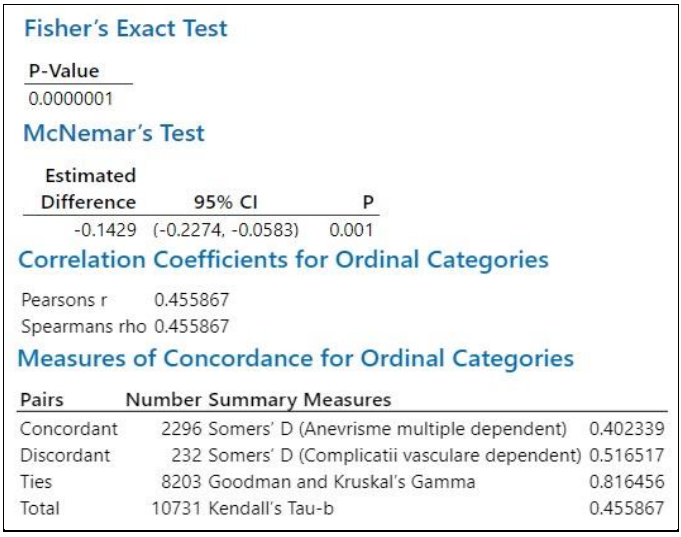

Fig. 2. Association tests between multiple intracranial vascular lesions and vascular complications development in patients who suffered A SAH. P values < 0.005 indicate that the null hypothesis is false and thus there's very little change that the investigator be wrong when assessing there is a correlation between the variables tested.
Results based on personal study using *Minitab software*

TABLE II.

Summary of statistical analysis parameters run for each vascular complication. Results based on personal study using *Minitab software*

Complications	95% C.I. - *McNemar* test	*McNemar* test – p value	*Fischer's* exact test - p value	Pearson R coefficient value
Rebleeding	(0.1416, 0.2938)	p<0.005	0.0456002	0.196447
Symptomatic vasospasm	(-0.1378, 0.0562)	0.461	0.0199008	0.200100
Ocular complications	(0.1123, 0.2687)	p<0.005	0.0216543	0.212052
Intracerebral hematoma	(0.1098, 0.2711)	p<0.005	0.100841	0.142317
Hematoma at puncture site	(0.1502, 0.2987)	p<0.005	0.0137661	0.253448
Re-permeabilization aneurysm	(0.1416, 0.2938)	p<0.005	0.0456002	0.196447
Transient ischemic stroke	(0.1477, 0.3013)	p<0.005	0.148593	0.141566
Thrombosis of cerebral arteries or veins	(0.1269, 0.2813)	p<0.005	0.0319193	0.202323
DVT / PE	(0.1329, 0.2888)	p<0.005	0.0945758	0.154953

We obtained further positive but weak correlations, with a *Pearson* R coefficient greater than 0.2 and less than 0.4 for the following associations: belonging to the group of subjects with multiple intracranial vascular malformations and aneurysmal rebleeding, aneurysmal re-permeabilization, developing deep venous thrombosis (DVT) or pulmonary thromboembolism (PE) during hospitalization for SAH, intracerebral hematoma formation and transient ischemic attacks (TIAs) development during hospitalization for SAH.

The one-way *ANOVA* test was run for the comparison of the averages of 2 groups: the group of subjects with a single aneurysm and the group of subjects with multiple cerebral vascular malformations, respectively, in terms of functional status scores at discharge and follow-up visit (3-6 months).

When calculating the average *Glasgow Outcome Score* (GOS) at discharge, we obtained a higher average value among those with a single intracranial vascular malformation than for those with multiple lesions of this type (3,865 *vs.* 3,694), the difference being 0.171.

A similar positive difference of 0.139 was obtained for the GOS averages at follow-up visit (4,306 *vs.* 4,167). However, the value of the parameter F being subunit in both cases (F = 0.50 for GOS at discharge and 0.34 for GOS at follow-up) and providing p values greater than $\alpha = 0.05$ (p = 0.428 and p=0.559 respectively), means that the null hypothesis cannot be rejected, the conclusion being that we do not have enough evidence that there is a statistically significant difference between the means of the two groups regarding GOS values at neither discharge, nor follow-up (tab. III).

TABLE III.

ANOVA* one-way test results for the average GOS at discharge and follow-up for the two groups. Results based on our study using *Minitab software

ANOVA one-way, α=0.05	GOS at discharge	GOS at follow-up
F parameter	F=0.50<1	F=0.34<1
p value	p=0.428	p=0.559

DISCUSSION

Comparing the two groups of subjects in terms of complications - those with a single aneurysm and those with multiple vascular intracranial aneurysms/ malformations, we can make the following statements after running analytical statistics tests:

• Subjects with a single intracranial vascular malformation suffered to a lesser extent from vascular complications such as those described;

• Strong positive correlations were obtained between belonging to the group with multiple intracranial malformations and the risk of developing: Hematoma at the site of the arterial puncture deep venous thrombosis (DVT) / pulmonary thromboembolism (PE), re-permeabilization of the previously secured aneurysm, ocular complications (bleeding), symptomatic vasospasm, hematoma at arterial puncture site;

• Positive correlations, although weaker than those mentioned in the previous paragraph, were obtained between belonging to the group with multiple intracranial malformations and the development of the following: cerebral arteries or veins thrombosis, intracerebral hematoma, symptomatic vasospasm, TIAs.

Regarding the GOS on discharge and follow-up visit (3-6 months), the average scores of the group with a single aneurysm was higher than that of the group with multiple cerebral vascular malformations, but the statistical significance of these results could not be demonstrated.

CONCLUSIONS

The nature of multiple intracranial vascular lesions in the subjects of the current study cannot be attributed to connective tissue disease (6), none of the 36 subjects being known to have had such a condition and showing no clinical signs to suggest such a pathology.

Given these aspects, it can be stated that the profile of these complications suggests, rather than a defective structure of the vascular wall, a defect in the intimal endothelial layer of the vascular wall. This observation is made due to the fact that some complications such as thrombosis, ischemia, bleeding - are closely related to the reactivity of the vascular endothelium and given that this layer of endothelial cells is the source of synthesis and release into the systemic circulation of a large number of prothrombotic and antithrombotic factors, and proinflammatory and anti-inflammatory factors whose imbalance favors and promotes such complications (7).

Whether the vascular wall or intimal layer of cells defects are congenital or achieved, pursuing further studies aimed at them is equally important because it would provide for potential targeted therapy. Given the previous observation, as well as the model of the rate of occurrence of vascular complications among the presented subjects, we consider it imperious that further studies be conducted in order to investigate issues related to vascular endothelium and angiogenesis in patients with simultaneous intracranial vascular malformations (8).

There are recent studies regarding post-SAH vascular endothelium and its connection to local phenomena such as vasospasm, early brain injury, disruption of the blood-brain barrier and cytotoxic and vasogenic edema (9), but vascular complications at other sites (ocular bleeding, DVT/PE, re-permeabilization of the aneurysm) are still poorly studied (6).

Furthermore, if the role of the endothelial cells in promoting local destructive phenomena is well established, then what happens if SAH occurs on previously dysfunctional ves-

sel wall or endothelium? We did not find recent data in the literature that refer to the quality of the arterial wall or the intrinsic characteristics of the vascular endothelium and its role in the prognosis of patients with SAH.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

BIFIDOBACTERIUM LONGUM: PROTECTION AGAINST INFLAMMATORY BOWEL DISEASE

The prevalence of inflammatory bowel disease is generally associated with the change of the immune system and gut microbiota. Bifidobacterium longum is a symbiotic bacterium existed in the human gastrointestinal tract and has been demonstrated to be capable of relieving colitis in mice and can be employed as an alternative or auxiliary way for treating inflammatory bowel disease. Bifidobacterium longum can change the structure of the gut microbiota, induce and regulate immune responses, and reduce the expression of inflammatory cytokines and reactive oxygen species in the intestine. Besides, it can also maintain the normal intestinal barrier function by increasing the expression of the tight junction protein. Therefore, Bifidobacterium longum has great potential and can be used as a prevention, replacement, or adjuvant treatment for inflammatory bowel disease. (Yao S, Zhao Z, Wang W, Liu X. Bifidobacterium Longum: Protection against Inflammatory Bowel Disease. J *Immunol Res.* 2021 Jul 23;2021:8030297).

BEYOND DYSPHAGIA: RESULTS OF A SURVEY AMONG ROMANIAN GASTROENTEROLOGISTS REGARDING THEIR DIAGNOSIS AND MANAGEMENT OF EOSINOPHILIC ESOPHAGITIS

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BEYOND DYSPHAGIA: RESULTS OF A SURVEY AMONG ROMANIAN GASTROENTEROLOGISTS REGARDING THEIR DIAGNOSIS AND MANAGEMENT OF EOSINOPHILIC ESOPHAGITIS (Abstract): Eosinophilic esophagitis (EoE) is a chronic, immune-mediated disease characterized by symptoms of esophageal dysfunction and eosinophil-predominant inflammation on esophageal biopsies. EoE prevalence increased in recent years and nowadays it is recognized as a common cause of dysphagia and food impaction. Despite the publication of updated guidelines, multiple studies reported variable clinical practices regarding the management of EoE patients. The **aim** of this study was to assess the adherence to published guidelines and the current clinical management of EoE among gastroenterologists from three academic centers in Romania. **Material and methods:** We performed a cross-sectional study of 62 gastroenterologists using a 12-item questionnaire probing the clinicians' understanding of EoE diagnosis and therapeutic guidelines. The online survey was conducted between 15th of February and 15th of March 2022 and the data was processed using SurveyPlanet. **Results:** The overall completion rate was 100% and the mean time of completion was 8.2 minutes. Most participants (70.9%) were gastroenterologists from Institute of Gastroenterology and Hepatology in Iasi Romania. The rest were from academic centers like Bucharest (19.3%) and Timisoara (9.6%). Regarding the understanding of the published EoE guidelines, only 30 respondents (31.6%) were aware that dysphagia and food impaction can be suggestive of EoE and are required diagnostic criteria. All the gastroenterologists agreed that eosinophil-predominant inflammation on esophageal biopsy is a mandatory criterion for EoE diagnosis. The exclusion of secondary causes of esophageal eosinophilia was considered necessary for EoE diagnosis by 42 survey participants (48.3%), while the other gastroenterologists found the differential diagnosis helpful, but optional. The majority agreed to the necessity of a minimum of 6 esophageal biopsies in order to increase the diagnostic yield of EoE, while 16.1% thought a minimum of 2 biopsies to be enough. The preferred first line treatment was proton pump inhibitors (PPI) for 41.9 % and topical steroids for 48.4 % of respondents. Twenty-nine participants (46.8%) considered follow-up with endoscopy and biopsies after a course of 6-12 weeks of therapy in order to evaluate remission, while 22 (35.5%) would not repeat endoscopy in case of symptom resolution. **Conclusions:** Among gastroenterologists, the adherence of Romanian gastro-enterologists to published EoE guidelines seems to vary considerably, especially regarding PPI use and assessing response to therapy. Targeted strategies are needed to reduce discrepancies in clinical practice, starting with interventions to increase clinical awareness in order to optimize the management of EoE patients. **Keywords:** EOSINOPHILIC ESOPHAGITIS, DYSPHAGIA, ESOPHAGEAL BIOPSIES, GUIDELINES ADHERENCE.

Eosinophilic esophagitis (EoE) is a chronic, immune-mediated condition that features symptoms of esophageal dysfunction such as dysphagia and food impaction in adults. The first description of EoE as a distinct entity, different to gastroesophageal reflux disease and achalasia, was made by Attwood *et al.* in 1993 (1). Since then, the epidemiology of EoE has evolved substantially and multiple studies have been reporting increased prevalence (2-4), making so that EoE is now recognized as a leading cause of dysphagia. For instance, according to a study by Prasad et al., the EoE incidence in Minnesota, USA, rose from 0.35 cases/100,000 people to 9.5 cases/100,000 people over a 15-year period (2). Similarly, in Denmark, Dellon *et al.* reported an increased annual incidence rate from 0.13/100.000 in 1997 to an annual incidence rate of 2.6/100,000 in 2012 (3).

The first consensus guidelines for the diagnosis and treatment of EoE were published in 2007 and subsequently updated in 2018 and then in 2020 (5-7). The EoE diagnosis is based on symptoms of esophageal dysfunction and esophageal mucosal biopsies with ≥15 eosinophils per high power field (eos/HPF). Until recently, consensus guidelines recommended as the first diagnostic step a trial of proton pump inhibitor (PPI). The latest guidelines now recommend PPI therapy not as a diagnostic criterion, but rather a first-line therapeutic agent in newly diagnosed patients with EoE (6, 7). Topical steroids and elimination/elemental diets can be also considered treatment options. With regard to treatment endpoints, the most commonly assessed outcome is histological, clinical and endoscopic remission.

Despite the publication of updated guidelines, clinical practice related to the management of EoE patients has been proven variable (8-10). In this context, we aimed to assess gastroenterologists´ adherence to the latest guidelines and the current clinical management of adult EoE patients in three academic centers from Romania.

MATERIAL AND METHODS

This was a cross-sectional study into the adult EoE patient evaluation and management practices of Romanian gastroenterologists using a web-based survey conducted between 15th of February and 15th of March 2022.

The survey instrument consisted of 12 targeted questions about the respondents' knowledge of published guidelines related to the diagnosis and management of EoE, and also scenario-based clinical judgment questions. The first six questions assessed the physicians' general perception regarding the clinical symptoms, the endoscopic and the histologic diagnosis of EoE. The next questions focused on the therapeutic management and follow-up of EoE patients.

Participation was voluntary. The survey could only be accessed via an email link, and all responses were anonymous. Data recording and processing was performed using SurveyPlanet. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

The web-based survey was completed by 62 gastroenterologists with a completion rate of 100% and a median completion time of 8.2 minutes. Survey respondents were mostly female (59.6%). The majority (70.9%) were gastroenterologists from the

Institute of Gastroenterology and Hepatology in Iași, Romania. The others were from academic centers like Bucharest (19.3%) and Timisoara (9.6%).

The number of EoE patients managed annually by survey participants differed from 1 to 5 patients in 56.5% of respondents, 6-19 patients in 4.8%. Only 1 participant reported managing between 20-50 patients with EoE annually.

EoE diagnosis

Concerning the understanding of EoE published guidelines, 31.6% of gastroenterologists founds the clinical symptoms of dysphagia and food impaction necessary for diagnosis, while 65.3% reported that symptoms were helpful, but not mandatory clues. However, all the participants agreed that the presence of eosinophil-predominant inflammation on esophageal biopsies (>15 eosinophils/high-power field) was a required criterion for a definitive diagnosis of EoE. Likewise, almost all gastroenterologists (82.3%) agreed to the necessity of a minimum of 6 esophageal biopsies taken from the proximal and distal esophagus in order to increase the diagnostic yield of EoE. In addition, only 48.3% of participants considered it of major importance to exclude other causes of esophageal eosinophilia, including gastroesophageal reflux disease, and 34.5% did not find this necessary for EoE diagnosis (fig. 1).

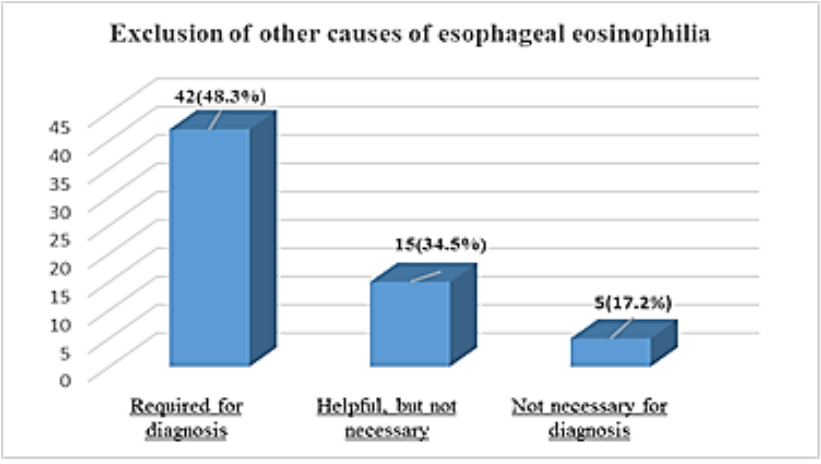

Fig.1. Knowledge of EoE published guidelines regarding diagnosis

EoE treatment and monitoring

When asked about treatment options for patients diagnosed with EoE, 41.9% of respondents reported using as first-line therapy a high-dose PPI regimen, while 48.4% indicated the use of topical steroids. Only 8.1% of participants preferred to start the patient on an elemental/elimination diet (fig. 2). In case of esophageal stricture, management by means of endoscopic dilation was considered safe and efficient by 56.5% of gastroenterologists. However, 43.5% of participants associated endoscopic dilation with a higher risk of perforation. In addition, when suspecting EoE, 67.7% of participants considered an initial trial of PPI useful before trying other diagnostic strategies.

In order to determine the success of therapy, 35 gastroenterologists (56.5%) considered it imperative to repeat endoscopy with biopsies to assess histologic remission, while 19 (30.6%) would not repeat endoscopy in the presence of symptoms resolution, and 8 (12.9%) would repeat endoscopy only to assess endoscopic remission (fig. 3).

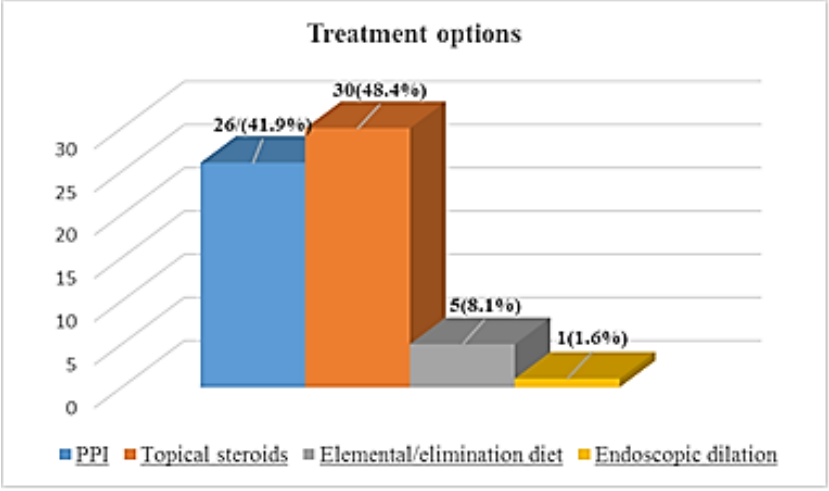

Fig. 2. Treatment options for EoE patients

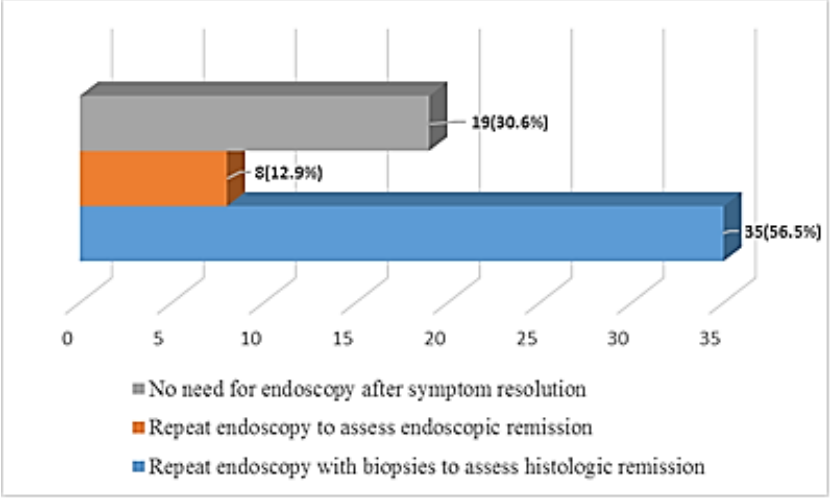

Fig. 3. Assessing the response to therapy

Last but not least, about monitoring patients' responses to therapy, 29 survey participants (46.8%) would consider follow-up with endoscopy and biopsies after a course of 6-12 weeks of therapy in order to evaluate remission, while 22 (35.5%) would not repeat endoscopy in the presence of symptom resolution.

DISSCUSION

To our knowledge, this is currently the first survey regarding the EoE diagnosis and management practices of gastroenterologists in our country. Given the indications of variable EoE management internationally despite updated published guidelines, our study provides insight into the adherence of Romanian gastroenterologists to available EoE guidelines.

While there is a lack of epidemiological data of EoE in Romania, our results suggest a possible low incidence of EoE cases, as more than half of the participating gastroenterologists reported diagnosing a maximum of five new EoE patients annually.

When a patient presents complaining of dysphagia or food impaction, only 31.6% of gastroenterologists are aware that these symptoms can be suggestive of EoE, and that they are, in fact, compulsory diagnostic criteria for EoE. These findings indicate a real need for to improve clinical awareness to avoid misdiagnosis.

Regarding the clinicians' knowledge of published guidelines, our survey further showed that, while all survey participants agreed that eosinophil-predominant inflammation on the esophageal biopsy is a mandatory criterion for EoE diagnosis, only 48.6% considered it necessary for diagnosis to exclude other causes of esophageal eosinophilia, including gastroesophageal disease. This emphasizes the importance of a complete and accurate differential diagnosis in order to confirmed EoE.

Despite the updated published guidelines that underline the need to remove PPI trials from the diagnostic criteria (6, 7), our survey revealed that approximately 70% of gastro-enterologists would still recommend a trial of PPI before any other strategies in case of suspected EoE.

Concerning treatment options, PPI and topical steroids are recommended in relatively equal proportions according to our survey, which is in accordance with existing guidelines (6, 7). However, very few Romanian gastroenterologists would initiate an elemental/elimination diet as first-line treatment for EoE. This reluctance may be justified by the arguably poor compliance of Romanian patients to dietary restrictions and also by the current shortage of trained nutritionists in our country.

Multiple studies reinforce international guidelines with regard to the need of clear therapy endpoints for patients diagnosed with EoE, histological remission being the most important one (6, 7, 11-13). Our survey revealed that most of the participating gastroenterologists (56.5%) considered it essential to repeat endoscopy with esophageal biopsies in order to assess histological remission. However, there is still a sizeable proportion of healthcare providers (30.6%) who do not consider the endoscopic reevaluation necessary in case of symptom resolution. These variations in Romanian practitioners' adherence to guidelines are important indicators of EoE patient follow-up practices that prioritize symptomatic manifestations.

CONCLUSIONS

Our survey found differences in the diagnosis and management of patients with EoE in Romania, particularly regarding the use of PPI trials in case of suspected EoE, and also in appreciating the need of repeat endoscopies with histology to monitor response to therapy. As these findings are in contrast with the latest international guidelines, our survey highlights the need for intensified education and development of targeted strategies in order to raise clinicians' awareness and optimize the clinical management of EoE patients.

CONFLICT OF INTEREST AND FUNDING

The authors declares that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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URETEROHYDRONEPHROSIS AND KIDNEY STONE COMPOSITION: POTENTIAL PREDICTING FACTORS FOR THE FAILURE OF *IN SITU* EXTRACORPOREAL LITHOTRIPSY

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URETEROHYDRONEPHROSIS AND KIDNEY STONE COMPOSITION: POTENTIAL PREDICTING FACTORS FOR THE FAILURE OF EXTRACORPOREAL LITHOTRIPSY (Abstract) **Aim:** The objective of our study was to identify the factors that predict the failure of extracorporeal lithotripsy in the treatment of ureteral stones, with reference to the ureteral obstruction and composition of stones determined by infrared spectroscopy. **Material and methods**: We conducted a retrospective study, evaluating stone-free rates of extracorporeal lithotripsy in patients with ureteral lithiasis. The stone examination was made by infrared spectroscopy. **Results**: 215 patients were included in this study. The overall stone-free rate in this study was 79.06%. The presence of ureteral obstruction consecutive to stones correlates with lower stone-free rates in comparison to patients without ureterohydronephrosis. Calcium oxalate monohydrate stones had lower fragmentation rates (37.5%) compared to calcium oxalate dihydrate (84.69%) and uric acid stones (85.71%). In this study, age and gender are not factors that influence the effectiveness of extracorporeal lithotripsy. **Conclusions**: Extracorporeal shockwave lithotripsy is an effective method to manage ureteral lithiasis, but it is limited by numerous factors: body mass index, the presence of ureteral obstruction, as well as by the biochemical composition of stones. Impacted stones for a long period and the main component of calcium oxalate monohydrate have fewer chances to be successfully fragmented. **Keywords**: SHOCK WAVE LITHOTRIPSY, URETERAL STONES, STONE COMPOSITION, URETERAL OBSTRUCTION, INFRARED SPECTROSCOPY.

Urinary lithiasis is one of the most common chronic urological conditions with a prevalence in Europe estimated at approximately 4% (1). Despite the important technological progress in endourology, extracorporeal shock wave lithotripsy (ESWL) is still one of the most important methods used in managing kidney stone disease that is currently in the arsenal of urologists, due to its safety profile and minimal invasiveness.

The European Association of Urology Guidelines currently recommends ESWL for the treatment of lithiasis, regardless of a stone's topographical in the ureter.

The efficiency of *in situ* ESWL for ureteral stones has been reported by various authors as ranging between 80-95% (2, 3, 4).

In addition to the technical parameters of the lithotripter, the efficiency of ESWL is conditioned by anatomical factors (body mass index, skin-to-stone distance, and ureteral wall thickness) and by stone properties (stones' chemical composition, the degree of impaction in the ureteral mucosa).

The influence of the ureteral obstruction on the effectiveness of ESWL for ureteral stones is still a controversial subject and this become one of the main goals of our research.

MATERIAL AND METHODS

We have conducted a retrospective study from January 2021 to January 2022, that included patients treated in the Department of Extracorporeal Lithotripsy of "Dr. C. I. Parhon" Hospital in Iasi. All ESWL procedures were performed by a urologist with significant experience in this field.

The patients were investigated by ultrasound to determine the degree of ureteral obstruction and, if possible, to determine the stones' size and position. Intravenous urography was performed in selected patients. The exclusion criteria were the presence of a ureteral stent, blood clotting disorders, inflammatory syndrome, and multiple ureteral calculi.

The procedures were performed with Dornier Sigma Compact electromagnetic lithotripter, and the stones were detected by X-Ray. The maximum number of shock waves per session was 2900 with a frequency of 70 shock waves per minute. Local analgesia was obtained by topical application of a gel containing piroxicam, lidocaine and cyclobenzaprine, 20 minutes before the procedure. Only in 15 cases, upon request, we have administrated 1 ampoule of Tramadol, 100 mg intramuscularly in order to continue the procedure.

The symptoms associated with the removal of lithiasis fragments were of moderate intensity, under treatment with non-steroidal anti-inflammatory drugs and alpha-blockers (Tamsulosin) and they did not require a form of urinary drainage (ureteral stenting or percutaneous nephrostomy).

The postprocedural evaluation was conducted 3-4 weeks after extracorporeal lithotripsy and it included control reno-vesical radiography (KUB) and ultrasound. The presence of fragments larger than 7 mm with or without ureterohydronephrosis after lithotripsy was considered a failure of treatment and the patients accepted the second session of ESWL or retrograde ureteroscopy.

Stones' composition was determined by infrared spectroscopy (FTIR) with the Brucker Alfa II spectrophotometer.

The ethical approval for this study was obtained from the Institutional Ethics Committee of "Dr. C. I. Parhon" Clinical Hospital (No. 3530/ 06.05.2022). All the methods that we used comply with relevant guidelines and regulations.

The results were processed and analyzed using statistical *SPSS software version 27.0*, IBM Corporation. Chi-squared test (χ^2) and t-test were applied. A result of $p<0.05$ was accepted as statistically relevant.

RESULTS

During the above-mentioned period, 319 patients with ureteral lithiasis were evaluated, but after applying the exclusion criteria, 215 patients were included in our study, aged 19-73 (the average age was 50.32, SD 12.14 years).

Table I shows the results of the compar-

ative analysis of the two groups, related to the demographic characteristics, the chemical composition of the stone, the location of the stone and the personal history of urinary lithiasis. Among the six analyzed elements, four reached the limit of statistical significance (body mass index, degree of ureteral obstruction, stone's composition and location in the ureter), one element (history of the urinary stones) had a borderline value, and one element was not statistically significant (patient's gender).

TABLE I.

The results of the comparative analysis of the patient's demographic and medical characteristics

	Stone-free group (n=170)		Failure group (n=45)		Total (n=215)		χ^2	p-value
	N	%	n	%	n	%		
Gender								
Male	98	57.64	21	46.66	119	55.34	1.73	0.1876
Female	72	42.35	24	53.34	96	44.66		
Body Mass Index (kg/m^2)								
Normal	45	26.47	6	13.33	51	23.72		
Overweight	87	51.17	13	28.88	100	46.51	21.38	<0.001
Obese	38	22.35	26	57.77	64	29.76		
Obstruction degree								
Without obstruction	24	14.11	2	4.44	26	12.09		
Ureterohydronephrosis I	115	67.64	24	53.33	139	64.65	12.68	0.0017
Ureterohydronephrosis II	31	18.23	19	42.22	50	23.25		
Kidney Stone Composition								
COM								
COD	12	7.05	20	40.44	32	14.88		
UA	83	48.82	15	33.33	98	45.58	66.03	<0.001
CA	18	10.58	3	6.66	21	9.76		
STRU	53	31.17	6	13.33	59	27.44		
	4	2.35	1	2.22	5	2.32		
History of lithiasis								
First stone	53	31.17	21	46.66	74	34.41	3.78	0.0517
Recurrent stone	117	68.82	24	53.33	141	65.58		
Ureteral location								
Pelvic ureter	38	22.35	17	37.77	55	25.58		
Iliac ureter	10	5.88	6	13.33	16	7.44	8.74	0.012
Lumbar ureter	122	71.76	22	48.88	144	66.98		

*Significant (p-value<0.05). Chi-Square tests were used. COD – Calcium Oxalate Dihydrate; COM – Calcium Oxalate Monohydrate; UA – Uric Acid; CA – carbapatite; STRU – Struvite.

Figure 1 shows stone-free rates according to the chemical composition determined spectroscopically. We noticed that stones with COM as the main component have the lowest probability of being stone-free, while for the other types of stones, the rates of fragmentation are similar. No patients with cystine or brushite calculi were reported in this group.

Table II shows no significant differ-

ences regarding certain parameters: patients' age, irradiation dose and the number of sessions required to reach stone-free status, between the two groups of patients. On the other hand, patients in whom ESWL did not result in stone fragmentation had an average stone size of 10.37 mm compared to 8.97 mm, the difference being statistically significant (t=2.556, p=0.013).

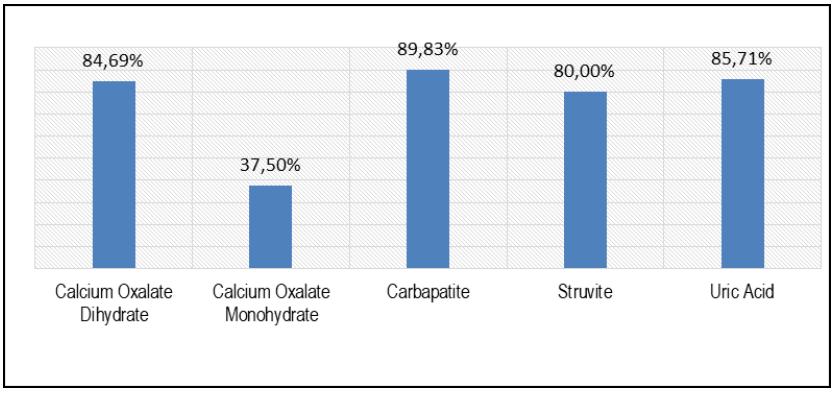

Fig. 1. Stone free rate according to stone composition FTIR determined

TABLE II.

Analysis of the numerical variables related to the postprocedural stone-free rates

	Stone-free group (n=170)		Failure group (n=45)		Total (n=215)		
	Average	Standard Deviation	Average	Standard Deviation	t	p	95% CI
Age (years)	50.45	12.269	49.82	11.787	0.313	0.755	-3.349 ÷4.599
Stone size (mm)	8.976	2.847	10.378	3.372	2.556	0.013	0.3054 ÷ 2.4972
Body Mass Index (kg/m^2)	27.7405	5.06543	29.2520	3.94513	2.144	0.035	0.11047 ÷ 2.91259
Radiation dose (µG*m$^{2)}$	3079.74	2407.127	3123.52	1582.408	0.146	0.884	637.778÷550.219
Number of sessions	1.42	0.495	1.53	0.505	1.312	0.194	-0.279÷0.058
*significant (p-value<0.05). The t-student was used. CI – Confidence Interval.							

DISCUSSION

In this study, we tried to identify the factors that can be associated with the failure of first *in situ* ESWL in the treatment of ureteral stones, especially the presence of the ureteral obstruction and the specific accurate composition of stones.

Despite the important progress made in the field of endourology, ESWL is still one of the most feasible ways to manage ureteral stones, due to its low costs and favorable clinical results (7).

Various studies regarding the success of ESWL for ureteral stones have been pub-

lished. The data published by Seitz *et al.* reveal that the general stone-free rate obtained in a group of 130 patients with ureteral lithiasis was 77.4%, but another research, published by Mogensen *et al.* , found much higher rates, respectively 91.1% for lumbar ureteral stones, and 90.9% in the case of stones located in the iliac or pelvic segment of the ureter, whereas similar success rates were demonstrated by Chang *et al.* , respectively 81.9% (8). The stone-free rate for the patients included in this study is 79.06%, falling within the reference ranges of similar studies.

Previous studies have demonstrated that the success of ESWL depends on numerous factors, among which the most important are stone location and density, skin-to-stone distance (SSD), and BMI. As for the influence of age on ESWL success rate, in a comparative retrospective study, Halachmi *et al.* found stone-free rates of 91% for elderly patients and young patients. The efficiency profile of ESWL in elderly patients is also proved by data published by Delakas *et al.* (9, 10).

Yet, the research conducted by Ikegaya contradicts this statement: its results prove that the success rate of ESWL in elderly patients is lower in comparison to young patients (11). Ng *et al.* found that lithotripsy is less effective in elderly patients with renal lithiasis, but the differences are not statistically significant concerning ureteral lithiasis (12). In our study, we did not find significant differences regarding age in the chances for stone-free status (p=0.755)

In addition, we also found that there is no statistically significant difference in the correlation between personal history of lithiasis and the stone-free rate after ESWL, as the value obtained is borderline (p=0.0517).

Previous studies have demonstrated a strong relationship between the efficiency of stone fragmentation by ESWL and patients' BMI. In a group of 100 patients with ureteral stones measuring 5-10 mm, Pareek *et al.* found that the BMI is an independent predictive factor for the stone-free rate and that obese patients most likely need an endoscopic intervention in the context of the failure of ESWL (13). These data are also supported by Yang *et al.* , who demonstrated, in a group of 203 patients, that extracorporeal lithotripsy's probability of success is twice lower in the case of obese and overweight patients, in comparison to normal weight patients (14). A study conducted in France showed that the efficiency of ESWL in obese patients, with an average BMI between 35 and 40 kg/m^2, is around 60%, and it decreases in direct proportion to its increase. For patients with a BMI of 45-50 kg/m^2, the efficiency is 33.3%, and for patients with BMI greater than 50 kg/m^2, the efficiency is only 20% (15). Paradoxically, Delakas *et al.* , in their studies, note that a BMI above normal values does not negatively influence the efficiency of ESWL (10).

In this group of patients, we observed that the highest stone-free rate was obtained in patients with stones located in the lumbar segment of the ureter (84.72%) in comparison to stones located in the iliac ureter (62.5%) and the pelvic ureter (69.09%), respectively. The data identified by us are also supported by other studies in the literature. Delakas identifies a stone-free rate of 61.5% for stones located at the level of the pelvic ureter, 75.6% for iliac stones and 82.9% for lumbar ureteral stones (10). However, there is no consensus

in the literature about the importance of stones' location. Kim *et al.* found that there are no significant differences between the stone-free status and the location of the stone in the ureter (16).

One of the most important factors that condition the fragmentation and, consequently, the clearance of fragments, is the chemical composition of the stones. The concept of "stone fragility" was introduced by Dretler who found that the specific density of calcium oxalate monohydrate stones is 0.851 g/cm^2, for calcium oxalate dihydrate stones, 0.715 g/cm^2, whereas for uric acid it is 0.226 g/cm^2; further, he demonstrated that the most difficult fragmentation is found in patients with stones of calcium oxalate monohydrate and brushite, and the easiest fragmentation is found in stones made of uric acid and calcium oxalate dihydrate(17).

In this study, the analysis of the biochemical composition of stones was made by infrared spectroscopy in "Michel Daudon-Spectroscopy Center", "Dr. C. I. Parhon" Clinical Hospital, Iasi.

The vast majority of samples obtained by ESWL were of small size and the results implied only a global analysis (it was not possible to identify the specific composition of the core of the stone). We found that only 37.5% of the patients in whom the spectroscopic analysis showed the presence of COM were stone-free by ESWL. Except for this biochemical type of stone, the other crystalline species had stone-free rates of over 80%. It is noteworthy that there were no patients with brushite or cystine stones in this study. Our data on the poor performance of ESWL on the stones of COM differ from the data reported by Seitz, who found approximately similar fragmentation rates for both varieties of calcium oxalate (18).

In our study, 13.02% of the patients who underwent *in situ* ESWL did not have ureterohydronephrosis, and 92.3% of these patients were stone-free after the procedure, compared to 82.73% of those with grade II obstruction and 62% in the case of those with grade III ureterohydronephrosis. Similar studies on the influence of obstruction on the effectiveness of ESWL are contradictory. Thus, in a comparative study on the effectiveness of lithotripsy in patients with ureterohydronephrosis, Chang *et al.* find that the obstruction degree and the effectiveness of the procedure are directly correlated. Patients with high degrees of obstruction (III and IV) had significantly lower stone-free rates compared to patients with lower degrees of ureteral obstruction (19). The research of Kirkali *et al.* reveals that the ureteral obstruction is not a factor that negatively influences the disintegration of stones or the clearance of fragments resulting from extracorporeal lithotripsy. The two compared groups achieve similar stone-free rates, respectively 90% in the case of patients without obstruction and 85% in the case of those with ureterohydronephrosis (20). This hypothesis is also supported by the studies conducted by other authors who find that the presence of obstruction is not associated with a decrease in the stone-free rate, nor does it influence the number of pulses required to achieve fragmentation (19, 21).

Another factor with predictive value regarding the success of ESWL is represented by the presence of the ureteral stent. In a prospective randomized trial, Chandhoke *et al.* found that there are certain benefits of pre-ESWL ureteral stenting, especially the decrease in the number of colic episodes

and the number of post-procedural hospitalizations (22). Despite the benefits related to the prevention of complications, data available in the literature has demonstrated that the presence of JJ stents inserted before ESWL does not bring additional advantages. While some studies have demonstrated that ureteral stents do not influence the fragmentation rate (23, 24), most studies conducted on large groups of patients have demonstrated that the presence of ureteral stents correlates with lower rates of stone fragmentation and subsequent lower stone-free rates (25, 26, 27). The explanation is related, on the one hand, to the absence of the liquid interface between the calculus and the shock wave and on the other hand to the energy absorption by the stent (23, 28).

One of the most important limits of our study is the lack of computed tomography investigation of patients with ureteral lithiasis. It is demonstrated that the success of ESWL can be predicted by the determination of Hounsfield Units (HU). Thus, research dedicated to this topic has demonstrated that stones of sizes ranging between 10-20 mm and of densities higher than 1000 HU are associated with high probabilities of failure of ESWL (29). Joseph *et al.* demonstrated that the probability of fragmentation is inversely proportional to stone density: 55% for stones >1,000 HU, 86% for stones 500-1,000 HU and 100% for stones < 500 HU (30). The patients in our study were evaluated by KUB, which is a less reliable method to predict fragmentation. Krishnamurthy's results showed that patients in whom radiography revealed a calculus with a higher radiodensity than the one of the 12th rib, had a stone-free status of 60% (31). Also, we were not able to determine the skin-to-stone distance, which is an important predictor of the effectiveness of lithotripsy. Pareek *et al.* estimated that patients in whom this parameter exceeds 10 cm have the highest probability of treatment failure by ESWL (32). Our goal in the future is to evaluate these computer-tomographic parameters and correlate them with stone hardness and composition.

CONCLUSIONS

In situ extracorporeal lithotripsy is an efficient and safe method for the therapeutic management of patients with ureteral lithiasis, with better results if the procedure is performed as soon as possible after the onset of obstruction. In our study, we found that patients who had associated significant ureterohydronephrosis had lower stone-free rates after the procedure, compared to patients without ureteral obstruction. Regarding stone composition, patients with calcium oxalate monohydrate lithiasis had significantly lower stone-free rates in comparison to patients with stones made of calcium oxalate dihydrate, carbapatite or uric acid. We consider that the evaluation of stone density by computer tomography is necessary to assess the probability of fragmentation, but it cannot replace the morphological and spectroscopic analysis for the initiation of a targeted metaphylactic plan.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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ROLE OF THE PROSTHETIC MEDICAL DEVICES IN MANAGEMENT OF ABDOMINAL PARIETAL DEFECTS

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ROLE OF THE PROSTHETIC MEDICAL DEVICES IN MANAGEMENT OF ABDOMINAL PARIETAL DEFECTS (Abstract): Management of abdominal parietal defects is a major public health concern, both nationally and internationally, due to morbidity, mortality, loss of function, decreased quality of life and loss of work capacity. **The aim** of this retrospective study is driven by the current possibility of treating abdominal parietal defects by prosthetic medical devices with various high-performance synthetic materials. **Material and methods:** The approach is from a statistical and multifactorial point of view in patients with abdominal parietal defects treated with prostheses in the I and II Surgery Clinics of "Sf. Spiridon" County Clinical Emergency Hospital Iaşi for a period of 15 years. The information was retrospectively obtained from the register of the hospital secretary and from the observation sheets, analyzing the cases on the basis of the multiple criteria. **Results:** The statistical analysis of the selected cases revealed that at a number of 5865 of abdominal wall defects surgeries that were performed in our clinics between 01.01.2006–01.07.2021 we recorded 3029 inguinal hernias (52%), 220 femoral hernias (4%), 796 umbilical hernias (13%) and 1820 incisional hernia (31%). The information on the main diagnosis associated with the 1820 subjects shows that most patients were hospitalized with the diagnosis of ventral hernia, 1122 cases without occlusion or gangrene (61.64%), 522 cases with obstruction (28.68%) and 176 cases with gangrene (9.6%). Obesity was the most common factor of risk for an abdominal wall defect with an index of 29%. The highest incidence of the develop of an incisional is reported on the midline, respectively 74%. **Conclusions:** Management of abdominal parietal defects is a major public health concern. Early diagnosis, easily accessible health facilities and health education are important to prevent complications and improve quality of life. **Keywords:** ABDOMINAL WALL DEFECTS; HERNIA; INCISIONAL HERNIA; PROSTHETIC MEDICAL DEVICES.

Management of abdominal parietal defects is a major public health concern, both nationally and internationally, due to morbidity, mortality, loss of function, decreased quality of life and loss of work capacity.

The first description of a hernia appears in the Ebers Papyrus in Egypt in 1550 BC.

There is evidence of hernia surgery since antiquity, with Hippocrates describing the umbilical hernia in 460-374 BC. This pathology is an ongoing concern, with the development of multiple surgical techniques over the years. The "modern" era of surgical treatment of hernia begins in 1958, when the procedure with polyethylene mesh (Marlex-50) was introduced by FC Usher, used in the case of inguinal hernias, and in 1984 Liechtenstein established the gold standard with the technique "tension-free", followed in 1992 by the development of laparoscopic techniques (1).

There is no universally valid definition of abdominal parietal defect, this may include recurrent or non-recurrent hernias, incisional hernias and eviscerations. A hernia is defined as the protrusion of a viscera, or anatomical tissue, through an existing or acquired orifice in the abdominal wall. Incisional hernia refers to abdominal wall hernia at the site of a previous surgical incision (2). Of the total interventions for the surgical treatment of parietal defects, in 60% of cases the prosthetic material used is mesh type and in 40% combined systems are used (3).

Incisional hernia develops in 11-20% of patients with laparotomy history, complicated with incarceration in 6-15% of cases and strangulation in approximately 2%. The recurrence rate after primary suture surgery is cited in approximately 54% of cases, up to 58% for tissue procedures and 20% for alloplastic procedures (4).

MATERIAL AND METHODS

The purpose of this retrospective study is driven by the current possibility of treating abdominal wall defects by prosthetic medical devices with various high-performance synthetic materials. The approach is from a statistical and multifactorial point of view of the parietal defects treated by prosthetic medical devices in the period 01.01.2006–01.07.2021, in the I and II Surgery Clinics of "Sf. Spiridon" County Clinical Emergency Hospital Iaşi.

The information was retrospectively obtained from the register of the hospital secretary and from the observation sheets, analyzing the cases on the basis of the following criteria:

- Age
- Sex
- Associated diseases
- Type of prosthetic medical devices
- Number of hospital days
- Immediate, remote, local, general complications

Regarding the analysis of cases with postoperative incisional hernia, the following criteria were also taken into account:

- The size of the parietal defect
- Number of previous incisions
- Type of initial incision.

The data systematized in this study was used in the analysis of the incidence of abdominal parietal defects in relation to the number of laparotomies performed in the two clinics, risk factors for the occurrence of the defect, recurrence and the occurrence of postoperative incisional hernia. These values should not be considered as an assessment of maximum accuracy, as it should be borne in mind that some patients come from outside the hospital area and others, with laparotomy events performed in the two clinics, may have turned to other surgical services. Another category of patients is represented by those patients with well-tolerated small abdominal parietal defect who did not request surgical consultation, remaining outside the evidence that was analyzed in this study. All data was collected in full acknowledgement of GDPR regulations and confidentiality, from hospital registry (5, 6).

Statistical analysis was performed as

case-control analyses, without adjustment for multiple testing, with nominal significance defined as $p<0.05$. Continuous variables were described using ANOVA test. The quantitative differences between the two groups were established with t-Student test. The categorial demographic data were compared using the Chi-2 test. The ROC curve (Receiver Operating Characteristics) helps us to measure the efficiency of a model, by drawing the specificity / sensitivity balance as a prognostic factor. The larger the area under the curve (the maximum is 1) the better the model.

RESULTS

The statistical analysis of the selected cases revealed that at a number of 5865 of abdominal wall defects surgeries that were performed in our clinics between 01.01.2006–01.07.2021 we recorded 3029 inguinal hernias (52%), 220 femoral hernias (4%), 796 umbilical hernias (13%) and 1820 incisional hernia (31%), (fig. 1).

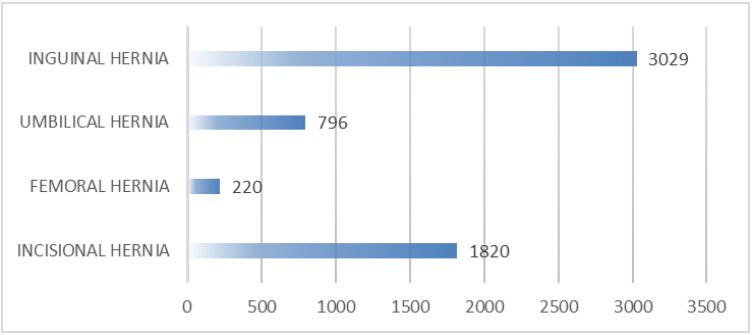


Fig. 1. This is a figure. Schemes follow the same formatting.

The data was analyzed in relation to the main local and systemic risk factors that interfere with the occurrence of the parietal defect.

Risk factors in the appearance of abdominal wall defects

• *Age and sex*

Patient gender data show the numerical difference between the sexes, which corresponds to a ratio of 2: 1 in favor of women in incisional hernia cases. This explained by the more frequent presence in females of risk factors conductive to postoperative parietal defects: decreased muscle tone after multiple pregnancies, obesity, weaker musculoskeletal layers, more frequent surgery on the lower abdomen as pelvic surgery, caesarean section, hysterectomies (tab. I).

TABLE I.

Incidence of inguinal hernias according to demographical data

	All groups N=5865	Incisional hernia N=1820	Other abdominal parietal defects N=4045	P values
Demographic data				
Age, mean±SD y (limits)	60.56±11.37 (17-98)	61.85±9.24 (30-96)	59.97±12.17 (17-98)	0.001*
Female, n(%)	2083 (35.5%)	1195 (65.7%)	888 (22.0%)	0.001**
≥ 60 years	3163 (53.9%)	1085 (59.6%)	2078 (51.4%)	0.001**

* t-student test ** chi2 test.

In inguinal hernia cases the main reason why men are more prone to inguinal hernias, both direct and indirect is due to reproductive anatomy and the inguinal canal in women is narrower., with a ratio of 8:1 (7.6:1) in favor of men. The umbilical hernia has a ratio of 1:1, and the femoral hernia has a ratio of 4:1 (3,6:1) in favor of women, because of the wider shape of the female pelvis (p=0.001) (fig. 2).

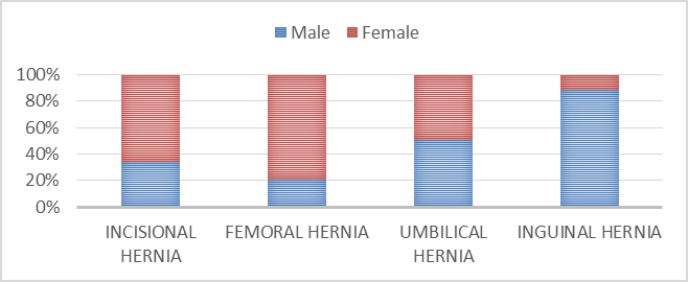


Fig. 2. Sex distribution by diagnosis.

The average age was highest in the group with femoral hernia and the lowest in the group with umbilical hernia (67.13 vs 57.97; p=0.001) (fig. 3).

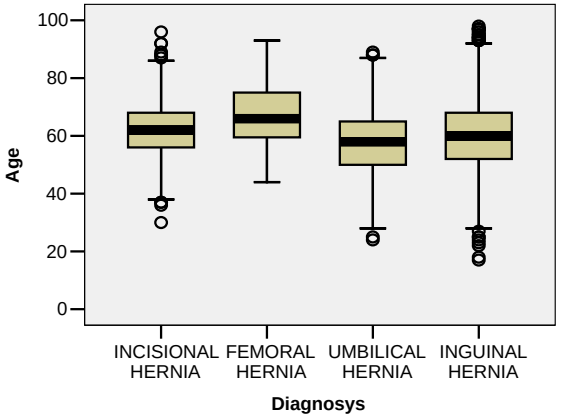


Fig. 3. Mean age by diagnosis.

Associated diseases

A hernia is often caused when there is a history of a hernia in the family. Straining your muscles due to intense weightlifting or coughing and even pregnancy may cause a hernia. When there is pressure on the abdominal area can lead to a hernia. Even issues like constipation, urinary obstruction along with chronic cough are risk factors. Obesity is also a major risk factor. As a hernia is caused when the tissue or part of muscle pushes out, damage to the elasticity and the strength of the tissue can also be a factor causing a hernia. Also, it may be due to residual embryological channels which is generally what a person is born with,

causing a congenital hernia.

Organic strains were found in a percentage of 62% being represented by various organo-systemic and metabolic disorders (fig. 4). Among the organic disfunctions mentioned, obesity was the most common with an index of 29%. Data on organo-systemic and metabolic disorders as individual risk factors provide an incomplete picture of their influence on the frequency of postoperative events. For a more rigorous interpretation it would be necessary to take into account their cumulative and synergistic effects.

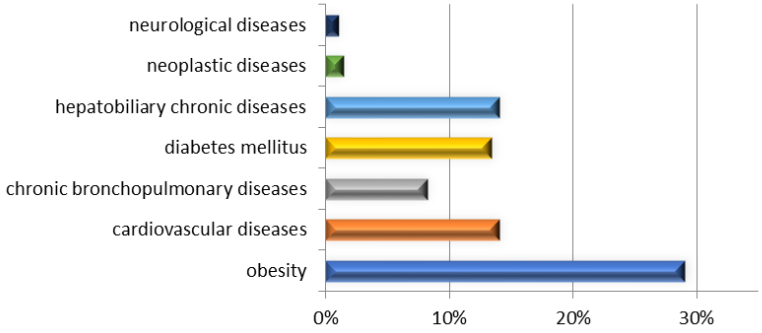


Fig. 4. Organo-systemic and metabolic disorders.

Obesity occurs more frequently under 60 years, muscle-aponeurotic structures begin to lose strength (table 2), chronic cardiovascular, lung disease and diabetes occur more frequently, and in women, the onset of menopause can trigger endocrine and metabolic disorders with a resounding decrease in the strength of supporting connective tissue (tab. III).

The repair of skin defects is incomplete and unfeasible in the elderly where epithelialization is delayed and limited, the vascular network recovers slowly and poorly, and the basement membranes recover abnormally. The scar tissue of the old man is younger compared to that of the body's original skin, the scar remaining immature for a long time.

TABLE II.

Organo-systemic and metabolic disorders by age group in patients with incisional hernia

Organo-systemic and metabolic disorders	< 60 years (n=625)	≥ 60 years (n=1195)	P values for Chi² test	OR	CI 95%
Obesity	369 (50.2%)	519 (47.8%)	0.321	1.06	0.95-1.18
Cardiovascular	359 (48.8%)	513 (47.3%)	0.513	1.04	0.93-1.16
Lung disease	228 (31.0%)	325 (30.0%)	0.628	1.03	0.91-1.16
Diabetes	340 (46.3%)	483 (44.5%)	0.464	1.04	0.93-1.17
Hepatobiliary	266 (36.3%)	402 (37.1%)	0.709	0.98	0.87-1.10
Neoplasic	126 (17.1%)	190 (17.5%)	0.838	0.99	0.85-1.14
Neurological	127 (17.3%)	192 (17.7%)	0.818	0.98	0.85-1.14

TABLE III.

Organo-systemic and metabolic disorders by sex in patients with incisional hernia

Organo-systemic and metabolic disorders	Female (n=1195)	Male (n=625)	P values for Chi² test	OR	CI 95%
Obesity	584 (48.9%)	304 (48.6%)	0.962	1.00	0.82-1.20
Cardiovascular	585 (49.0%)	287 (45.9%)	0.218	1.04	0.98-1.11
Lung disease	373 (31.2%)	180 (28.8%)	0.287	1.04	0.97-1.12
Diabetes	541 (45.3%)	282 (45.1%)	0.951	1.01	0.94-1.07
Hepatobiliary	441 (36.9%)	227 (36.3%)	0.806	1.01	0.94-1.08
Neoplasic	200 (16.7%)	116 (18.6%)	0.331	0.96	0.87-1.05
Neurological	203 (17.0%)	116 (18.6%)	0.404	0.96	0.88-1.05

The period of maturation and integration of the scar is double or even triple in duration in the elderly, and the resulting scar is worse than in the young, the evolution being able to be interrupted by lesions with secondary healing.

• *The influence of number of previous interventions in incisional hernia cases*

From a total of 1820 patients, 46.3% had previously undergone two or more surgeries on the abdominal wall for various conditions, all with at least one attempt at surgery to treat an incisional hernia by simple parietal suture and autoplastic methods or by prosthetic medical devices.

The number of surgeries performed on the abdominal wall recorded in the history of patients in the study group are shown below (fig. 5).

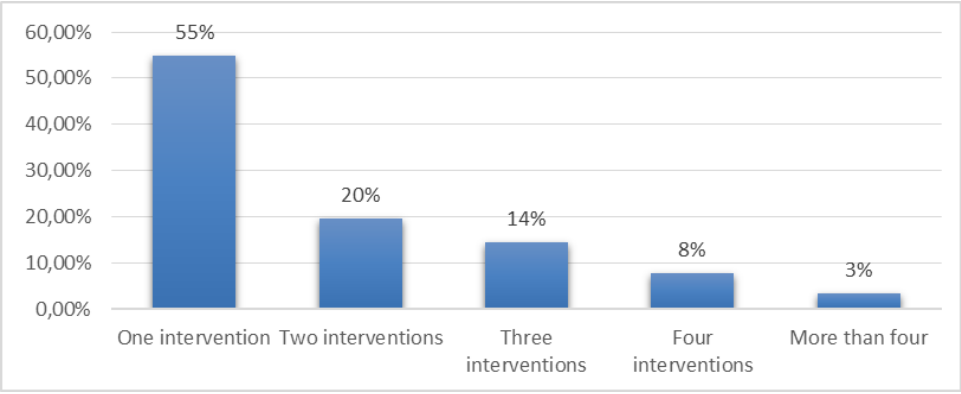


Fig. 5. Distribution of incisional hernia cases by history of previous surgeries.

• *Topography of the parietal defect*

The approach to resolving the condition of origin of the current parietal defect generally followed the place of establishment of the incisional hernia.

Some topographic regions predispose to parietal scarring defects either by the distribution of aponeurotic-fascial structures in the superficial planes and enduring a constantly positive intra-abdominal pressure (sub umbilical region), or by high tension in the scar caused by the presence

of close bone marks. However, the highest incidence is reported on the midline, respectively 74%. This is explained by the fact that the median laparotomy offers the best comfort in resolving surgical emergencies, being preferred by most surgeons. The topography of the parietal defect overlapped in most patients (~97%) with that of the last laparotomy performed.

The information on the main diagnosis associated with the 1,820 subjects shows that most patients were hospitalized with the diagnosis of ventral hernia, 1,122 cases without occlusion or gangrene (61.64%), 522 cases with obstruction (28.68%) and 176 cases with gangrene (9.6%).The appearance of the current event finds its explanation in a series of elements related to:

• Conditions in which the laparotomy (emergency or election) was performed.

• The lesions encountered and how to resolve them.

• Incidence of the infectious process and contamination or not of the parietal wound.

• Quality of parietal strength structures.

• Anesthetic conditions under which the operation was performed,

• Reaction to the suture material used (intolerance, granulomas).

The recurrence of the events poses problems of surgical resolution of the musculo-aponeurotic gap and difficulty in choosing the right method of restoring the wall to avoid a new recurrence. It, and especially the multi-recurrence, draws attention to the cumulation in the same patient of several local and general risk factors that must be taken into account when choosing the treatment method.

A new attempt to repair the wall by the same methods used previously without results is illusory and will further compromise the local anatomical structures.

Regarding recurrence, in just over half of the cases there were primary events (1,010 and 55.5%, respectively) and a number of 810 patients had multiple recurrent incisional hernia. In 18% of patients, it was intervened in the antecedents 2 or more times by classical methods of suturing the edges of the parietal defect.

• *The size of the parietal defect*

Another risk factor that may influence the therapeutic outcome is the size of the parietal defect (fig. 6).

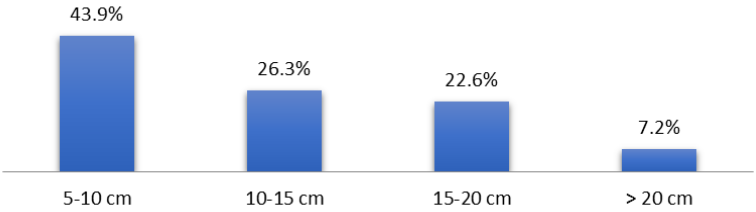


Fig. 6. Distribution of incisional hernias according to the size of the parietal defect.

About 7% of patients with incisional hernia were over 60 years old, female, and had complications (tab. IV).

ROC curve not confirmed age (AUC=0.479; IC95%: 0.453-0.506; p=0.131), gender (AUC=0.498; IC95%: 0.471-0.525; p=0.875) and presence of complications (AUC=0.494; IC95%: 0.468-0.521; p=0.685) as good predictors of a high size of parietal defect (fig. 7).

TABLE IV.
Size of parietal defect in relation with characteristics in patients with incisional hernia

Characteristics	Size of parietal defect				P value for Kruskal-Wallis test
	> 20 cm	15-20 cm	10-15 cm	5-10 cm	
Age ≥ 60 y	78 (7.2%)	261 (24.1%)	284 (26.2%)	462 (42.6%)	0.439
Female	83 (6.9%)	271 (22.7%)	317 (26.5%)	524 (43.8%)	0.898
Complicated	55 (7.5%)	159 (21.7%)	192 (26.2%)	328 (44.7%)	0.659

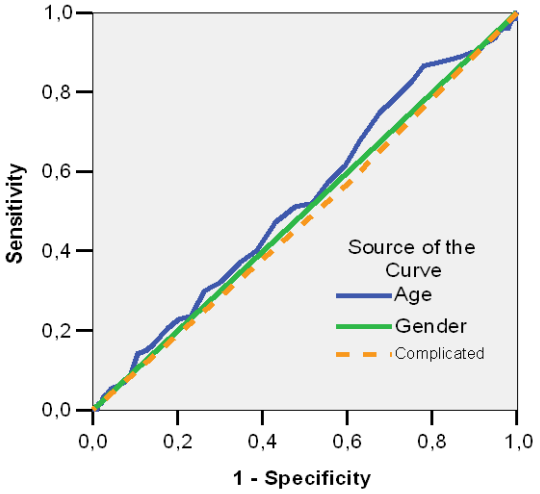


Fig. 7. ROC curve. Epidemiological data predictors of parietal defect size.

Prosthetic methods and techniques used

The chosen method depends mainly on the size of the parietal defect, but with the diversification of the available prosthetic materials, it became necessary to correlate the characteristics of the mesh with the technique of abdominal repair.

Polypropylene nets (PP) are the most widely used. It does not stimulate the immune response or rejection, being quickly incorporated by fibroblasts and granulation tissue that passes easily and fills the spaces between the nodes. Due to the low porosity, the chances of bacteria colonizing are low and the infections exceptional.

Multifilament polyester (PET) yarns are made of soft, fine, soft, durable and elastic nets. In addition, they can be adapted to all shapes and surfaces. The structure of the thread is responsible for the greater tissue inflammatory response than that of the propylene mesh.

In order to eliminate material incompatibilities, it is recommended that the non-absorbable nets (PP, PET) be sutured with non-absorbable threads, preferably from the same material from which the mesh is made.

The most commonly used composite mesh are the double layer: PP with Vicryl

type layer (resorbable copolymer based on glycolic acid and lactic acid) or with non-absorbable layer of e-PTFE (expanded polytetrafluoroethylene-expanded Teflon). Such nets have the advantage of weak adhesions that are established with the abdominal organs when they come in contact with them, but they are more expensive.

The treatment of events in the case of the patients in the analysis group was mainly based on polypropylene prosthetic materials several polyester mesh and more recently, composite mesh, implanted at different stratigraphic levels.

We treated 6 selected cases of incisional hernia by laparoscopic abord. The incisional hernia was positioned in 2 cases in the abdominal midline, 2 cases in the right inguinal fossa, and 2 cases in the umbilical region.

The patients were placed in the lithotomy position and then in the Trendelenburg position (30°). An open technique of creation of pneumoperitoneum was used, at a 12-14 mmHg intra-abdominal pressure. The vital signs (ECG, non-invasive blood pressure measurement, inspired O_2 concentration, tidal volume, airway pressure, pulse oximetry, capnography, temperature) were recorded during surgery, but there was no change in vital parameters after the abdomen was insufflated with carbon dioxide at this pressure. The video monitor placed on an instrument tower at eye level, on the right side of the patient, in front of the main surgeon. To facilitate instrument manipulation along with adequate visualization during laparoscopy, trocars usually are placed in triangular fashion termed triangulation, as far as possible from the site of expected adhesions. The 11 mm optical trocar was placed in the left hypochondrium, as far as possible laterally from the defect to provide clear visualization of the defect margins, and 3 other trocars (10 mm and 2 of 5 mm). Adhesiolysis was limited to the expected landing zone of the mesh on the peritoneal surface of the abdominal wall (fig. 8).

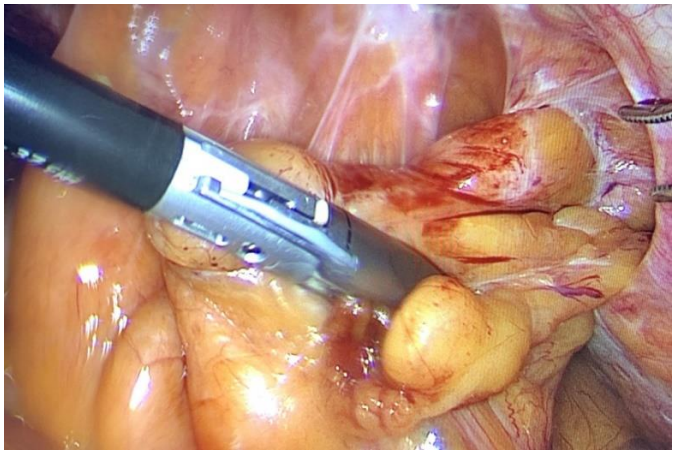


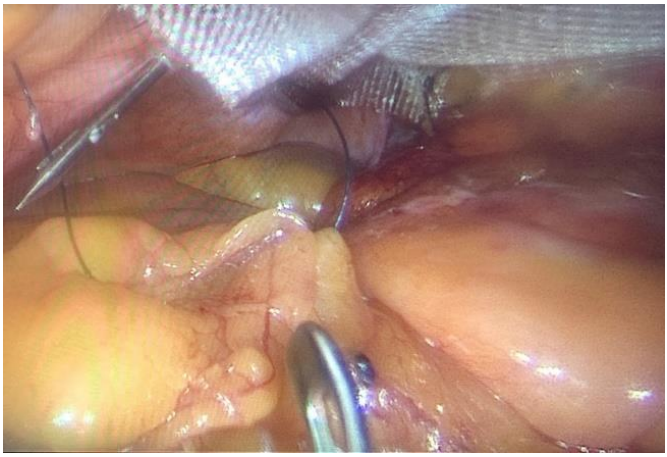
Fig. 8. Adhesiolysis of the incisional hernia.

We used polypropylene prosthetic medical device introduced in the abdomen through the 10-mm trocar site, avoiding contact with the skin. The omentum was used as a protective layer over the viscera in repair of the incisional hernias using on-lay technique.

Sutured was performed with an overlap of 3-5 cm of the borders by using a non-absorbable suture material trans-percutaneous in 4 patients, using a Reverdin needle (fig. 9A) and metallic tackers fixation in 2 patients placed at intervals of 1.5 cm (fig. 9B).

The interventions were uneventful, without devitalized or injured small intestine during de dissection of the incisional hernia sac.

A.



B.

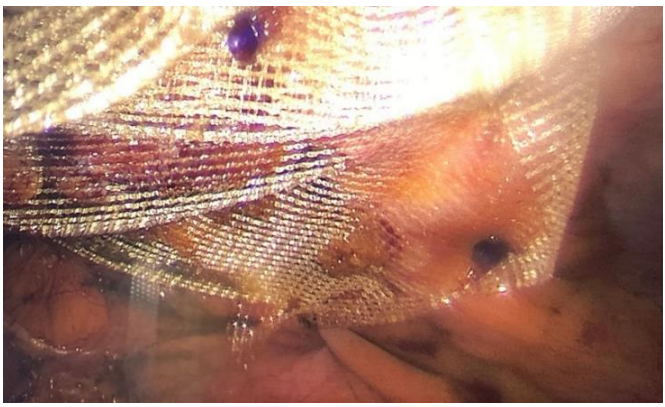


Figure 9. A. Trans-percutaneous suture of the prosthetic medical device.
B. Metallic tackers fixation of the prosthetic medical device.

Postoperative morbidity

Postoperative morbidity was divided into three evolutionary stages: (i) immediate morbidity (first 48 hours postoperatively); (ii) early morbidity (first 30 days postoperatively); (iii) late morbidity (more than 30 days after surgery).

- Immediate morbidity - the immediate postoperative course was simple in 1,743 patients (95.8%) and with general and local complications in 76 cases (4.2%).
- The general complications registered a percentage of 3.8% (N=3) being represented by:
- 32 postoperative respiratory failures in patients with known chronic bronchopulmonary disease, which required respiratory surveillance.

- 16 dynamic postoperative ileus remitted after medical treatment.
- 24 febrile ascents (38 ° C) in patients who subsequently had suppuration of the surgical wound.
- Early morbidity - overlaps, over time, with postoperative hospitalization- in the first 30 days after the operation, 1678 patients (92.2%) had a simple evolution, and 142 (7.8%) developed general and local complications.

The death occurred in 4 patients. However, the patients were with associated: severe sepsis, postoperative parietal hematoma, acute postoperative coronary syndrome, mechanical ventilation-associated tracheobronchitis, left carotid chronic ischemic stroke, right hemiplegia, atrial fibrillation, essential hypertension, acute renal failure and acute renal failure.

Local complications occurred in 64 (12.8%) patients. In most of them (N = 35; 7%) the mesh was implanted supra-aponeurotic, and the complications, which manifested themselves in the form of serous-hematic collections in the vicinity of the prosthetic medical device and wound infections, are as follows:

- 29 subcutaneous hematomas (1.6%);
- 40 subcutaneous seromas (2.2%);
- 58 infections of the surgical wound (3.2%).

The therapeutic conduct was simple, consisting of surgical drainage of blood collections through the surgical wound, evacuation of serous collections by repeated punctures and direct drainage of septic collections.

Late morbidity was assessed after 30 days postoperatively, taking into account the period 01.07.2011-01.11.2021. The observation sheets of 192 patients who returned for the application of the therapeutic conduct were also analyzed at the Clinic I and Clinic II Surgery of the "St. Spiridon" Hospital. It should be noted that the number of cases of late complications may be higher because it cannot be ruled out that some patients may have turned to other surgical services for possible complications, patients who could not enter the monitoring of this study.

Patients who presented during the period 01.07.2011-01.12.2020 presented for the following complications:

65 (3.6%) patients presented with chronic local suppurations.

149 (8.2%) patients experienced recurrence of the incisional hernia.

14 (0.8) patients with intestinal obstruction formed by adhesive flanges.

Of the late complications, 75 were operated on and 32 parietal suppurations were cured by simply draining the septic collection, followed by secondary suturing of the dehiscent wound. In 3 patients with recurrent eventration, surgery was contraindicated due to multiple organic stains.

The following interventions were performed:

- 12 intestinal obstructions resolved by lysis of the adhesions. The prosthetic graft being integrated in the abdominal wall, the suture of the wound edges was made in the subtotal plane, disregarding the existence of the in the wall.
- 15 chronic suppurations with intolerance to the synthetic material benefited from the total ablation of the prosthetic medical device placed supra-aponeurotic.
- one chronic suppuration benefited from the partial excision of the mounted above the aponeurotic tissue and the installation of a new preperitoneal prosthetic medical device (a la Rives) which in turn was not tolerated and also partially excised,

the wall being partly sutured.

- patients who had recurrence of the eventration benefited from a new prosthetic medical device with the fit of the preperitoneal or intraperitoneal prosthetic medical device, as the case may be.

Analyzing late complications depending on the stratigraphic level of medical device implantation, it can be seen that most of them appeared after the implantation of the prosthetic medical device above the aponeurosis. Thus, out of a number of 192 events that presented for late complications, 107 patients (55.7%) had the prosthetic medical device implanted above the aponeurosis, and the remaining ones had the net implanted in the deep planes of the wall.

The duration of postoperative hospitalization was on average 9.2 days and was influenced by the associated morbidity. Of the 1820 cases analyzed in only 9 patients, the postoperative hospitalization period exceeded 30 days.

The representation of the data on the number of hospitalization days of the patients in the analyzed group shows that the number of those who resumed their activity after the first 10 days after surgery is significantly higher than those who required longer hospitalizations.

The condition at discharge is an indication of the postoperative evolution and from the data recorded in the 1820 patients it can be seen that the percentage of surgically cured (96.6%) is an argument in favor of the alloplastic cure of postoperative events.

DISCUSSION

Hernia is a common condition with an incidence of 2-15% (7). The World Society of Emergency Surgery (WSES) broadly categorizes abdominal wall hernias into groin hernias and ventral hernias based on anatomical location. Groin hernias are located at the bottom half of the body and includes these types of hernias: indirect inguinal, direct inguinal, and femoral hernias. Ventral hernias encompass the other types of hernias which include umbilical, epigastric, Spigelian, lumbar, and incisional hernias (8).

In our study the statistical analysis of the selected cases revealed that at a number of 5865 of abdominal wall defects surgeries that were performed in our clinics between 01.01.2006–01.07.2021 we recorded 3249 groin hernias, 3029 inguinal hernias (93%), 220 femoral hernias (7%). A similar study that analyzed a total of 46,717 persons operated for a groin hernia found that 97% of all groin hernia repairs were inguinal hernias and 3% femoral hernias (9). Inguinal hernia is the most frequently diagnosed hernia and during their lifetime one third of males are diagnosed with an inguinal hernia. The age distribution is bimodal with the highest incidence in childhood and after 50 years of age (10). Referring to the incisional hernia we found that is more frequent in females, with a ratio of 2: 1 in favor of women. In a study performed of a total of 368 incisional hernias that been operated in the First Surgery Clinic, University Emergency Hospital Bucharest, female sex was predominant (81.25%) (11).

The distribution by decades of age reveals a higher incidence of events within the 5th and 6th decades of life. The age distribution is bimodal with the highest incidence in childhood and after 50 years of age (10).

Organic strains were found in a percentage of 62% being represented by various organo-systemic and metabolic disor-

ders Among the organic dysfunctions mentioned, obesity was the most common with an index of 29%. The literature also cites that obesity is the main cause on abdominal wall defects (12-16).

The highest incidence is reported on the midline, the influence of the topography of the parietal defect is explained by the fact that the median laparotomy offers the best comfort in resolving surgical emergencies, being preferred by most surgeons. A systematic literature search performed concluded that he risks of developing an incisional hernia after primary elective median laparotomy is reported in the literature as being between 5 and 20% (17).

Patients requiring emergent surgery for hernia vary widely in presentation and management, and the variation in timing of urgent surgery impacts surgical outcomes. The small percentage cases with gangrene (9.6% cases with gangrene) reveals the prompt urgent surgical intervention. The literature cites that delay in surgery for emergent hernias increased the odds of major morbidity but not mortality, patients presenting with hernia and an indication for urgent surgical intervention may benefit from an operation as soon as feasible (18).

Referring to the treatment, the evaluation of the benefits and harms of repair techniques in adults, specifically comparing closure with mesh versus without mesh, most authors decided that mesh repairs probably reduce the rate of hernia recurrence, and reduce visceral or neurovascular injuries, making mesh repair a common repair approach. Mesh repairs may result in a reduced length of hospital stay and time to return to activities of daily living. Non-mesh repair is less likely to cause seroma formation and has been favored in low-income countries due to low cost and reduced availability of mesh materials (19-23).

Controversy exists regarding the outcomes following ventral hernia repair with polypropylene (PP) or polyester (PET) mesh. Some studies suggest that mesh material does not affect recurrence or infection in ventral hernia repair and that surgery can be safely performed with both PP and PET mesh (24-27), other authors polyester mesh placed during ventral hernia repair results in acceptable infection rates, and no direct bowel complications or fistulas (28).

Although there was a trend toward more recurrence with suture repair in our study, this was not statistically significant. Mesh repair was associated with seromas (2.2%) and infections of the surgical wound (3.2%) but is not statistically different from other studies (29).

Analyzing late complications depending on the stratigraphic level of prosthetic medical device implantation, it can be seen that most of them appeared after the implantation of the prosthetic medical device above the aponeurosis. Meshes implanted intraperitoneally are known to cause adhesions potentially resulting in complications such as chronic pain, enterocutaneous fistula, or mesh infection. A study was performed on rats, on a model mimicking a clinical situation like hernia repair by intraperitoneally implanted meshes when mesh has contact with normal and with de-peritonealized intestine. The high adhesion scores of rats with local de-peritonealization compared with the low scores of animals with intact peritoneum indicate that the integrity of intestinal peritoneum is a decisive factor for adhesion formation (30). There is no single treatment modality to prevent adhesion formation after abdominal wall defect repaired with prosthetic materials (31-33).

CONCLUSIONS

Abdominal hernia is a common problem in the general population. Abdominal hernias are more common in men than women, there is an obvious relationship between obesity, history of abdominal surgery, history of abdominal trauma, family history and hernias. Early diagnosis, easily accessible health facilities and health education are important to prevent complications and improve quality of life.

Based on the currently evidence in midline closure after elective laparotomy in the small bites technique can be recommended to significantly reduce the rate of incisional hernia. The additional use of a prophylactic mesh in high-risk patients can significantly reduce the occurrence of incisional hernia.

Mesh repair has a small reduction in recurrence rates compared with suture repairs for primary ventral hernias, but an increased risk of seroma and infection of the surgical site was observed.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest, and they received no funding regarding this scientific research.

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VILAZODONE ENHANCES THE ENDURANCE CAPACITY AND IMPROVES THE BIOLOGICAL CHANGES IN TREADMILL TEST IN RATS

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VILAZODONE ENHANCES THE ENDURANCE CAPACITY AND IMPROVES THE BIOLOGICAL CHANGES IN TREADMILL TEST IN RATS (Abstract): The **aim** of our study was the evaluation of the vilazodone (VLZ) influence on the endurance capacity and biological modifications in rats subjected to forced exercise. **Material and methods:** For the experiment, 24 white Wistar rats, were randomly assigned into four groups. The substances were administered orally, in a single daily dose, for 14 consecutive days: Group 1 (Negative control): distilled water 0.3 mL/100g body without effort; Group 2 (Positive control): distilled water 0.3 mL/100g body subjected to effort; Group 3 (Mg): 200 mg/kg body magnesium chloride; Group 4 (VLZ): 20 mg/kg body vilazodone. The rats motor behavior and the endurance capacity were evaluated using the treadmill test, and some blood parameters, estimating the stress influence were assessed: The investigation protocol was approved and implemented according to recommendations of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi Committee for Research and Ethical Issues. **Results:** The administration of VLZ resulted in a prolongation in distance traveled, an increase in the number of touches the opposite end of belt and a diminution in the number of delivered shocks. Moreover, it decreased the blood levels of glucose and cortisol, and increased the serum values of b*rain derived neurotrophic factor*. The use of VLZ was accompanied by an intensification of anti-oxidant enzymes activity, respectively a decrease of the oxidative stress marker level in blood. Its effects were less intense, when compared to Mg. **Conclusions:** The treatment with VLZ improved the physical performances and the endurance capacity in this experimentally-induced stress model in rats. The use of this antidepressant drug recovered the biochemical changes and produced antioxidant effects in animals subjected to forced exercise in treadmill test. **Keywords:** VILAZODONE, TREADMILL TEST, RATS, STRESS, BDNF.

Currently, the most commonly prescribed therapy for affective disorders is based on active agents that block the reuptake or degradation of monoamines at

the synapse. The administration of such drugs is associated with a variable response and long period (weeks to months) between the onset of administration and the achievement of clinical results (1). Paradoxically, the neurochemical effects of typical antidepressants are measurable in the days immediately following treatment initiation (2). This discrepancy suggests that, in addition to increasing serotonin and/or noradrenaline levels, classical antidepressants seem to be involved in the modulation of other cellular pathways to achieve the therapeutic effect (3).

A new theory of antidepressant function suggests that selective serotonin reuptake inhibitors create a window for change by increasing brain plasticity and increasing the patient's susceptibility to factors in their environment (4-6). Classical antidepressants, such as selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors, and monoamine oxidase inhibitors, therapeutic agents that participate in the modulation of brain monoamine levels, have limited efficacy and a delayed therapeutic response of several weeks or months (2).

The relatively new antidepressant drug, vilazodone (VLZ) is an indolylalkylamine derivative with a dual mechanism of action consisting of partial 5-HT1A receptor agonist and selective serotonin reuptake inhibitory activity. It does not bind with high affinity to norepinephrine or dopamine reuptake sites (7-9). VLZ is used for the treatment of major depressive disorder in adult patients (10). It also, has been found to improve mental and somatic symptoms in generalized anxiety disorder (7). Due to its unique mechanism of action, VLZ has the following therapeutic benefits: faster onset, higher efficacy, and lower risks of adverse events compared to currently used antidepressants, especially lower sexual side effects (11).

The purpose of our experimental research was the evaluation of the VLZ influence on the endurance capacity and biological modifications in rats subjected to forced exercise.

MATERIAL AND METHODS

Substances

VLZ (≥98% HPLC, molecular weight 477.99 g/mol, catalogue code: SML1098), magnesium chloride (anhydrous, ≥98%, molecular weight 95.21 g/mol, catalogue code: M8266), saline solution (molecular weight 18.02 g/mol, catalogue code: 07-6061) were bought from Sigma-Adrich Chemical Co (Steinheim, Germany). Substances were dissolved in saline solution, prepared proximately before the administration.

Laboratory animals

White Wistar rats (weighting 200-250 g, 6 months), healthy, non-genetically modified, with equal repartition by sexes were procured from the National Medical-Military Institute "Cantacuzino" for Research and Development, Băneasa Resort, Bucharest, Romania through the biobase of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania. The rats were kept in standard laboratory conditions (with constant temperature of 21°C ± 2°C, relative humidity of 50-70% and alternating lighting regime (light/dark ratio = 12 hours/12 hours), with standard food pellets and water *ad libitum*.

Treadmill test

The animals were randomly assigned into 4 groups of 6 rats each, and treated orally (using an esogastric tube), in a single daily dose, for 14 consecutive days, according to the following protocol: Group 1 (Negative control): distilled water 0.3 mL/100 g body without effort; Group 2 (Positive control): distilled water 0.3 mL/100 g body subjected

to effort; Group 3 (Mg): 200 mg/kg body magnesium chloride; Group 4 (VLZ): 20 mg/kg body vilazodone. During the experiment, the normal behavior of the laboratory animals was observed, by monitoring the general condition, posture, spontaneous behavior, movements, orientation ability, hair appearance, breathing, the reaction to various stimuli. All behavioral disturbances, changes in posture or reactions to handling, abnormal movements, stereotypic behaviors (excessive grooming, repeated circular movements), and any other bizarre behavior (self-mutilation, walking backwards) were noticed. On the 14th day of the experiment, 15 minutes after the administration of the substances, the animals from the last three groups were subjected to treadmill test. Magnesium chloride (Mg) was considered a positive control substance, with known effects on this behavioral model in rats.

This experimental model is part of the group of classical behavioral tests, being used to evaluate the motor function of laboratory animals and their ability to endure sustained physical effort. The treadmill for testing laboratory animals is a device that forces them to run on a rotating belt at a constant speed, having the same operating principle, as the treadmill used by humans for to practice physical exercises (12). The device allows the application of additional electrical stimuli (with constant intensity from 0 to 2 mA, called - electric shocks). These shocks push the animal to continue traveling, when it tends to stop, or when it stumbles and falls on the moving (12, 13). For each experimentation session of 8 minutes, the following were monitored: total distance covered; the number of applied electric shocks, the time of application of the electric shocks; the length of time the animal resists until the application of the first electric impulse; the number of touches of the opposite end of the conveyor belt.

Laboratory investigations

To compare the influence on the on the biological constants, we used the negative control group with rats not subjected to physical exercise. The blood samples (0.3 mL) were collected from the lateral tail vein after performing the exercise test (13). For the laboratory investigations the appropriate analyzers and kits to measure the blood level of glucose (Hematology Analyzer 5 DIFF model BF-5180, DIRUI, Istanbul, Turkey), cortisol (by electrochemiluminescence immunochemical method), and b*rain derived neurotrophic factor* (*BDNF*) (by ELISA method – using a specific kit) (15). The oxidative stress was evaluated (using a Shimadzu 1800 spectrophotometer, Kyoto, Japan, using the specific RANSOD kit from RANDOX Laboratories Ltd., Warsaw, Poland) by estimating the activity of: malondialdehyde - MDA (using the thiobarbituric acid method); glutathione peroxidase - GPx (using the dithio-nitrobenzoic acid method). The blood levels of superoxide dismutase - SOD, were assessed according to a method based on inhibiting the reduction of nitro blue tetrazolium with xanthine-xanthine oxidase, used as a superoxide generator.

The obtained results were expressed as arithmetic mean ± the standard deviation (S.D.) of the mean values and statistically analyzed using *SPSS* software for Windows EXCEL program (New York, Unites States) and ANOVA method. The *p* (probability) coefficient values lower than 0.05 were considered statistically significant compared to the control group.

The experimental studies were carried out after approval by the Ethics Committee of the University (Certificate No. 25/14.07.2020), in strict accordance with the international ethical regulations regarding work on laboratory

animals. The research methodology was established in accordance with international and national standards (16).

RESULTS

The treatment with Mg was linked to an increase in distance run (146.17±8.26 meters), statistically significant (**p <0.01) compared to distilled water group subjected to effort (107.17±8.16 meters). The administration of VLZ was accompanied by a substantial (*p <0.05) prolongation in distance traveled (124.83±3.49 meters) when compared to control group, but a little shorter than the one traveled by Mg group, the same time interval in treadmill test (fig. 1a).

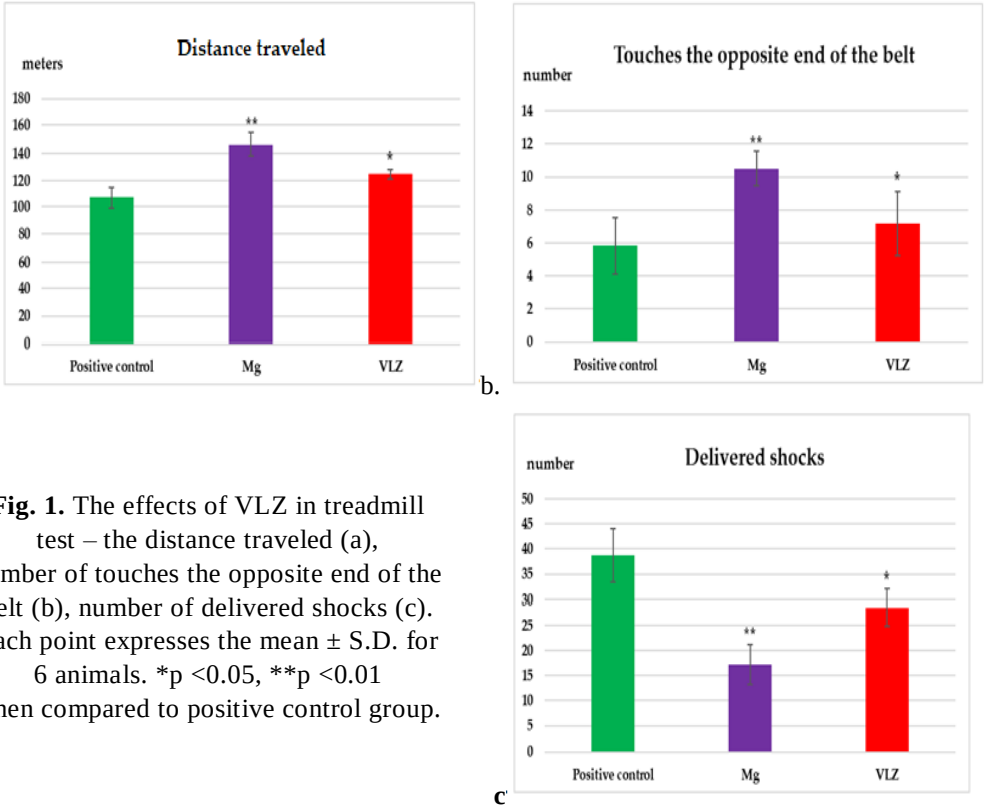

Fig. 1. The effects of VLZ in treadmill test – the distance traveled (a), number of touches the opposite end of the belt (b), number of delivered shocks (c). Each point expresses the mean ± S.D. for 6 animals. *p <0.05, **p <0.01 when compared to positive control group.

The treatment with Mg was correlated with a considerable (**p <0.01) increase in the number of touches the opposite end of the belt (4.83±1.72), compared to positive control animals (10.5±1.05). Oral administration of VLZ for 14 days led to an important (*p <0.05) increase in the number of touches (7.17±1.94) when compared to control group, but its effects were less intense than those of Mg group, the similar session in treadmill test (fig. 1b).

The administration of Mg was associated by a noticeable (**p <0.01) reduction in the number of delivered electric impulses (17.17±4.02), when compared to animals from distilled water group (38.83±5.31). The treatment with VLZ for 2 weeks resulted in an obvious (*p <0.05) decrease in

the number of needed shocks (28.50±3.62) comparing with positive control group, but, lower than those counted for Mg group, the same training session in forced exercise test (fig. 1c).

The use of Mg considerable (**p <0.01) increased the time need to first shock delivery (221 ± 8.65), and markedly shorten the time of providing electric stimuli (17.67 ± 2.80), comparing with positive control (134.33 ± 12.48, respectively 38.17 ± 4.17). The subacute administration of VLZ for 14 days caused an important (*p <0.05) prolongation of the time need to first shock use (184.67 ± 7.66), and a reduction in the time of providing shocks (31.17 ± 4.71), when compared to positive control (134.33 ± 12.48, respectively 38.17 ± 4.17) (tab. I).

TABLE I.

The effects of VLZ in treadmill test – time to first shock delivery, time of providing shocks (seconds). Each point expresses the mean ± S.D. for 6 animals. *p <0.05, **p <0.01 when compared to positive control group.

Groups	Time to first shock delivery	Time of providing shocks
Positive control	134.33 ± 12.48	38.17 ± 4.17
Mg	221 ± 8.65**	17.67 ± 2.80**
VLZ	184.67 ± 7.66*	31.17 ± 4.71*

TABLE II.

The effects of VLZ on blood levels of glucose, cortisol and BDNF in treadmill test in rats. Each point expresses the mean ± S.D. for 6 animals. *p <0.05, **p <0.01 when compared to positive control group, ♦p <0.05, ♦♦p <0.01 versus negative control group.

Groups	Glucose (mg/dL)	Cortisol (pg/mL)	BDNF (pg/mL)
Negative control	88.50 ± 6.24	0.14 ± 0.01	575.67 ± 47.25
Positive control	116.33 ± 1.53♦♦	0.19 ± 0.02♦	477.50 ±33.23♦♦
Mg	93.33 ± 8.02*	0.16 ±0.03	569.50 ±31.82*
VLZ	94.33 ± 3.06*	0.16 ±0.01	560.67 ±12.10*

An increase in blood glucose values was highlighted in the positive control group (116.33±1.53 mg/dL), statistically significant (♦♦p <0.01) compared to the negative control animals (88.50±6.24 mg/dL). The treatment with Mg for 14 days was associated with a significant reduction (*p <0.05) of blood glucose (93.33±8.02 mg/dL) compared to distilled water group subjected to exercise. The use of VLZ produced the relevant decrease (*p <0.05) in blood glucose level (94.33±3.06 mg/dL) compared to positive control, but less intense than noted for Mg group. In rats subjected to exercise stress, a significant increase (♦p <0.05) of the serum cortisol level (0.19±0.02 pg/mL) compared to animals without exercise (0.14±0.01 pg/mL) was detected. Mg caused a slight decrease in the cortisol level (0.16 ± 0.03 pg/mL), but without significance compared to positive control animals. The administration of VLZ led in a slight decrease in the blood value of cortisol (0.16±0.01 pg/mL), non-significant when compared to the positive control group (tab. II). The physical exercise induced a substantial de-

crease (♦♦p <0.01) in blood levels of BDNF (477.50±33.23 pg/mL), compared to the negative control group (575.67±47.25 pg/mL). The administration of Mg determined a relevant increase (**p <0.01) in the value of BDNF in the blood (569.50 ± 31.82 pg/mL), compared to distilled water group subjected to exercise. VLZ use led to an increase in serum BDNF (560.67±12.10 pg/mL), statistically significant (**p <0.01) compared to the positive control group, but weaker than produced by Mg (tab. II).

TABLE III.

The effects of VLZ on the SOD, MDA and GPx activity in treadmill test in rats. Each point expresses the mean ± S.D. of mean, for 6 animals in a group. *p <0.05, **p <0.01 when compared to positive control group, ♦p <0.05, ♦♦p <0.01 versus negative control group.

Groups	SOD (µg/mL)	MDA (nmol/L)	GPx (pg/mL)
Negative control	24.33 ± 2.46	27.83 ± 3.16	321.17 ± 33.27
Positive control	15.00 ± 2.83♦♦	38.50 ± 2.40♦♦	194.00 ± 35.36♦♦
Mg	23.00 ± 1.41*	26.65 ± 3.46**	311.50 ±113.84**
VLZ	20.83 ± 3.37*	29.57 ± 3.54*	307.67 ± 75.65**

A significant (♦♦p <0.01) decrease in SOD activity (15.00±2.83 µg/mL) compared to the negative control group (24.33 ± 2.46 µg/mL) was revealed in positive control animals in the treadmill test. The use of Mg was correlated with an obvious enhancement (*p <0.05) of SOD activity (23.00±1.41 µg/mL), compared to positive control group. The administration of VLZ was followed by marked intensification (*p <0.05) of SOD activity (20.83±3.37 µg/mL), when compared to the positive control group, but with less importance that observed for Mg group (tab. III). The positive control animals showed a considerable increase (♦♦p <0.01) of MDA activity (38.5±2.40 nmol/L), compared to the negative control (27.83 ± 3.16 nmol/L). The treatment with Mg induced an obvious diminution (**p <0.01) in the serum values of MDA (26.65±3.46 nmol/L), comparing with distilled water group with effort. The use of VLZ was accompanied by a notable (*p <0.05) decrease in the blood level of MDA (29.57 ± 3.54 nmol/L), compared to positive control group (tab. III). Subjecting control rats to physical exercise resulted in an obvious decrease (♦♦p <0.01) of GPx values (194.00 35.36 pg/mL), compared to the negative control animals (321.17±33.27 pg/mL) (Table 3). Mg caused a significant increase (**p <0.01) in the serum level of GPx (311.50±113.84 pg/mL), compared to distilled water group subjected to exercise (Table 3). The treatment with VLZ resulted in the increase of GPx values in blood (307.67±75.65 pg/mL), with statistical significance (**p <0.01) compared to positive control animals (tab. 3).

DISCUSSION

The administration of VLZ, during 14 consecutive days was associated by an increase in endurance capacity to forced exercise, objectified by the prolongation the distance traveled by the animal on the treadmill, the increasing in the number of touches the opposite end of belt, and a decreasing in the number of delivered electric stimuli. Biochemical analysis showed

that the treatment with VLZ significantly improved the glycaemia and decreased the serum value of cortisol. The effects of this antidepressant drug on these two parameters were of lower intensity than those induced by Mg in rats.

We investigated the modification of BDNF blood values in rats subjected to forced exercise, and the influence of the administration of VLZ. In our experimental condition the use of VLZ was followed by a substantial increase in the serum level of BDNF, *thus suggest its obvious stress-reducing effects in rats exposed to physical effort. Its effects were less intense when compared to Mg.* BDNF plays a critical role in the development of mental illnesses such as depression and anxiety (17). Previous research has also demonstrated the relationship between stress and BDNF expression in many critical brain regions (18). Clinical studies report that serum BDNF levels are decreased in depressed patients and then increased in response to treatment with typical antidepressants. Reduced BDNF is also observed in post-mortem tissue of untreated depressed individuals (19). Physical activity has been shown to increase BDNF mRNA expression in multiple brain regions, especially in the hippocampus, which is consistent with the delay in the antidepressant behavioral response in experimental rodent models (20). Recent studies have highlighted the fact that the induction of BDNF expression in the hippocampus is important in the manifestation of the effectiveness of antidepressant treatment, and its repression seems to have a determining role in the pathophysiological mechanisms of depression, in various animal models (21). Conversely, sustained induction of BDNF in the nucleus accumbens, which persists for at least one month after chronic stress, mediates depression-like behavioral abnormalities, while reversal of this effect induces an antidepressant response (22-24).

In response to endurance training in rats, free radicals are generated due to oxidative stress. The assessing of blood levels of MDA, GPx and SOD offers the possibility of estimation of VLZ influence on the cellular oxidative processes. In animals exposed to forced exercise training high levels of MDA in blood, as well as a diminution in SOD and GPx activity was detected. The improvement of the changes observed in these parameters, following the use of VLZ, suggests its influence of reducing oxidative stress. *The use of VLZ was accompanied by an intensification of antioxidant enzymes activity, respectively a reduction of the serum value of* MDA. *Its effects were less intense, when compared to magnesium.*

CONCLUSIONS

The treatment with VLZ improved the physical performances and the endurance capacity in this experimentally-induced stress model in rats. *The use of this antidepressant drug recovered the biochemical changes and produced antioxidant effects in animals subjected to forced exercise in treadmill test.*

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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THE PROINFLAMMATORY ROLE OF SERUM IL-6 IN ACUTE OTITIS MEDIA IN CHILDREN

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THE PROINFLAMMATORY ROLE OF SERUM IL-6 IN ACUTE OTITIS MEDIA IN CHILDREN (Abstract): Acute otitis media (AOM) represents the leading cause of health care visits and antibiotic prescriptions in children. In order to reduce the unnecessary antibiotic prescriptions and to avoid the burden of potential complications, new biomarkers may be useful for the diagnosis and follow-up of children with AOM. **Material and methods**: We performed a prospective study in pediatric patients with AOM hospitalized in a tertiary hospital in Iasi, Romania, during a 1-year period (between November 2021 and November 2022). We assessed the utility of serum interleukin-6, S100A12 protein, ESR (erythrocyte sedimentation rate) and CRP (C-reactive protein) in the diagnosis of children with clinical signs of AOM. **Results**: A total of 88 children, aged between 2 months old to 17 years old were included (46 with AOM and 42 healthy controls). The differences between the control group and AOM group were statistically significant ($p<0.001$) for ESR, CRP and IL-6 (median values being much higher in the case of patients with AOM). The differences between the control group and the one with AOM were only random ($p=0.326 > 0.05$) in case of S100A12 protein. ESR > 27 mm/hour was associated with a 2 times higher risk (RR=2.022) of having concomitant adenoiditis. **Conclusions**: IL-6 could offer a powerful tool for the objective evaluation of AOM. Further research is needed to fully elucidate the role of IL-6 in AOM and to determine whether it may be a useful biomarker for predicting treatment outcomes. **Keywords**: ACUTE OTITIS MEDIA, CHILDREN, IL-6, S100A12 PROTEIN.

Acute otitis media (AOM) is defined by the American Academy of Pediatrics as the rapid onset of signs and symptoms of inflammation in the middle ear, with or without otorrhea (1). In children, AOM represents an important healthcare issue due to high rates of antibiotherapy (2). Despite overall reduction in the incidence of AOM after the introduction of pneumococcal conjugate vaccines, Hu *et al.* reported at least one episode of AOM in 28.6% to 31.5% of the children younger than 2 years old in 2022 (3). In the United States, about 60% of children experience an episode of AOM before the age of 3 (4). In this context, AOM still represents the leading cause of health care visits and antibiotic prescriptions (5), with additional burden arising

from long-term complications, as hearing loss that impacts language acquisition and educational outcomes (6).

Currently, the diagnosis of AOM is based on signs of inflammation on otoscopy combined with clinical history and presenting signs and symptoms (7). As these methods may have a certain grade of subjectivity derived from the healthcare professional, but also from the perception of the parents on the pain and discomfort of their child, a more objective approach may be welcome. Microbiological culture and antibiotic sensitivity testing is useful in order to justify the antibiotic prescription, but the available guidelines do not routinely recommend performing a tympanocentesis (8). Also, blood tests (complete blood count, acute phase reactants such as C-reactive protein, erythrocyte sedimentation rate and fibrinogen, cultures) are performed only under specific circumstances (recurrent AOM, complications or treatment failure, follow-up) (9).

Recent discoveries emphasized the potential role of cytokines, a group of glycoproteins, in the modulation of inflammatory and immune reactions in AOM. Interleukin-6 (IL-6), an inflammatory mediator with pleiotropic effects, is highly produced during the initial stage of inflammation (10). Higher levels of serum IL-6 were found at the moment of diagnosis in children with AOM due to *Streptococcus pneumoniae*, but these results are difficult to extrapolate to other populations as the study sample comprised only 12 children (11). A less-studied molecule in the context of inflammation associated to AOM is S100A12 protein, also known as calgranulin C. Markedly overexpressed levels of S100A12 protein have been reported in inflammatory compartments, but the potential usefulness in clinical practice in the context of AOM has not yet fully established (12).

In order to reduce the unnecessary antibiotic prescriptions and to avoid the burden of potential serious complications, new biomarkers may be useful for the diagnosis and follow-up of children with AOM. Our aim was to assess the utility of serum interleukin-6 and S100A12 protein in the diagnosis of children with clinical signs of AOM.

MATERIAL AND METHODS

We performed a prospective study in pediatric patients with AOM hospitalized in "Sf. Maria" Emergency Hospital for Children, a tertiary hospital in Iasi, Romania, during a 1-year period (between November 2021 and November 2022). The study protocol was reviewed and approved by the Ethical Committees of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania (No. 12292-22.07.2020) and of "Sf. Maria" Clinical Emergency Hospital for Children, Iasi (No. 34306/ November 5, 2020). Written informed consent was obtained from the parents of all study participants.

We included the children aged 0-18 years old who were diagnosed with AOM in the ENT Department based on the following criteria: acute onset of the symptoms (fever, irritability, otalgia or otorrhea) and at least 2 signs of middle ear effusion confirmed by otoscopy (bulging or erythema of the tympanic membrane, air-fluid level behind the tympanic membrane, limited or absent mobility of tympanic membrane). Only children with a recent onset of AOM symptoms (the first 48 hours) who did not receive any antibiotic treatment were considered for our analysis. Patients with acute comorbidities which might have triggered a more powerful inflammatory

response - e.g., lower respiratory tract infections, gastroenteritis, urinary tract infections, meningitis or with chronic systemic conditions - e.g., diabetes mellitus, cancers or autoimmune disorders were excluded from our sample. We also set up a control group with children who presented in our hospital for a routine check-up, with no current infection or inflammatory disease.

At hospital admission, 3 mL blood samples were obtained from both AOM and control groups and collected in heparin-free tubes at room temperature. After centrifugation at 2000 x g for 15 minutes at 4 °C, we obtained serum samples which were immediately stored at – 80 °C. The Enzyme-Linked ImmunoSorbent Assay (ELISA) method was used to measure the IL-6 (code E-EL-H0102 ELISA Kit) and S100A12 protein (code E-EL-H1293 ELISA Kit) levels. Both kits were provided by Elabscience, United States of America. The micro-ELISA plate has been pre-coated with an antibody specific to Human IL-6 and Human S100A12, respectively. The samples were added to the micro-ELISA plate wells and combined with the specific antibody using the Sandwich-ELISA principle. Then, a biotinylated detection antibody specific for Human IL-6 and Human S100A12, respectively with an Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away, and the substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of stop solution which turned the color into yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. As the OD value was proportional to the concentration of IL-6 and S100A12 protein, respectively, we calculated these concentrations in the samples by comparing the OD of the samples to the standard curve.

Besides demographic data (age, gender), we also recorded data regarding significant commorbidies (adenoiditis), the hospitalization length, the potential complications and the readmissions to hospital in the following 5 months. We have also analyzed biologic markers - C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in both AOM and control group. The normal range provided by the laboratory of our hospital is less than 5 mg/L for CRP and less than 12 mm/hour for ESR.

Data obtained were analyzed using specific statistical functions provided by SPSS software, as the non-parametric Mann-Whitney U test, as well as Spearman's rank correlation coefficient. P values < 0.05 were considered statistically significant.

RESULTS

A total of 88 children aged between 2 months old to 17 years old were included in our analysis, among which 46 were diagnosed with AOM and 42 were healthy controls presented to our hospital for a routine check-up. The descriptive statistics revealed significantly higher values for ESR, CRP and IL-6 in children with AOM than in controls (tab. I). The distributions of the ESR, CRP, IL-6 and S100A12 protein in controls and cases are represented in first figure.

Our data were not normally distributed, and therefore, instead of using the t-test for comparing independent samples, we needed a non-parametric alternative test, which in this case was the Mann-Whitney U Test (tab. II), which does not require the assumptions of normality of variables and homogeneity of variances. The test showed that the differences between the control

group and AOM group are statistically significant ($p<0.001$) for ESR, CRP and IL-6 (median values being much higher in the case of patients with AOM, which is also illustrated in fig. 1). The values of the Rank-Biserial Correlation indicator showed an important effect of these differences between the control group and the otitis group for ESR, CRP and IL-6, their magnitude being above the threshold of 0.50. The differences between the control group and the one with AOM were only random ($p=0.326 > 0.05$) in case of S100A12 protein.

TABLE I.

Descriptive statistics of the main parameters analyzed in AOM cases and healthy controls

	ESR (mm/h)		CRP (mg/dL)		IL-6 (pg/mL)		S100A12 protein (pg/mL)	
	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
Median	5	27	2	13.920	3.885	118.815	9.495	9.795
MAD*	3	13	1	8.680	0.765	45.185	1.645	1.945
Shapiro-Wilk	0.912	0.886	0.942	0.762	0.749	0.83	0.946	0.935
P-value of Shapiro-Wilk	0.003	< .001	0.034	< .001	< .001	< .001	0.047	0.012
Minimum	2	10	0.100	1.38	2.88	44.1	1.360	0.4
Maximum	14	89	6	152.43	14.34	443.3	14.840	14.71

* MAD, median absolute deviation

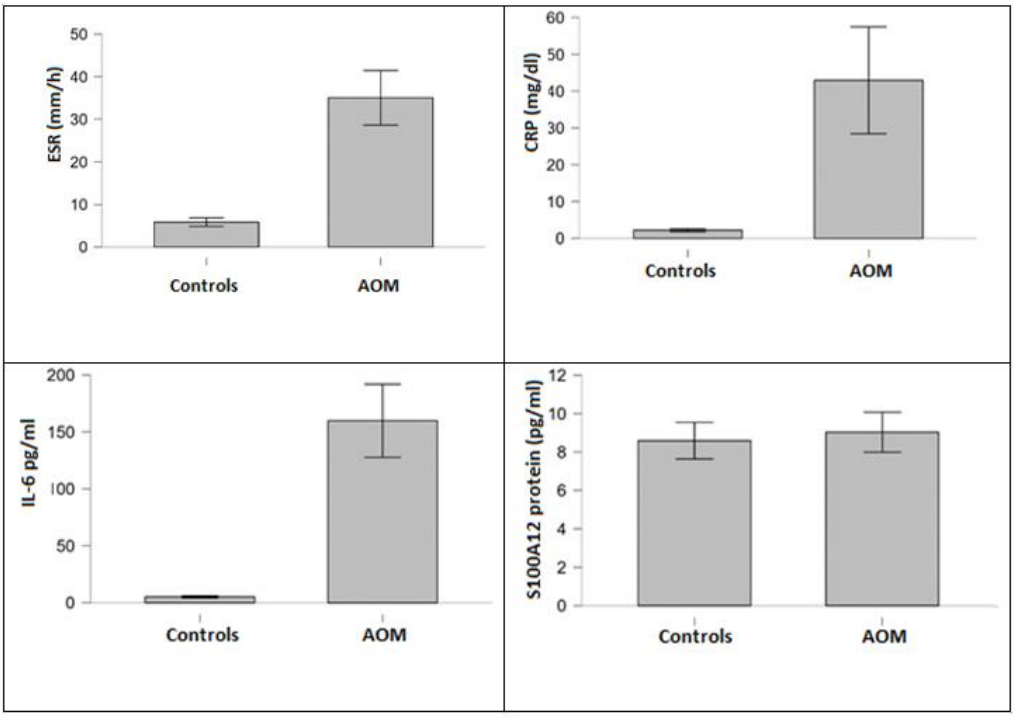

Fig. 2. Distribution of the ESR, CRP, IL-6 and S100A12 protein in controls and cases

As the assumption of normality of the sample was previously violated (the Shapiro-Wilk test registers statistically significant values in all situations, respectively $p < 0.05$), we used Spearman's correlation coefficient instead of Pearson's coefficient to highlight the existing correlations between ESR, CRP, IL- 6 and S100A12 (tab. III). A matrix of correlation coefficients between the 4 variables, which also contains the level of statistical significance associated with each variable was generated (tab. III). A strong direct (positive) relation between CRP and ESR ($rs = 0.630$, $p < 0.001$) and a direct link of medium intensity between IL-6 and ESR ($rs = 0.3090$, $p < 0.05$) were observed. No statistically significant correlation was revealed between the other pairs of variables.

The association between the presence of adenoiditis and the value of ESR > 27 mm/hr was statistically significant ($\chi^2=5.841$, $p<0.05$). Moreover, an ESR > 27 mm/hr was associated with a 2 times higher risk (RR=2.022) of having concomitant adenoiditis (fig. 2).

Table II.

Mann-Whitney U test

	W df	p	Hodges-Lehmann Estimate	Rank-Biserial Correlation	SE Rank-Biserial Correlation
ESR	9.000	**< .001**	-22.000	-0.991	0.123
CRP	37.000	< .001	-11.840	-0.962	0.123
IL-6	0.000	< .001	-114.324	-1.000	0.123
S100A12	848.000	0.326	-0.650	-0.122	0.123
Note. For the Mann-Whitney test, effect size is given by the rank biserial correlation.					

TABLE III.

Correlation Matrix for AOM group

		ESR	CRP	IL-6	S100A12 protein
ESR	Spearman's rho	—			
	p-value	—			
CRP	Spearman's rho	0.630	—		
	p-value	< .001	—		
IL-6	Spearman's rho	0.309	0.261	—	
	p-value	0.037	0.080	—	
S100A12 protein	Spearman's rho	0.195	0.185	0.229	—
	p-value	0.193	0.219	0.125	—

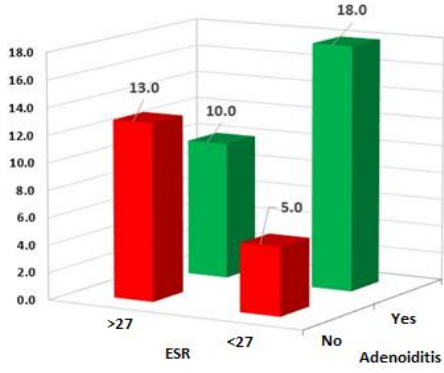

Fig. 2. Correlations between adenoiditis and ESR>27 mm/h

No statistical significance was found between adenoiditis and CRP > 50 mg/L (χ^2=2.741, p=0.098) or between bilateral AOM and CRP > 50 mg/L (χ^2=0.063, p=0.801).

The average hospitalization length was 5 days and no serious complications or readmissions to hospital have occurred in the following 5 months in the AOM group.

DISCUSSION

The role of IL-6 in the diagnosis of AOM

IL-6 is a pleiotropic cytokine, commonly referred as a primary mediator of the acute phase response, which plays an important role in the inflammatory reaction to bacterial infections (13). Up to the present, its important role in inflammation, innate and adaptative immune responses has been recognized in various studies (14, 15). During the resolution phase of the infections, IL-6 promotes the reduction of the neutrophil infiltrate and initiates an acquired immune response (16). In contrast to other cytokines, the production of IL-6 may continue even after the initial stimulus has been removed, being eliminated in a lower rate than other cytokines (17). This may explain why higher levels of IL-6 were found in the sera from patients with AOM in the early phases of the disease in comparison with healthy controls.

Several studies have investigated the role of IL-6 in AOM in children, but the exact role of IL-6 in the pathogenesis of AOM has not been clearly established yet. However, it was almost 35 years ago when Heikkinen *et al.* already reported that low levels of IL-6 cannot solely exclude the bacterial etiology of acute otitis media (18). Our results regarding IL-6 serum levels are in accordance with those of Scharer *et al.*, who found higher mean concentrations of IL-6 in a sample of 10 children with AOM compared to healthy controls (p = 0.05) (11). Also, our findings were consistent with another study performed in a sample of 14 children with AOM (19). However, these results are difficult to interpret and extrapolate as in pediatrics, there are often difficulties in developing studies on larger samples.

The optimal cut-off value for serum IL-6 to discriminate AOM from healthy controls has yet to be clearly established. However, the role of IL-6 has been better described in chronic otitis media. For instance, in a study comparing the role of interleukin 1α (IL-1α), IL-6 and interleukin 8 (IL-8) in the chronic evolution of otitis media, Serban *et al.* reported that IL-6 serum values recorded the highest mean level in chronic suppurative otitis media ranging from 57.23 to 193.33 pg/mL (20). In our sample, the serum IL-6 levels ranged from 44 to 443.3 pg/mL, with a medium level of 118.815 pg/mL, which might suggest that higher levels of IL-6 can be correlated to acute rather than chronic otitis media. It has been shown that IL-6 levels also decrease after antibiotic treatment, suggesting that IL-6 is involved in the response to bacterial infection (11).

In vitro studies have shown that IL-6 is produced by middle ear epithelial cells and fibroblasts in response to bacterial components, such as lipopolysaccharides (LPS), and that IL-6 production is enhanced by other cytokines, such as TNF-α and IL-1 β. IL-6 can also stimulate the production of other cytokines, such as IL-8, which promote neutrophil recruitment and activation in the middle ear (21). The role of IL-6 in AOM has been also investigated in animal models, such as mice and chinchillas. In these models, IL-6 levels were found to be elevated in the middle ear after bacterial

inoculation, and IL-6 blockade with antibodies or genetic deletion resulted in reduced neutrophil recruitment and less severe middle ear inflammation (22). IL-6 has also been investigated as a potential therapeutic target in AOM. Blockade of IL-6 signaling has been shown to reduce middle ear inflammation and improve clinical symptoms in animal models (23).

S100A12 protein in the diagnosis of AOM

Firstly, described in 1995 by Guinard *et al.* (24), S100 calcium-binding protein A12 (S100A12 protein), also known as calgranulin C, MRP6, or EN-RAGE (extracellular newly identified receptor for Advanced glycation end products binding protein), belongs to the S100 family of low molecular weight proteins (25). S100A12 protein is predominantly localized in the cytoplasm of myeloid cells such as neutrophils (26), but recent data suggest that, under inflammatory conditions, it can also be present in epithelial and endothelial cells and in pro-inflammatory macrophages (27). Besides its role in calcium homeostasis, S100A12 protein express chemo-attractive properties to immune cells, leading to a pro-inflammation state via TLR-4 and RAGE pathways (28). Recent data reveals the role of S100A12 protein in the evaluation and assessment of several inflammatory disorders, including Behçet's disease (29), familial Mediterranean fever (30), Kawasaki's disease (31), inflammatory bowel disease (32) and AOM (33).

Several studies depicted its antimicrobial role, mainly via a nutritional immunity – based mechanism, as essential transition metals for the growth and resistance of the microorganisms are depleted during the infections. For instance, S100A12 protein exerts inhibitory activity against *Campylobacter jejuni* (34) and *Helicobacter pylori* through a zinc+-dependent sequestration (35). Moreover, S100A12 expresses antiparasitic properties, by enhancing the production of superoxide possibly through the binding of copper (36).

Contrary to our results, higher levels of S100A12 protein were reported in children with AOM than in healthy controls. According to Liu *et al.*, increased serum levels of S100A12 protein were found at the onset of acute otitis media due to *Streptoccocus pneumoniae* or nontippable *Haemophilus influenzae* (37). Upper respiratory viral infections which may coexist during the episodes of acute otitis media did not significantly influence the concentrations of S100A12 protein, but the authors noticed a decreasing level of S100A12 protein in children who recovered from acute otitis media (37).

Up to the present, no consensus regarding the reference values for serum levels of S100A12 protein has been reached. Using enzyme-linked immunosorbent assay (ELISA), several authors reported mean serum S100A12 protein levels in healthy adults ranging from 10.7 to 75.0 ng/mL (38-41). Liu *et al.* were the first to provide a reference value for serum S100A12 protein levels in children: the mean serum concentration was 12.1 ng/mL ± 19.8 ng/mL in normal healthy subjects and 36.7 ± 72.4 ng/mL in children with acute otitis media (37). More recently, Hinze *et al.* reported a mean value of 44 ng/mL for S100A12 protein levels in healthy individuals aged between 4 to 20 years old (42). Recently, Pichichero *et al.* proposed a biomarker score based on age at diagnosis and serum concentrations of S100A12 protein and IL-10, which enables the clinicians to distinguish between upper respiratory infections with and without middle ear involvement

(43). However, further studies are required to fully understand the mechanisms involved in the activation of S100A12 protein in AOM and its implications in the development of the disease.

Limitations of the study

There are a number of limitations in this study that ought to be acknowledged. A major limitation of this study is the small sample size, but in pediatrics it is often difficult to recruit a high number of participants for research purpose basically due to ethical issues (44). Moreover, we recruited less patients than expected probably due to the lower incidence of the AOM in the Covid-19 era. There are several studies reporting a reduction in the incidence rate of AOM during the pandemic era and probably the protective measures we adopted to combat Covid-19 (45, 46). Another limitation is the lack of serum re-evaluation after the antibiotic treatment. This was mainly due to the favorable evolution of AOM cases, which did not require another blood sample to confirm the efficacy of the treatment. Also, it would have been useful to have the microbiological spectrum of the middle ear effusions, but a tympanocentesis was not routinely performed as it is an invasive procedure which weather was not recommended by the ENT specialist, or the parents were often reluctant to accept additional or invasive investigations.

CONCLUSIONS

Our study reveals that the inflammatory response related to AOM may be rather specific, than global, as the serum levels of IL-6 were higher in AOM group than in healthy controls ($p<0.001$), but with no significant difference regarding serum S100A12 protein between the two groups. Therefore, IL-6 could offer a powerful tool for the objective evaluation of AOM. IL-6 plays an important role in the pathogenesis of AOM in children, through its pro-inflammatory and immune-modulatory effects. Further research is needed to fully elucidate the role of IL-6 in AOM and to determine whether it may be a useful biomarker for predicting treatment outcomes.

Although it is only theoretical at this point, this different response may be specific to the children prone to AOM, and further studies are needed in order to establish if the risk of developing AOM is related to specific cytokine responses. Understanding the intracellular intricacies in relation to inflammation may contribute to newer therapies for AOM and even to the reduction of the complications and economic burden. We are hopeful that advances in the understanding of the inflammatory background of AOM may provide opportunities to create more targeted and effective treatments in the future.

Abbreviations

AOM: acute otitis media, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, HRP: Avidin-Horseradish Peroxidase, IL-1α: Interleukin-1α, IL-1β: Interleukin-1β, TNF-α: Tumor necrosis factor, IL-6: Interleukin-6, IL-8: Interleukin-8, OD: optical density, LPS: lipopolysaccharides.

CONFLICT OF INTEREST AND FUNDING

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All the authors had equal contributions

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committees of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania (No. 12292-22.07.2020) and of "Sf. Maria" Clinical Emergency Hospital for Children, Iasi (No. 34306/November 5, 2020).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical issues.

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THE FOLLOW-UP OF PATIENTS WITH SURGICAL SOLITARY KIDNEY - THE UNDER-ESTIMATED ROLE OF COMORBIDITIES

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THE FOLLOW-UP OF PATIENTS WITH SURGICAL SOLITARY KIDNEY-THE UNDERESTIMATED ROLE OF COBORBIDITIES (Abstract): In order to customize the follow-up plan and lower the risks of end-stage renal disease progression (ESRD), we set out to create a monitoring program for patients with surgical solitary kidneys. Additionally, by determining the underlying etiology and comorbidities (such as pre-existing CKD or Diabetes). 91 patients have been found who satisfy the inclusion requirements. **Material and methods:** There were 51 ladies and 40 guys among them. They ranged in age from 20 to 90. The average age was 60.69 years (SD +/- 15.87). There was no difference in the mean age between the genders (63.22 *vs.* 58.71, p=0.08). Kidney cancer (50.54%), upper urothelial tract carcinoma (25.27%), pyonephrosis (17.58%), and kidney trauma (6.59%) were the primary pathologies for which surgery was performed. The patients were also arbitrarily separated into three age groups: Group A, 20-40 years old; Group B, 41-60 years old; and Group C, over 60 years old. **Results:** One month after discharge, C-reactive protein increased (p=0.05), and eGFR decreased significantly (p=0.003). The most common comorbidity (74.72%; n=68) was arterial hypertension. Of these, 51.47% (n=35) have diabetes mellitus concurrently. Diabetes was the second-highest occurrence, with 49.45% (n=45) of patients receiving therapy for it. Also, at the time of surgery, 12.08 % (n=11) had CKD, according to KDIGO definition. Since acquired solitary kidney patients are more likely to experience rapidly declining renal function, they require continuous monitoring. **Conclusions:** Younger individuals are more prone to develop CKD slowly. Therefore, the clinician has to monitor issues like kidney stones or urinary tract infections. **Keywords:** SURGICAL SOLITARY KIDNEY, COMORBIDITIES, CHRONIC KIDNEY DISEASE.

In patients with acquired solitary kidneys, reduced renal mass causes higher intraglomerular pressure and glomerular hyperfiltration, including individuals who have undergone unilateral nephrectomy for living kidney donation, renal cancers, or trauma. These physiological modifications to the single kidney may aggravate other inherited and pre-existing diseases that might increase glomerular disorders or establish susceptibility to them, leading to adverse renal outcomes. According to Brenner *et al.*'s hypothesis from the 1980s, a patient is more likely to develop hyper-

tension, proteinuria, and a long-term reduction in glomerular filtration rate (GFR) if they have fewer nephrons (1). A growing body of research indicates that a congenital or acquired decrease in renal mass is associated with a higher risk of renal impairment. Nephrosclerosis and nephron hypertrophy might have a significant role in CKD and mortality. However, studies on the effects of these microstructural traits have only been conducted on tiny tissue specimens from people chosen for either having healthy kidneys or renal disease. To investigate kidney structure-outcome relationships, patients with radical nephrectomy (often renal cancer) represent a special cohort. These people are chosen neither for the lack of nephropathy (such as live kidney donors) nor for the presence of nephropathy (such as those who have had a nephrologist perform a biopsy), despite the fact that they frequently have major comorbidities. We aimed to develop a monitoring program for patients with solitary kidneys in order to personalize the follow-up scheme, thus minimizing the risks of end-stage renal disease progression (ESRD) based on comorbidities at the moment of nephrectomy and after. (e.g., Diabetes Mellitus-DM, pre-existing chronic kidney disease-CKD)

MATERIAL AND METHODS

Even if, at first glance, the patient with a solitary kidney, especially those who do not have surgical history, should be monitored by a nephrologist, we believe that multidisciplinary management is needed. We initiated a prospective follow-up program for this group of patients in our hospital. We enrolled patients with surgically single kidneys (after high urothelial tumors, parenchymal tumors, pyonephrosis, renal trauma, etc.) who underwent nephrectomy between February 2022 and February 2023. Exclusion criteria have been represented by: pregnancy, age under 18 years, and lack of compliance. We collected demographic data, personal history, chronic medication, and biochemical parameters. The last item was evaluated in patients who underwent nephrectomy before the surgery, after one week, and after one month. We realized the statistical analysis using the ANOVA, chi-square, and t-Student tests. A p-value of < 0.05 was considered statistically significant. We have also used *IBM SPSS Statistics version 27.0.1.0* for our statistical analysis.

RESULTS

We have identified 91 patients who meet the inclusion criteria. Of these, 40 were males, and 51 were females. They were aged between 20 and 90 years. The mean age was 60.69 (SD+/- 15.87) years. The mean age was not different between the genders (63.22 *vs.* 58.71, p=0.08). The evolution of the biochemical parameters preoperatively, before discharge, and one month after discharge is represented in first table.

The main pathology for which surgery was performed was kidney cancer (50.54%), upper urothelial tract carcinoma (25.27%), pyonephrosis (17.58%), and kidney trauma (6.59%). We also arbitrarily divided the patients into three age groups: Group A, 20-40 years; Group B, 41-60 years; and Group C >60 years old.

We compared the values of serum hemoglobin, creatinine, C-reactive protein, and eGFR between groups, as shown in Table II.

We have identified the several comorbidities. Arterial hypertension was the most frequent encountered, 74.72% (n=68) patients were diagnosed. Of which 51.47%

(n=35) simultaneously having diabetes mellitus. This condition was the second most frequent encounter, 49.45% (n=45) of patients receiving treatment for it. Both are well known kidney injury elements even in patients without a history of nephrectomy. Before surgery, 12.08 % (n=11) had CKD, according to KDIGO definition (<60 mL/min/1.73 m^2 in the last three months). As shown in Table II, most patients diagnosed with this conditions had over 60 years of age (group C).

TABLE I.

Evolution of biochemical parameters

	Preoperatively	Before discharge	One month after discharge	p
Hemoglobin (g/dL)	11.96 (+/-2.51)	10.85 (+/-2.18)	11.76 (+/-1.65)	0.005
Serum creatinine (mg/dL)	1.78 (+/-2.04)	1.67 (+/-1.58)	1.46 (+/-0.71)	0.600
Serum urea (mg/dL)	45.38 (+/-25.09)	44.10 (+/-24.82)	41.39 (+/-19.59)	0.680
C-reactive protein (mg/dL)	40.59 (+/-64.13)	75.45 (+/-76.46)	18.58 (+/-26.86)	0.001
eGFR (mL/min/1.73m^2)	65.54.(+/-32.34)	56.70 (+/-22.40)	59.25 (+/-26.24)	0.130

TABLE II.

Biochemical parameters one month after discharge according to the age-group

	Group A	Group B	Group C	p
No of patients	11	28	52	
Hemoglobin (g/dL)	11.95(+/-1.72)	12.07(+/-1.84)	11.55(+/-4.5)	0.640
Serum creatinine (mg/dL)	1.02(+/-0.2)	1.41(+/-0.71)	1.58(+/-0.76)	0.270
C-reactive protein (mg/dL)	12.58(+/-8.76)	12.16(+/-9.65)	22.19 (+/-33.52)	0.050
eGFR (mL/min/1.73m^2)	95.67(+/-7.94)	63.89(+/-31.82)	46.41(+/-21.6)	0.003
Arterial hypertension (n)	2	16	50	0.0001
Diabetes mellitus (n)	2	11	32	0.034
CKD	1	3	7	0.280

DISCUSSION

In the past decades, much research has been done on the possibility of reduced renal function in solitary kidneys. Numerous clinical studies, mostly on people with congenital renal illness, have been undertaken to validate physiopathological results in animal models. Congenital solitary kidney patients had a considerably increased probability of needing dialysis, according to a retrospective assessment by Sanna-Cherchi *et al.* (2). This finding raises the possibility that subclinical solitary kidney anomalies are to blame for the worsened renal outcome. Previous observational studies have looked at the short- and long-term renal outcomes of living kidney donors. According to certain longitudinal investigations of living kidney donors, renal function had been well-maintained following unilateral nephrectomy, with a mean follow-up duration of 10.7 years to 25 years (3). Studies have shown that individuals with solitary kidneys have an in-

creased risk of developing kidney stones compared to those with two functioning kidneys. The exact incidence can vary widely, but research suggests the risk is significantly higher in this population.

CKD progression in patients with solitary kidneys has been studied, especially in children. Groen In't Woud S *et al.* evaluated 944 patients (children) diagnosed with a single functional kidney (congenital and acquired) (4). At the end of the study, 553 (59%) patients presented at least one indicator for renal failure (≥1 indicator of kidney injury), and 255 (27%) presented at least one indicator for severe renal failure. Akyol *et al.* in a cohort of 101 children, 71 with congenital single kidney (of which 55 with unilateral renal agenesis and 16 with polycystic dysplastic kidney) and 30 with acquired single kidney (17 with unilateral nephrectomy for renal tumor and 13 urological structural anomalies) highlighted renal injury in 9 (9%) of the patients and a positive correlation between uric acid concentration and renal injury. In adults, Argueso *et al.* performed a study on 157 patients with unilateral renal agenesis (5). The authors showed that 7 (19%) of 37 tested patients developed proteinuria (>150 mg/24h), 22 (47%) of 47 tested patients developed hypertension, and in 4 (13%) of 32 tested patients, renal function (creatinine) it degraded. At the end of the study, 43 (27%) patients died, 6 (4%) were due to renal failure. Xu *et al.* studied 118 patients with unilateral renal agenesis. At the initial evaluation, 14 (11.9%) had additional abnormalities, 15 (12.7%) had a heredocolateral history, and 30 (25.4%) presented with renal failure. They concluded that renal failure is common in patients with unilateral renal agenesis and that family history is a risk factor for severe renal failure (6).

Although, according to Worcester *et al.*, nephrectomy does not negatively impact stone disease, it may decrease renal function among younger males(7). In our study, patients over 60 years had more often comorbidities that could lead to kidney stones (8). Some of these conditions are known to worsen CKD. Kiberd *et al.* evaluated 381 transplant patients, in which they diagnosed 111 (29%) cases of diabetes, of which 17 were considered to have pre-existing diabetes, and 94 were new cases (31 transient, 63 fixed) (9). Alfandary *et al.* evaluated 353 patients with solitary functional kidneys (10). Compared to the control group, the prevalence of renal injury is higher at 42.2% *vs.* 23.5%, hypertension at 31.7% *vs.* 23.1%, proteinuria at 18.2% *vs.* 0.4%, and eGFR at 12.0% *vs.* 0.1 %. They concluded that a solitary kidney is associated with a significant risk of renal injury. Obesity is also a major modifiable factor for kidney injury.

According to Ellis *et al.*, nephrectomy can have unfavorable effects and induce progressive eGFR drop in individuals with preoperative CKD and in patients without preoperative CKD who later develop postoperative CKD. This is due to poor nephron mass reduction adaption or underlying kidney injury. This damage has been recognized in patients with clinically-evident CKD prior to nephrectomy. This damage may not be present, may only be modest, and may never have become symptomatic in individuals who did not have clinically obvious CKD prior to nephrectomy. Nephrectomy alters this, and individuals are then categorized as having CKD. The difference in overall survival between the two groups (patients with CKD before surgery and patients with incident CKD after nephrectomy) could be attributed to lead-time

bias if patients who develop CKD after nephrectomy are thought to have had subclinical CKD before surgery. The sole difference between subclinical and clinically-evident CKD, assuming the underlying pathogenic mechanisms are thought to be fundamentally comparable, is the timing of the first occurrence. individuals with CKD that is clinically obvious before nephrectomy are more likely to have an earlier start of the underlying disease that causes CKD, making them more likely to die as a result of CKD than individuals who do not have CKD that is clinically evident until after nephrectomy (11).

The third underlying disease that led to nephrectomy was lithiasic pyonephrosis. Considering that kidney stones have a complex etiology and often we encounter a so-called "metabolically active lithiasis," the follow-up of the patients is even more justified (12). A screening of lithiasic patients could prevent hydronephrosis episodes, which, over time, have a negative impact on kidney parenchyma. Often hydronephrosis is accompanied by urinary tract infection, especially in high-volume stones (13,14). According to Naqvi *et al.*, infection outcomes in CKD patients are 3 to 4 times worse than in healthy subjects (15). Also, according to Shang *et al.*, patients with kidney have a 1.47 relative risk of developing CKD (16).

In light of this, the following pertinent query is: How does the mortality risk for patients with new-onset CKD following surgery compared to that of individuals with medical causes of CKD who do not have nephrectomy? Demirjian *et al.* partially addressed this using the Cleveland Clinic Surgical Registry dataset, comparing all-cause mortality and non-renal cancer mortality in patients with "medical/surgical" and "surgical" CKD (n=1,097 and 1,053, respectively), as well as a cohort of 42,658 patients with CKD that wasn't brought on by nephrectomy and were treated by nephrologists at the Cleveland Clinic (17). The claim that CKD brought on by surgical nephron reduction has less clinical importance than CKD brought on by other sources has a problem. Clinical recommendations' thresholds for CKD stages are based on the facts that 60 mL/min per 1.73 m^2 is almost half of the average person's maximal physiological eGFR, can be correctly discriminated against using estimating equations, and is linked to a higher risk of adverse outcomes (18).

In our opinion, patients who are more likely to develop lithiasis should be evaluated at 3 months. For those with urinary tract infections, monthly after administration of the treatment according to urine culture and antibiogram, while for those with associated pathologies (hypertension, diabetes etc.), six months evaluation is the most cost-efficient, with subsequent monitoring depending on the clinical manifestations.

CONCLUSIONS

Patients with acquired solitary kidneys need close follow-up because they are prone to rapidly decreasing renal function. Although, according to our data, serum creatinine and eGFR do not have a significant modification, one month after patients discharge, degradation will likely occur over time. In younger patients, CKD is likely to develop slower; the clinician needs to follow up on the conditions like kidney stones or urinary tract. We suggest that patients who are more likely to develop lithiasis should be evaluated at three months. In our opinion, the most important comorbidity that should be followed is

urinary tract infection, the doses and duration of the treatment being adapted for eGFR, but the others should not be neglected. patient education to avoid as much as possible any trauma to the single kidney must be included in the therapeutic plan.

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TRANSTHYRETIN CARDIAC AMYLOIDOSIS: PARTICULARITIES IN NORTH-EASTERN ROMANIA

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TRANSTHYRETIN CARDIAC AMYLOIDOSIS: PARTICULARITIES IN NORTH-EASTERN ROMANIA (Abstract): Transthyretin cardiac amyloidosis (ATTR-CA) is characterized by progressive thickening of both ventricles and interatrial septum. The diagnostic algorithm is based on a correlation between biomarkers, electrocardiography, ultrasonography and bone scintigraphy criteria. However, epidemiological data may offer a glimpse for a possible ATTR-CA even before other investigations. The aim of this study is to determine the profile of the ideal ATTR-CA patient based on patient's epidemiology. **Materials and methods:** 72 patients (suspected, accidentally diagnosed or diagnosed with cardiac amyloidosis) over the course of 7 years from the archive of the Nuclear Medicine Laboratory of "Sf. Spiridon" County Clinical Emergency Hospital were analyzed. ATTR-CA diagnoses was made by bone scintigraphy, reducing the group to 20 patients. Epidemiological parameters included: patient's gender, age, height, weight and provenance region. **Results:** Gender classification revealed an equal distribution among ATTR-CA positive patients, aged between 30-89 years old and from Iași County. Endomyocardial biopsy (gold standard method) is an invasive and not widely accessible method. Correlation between biomarkers, electrocardiography, ultrasonography and bone scintigraphy criteria represents the alternative method for diagnosing this rare disease. However, a profile of the standard ATTR-CA patient based on epidemiological data may offer a possible insight for directing the diagnosing algorithm to include this pathology. **Conclusions:** Epidemiological data may offer a possible profile of the standard patient that can be suspected of ATTR-CA, therefore increasing the accuracy of diagnosing this disease. **Keywords**: ATTR-CA, EPIDEMIOLOGY, BONE SCINTIGRAPHY.

Amyloidosis is considered to be a rare and hard to diagnose protein deposition disease. Its main characteristic is the accumulation in different organs and tissues of amyloid resulting, eventually in organ failure. There are more than 30 amyloidogenic proteins, however, only six can affect the normal function of the myocardium (1, 2, 3, 4). Cardiac amyloidosis is considered to be the most prevalent cause of restrictive cardiomyopathy and therefore being associated with heart failure but with preserved ejection fraction. An analysis made in the US over the course of 12 years has determined an increase in the prevalence of this disease, from 8 to 17 per 100,000 people,

with an incidence from 16 to 55 per 100,000 person-years. However, this increase can be explained by the increase awareness and improved diagnostic methods (5). The most frequent forms of cardiac amyloidosis are represented by transthyretin cardiac amyloidosis (ATTR-CA) or light chain (AL) (4).

ATTR-CA is characterized by the buildup of amyloid in both ventricles and inter-atrial-ventricular septum. Untreated, it can be fatal. The mechanism is based on misfolding the transport protein transthyretin resulting in its inability to perform this task and to deposit into different organs and tissues. ATTR-CA presents two subtypes: wild type (ATTRwt) and hereditary or mutation (ATTRh/ATTRm) (6) (fig. 1).

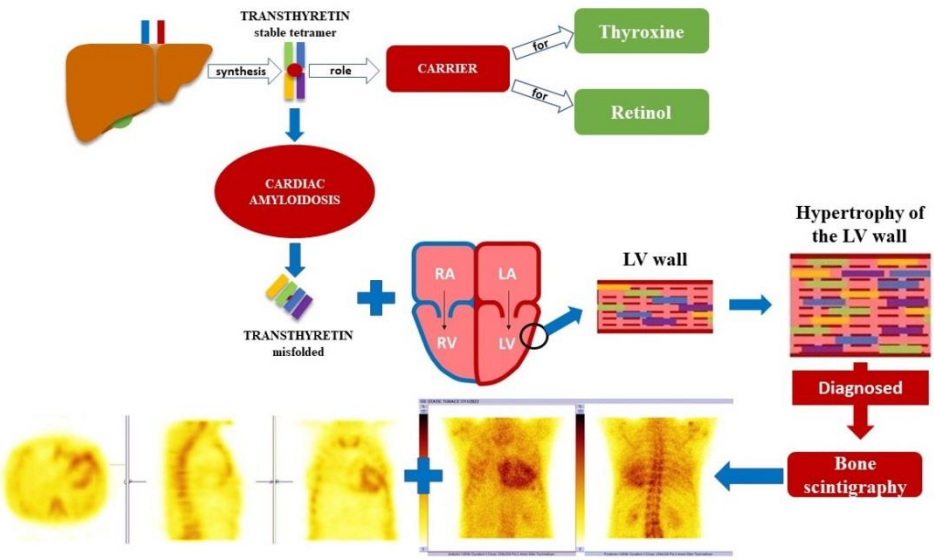

Fig 1. ATTR-CA mechanism and the relation with the functional image detection; (RA - right atrium; LA - left atrium; RV - right ventricle; LV - left ventricle)

The difficulties in predicting the incidence and prevalence for this disease are related to the complexities of the diagnostic process and not being able to rely on only one investigation to give the exact diagnosis all the time. Nevertheless, increased awareness for this pathology has proven to be important in the development of future medical therapies, such as the Transthyretin Amyloidosis Outcomes Survey which aims to characterize the course of the disease and the distribution worldwide. In the United Kingdom, over the past 2 decades, potential ATTR-CA cases have increased from 2% - 23.7% of new referrals, many of them being ATTRwt (5). A survey made an Italy which was based on 5315 ultrasonographies of patients over the age of 55 years old demonstrated a prevalence of 1%. However, by adding a few additional criteria such as increased wall thickness, a left ventricle ejection fraction over 50% and at least one ultrasonography red flag criteria for infiltrative disease, the prevalence was modified to 23.5% (7).

TTRwt affects especially the male pop-

ulation, presenting a male-female ratio of 25-50:1 (5, 8).

According to some articles ATTRwt affects approximately 25% of Finland's population and only 13% of Spain's. The ATTRwt positive patients in these articles, age wise, were over 85 years old (2, 9-11). Approximately 1 in 4 diagnosed patients is over 85 years old, presenting an average age of diagnosis of over 60 years old (5). The prevalence for this subtype being between 8-16%: 13% in patients with heart failure but with preserved ejection fraction; 16% in patients with aortic valve stenosis replacement; 30% in the subgroup of low-flow low-gradient stenosis and 5% of patients with hypertrophic cardiomyopathy (2, 9-12).

For ATTRh, the exact prevalence can be difficult to determine, mainly due to the variable geographical distribution of the mutation (6). From an epidemiological point of view, most of the patients are of male gender and with an average age between the 3rd and 8th decade. Mean survival rate depends on the mutation, however, it is considered to be approximately 3-12 years (13). However, in Europe it considered to be less than 1 in 100,000 and in Japan 1 in 1,000,000 (6). The most common mutations in ATTRh are: Val50Met and Val142Ile. Val50Met is found in male patients (male-female ratio of 2:1) ages ranging from 30-40 years in Portugal and 50-60 years in Sweden. It is considered to be endemic in Portugal, Sweden, Japan and Majorca (12). Untreated, mean survival rate is 26-46 months. It is considered that the origins of this particular subtype to be British, Cypriot, French, Greek, Italian, Japanese (5). Val142Ile is considered to be the most common variant worldwide presenting a prevalence of approximately 3.4% of African Americans. The origins seem to be from Africa, America and the Caribbean. The average age is over 65 years old: 20-39 years in Portugal, Sweden, Greece and Cyprus; 30-49 and over 60 years in Japan; average of 68 years in Britain, average of 64 years in France; average of 51 years in Italy (5, 6, 12, 14).

Due to the fact that ATTR-CA is a rare pathology, even with up-to-date methods for detection, any additional information can prove useful. The aim of the study is to determine ideal profile of the ATTR-CA patient based on the epidemiology data, for a more accurate detection.

MATERIALS AND METHODS

We performed a retrospective study based on data from the archive of the Nuclear Medicine Laboratory of "Sf. Spiridon" County Clinical Emergency Hospital, Iasi. Inclusion criteria were as follows: patients with the referral diagnosis of possible cardiac amyloidosis, positive bone scintigraphy for ATTR-CA, accidental diagnoses of ATTR-CA after bone scan. The study was made over the course of 7 years (2015-2022). Acquired epidemiological data included: age, gender and provenance region (in accordance with the identification card at that time).

ATTR diagnoses was determined after bone scintigraphy using the Perugini visual score and a semiquantitative method based on heart to contralateral and heart to rib ratios. All positive patients were recommended to undergo genetic testing.

All the patients were required to sign an informed consent in which full confidentiality was provided in case of publication. The

protocols that were used, were approved by the Ethics Committee of both the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania and "Sf. Spiridon" County Clinical Emergency Hospital, Iasi and therefore presented in the informed consent signed by the patient.

RESULTS

68 patients with the referral diagnosis of cardiac amyloidosis were initially included in the study. Bone scan diagnosed 16 patients with ATTR-CA. The remaining patients were classified as non-ATTR-CA patients that required further investigation to determine the diagnosis, 4 patients being eventually diagnosed with AL cardiac amyloidosis. To the ATTR-CA positive group, another 4 patients were added due to accidental diagnosis (other referral diagnosis) (fig. 2).

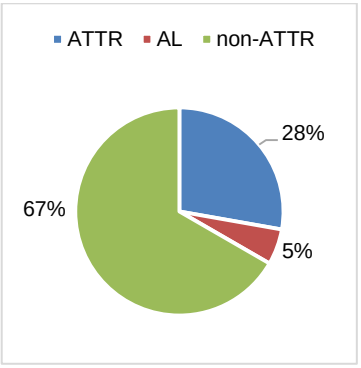

Fig 2. Patient distribution in accordance with the bone scan result (72 patients)

Gender and Age

Gender classification revealed an equal distribution among ATTR-CA positive patients: 10 male and 10 female patients (prevalence = 50%). Patients age varied from 35-89 years old. A more accurate distribution by age was obtained by classifying the patients per age decade (fig. 3).

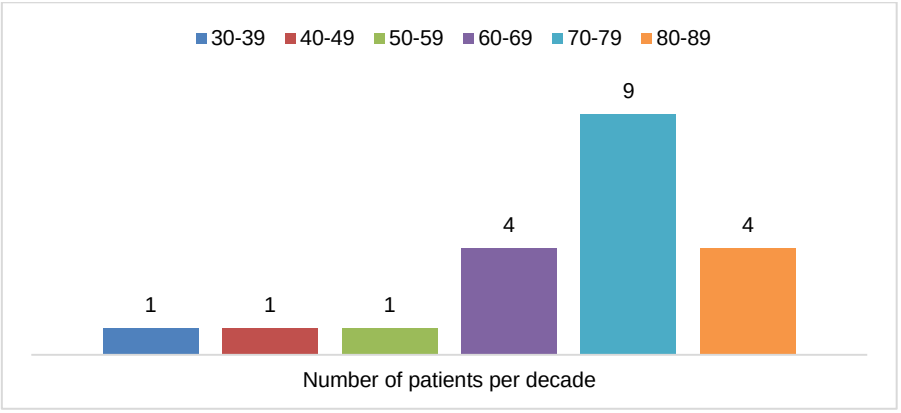

Fig 3. Patient distribution by age decade

Most of the patients were between 70-79 years old (prevalence = 45%).

Provenance region

All the studied patients were from the north-eastern region of Romania. Therefore, the ATTR positive patients were from the same region. Patient distribution was determined per county and not per city due to the fact that not all of the patients from one county were also from the same city (tab. I).

TABLE I.

Positive patient distribution per county and per total county population

County	Suceava (SV)	Bacău (BC)	Neamț (NT)	Vaslui (VS)	Iași (IS)	Botoșani (BT)
Positive patients per county	3	4	3	3	7	0

Most of the patients were from Iași County. (prevalence per positive patients = 35%)

Genetic testing

Genetic testing was performed only by 7 patients out of the entire ATTR positive group out of which 3 were diagnosed with ATTRwt and 4 with ATTRm (3 patients - GLU74GLN; 1 patient - VAL50MET).

Patient's particularities and correlations

Due to the fact that there is an equal number of males to female patients, the first filter that can be applied refers to the age of the patient. The results were as follows (tab. II):

TABLE II.

Correlation between gender and patients' age

Patients gender	Decades (years)					
	30-39	40-49	50-59	60-69	70-79	80-89
Female	1	1	1	1	4	2
Male	0	0	0	3	5	2

The majority of the ATTR positive patients where aged between 70-79 years old. Correlating this age with the gender revealed that most of them were males (male to female ratio = 1.25). The provenance region analysis determined that the focal area is Iași County. Genetic testing was also correlated with the patient's epidemiology data. For ATTRh, most of them where female aged between 35-52 years old. Provenance regions where Bacău (1 patient), Suceava (2 patients) and Iași (1 patient). For ATTRwt, most of the patients were females aged between 72-89 years old. Provenance regions where Bacău (1 patient), Iași (1 patient) and Vaslui (1 patient).

DISCUSSION

Over the years, ATTR-CA has proven to be a challenge for the practicing physician. The gold standard method for detecting cardiac amyloidosis refers to endomyocardial biopsy. However, this method is invasive and not widely available. To that end, the European Society of Cardiology offers an alternative for diagnosing this pathology, namely a correlation between biomarkers (NT-proBNP, troponin T) electrocardiography (micro voltage, left ventricular hypertrophy, pseudo infarct pattern, conduction and rhythm disorders), ultrasonography (left ventricular concentric hypertrophy; preserved left ventricular systolic function and ejection fraction; depressed longitudinal strain in the basal and midventricular sections with preservation of the apical one, reduced global longitudinal strain with apical sparing providing a specific appearance of "cherry on top" or "cupcake") and bone scintigraphy criteria (Perugini visual score and semiquantitative analysis). For positive cases, genetic testing is indicated for subtype determination. For negative results, free light chain dosage should be considered. The "red flags" offer a profile for the best candidate for cardiac amyloidosis, however, this profile must be sustained by other investigation methods (15).

In this research all of the patients were screened for a possible cardiac amyloidosis in the cardiology department, final diagnosis for ATTR being made by bone scan. To that end the researched focused to determine the characteristics from an epidemiological point of view of the ideal profile of the ATTR-CA patient. By evaluating the gender and age we were able to determine a possible profile for the ATTR-CA patient and compared our results to those present in the literature.

According to the "red flags", the ideal patient should be of male gender and over 65 years old. In our study, we obtained an equal number of ATTR positive patients regarding the gender (16). This can be explained by increased awareness of this disease in the last few years. Regarding the age of the patients, most of our patients were between 70-79 years old. Compared to the literature, these patients were detected in a later stage.

The provenance region represents one of the most important first steps that can determine the practicing physician to consider cardiac amyloidosis as a possible diagnosis. The obtained results revealed that in the North-Eastern region of Romania, the most affected county was Iași, followed by Suceava, Bacău and Neamț. The focal area for ATTR-CA in Romania is considered to be Suceava county (16). The provenance region of our group was based on the identification card, so it is conceivable that a change of address may have taken place at some point resulting in a balance shift towards a new region.

Based on the epidemiological data available data until this point the ideal ATTR-CA patient can be of either gender, aged between 70-79 years old and from Iași county.

However, some of the positive patients underwent genetic testing for subtype determination. Even though, the number of patients that underwent this test is small, it may offer an insight for a future research project. As a result, it was determined that out of the group of 20 ATTR positive patients, only 7 underwent genetic testing. Out of these 7 patients, 3 of them were diagnosed with ATTRwt and 4 of them with ATTRh (3 patients with GLU74GLN and 1 patient with VAL50MET). By applying the epidemiological filters stated above to these patients the results were modified. Therefore, for ATTRh, most of the patients were females, aged between 35-46 years old and from Suceava county. For ATTRwt most of the patients were females, aged between 72-89 years old. The provenance region, presented an equal distribution among Iași, Bacău, and Vaslui. These results underline the fact that for the hereditary subtype, the focal region is Suceava county, while for the wild type, this region is not included.

Therefore, if a patient, regardless of the gender, with an age between 35-46 and 70-79 years old and with a provenance region of either Iasi or Suceava county, then ATTR-CA may be suspected as a possible diagnosis. The two different intervals regarding the patients age, may be explained by the early onset of ATTR-CA symptoms in patients with the ATTRh subtype.

It must also be mentioned that due to the small number of positive patients, the risk of bias in our study may be present. The risk factor may require further validation in the future.

Nevertheless, a proper investigated background that includes the provenance region, in association with the gender and age interval for ATTR-CA may steer the practicing physician to consider this pathology as a possible diagnosis.

CONCLUSIONS

The first step in the diagnostic algorithm is the patient's epidemiology data. In this article, we tried to correlate the epidemiological data for ATTR-CA patients in order to give the practicing physician an edge. Therefore, a patient aged between 35-46 (especially ATTRh) and 70-79 years old, regardless of the gender coming either from Iași or Suceava should be considered as possible candidates for ATTR-CA. Based on the different age intervals an indication for the possible subtype involved can also be considered: ATTRh can be detected in an earlier stage compared to ATTRwt. Any kind of advantage in the detection algorithm of this rare disease should be taken into consideration for an early and more accurate diagnosis.

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All authors had equal contribution.

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LONG-TERM RESULTS AFTER PRETARSAL FIXATION OF GOLD WEIGHTS IN FACIAL NERVE PALSY

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LONG-TERM RESULTS AFTER PRETARSAL FIXATION OF GOLD WEIGHTS IN FACIAL NERVE PALSY (Abstract) Chronic corneal exposure after facial palsy is a clinically condition requiring immediate treatment. The most effective and simple solution is the gold weight implant into the upper eyelid. **Aim**: to evaluate our long-term results using this method. **Materials and methods:** This is a retrospective study of the outcomes after pretarsal placement of gold weights in 20 patients operated by one surgeon from 2002 to 2020. **Results:** A total of 20 patients were included in the study. Follow up period ranged from 5 to 21 years. None of them have postoperative infections, ptosis, keratopathy, nor have they reduced visual acuity. Fifteen out of twenty patients have the implant in an adequate position, with appropriate lid closure. Two patients developed minimal implant migration and slight asymmetry, but implant remained functional and in place. Three patients developed important implant migration leading to extrusion. **Conclusions:** The results of our long-term study are similar to those revealed in the literature. Considering the favorable post-operative outcomes, we would recommend this technique for using either as a self-standing procedure or in conjunction with other static or dynamic procedures in facial palsy. For long term results and for improved migration and implant extrusion rates we recommend under correction by choosing to place the lighter implant instead of the "perfect", heavier implant. **Keywords:** FACIAL PALSY, CORNEAL EXPOSURE, GOLD WEIGHTS.

Chronic corneal exposure is a clinically condition requiring immediate and effective treatment. The primary cause of corneal exposure and lagophthalmos is facial nerve paralysis, occurring after facial nerve damage caused by trauma, surgery, infection, tumor, cerebrovascular events, or Bell's palsy (1). The dysfunction of the facial nerve severely impacts patient's emotional and psychological status, due to significant changes in the facial symmetry, having negative impact on the patient's quality of life. Ocular manifestations related to corneal exposure lead to cornea dryness, causing ocular discomfort, reduced vision, corneal ulcers, infection, and perforation. Maybe the most effective and simple solution for correction of paralytic lagophthalmos is the gold weight implant into the upper eyelid, associated or not to more complex, dynamic, or static facial reanimation method. The aim of this study is to evaluate our long-term results using this method.

MATERIALS AND METHODS

This is a retrospective study of the surgical outcomes after pretarsal placement of gold weights in 20 patients operated by one surgeon from 2002 to 2020. The collected data were the cause of facial palsy, the delay form the installation of the palsy to the implantation, the implantation technique, the weight of gold implant, additional lid and /or facial surgeries, and the post-operative complications. Results and evolution were photographically documented.

The gold weight implants used were either handcrafted (18 cases) or readily available (2 cases, MedDev Corporation) having a predefined weight from 1.2 to 1.8 grams with increments of 0.2 grams. In each case the preoperative calibration was performed using a Standard Tantalum Weight Sizing Set (MedDev Corporation).

Surgical technique is simple and straightforward. The patient is positioned supine with the head elevated at 30 degrees. We used local anesthesia (Lidocainum or Bupivacainum and adrenaline 1/100,000 injected subcutaneously), without sedation. Incision line is marked 6 mm from lid margin. A 1.5 cm long supra-tarsal incision is made through the skin and orbicularis oculi muscle, creating a pocket over the tarsal plate. The gold implant is centered and fixed to tarsal surface with three 6.0 polypropylene sutures. Eyelid eversion was performed to check the conjunctiva integrity. The wound was then closed in two layers with 6.0 resorbable sutures for the orbicularis and 6.0 nylon for the skin. The skin sutures were removed after 5-7 days. The patients were discharged same day. Follow-up was carried out at 48 hours, one week, one month, three months, six months, and on a yearly basis. The implant position and closure of the palpebral slit are checked on each examination by the plastic surgeon and corneal integrity is verified once a year by the ophthalmologist.

RESULTS

A total of 20 patients undergoing upper eyelid loading for lagophthalmos were included in the study. Follow up period ranged from 5 to 21 years. The mean age of our sample was 58 years, with an age range from 34 to 82 years. We had equal male / female distribution. The most common cause of the facial palsy was tumor in 16 patients including acoustic neurinoma (9 cases), cystic parotidean adenoma (3 cases), cholesteatoma (2 cases), and squamous cell carcinomas (SCC) (2 cases). Three cases of facial paralysis were post-traumatic, and one case was congenital. All patients had unilateral disease. All were operated after at least one year after facial palsy instalment. Twenty-seven operations were performed (20 for implantation, 4 for repositioning and 3 for final removal). We used 1.2 g implants in 2 cases, 1.4 g in 7 cases, 1.6 g in 8 cases, and 1.8 g in 3 cases (fig. 1).

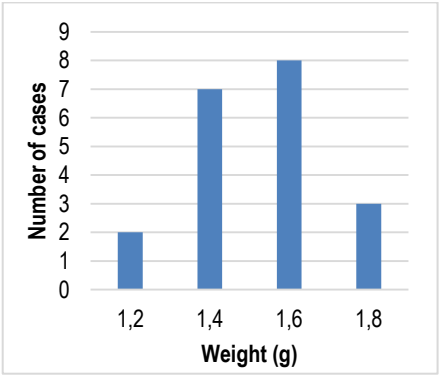

Fig. 1. Distribution of cases according to the weight of the implant

At the moment of the eyelid implant, 12 of the patients included in the study had already undergone at least one surgery on the eyelid or facial area in other hospitals (static only). Gold weight implantation was the only therapeutic option for 10 patients which denied any other type of surgical treatment for facial reanimation. Six patients needed dynamic reconstruction techniques (2 facial nerve reanimation, babysitter procedure with cross face nerve grafts, one with temporal tendon transfer, 2 with facial nerve grafting, and one with primary neurorrhaphy). Four patients needed additional static canthopexy for medial paralytic ectropion of the lower eyelid.

No intra-operative or early postoperative complications were registered. None of the patients have postoperative infections, ptosis, keratopathy, nor have they reduced visual acuity. Fifteen out of twenty patients have the implant in an adequate position, with appropriate lid closure since the surgery up to the latest follow-up. Two patients developed minimal implant migration and slight asymmetry, but implant remained functional and is still present. Three patients developed important implant migration leading to extrusion.

DISCUSSION

The eye closing inability results from the unopposed action of the *levator palpebrae superioris* muscle on the upper eyelid, which is normally contracted by the function of the orbicularis oculi muscle. The paralyzed eye can be conservatively managed with ointment, eye drops, moisture chambers, and lid taping. However, these methods may be difficult and cumbersome for most of the patients, and they provide suboptimal corneal protection. Ideally this should be only a temporary situation until the facial nerve is reconstructed by a dynamic method and the eye closure is restored. Unfortunately, and somehow inexplicably, there is still a vast majority of patients with long standing well documented facial palsies waiting for facial regeneration for many years. Moreover, they seem reluctant to complex functional surgeries preferring minimal interventions. Options for surgical management of the paralyzed eyelid in such cases include a variety of static techniques with variable degrees of success, such as tarsorrhaphy, canthoplasty, *fascia lata*, and silicone slings, palpebral springs. Very popular in Romania seems to be the simplest static methods like tight tarsorrhaphy (fig. 1) which might provide significant changes regarding corneal exposure, ocular surface symptoms but lead to poor aesthetics results or even disfigurements.

Weight implantation in the upper eyelid for treatment of the lagophthalmos in cases of facial paralysis was first described in 1950 by Sheehan *et al.* (2), and it was first used in 1958 by Illig *et al.* (3). Folded tantalum is the first material used as an implant. Gold was first used as a lid load in 1966, and it seemed to be “the most practicable material with its great range of colors and minimal reactivity in the tissues” (4). More materials emerged and have been used, such as stainless-steel mesh, lead, platinum and hyaluronic acid gel (2, 4, 5) The gold implant has the advantage of being relatively inert, providing an aesthetic camouflage, high density, and nonallergenic features. The complications of these implants include migration, soreness, persistent lagophthalmos (generally while sleeping), extrusion, and ptosis (6-10). Recently, platinum was used for lid loading as an alternative to the gold implant (11). Its advantages are the density (21.5 g/cm^3) which allows implants to be 10% smaller

than gold ones, better biocompatibility, flexibility and the reduced risk of extrusion and adverse reactions to the metal. However, despite these advantages, the gold implant remains the most used because of the accessibility and the price.

Although lid loading technique is simple the use of this method seems very limited in our country. We used gold weights for the management of the eyelid in facial palsy combining dynamic and static methods for lower middle and upper face with successful results.

All our patients had unilateral disease (figs. 2 A, B, C, and figs. 3 A, B, C). For the 20 eyelids reconstructed a number of 27 operations were performed (20 for implantation, 4 for repositioning and 3 for final removal). Seventeen patients had a single operation for implantation, 1 patient required 2 operation (implantation + removal), one patient had 3 operations (implantation, repositioning, and removal), and one patient underwent 5 interventions (implantation, 3 repositioning and 1 removal). All our patients in need for final removal arrived in clinic long time after implant exposure, and they denied any another implant or operation (figs. 4. A and B). In a retrospective cohort review on 104 gold weights in 94 eyelids, Rofagha (12) applied the Kaplan-Meier survival curve analysis and obtained 3% exposure rate at 12 months, 5% at 24 months, and 10% at 5 years, which increased to 35% at 153 months. Another study conducted by Kelly (13) reported a 68% complication rate, and the extrusion rate was 43 %. Our group shows a 15% extrusion rate after 5 - 21 years follow up. Since in our experience extrusion occurred secondary to a long period in which the patient neglected the doctor's recommendations in our opinion this complication is more related to the characteristic of the patient's skin and to the low adherence to treatment and less related to surgical technique.

Preoperative calibration (Standard Tantalum Weight Sizing Set, MedDev Corporation) was performed to establish the weight that induces the reduction of lagophthalmos without creating ptosis. Adhesive taped predetermined weights were attached to the pretarsal portion of the upper lid, while the patient was relaxed in a sitting position. We always start with the 1.2 grams weight for 3 to 5 minutes. The weight was increased by 0.2 grams until the proper result was obtained.

Fig. 1. A before implant, corneal exposure and tarsorrhaphy; B, C, eye closure after gold weight implant.

Fig. 2. A before implant, corneal exposure and tarsorrhaphy; B, C, eye closure after gold weight implant.

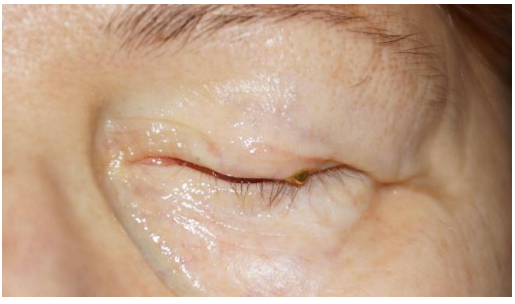

Fig. 3. A. Extrusion, before removal

Fig. 3. B. Extrusion, before removal

Corneal exposure was present in all patients before surgery. Postoperative, all twenty patients presented reduced corneal staining and within 2 to 4 weeks the corneal staining faded. Compared to the study conducted by Harrisberg *et al.* (6) our re-

sults are similar regarding the sustaining of corneal function and ocular comfort with the use of the lid gold implant. The corneal protection was maintained during the follow-up period. Although other authors (14, 15) aim a decrease in lagophthalmos from 4 to 5 mm preoperatively to 0.5 mm postoperatively, in our group, the rate of remaining long term lagophthalmos is 0.5 to 1.5 mm in all the cases as we aimed under correction allowing a slight corneal exposure (figs. 2 and 3) especially in previously operated cases. We consider that a smaller weight has les chances of extrusion.

The surgical technique evolved from the originally subcutaneous implantation of the gold weight 3,4 which was quickly waived due to the high rate of complications in favor of pre-tarsal fixation. Since there is a lack of evidence for the superiority of one technique of pretarsal fixation over another we used in all the cases the same technique: the size of the pocket is small enough to fit to the size of the gold weight and we place the knots of three permanent sutures into the holes of the plate. Migration is only possible if at least one of the 3 permanent suture points breaks. In a metanalysis Schrom *et al.* (7) highlights a complete eyelid closure rate of 84% but with a fairly high rate of complications which encourages the oculoplastic surgeons to consider alternative methods in terms of surgical technique. In our experience we achieved eyelid closure in 100% of the cases but with the same relatively high rate of complications, 15% (extrusion) compared to 6.8% extrusion and 6,4% migration.

Although lower placement of the gold weight might lead to a better functional result, we favor upper implantation since it seems to us it causes less damage to the eyelid tissues with less chances of extrusion. Nowak-Gospodarowicz (16) considers that combining lower placement of the gold weights about 2 millimeters above the eyelash line with absorbable sutures should be avoided due to the high risk of complications.

Preoperatively, all patients complained of foreign body sensation and ocular discomfort despite the use of lubricating eye drops and eye closure during the night. After upper lid loading, all 20 patients discontinued to use lubricant eye drops after a couple of postoperatively but used external eyelid closure during the night for several years. On the long run, out of the 20 patients operated, 3 had a remaining lagophthalmos (2 mm) located exclusively medial due to the ectropion which left a portion of the medial sclera exposed. The lagophthalmos was not present in patients which accepted lateral canthopexy. Comparatively, after placement of 104 gold weights in 94 eyelids, Rofagha and Seiff (12) obtained decreased lagophthalmos in 88 patients, and in the case of the remaining 6 patients, no improvement in lagophthalmos was observed. The principal cause for persistent lagophthalmos in their study was the eyelid scaring from other surgery or prior spring removal.

CONCLUSIONS

Gold weight implantation in patients with facial paralysis is a simple and reliable procedure with significant reduction in paralytic lagophthalmos and improvement of corneal coverage and obvious long-term cosmetic benefits. The results of our long-term study are similar to those revealed in the literature. Considering the favorable post-operative outcomes, we would recommend this technique for using either as a

self-standing procedure or in conjunction with other static or dynamic procedures in facial palsy. For long term results and for improved migration and implant extrusion rates we recommend under correction by choosing to place the lighter implant instead of the "perfect", heavier implant.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors are responsible for the content of the article.

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GLIOMA-RELATED EPILEPSY - CHARACTERISTICS AND IMPLICATIONS ON TREATMENT AND PROGNOSIS IN THE CONTEXT OF MOST RECENT BRAIN TUMOR MANAGEMENT GUIDELINES

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GLIOMA-RELATED EPILEPSY-CHARACTERISTICS AND IMPLICATIONS ON TREATMENT AND PROGNOSIS IN THE CONTEXT OF MOST RECENT BRAIN TUMOR MANAGEMENT GUIDELINES (Abstract): Diffuse low-grade gliomas, neoplasms originating from glial tissue, exhibit a propensity to evolve into tumors of a higher grade over time. The management of low-grade glioma presents a multifaceted challenge in clinical decision-making, given its complex diagnostic profile. Numerous factors have been identified as critical in determining the outcomes of various treatment modalities and in serving as prognostic indicators with predictive significance. Among these, glioma-associated epilepsy stands out as a principal clinical indicator, playing a pivotal role in both the management strategies and prognostication post-treatment. **Materials and methods:** A retrospective analysis was conducted on a cohort of 38 patients diagnosed with low-grade glioma (LGG), World Health Organization (WHO) grade 2 or 3, who were treated within our neurosurgical department from 2013 to 2023. This study primarily focused on the occurrence of glioma-related epileptic seizures. Additionally, an extensive review of the pertinent literature was undertaken to enrich the analysis. The evaluation encompassed the assessment of Engel outcomes six months following surgical resection, along with the examination of the anti-seizure medication (ASM) regimens implemented. Furthermore, a comprehensive review and synthesis of the current National Institute for Health and Care Excellence (NICE) guidelines pertaining to the management of low-grade gliomas were conducted, highlighting the latest recommendations and therapeutic strategies. **Results:** Within the cohort of 38 patients diagnosed with glioma, 30 were definitively identified as having diffuse astrocytoma (grade 2 and 3), while the remaining 8 were diagnosed with oligodendroglioma. Among those experiencing glioma-related epilepsy (n=25), a management approach involving biopsy only was applied to 8% (n=2) of the cases, gross total resection (GTR) was performed on 40% (n=10), subtotal resection (StR) was administered to 32% (n=8), and partial resection (PaR) was executed on 20% (n=5) of the patients. The investigation did not uncover a significant correlation between the occurrence of seizures and variables such as tumor volume, rate of growth, or histological findings. However, a notable positive effect of the extent of surgical resection on patient outcomes was observed, as measured by the Engel classification system, six months subsequent to oncological intervention. **Discussion:** Several researchers have illustrated that the presence of seizures during the initial stages serves as a favorable prognostic indicator for both malignant progression-free survival and overall survival

in patients diagnosed with glioma. Furthermore, the manifestation of epilepsy as a presenting symptom significantly impacts the quality of life of individuals afflicted with glioma. **Conclusions:** Exploration into a more intricate approach to epilepsy surgery specifically targeting glioma-related seizures could yield considerable advantages, with the potential to enhance the quality of life for patients post-treatment. **Keywords**: GLIOMA-RELATED EPILEPSY, LOW-GRADE GLIOMA, EPILEPSY SURGERY, ENGEL CLASSIFICATION, NICE BRAIN TUMOR GUIDELINES.

INTRODUCTION

Diffuse low-grade gliomas, classified as tumors originating from glial tissue, possess the inherent capability to evolve into higher-grade malignancies over time (1, 2). Consistent with established guidelines for glioma management, the patient's treatment trajectory post-diagnosis is recommended to adhere to a predefined algorithm. This commences with diagnostic procedures, prioritizing imaging techniques for the presumptive identification of glioma. Initial imaging should include a comprehensive structural MRI protocol, incorporating T2-weighted, Diffusion-Weighted Imaging (DWI), Fluid-Attenuated Inversion Recovery (FLAIR), and T1-weighted sequences both pre- and post-contrast administration. A consensus on the management strategy should be sought through discussion within a multidisciplinary team upon the initial imaging diagnosis. Furthermore, the deployment of advanced MRI methodologies, such as MR perfusion and MR spectroscopy, is advocated where feasible, to facilitate the identification of potential high-grade transformations within gliomas that may initially present as low-grade on standard structural MRI scans (3) (fig. 1).

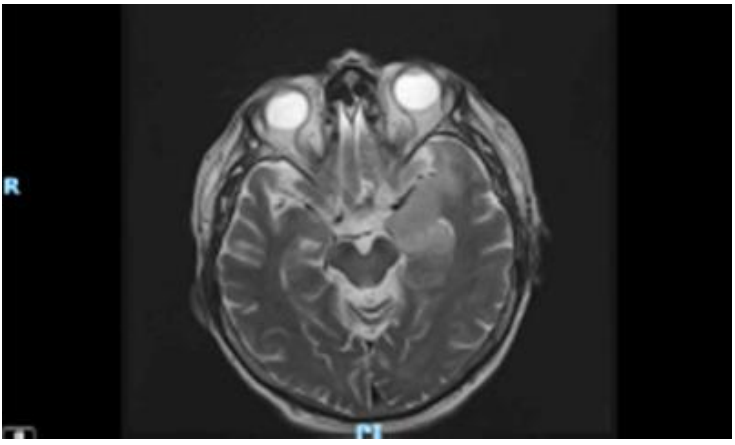

Fig. 1. Axial T2W images of 69M with WHO grade 2 diffuse astrocytoma (Left Temporal Lobe)

The management of glioma should incorporate surgical resection ideally within a six-month period following the imaging diagnosis. The primary objectives of the initial surgical intervention are to secure a histological diagnosis and to excise as

much of the glioma as is feasibly safe, subsequent to a thorough and thoughtful deliberation with the patient and their kin. In instances where surgical resection is deemed infeasible, pursuing a biopsy for histological determination is advised. Additionally, the option of active surveillance without histological confirmation may be considered, albeit exclusively for tumors exhibiting characteristic imaging signatures of low-grade tumors, such as Dysembryoplastic Neuroepithelial Tumors (DNET) or optic pathway glioma. Facilities specialized in neurosurgery for glioma treatment should be equipped with at least some advanced operational capabilities, including but not limited to, the ability to conduct awake craniotomies with language and functional monitoring, intra-operative neurophysiological monitoring, and intra-operative imaging guidance (3).

The surgical approach to glioma management necessitates the incorporation of various contemporary guideline recommendations to enhance treatment efficacy and patient safety. The use of 5-aminolevulinic acid (5-ALA)-guided surgery is recommended as a significant adjunctive measure to enhance the extent of resection during the initial surgery of gliomas that are radiologically suspected to be high-grade. Furthermore, the employment of intraoperative magnetic resonance imaging (MRI) is advocated to facilitate the precise removal of tumor tissue while concurrently preserving neurological function. The integration of intraoperative ultrasound and diffusion tensor imaging (DTI), alongside traditional neuro-navigation methods, is crucial for minimizing damage to critical neuronal pathways. Additionally, the execution of awake craniotomy stands out as a paramount technique for the maximal preservation of neurological functions, necessitating the involvement of neuropsychologists and speech and language therapists to ensure optimal outcomes. This comprehensive and nuanced surgical strategy underscores the importance of employing advanced technological interventions and multidisciplinary collaboration in the management of glioma, aiming to achieve the best possible patient-centric outcomes.

Within the realm of contemporary advancements in the treatment of brain tumors, laser interstitial thermal therapy (LITT) emerges as a noteworthy mention, having been progressively recognized as a minimally invasive therapeutic modality (4). Research conducted by Hawasli *et al.*, in 2013 (5), has validated LITT as a secure and efficacious ablative approach for addressing intracranial lesions, while also acknowledging the potential morbidities associated with its use, such as aphasia, hemiparesis, hyponatremia, meningitis, and deep vein thrombosis (DVT).

Moreover, the employment of molecular markers has become instrumental in the prognostication and therapeutic stratification of glioma. Notable markers include IDH1 and IDH2 mutations, ATRX mutations (pertinent to IDH mutant astrocytomas and glioblastomas), 1p/19q codeletion (indicative of oligodendrogliomas), histone H3.3 K27M mutations (associated with midline gliomas), and BRAF fusion and mutations (relevant to pilocytic astrocytoma) (3).

In the intricate landscape of glioma pathology, significant effort has been devoted to identifying and delineating factors that profoundly impact the quality of life of individuals diagnosed with this condition. Through a retrospective examination of cases handled within our neurosurgical

department, it has been observed that patients who presented with or developed epileptic seizures postoperatively experienced a notable deterioration in their quality of life. This adverse impact extends beyond physical health, exerting a considerable psychological burden on the affected individuals over both short and long-term periods.

Per the National Institute for Health and Care Excellence (NICE) guidelines, the management of epilepsy or seizure control is identified as one of the three paramount outcomes following glioma surgery, alongside progression - free survival and neurological function (assessed via the Neurological Function Scale or NIH Stroke Scale). These outcomes have been recognized as pivotal in assessing the risks associated with the decision to proceed with or forego tumor resection. Among these, progression-free survival is deemed to hold greater importance over overall survival, as it more accurately reflects the tumor-specific outcomes influenced by the decision to ablate the tumor (3).

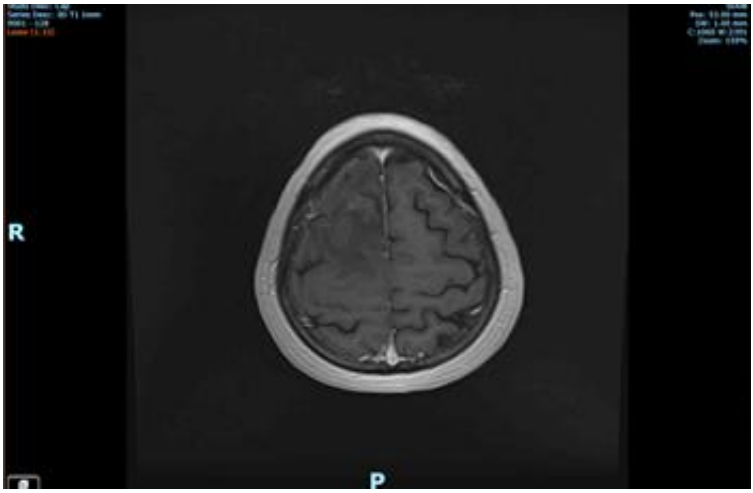

Fig. 2. Axial T2W images of 61F with WHO grade 3 astrocytoma (Frontal area)

Epileptic seizures presenting as initial symptoms significantly affect the quality of life for individuals diagnosed with glioma. Various studies have sought to identify factors influencing the incidence of epileptic seizures during the neurosurgical perioperative period, as well as potential interventions to enhance epilepsy-related outcomes in patients undergoing glioma surgical resection (6). Seizures typically manifest first in patients who are later diagnosed with glioma, particularly those with low-grade gliomas located in the frontotemporal regions, which are associated with an elevated risk of postoperative seizures. The presence of drug-resistant epilepsy (DRE) and notable peritumoral edema are linked to a higher occurrence of peri-operative seizures, whereas gross total resection has been shown to diminish the incidence of postoperative seizures (6) (fig. 2).

The Engel system is employed for classifying epilepsy outcomes following surgery, originally developed for assessing results in patients after epilepsy surgery (7).

TABLE I

Engel classification of postoperative outcome (Engel 1993) (7)

Class	Characteristics
Class I	Free of disabling seizures
	A: Completely seizure-free since surgery B: None disabling; simple partial seizures only since surgery C: Some disabling seizures after surgery, but free of disabling seizures for at least 2 years D: Generalized convulsions with AED discontinuation only
Class II	Rare disabling seizures (“almost seizure free”)
	A: Initially free of disabling seizures but rare seizures now B: Rare disabling seizures since surgery C: More than rare disabling seizures since surgery, but rare seizures for the last 2 years D: Nocturnal seizures only
Class III	Worthwhile improvement
	A: Worthwhile seizure reduction B: Prolonged seizure-free intervals mounting to greater than half the followed up., But not<2 years
Class IV	No worthwhile improvement

MATERIALS AND METHODS

A retrospective analysis was conducted on a cohort of 38 patients diagnosed with low-grade gliomas (LGG), WHO grades 2-3, who were treated in our neurosurgical department over the decade spanning 2013 to 2023. This analysis placed a particular emphasis on the incidence of glioma-related epileptic seizures, supplemented by a thorough review of pertinent literature on the subject. The study evaluated the Engel outcome six months post-resection, in addition to reviewing the anti-seizure medication (ASM) protocols employed.

Our neurosurgical department, situated within the Emergency University Hospital (SUUB) in Bucharest, serves an approximate population of one million individuals. Glioma cases are typically referred to our department either through the General Practitioner service or by direct admission via the Accident and Emergency Department. Upon a clinical and radiological presumptive diagnosis of a central nervous system (CNS) tumor, patients are integrated into a clinical pathway that includes Neurosurgery, Neuroradiology, and Oncology. This multidisciplinary team approach is facilitated by weekly meetings, known as the “Tumor Board,” at the Emergency University Hospital Bucharest.

The scope of our investigation encompassed a comprehensive review of clinical and imaging data, diagnostic approaches, and treatment modalities from 2013 to 2023. This review included an analysis of surgical procedure records, patient clinical notes, histology findings, and outpatient follow-up clinical data for all patients diagnosed with diffuse low-grade glioma. Parameters such as patient age, gender, initial presenting symptoms, lesion locali-

zation, eloquence, the occurrence of epileptic seizures, adjuvant treatments, as well as pre-operative seizure characteristics (type, duration, and frequency) and post-operative Engel classification outcomes were meticulously examined (fig. 3).

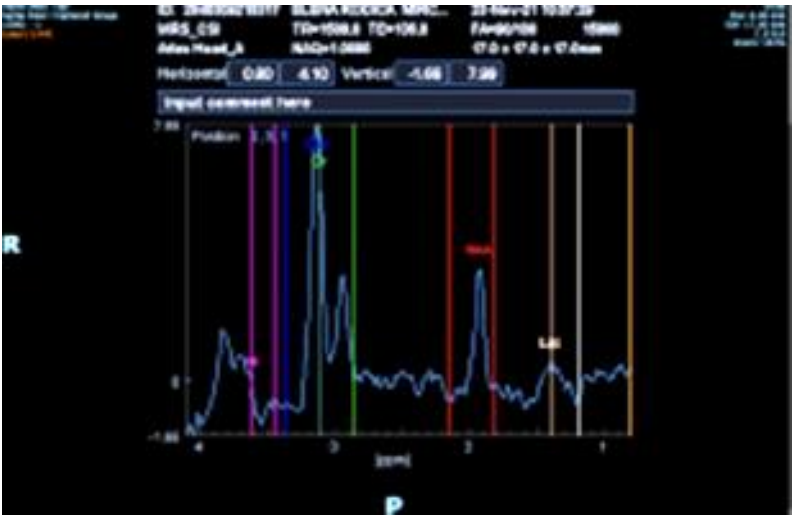

Fig. 3. MR Spectroscopy of 37F with WHO grade 3 oligodendroglioma

The analysis of preoperative and post-operative imaging focused on various dimensions, including the glioma's location, lateralization (dominant vs. non-dominant hemisphere), eloquence, and distinctive features observed in MR spectroscopy sequences, particularly the choline/N-acetyl aspartate (Cho/NAA) high ratio. Additionally, the study examined the correlation between the variability of peritumoral edema, as evidenced on imaging, and the incidence of epileptic seizures.

MRI examinations discussed in this research were conducted within the Radiology Department of the Emergency University Hospital Bucharest, serving both as an integral component of the preoperative diagnostic process and as part of the post-operative follow-up regimen.

RESULTS

Among the 38 patients diagnosed with glioma, 30 were conclusively identified with diffuse astrocytoma (grades 2 and 3), while 8 were diagnosed with oligodendroglioma. The median age of the cohort was 50 years old, with females comprising 60% of the patient population. Tumor localization was diverse: frontal lobe (n=24), temporal lobe (n=6), parietal lobe (n=6), and insular region (n=2). The majority of gliomas (n=28) were situated in the dominant hemisphere, with the remainder (n=10) in the non-dominant hemisphere. Presenting symptoms varied widely, including asymptomatic cases (n=3), motor deficits (n=16), visual deficits (n=2), seizures (n=25), aphasia (n=9), and intracranial hypertension (n=6).

Surgical interventions were categorized as follows: biopsy only (n=6), gross total resection (GTR) (n=14), subtotal resection (StR) (n=10), and partial resection (PaR) (n=8).

Regarding glioma-related epilepsy (n=25), management strategies included biopsy only (8%, n=2), GTR (40%, n=10), StR (32%, n=8), and PaR (20%, n=5). Seizure presentation varied from simple partial (focal) seizures to complex generalized tonic-clonic seizures, with seizure frequency ranging from daily to monthly prior to diagnosis. Beyond surgical resection, seizure management involved anti-seizure medication (ASM) from the initial presentation, adhering to standard guidelines. In complex epilepsy cases, specialized adjustments to ASM types and doses were necessary. The most responsive treatments involved Carbamazepine, Levetiracetam, Valproic acid, Topiramate, Phenytoin, or Lamotrigine, with combinations used for more complicated cases.

The study found no significant correlation between seizure presence and tumor volume, growth rate, or histological findings. However, the extent of surgical resection significantly influenced outcomes, as determined by the Engel classification system, six months post-treatment. Notably, 76% of patients undergoing maximal resection were seizure-free (Engel class IA) at the 6-month mark, whereas 20% of those with StR achieved Engel class IA, and 46% reached Engel Class IIB during the same follow-up period.

DISCUSSION

In examining the scholarly work pertaining to epilepsy associated with glioma, it is critical to acknowledge the nuanced impact of epilepsy on patients with glioma. Despite the adverse effects on quality of life, several pieces of research have underscored the prognostic significance of epilepsy in individuals with glioma (1). These studies underscore the necessity of further research into the prognostic implications of epilepsy in patients diagnosed with diffuse low-grade glioma, calling for extensive cohort analyses over extended follow-up periods. A notable study, utilizing a large patient cohort (1,509) from a multi-centric French national database, incorporated a variety of variables to explore both spontaneous and post-treatment seizure risk predictors, as well as the prognostic relevance of epileptic seizures. This research aimed to comprehensively assess the impact of epileptic seizures on the diagnosis and progression of diffuse low-grade glioma, in addition to evaluating the influence of such gliomas on the incidence of epilepsy, whether as a preoperative or postoperative condition (1).

Concurrent efforts are directed towards attaining a more profound and intricate comprehension of seizure mechanisms within the glioma context and the innovation of next-generation anti-seizure medications (ASM). Tripathi *et al.*, 2024 (8), delve into the proinflammatory immune dimension of epilepsy, contrasting it with the immunosuppressive milieu observed in patients experiencing glioma-related epilepsy. According to their findings, the epileptic condition characterized by excessive neuronal activity is proposed to encourage immunosuppression, thereby exacerbating glioma progression. The study highlights the IDH1 mutation’s role in metabolic modulation, offering a dual approach to counteracting immune suppression and diminishing seizure occurrences. This insight into the interplay between neuronal activity and immune responses presents a promising avenue for the development of advanced anticonvulsant therapies (8).

Pallud *et al*., 2014 (1) elucidated that risk factors for seizures in the initial stages of glioma are predominantly associated with the tumor's topographic location and its eloquence. Additionally, the presence of other symptoms, such as neurological deficits and increased intracranial pressure, does not favor the control of epileptic seizures, which, paradoxically, may not improve but rather deteriorate over the natural progression of diffuse low-grade glioma. The control of seizures post-treatment is likely influenced by the glioma's topographic characteristics and the extent of surgical resection. Notably, the initial presence of seizures is identified as a positive prognostic indicator for malignant progression-free survival and overall survival.

Avila *et al*., 2017 (9) posited that employing an epilepsy surgery strategy, including preoperative invasive recording in patients with brain tumor-related epilepsy (BTRE), could significantly aid in delineating the epileptogenic zone, potentially leading to enhanced Engel outcomes. Furthermore, for tumors located in the temporal lobe, such as dysembryoplastic neuroepithelial tumor (DNET) or ganglioglioma, a temporal lobectomy coupled with hippocamp-ectomy is suggested to yield a more favorable seizure prognosis than merely excising the lesion.

The approach to surgical management in glioma encompasses a paradigm distinctly different from that applied in focal non-tumor related epilepsy surgery, which often incorporates direct cortical recording or extra-operative invasive techniques. These methods are generally not utilized in glioma surgery, indicating a potential area for further research to ascertain their utility in enhancing seizure outcomes for glioma patients (9).

The exploration of a possible link between molecular markers and epilepsy in glioma remains a critical and imperative field of study. Some research suggests that seizures associated with glioma may not necessarily be triggered by specific tumor characteristics (10). It has been demonstrated that seizures typically originate from the peritumoral neocortex, where infiltrated glioma cells pervade (11).

Recent advancements in the identification of non-invasive pre-operative tumor markers have markedly progressed, poised to revolutionize the rapidly evolving cancer treatment paradigm. Innovations in imaging techniques, such as the apparent diffusion coefficient (ADC), cerebral blood volume (CBV), and the identification of T2FLAIR mismatch, are set to enhance the diagnostic capabilities in the pre-operative phase (12, 13) (fig. 4).

A pivotal concern in the management of gliomas pertains to the progression observed following radiotherapy treatment. This area of discussion is particularly relevant for cases that demonstrate radiological signs of tumor progression at varying intervals after undergoing radiotherapy, with or without surgical excision (14, 15). At this juncture, a critical question arises, one that is essential for determining the subsequent approach to patient care. This question stems from the challenge of distinguishing between radiation necrosis and actual tumor growth or progression, a task that often presents a complex diagnostic dilemma. The body of literature addressing this topic is extensive, highlighting the significance of this dichotomy. Indeed, the issue of differenti-

ating between radiation-induced changes and tumor progression is so critical that it warrants being the focal point of an entire research endeavor (16).

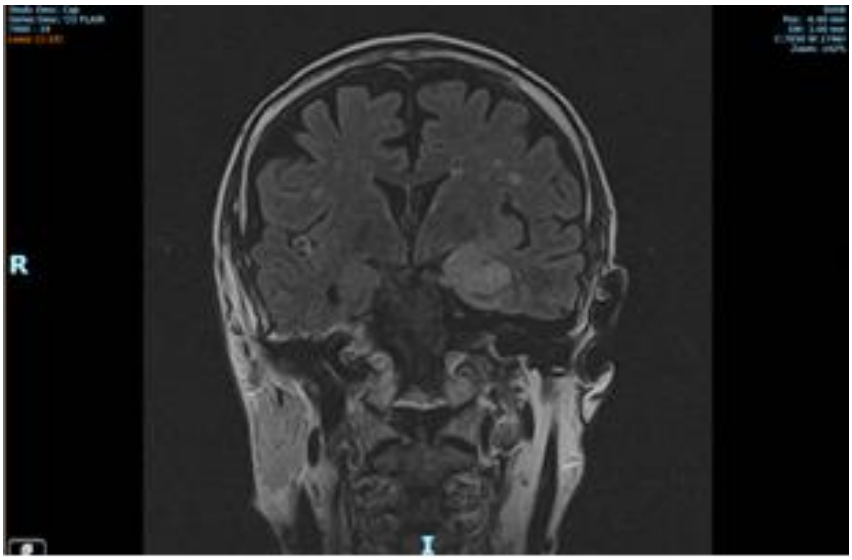

Fig. 4. Coronal FLAIR images of 69M with WHO grade 2 diffuse astrocytoma (Left Temporal Lobe)

CONCLUSIONS

Our examination of existing literature, coupled with a small-scale retrospective study, has provided us with clearer insights into the optimal strategies for addressing the challenging decision of whether and when to surgically intervene in cases of slow-growing low-grade glioma, particularly among relatively young patients exhibiting minimal or no neurological symptoms.

Maximal surgical resection offers not just substantial oncological advantages but also plays a significant role in influencing the epilepsy outcomes for patients diagnosed with diffuse low-grade glioma. A crucial benefit of early and maximally aggressive resection of low-grade tumors lies in the potential for controlling the glioma prior to its progression to a more advanced stage. This approach not only aims to enhance life expectancy but also to improve the quality of life for patients. Furthermore, surgical intervention stands as the sole method for obtaining tumor samples for molecular and histological sub typing, such as determining IDH status. This capability is immensely valuable for more accurately discussing prognoses or tailoring treatment strategies based on the tumor's specific characteristics.

Epilepsy has emerged as a pivotal factor in the intricate and often challenging diagnosis of glioma, serving variously as an early indicator and a quantifiable outcome following surgical resection.

CONFLICT OF INTEREST AND FUNDING

The authors have declared that there are no conflicts of interest in relation to this research, and no funding was secured for the study.

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COMPUTED TOMOGRAPHY *VERSUS* PCR TEST: EVALUATING THE DIAGNOSTIC PRECISION OF CHEST COMPUTED TOMOGRAPHY IN COVID-19 PATIENTS

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COMPUTED TOMOGRAPHY *VERSUS* PCR TEST: EVALUATING THE DIAGNOSTIC PRECISION OF CHEST COMPUTED TOMOGRAPHY IN COVID-19 PATIENTS (Abstract): The **aim** of the study is to analyze the lesions detected on chest computed-tomography in case of patients with suspicion of COVID-19 infection in comparison with RT-PCR (+), exploring the correlation between demographic factors and disease progression. **Materials and methods:** We retrospectively studied 830 patients suspected to have COVID-19 infection, who underwent computed tomography at "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania, from January to December 2021, and found 108 patients with lung lesions consistent with COVID-19 infection. We explored the correlation between demographic factors and disease progression, established CO -RADS classification and the correlation with PCR test. **Results:** Among 108 patients who had chest CT lesions consistent with COVID-19 infection, 92 patients had a positive RT-PCR test. Our study found a slight male predominance (54.63%), with moderate to severe cases more common among males, and an increased incidence in patients over 50, peaking in the 50-59 age group (33.33%). Severe cases being prevalent in the 60-69 age group (9.26%), suggesting a higher risk in these age ranges. Ground glass opacity was the most common CT finding (96.30%), while less common findings included pericardial effusion and halo signs (2.78%). A significant correlation was observed between CO-RADS classification and disease severity, with 53.70% of the most severe cases associated with CO-RADS 5. These findings suggest that CO-RADS classification helps also in assessing COVID-19 severity. **Conclusions:** Patients above 50 years old have a high risk of developing pulmonary lesions in case of COVID-19 infection; most of the patients with severe course were between 50 and 69 years old suggesting this age range is a risk factor. Ground glass appearance was the most common finding depicted on CT, seen in a majority of the cases, the other findings (pericardial effusion, halo sign and reversed halo sign) being less frequent. In our study the majority of the patients that had positive RT-PCR test, as well as severe disease, had also CO-RADS 5, which may suggest a positive correlation between the CO-RADS classification and the severity and the likelihood of having COVID-19 infection. **Keywords:** COVID-19, COMPUTED-TOMOGRAPHY, PULMONARY LESIONS, PCR.

Human coronavirus was first described in the 1960s (1). It was considered a causative agent for intestinal and upper respiratory tract infection both in animals and humans. However, before the outbreak of severe acute respiratory syndrome (SARS) in 2003 in China, it was not considered to be highly pathogenic to humans (2). Coronavirus belongs to the family of *Coronaviridae*, with the subfamily *Coronavirinae*. This member of the family is known to infect a broad range of hosts, resulting in a disease ranging from the common cold to severe and sometimes even fatal illnesses, such as SARS, MERS and, presently COVID-19(3).

This highly contagious infectious disease caused more that 2.9 million deaths worldwide until to April 2021, becoming the most consequential global health crisis in the world had since the influenza pandemic of 1918 (4). Beside the fact that COVID-19 primarily attacks the respiratory system causing cough, sputum production and shortness of breath (which remain the most common symptoms, followed by fever), it also can affect multiple other organs like the pancreas and induce pancreatic injury (producing acute pancreatitis) or even diabetes mellitus in due time (5, 6, 7).

CT scan aided in establishing the diagnosis and determining the severity of the disease, sometimes pulmonary lesions being visible even before the onset of symptoms. Studies found that ground-glass opacity is the most frequent finding on chest CT in COVID-19 infection, accounting for about 86% of the cases, consolidation (29%), mediastinal lymphadenopathy, nodules, cystic changes, and pleural effusion%; other characteristic pattern of the disease is the presence of bilateral lesions (76%), and mostly with a peripheral distribution (33%) (8,9,10).The evaluation of the lesions and CO-RADS staging can be easier if an artificial intelligence program is used (11).

Most of the times associated diseases like the cardio-vascular conditions (appearing frequently after the age of fifty) and the malignant tumors (which are encountered at any age) aggravate all the symptoms, knowing that the immune system is correlated with infections as well as in cancer (12, 13), Patients with diabetes mellitus have an increased risk of developing infections (14,15,16,17,18). Even though they had no prior history of diabetes, some COVID-19 patients acquired insulin resistance and raised blood glucose levels (14, 16, 19).

MATERIALS AND METHODS

Our retrospective study included 830 patients suspected to have a COVID-19 infection because they presented weakness, fever, and a new cough, and underwent computed tomography at “Sf. Spiridon” County Clinical Emergency Hospital, Iasi, Romania, from January to December 2021. Among the analyzed patients, we discovered 108 with age ranges from 19 to 92 years who had on CT pulmonary lesions consistent with SARS-CoV-2 infection; 92 of them were confirmed to have COVID-19 by positive RT-PCR tests.

The patient's data, including the computed tomography images, were collected from the medical records of Sf. Spiridon, Iasi. The CT scan images were reviewed by a radiologist with over 20 years of experience in the field.

For all patients, we analyzed the following demographic characteristics and CT scan findings: gender, age, location, and pattern of the pulmonary lesions. We established a CO-RADS classification, quanti-

fied the severity of the disease (mild, moderate, and severe) and correlated CT images with PCR test.

Computed tomography findings we considered included lung consolidation, ground glass appearance, halo sign, reversed halo sign, crazy paving, pleural effusion, and pericardial effusion.

The majority of the subjects (98) performed a non-enhanced chest CT, and therefore, no special preparation of the subjects was needed.

RESULTS

We evaluated 108 cases (average age 60.2 years), 59 males (54.63%) and 49 females (45.73%) who presented with chest CT lesions consistent with being determined by COVID-19 infection; among them, 92 patients had a positive RT-PCR test, 6 patients had a negative RT-PCR test, and 10 patients had no RT-PCR test documented (N/A). The patients in the study were divided into groups according to age and gender (tab. I).

TABLE I.

Prevalence of the disease correlated to age and gender

Age group	N	Fr (%)	Male	Fr (%)	Female	Fr (%)
19-29	1	0.93%	0	0.00%	1	0.93%
30-39	5	4.63%	3	2.78%	2	1.85%
40-49	14	12.96%	9	8.33%	5	4.63%
50-59	36	33.33%	20	18.52%	16	14.81%
60-69	25	23.15%	11	10.19%	14	12.96%
70-79	18	16.67%	10	9.26%	8	7.41%
80-89	8	7.41%	5	4.63%	3	2.78%
90-99	1	0.93%	1	0.93%	0	0.00%

RT-PCR results according to gender

Among the male patients, 48 patients (44.44%) had a positive RT-PCR test, 4 patients (3.70%) had a negative RT-PCR test, and 7 (6.48%) patients had no RT-PCR test documented. Among the female patients, 44 (40.74%) patients had a positive RT-PCR test, 2 (1.85%) patients had a negative RT-PCR test, and 3 patients (2.78%) had no RT-PCR test documented.

The disease severity according to the CT findings

The severity of the disease was assessed by examination of the chest CT images, evaluating the extension of pulmonary lesions. Accordingly, the patients were divided into 3 groups of severity: mild (30%), moderate (60%), and severe (more than 60%). In some patients, the severity could not be assessed (N/A). 27 patients (25.00%) had mild disease, 29 patients (26.85%) had moderate disease, and 32 patients (29.63%) had severe disease. In 20 patients (18.52%), the severity could not be assessed (N/A). In table II, we summarized the relationship between disease severity and age.

The severity of the disease in relation to the gender of the patients

Analyzing the severity of the disease for each gender revealed that only in cases of mild severity did we encounter more fe-

males (M/F = 10/17 patients); instead, when counting the moderate and severe cases, there were more males involved (M/F = 35/26) (tab. III).

TABLE II.

Disease severity according to the age groups

Age group	Mild N	Mild %	Moderate N	Moderate %	Severe N	Severe N	N/A-N	N/A %
19-29	0	0.00%	0	0.00%	0	0.00%	1	0.93%
30-39	1	0.93%	1	0.93%	2	1.85%	1	0.93%
40-49	6	5.56%	4	3.70%	3	2.78%	1	0.93%
50-59	10	9.26%	12	11.11%	9	8.33%	5	4.63%
60-69	5	4.63%	6	5.56%	10	9.26%	4	3.70%
70-79	3	2.78%	5	4.63%	7	6.48%	3	2.78%
80-89	2	1.85%	0	0.00%	1	0.93%	5	4.63%
90-99	0	0.00%	1	0.93%	0	0.00%	0	0.00%

TABLE III.

Correlation between the severity of the disease and the gender of the patient

Severity	M (n)	Male %	F (n)	Female %
Mild	10	9.26%	17	15.74%
Moderate	17	15.74%	12	11.11%
Severe	18	16.67%	14	12.96%
N/A	14	12.96%	6	5.56%

The pattern of the disease

CT describes the pattern of the lung lesions (ground glass lesions, pulmonary consolidation, reversal halo sign, halo sign, crazy paving) and the associated lesions pleural, and pericardial spaces (pleural effusion, pericardial effusion). The dominant lesion found was the ground glass opacity, present in 104 patients' CT (96.30%), and the second lesion was consolidation (38 patients, 35.19%). Other lesions in descending order are pleural effusion (13 patients (12.04%)), crazy paving (6 patients (5.56%), pericardial effusion, halo sign pattern, and reversal halo sign pattern, which were seen only in 3 patients for each of them (2.78%).

GGO being the dominant lesion seen on CT scans, all the other lesions found were combined with the ground glass appearance (fig. 1):

- 38 patients (35.19%) also had pulmonary consolidation, while the rest, 66 patients (61.11%), had no consolidation.

- 13 patients (12.04%) also had pleural effusion, while 91 patients (84.26%) with a ground glass appearance had no pleural effusion.

- 6 patients (5.56%) also had crazy paving, while 98 patients (90.74%) with a ground glass appearance had no crazy paving.

- 3 patients (2.78%) also had pericardial effusion, a reversed halo sign, and a halo

sign, respectively, while 101 patients (93.52%) with a ground glass appearance did not have such lesions.

Fig. 1. Chest computed tomography: bilateral lung ground glass opacities

CO-RADS classification

The COVID-19 Reporting and Data System (CO-RADS) is a computerized tomography (CT)-based system that assesses the suspicion of lung involvement in COVID-19 pneumonia patients. It should be noted that the confirmation of COVID-19 depends on an RT-PCR test. The CO-RADS classification provides a degree of suspicion for pulmonary involvement depending on the findings seen on a non-contrast enhanced chest CT. The grades range from very low (CO-RADS 1) to very high (CO-RADS 5). In addition, technically insufficient examination is stated as CO-RADS 0, and RT-PCR-proven SARS-CoV-2 infection is stated as CO-RADS 6.

In our study, applying the patients' CO-RADS classification revealed results ranging from CO-RADS 1 to CO-RADS 5. Most of them (58 patients, or 53.70%) had chest CT findings correlated with CO-RADS 5 (fig. 2).

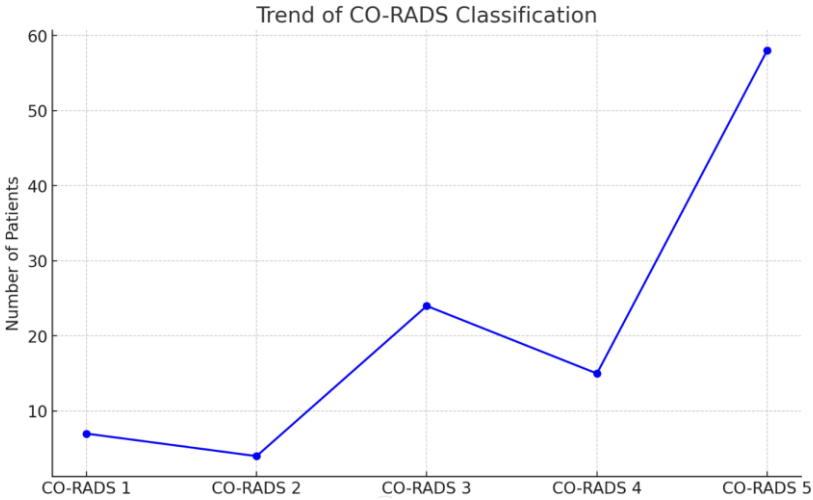

Fig. 2. Line chart illustrates the trend of patient numbers across different CO-RADS classifications

All the patients with CO-RADS 1 and CO-RADS 2 had positive RT-PCR tests. Most of the patients with CO-RADS 3, 4, and 5 had positive RT-PCR tests (23 pa-

tients, 16 patients, and 53 patients, respectively). One patient with CO-RADS 3, one from the group of CO-RADS 4, and six patients with CO-RADS 5 had a negative RT-PCR test. The correlation between CO-RADS score and RT-PCR test results are summarized in tab. IV.

The correlation between CO-RADS score and the severity of the disease

Among the 58 patients with a CO-RADS score of 5, 11 patients (10.19%) had mild disease, 19 patients (17.59%) had moderate disease, and 28 patients (25.93%) had severe disease (tab. V).

TABLE IV.

Summarize CO-RADS score in correlation with the RT-PCR test results

CO-RADS score	n	Freq (%)	Positive RT-PCR	Freq (%)	Negative RT-PCR	Freq (%)	Without RT-PCR test	Freq (%)
1	7	6.48%	2	1.85%	0	0.00%	5	4.63%
2	4	3.70%	2	1.85%	0	0.00%	2	1.85%
3	24	22.22%	19	17.59%	2	1.85%	3	2.78%
4	15	13.89%	14	12.96%	1	0.93%	0	0.00%
5	58	53.70%	55	50.93%	3	2.78%	0	0.00%

TABLE V.

CO-RADS score in correlation with the severity of the disease

CO-RADS score	1	2	3	4	5
N	7	4	24	15	58
Freq (%)	6.48%	3.70%	22.22%	13.89%	53.70%
Mild disease	0	0	10	6	11
Freq (%)	0%	0%	9.26%	5.56%	10.19%
Moderate disease	0	0	3	7	19
Freq (%)	0%	0%	2.78%	6.48%	17.59%
Severe disease	0	0	2	2	28
Freq (%)	0%	0%	1.85%	1.85%	25.93%
N/A	7	4	9	0	0
Freq (%)	6.48%	3.70%	8.33 %	0%	0%

DISCUSSION

The patients' age appears to play an important role, with a clear peak in the age group 50-59, which included 36(33.33%) patients. Studying the relationship between age and the severity of the disease shows that the majority of patients with severe disease are between 50 and 79 years old (26 patients from a total of 32 patients with severe disease), with only 1 patient with severe disease above 80 years old and 4 patients below 50 years old. The age groups 30-39, 50-59, and 70-79 included patients with the three levels of severity, and the distribution of the patients between the levels of severity was almost equal

without a predominant course. While the age group 40-49 had a peak of mild course, the age group 70-79 had a peak of severe course.

The study found a slight male predominance (54.63%), with moderate to severe cases more common among males, whom according to previous studies are considered a risk group. With regard to the severity of the disease in relation to gender, there is a little increase in the severe and moderate cases among the male gender, with 18 male patients (16.67%) versus 14 female patients (12.96%) in the severe course and 17 male patients versus 12 female patients in the moderate course. Furthermore, in the mild course, there is a small peak in the female gender when compared to the male gender, with 17 female patients, representing 15.74% of the total patients, and 10 male patients, representing 9.26% of the total patients.

Evaluating CT scan images in order to determine diseases' severity, we didn't depict notable differences among the three categories: mild, moderate, and severe disease, which represented 27 (25%), 29 (26.85%), and 32 patients (29.63%), respectively, but we also had 20 (18.52%), for whom the severity could not be assessed.

GGO was the most dominant lesion involving the lungs, appearing in 104 patients out of the total 108 patients. The second dominant lesion is pulmonary consolidation, which appears in 38 patients. Next was pleural effusion, seen in 13 patients; a crazy paving pattern was seen in 6 patients; while the other lesions - pericardial effusion, reversal halo sign, and halo sign-were seen in only 3 patients each.

Most of the patients had CO-RADS 5, accounting for 58 (53.7%) from the total 108 patients. The majority of the patients with CO-RADS 5 had positive RT-PCR tests and are 55 in number out of a total of 92 patients with positive RT-PCR tests.

CONCLUSIONS

COVID-19 infection has a slight prevalence among the male gender, most of the patients with moderate and severe courses are males, while the majority of the patients with mild courses are female.

There is a higher incidence in patients above 50 years, and a severe disease appeared above 60 years old, confirming that these age ranges are a risk factor.

Ground glass appearance was the most common finding seen in the CT scan of these patients, seen in a majority of the cases. Less common findings were pericardial effusion, the halo sign, and the reversed halo sign. Our findings show that the majority of the patients who had positive RT-PCR tests as well as severe disease also had CO-RADS 5, which may suggest a positive correlation between the CO-RADS classification, disease severity, and likelihood of having a COVID-19 infection.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research

ADDITIONAL INFORMATION

Patient consent was obtained for presenting this case in a medical publication to contribute to the expansion of the database on this pathology.

The ethics committee of the medical unit has approved the presentation of the case in a medical article in a medical publication while respecting personal data.

Financial relationships: All authors

Other information: All authors have declared that there are no other relationships or activities that have influenced the appearance of the published work.

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UNVEILING THE IMPACT OF INTRAUTERINE ADHESIONS ON INFERTILITY: A COMPARATIVE JOURNEY THROUGH PRIMARY AND SECONDARY INFERTILITY PATIENTS

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UNVEILING THE IMPACT OF INTRAUTERINE ADHESIONS ON INFERTILITY: A COMPARATIVE JOURNEY THROUGH PRIMARY AND SECONDARY INFERTILITY PATIENTS (Abstract): Intrauterine adhesions (IUAs) represent an important cause of infertility, having various effect in patients with primary and secondary infertility. The objective of the study is to evaluates the clinical, demographic, and intraoperative characteristics of patients with IUAs, focusing the correlation between hysteroscopic and laparoscopic tubal patency assessment and infertility type. **Materials and methods:** A retrospective descriptive study was conducted between 2016 and 2024 at two hospitals in Iasi, Romania: Clinical Hospital of Obstetrics and Gynecology "Elena Doamna" and "Cuza-Vodă". Medical records identified 134 patients with IUAs, of whom only 102 patients met the inclusion criteria and divided in two groups: primary and secondary cohorts. All patients underwent hysteroscopic adhesiolysis to restore the anatomy of uterine cavity, with perioperative tubal patency evaluation and confirmed laparoscopically in most cases. **Results:** Patients with IUAs and primary infertility were younger (31.66 *vs.* 34.13 years; p=0.023) and predominantly aged 25-34 years, whereas IUAs patients and secondary infertility was more common in those over 35 years. Secondary infertility subjects showed higher rates of repetitive dilation and curettage (77%, p=0.001), ectopic pregnancies (6.6%, p=0.04). Hysteroscopic and laparoscopic tubal patency revealed a higher patency rate in secondary infertility cases (90.2%) compared to primary infertility cases (73.2%, p=0.038). Bilateral positive patency through the laparoscopic Dye test was more common in primary infertility cases (61% *vs.* 41%, p=0.029). This study highlights distinct clinical and intraoperative characteristics between primary and secondary infertility in patients with AIUs. Secondary infertility was significantly associated with higher rates of previous D&Cs, increased hysteroscopic and laparoscopic tubal patency rates and ectopic pregnancies. **Conclusions:** This study emphasize the importance of tailored diagnostic and therapeutic approaches to optimize outcomes for patients with IUAs and infertility. **Keywords:** INTRAUTERINE ADHESIONS, HYSTEROSCOPY, HYSTEROSCOPIC ADHESIOLYSIS, LAPAROSCOPY, TUBAL PATENCY, INFERTILITY, ENDOMETRIAL RECEPTIVITY.

INTRODUCTION

The endometrium represents the internal layer of the uterus that is highly specialized for embryo implantation and sensitive to hormonal synthesis during the endometrial cycle. Ovarian hormones are essential for endometrial changes. If a pregnancy occurs during the window of implantation, the intrauterine microenvironment suffers specific changes due to progesterone secretion during the secretory phase to become more receptive for the implantation (1, 2). Numerous growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor are involved in the secretory phase, establishing changes in the proliferative endometrium, to become better vascularized and to remodel the maternal spiral arteries in order to decrease the vascular resistance and to increase the uteroplacental blood flow. The interplay between embryo and immune system is complex. The natural killer cells, cytokines and chemokines presents autocrine or paracrine properties and modulates the immune interaction in between to protect both the mother and the embryo but also to limit the trophoblastic invasion for a favorable implantation (3, 4) Modulatory factors like interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta) regulates the proliferation, migration and trophoblastic invasion and poses antiproliferative signals at the level of the uteroplacental interface to create a tolerant microenvironment. Genetic factors play an important role in endometrial repair conditions. Multiple genes and proteins have been implicated in cellular adhesion process and endometrial remodeling when pregnancy occur. Endocrine, neuronal, metabolic, immunologic, and mechanic factors harmonize together to develop the fertilized egg into a blastocyst and later to become an embryo but also, to establish a mature endometrium that allows proper implantation and following advance of early pregnancy (1, 2, 3).

The mechanisms by which the intrauterine adhesions (IUAs) affect the implantation of a pregnancy are not fully understood nor the factors implicated in its pathogeny. The precise process underlying modifications in endometrial repair is poorly knew. Understanding the molecular and cellular mechanisms activated following intrauterine surgery or trauma which are engaged in the endometrium regenerative pathways is essential since are playing a key role in endometrial receptivity, fertility or pregnancy complications (2). The effect of a mechanical trauma or an infectious process at the level of the endometrial basal layer may lead to blastocyst implantation failure, lessen of feto-uterine blood flow near the beginning or recurrent miscarriages. Intrauterine adhesions appear due to a disproportionate limited healing course that determines the rise of fibrous, thin or dense strips between the uterine walls which can induce partial or full obliteration of endometrial cavity or / and cervical canal.

The incidence and prevalence vary among population studied or geographic areas; The real incidence of IUAs is actually undetermined due to a sizeable number of cases that lack of symptoms or patient's neglection; besides, the paucity of diagnosis tools decreases the diagnosis rate in different geographic areas (5).

Any method of endometrial injury can be a cause for intrauterine adhesion formation. The etiology is multifactorial but the uterine curettage for different pathologies like miscarriage, abortion, postpartum placental fragments retention or curettage in non-pregnant patients are the most im-

plicated (6, 7, 8, 9). Others may be caesarean section, B-lynch suture, Bakri balloon application, hysterotomies, hysteroscopic surgery (10, 11), due to mechanical trauma of the endometrial tissue, uterine artery embolization for leiomyoma, endometritis or pelvic inflammation (4, 5, 12). The mechanical factors explain how intrauterine adhesions can affect the remodeling and maturation of the endometrium during the secretory phase. The damage of cellular elasticity and cytoskeletal architecture of the basal layer, resulting in endometrial fibrosis or adhesions formation, influence the adequate vascular reorganization, an essential stage for trophoblastic invasion. Genetic factors play an important role in endometrial repair conditions.

The objective of the study is to analyses the clinical and demographic characteristics, patient's history, and intraoperative findings in individuals with IUAs and infertility, aiming to summaries the etiology and risk factors and to identify features that influence diagnosis, management, and reproductive outcomes. A secondary objective is to corelate the hysteroscopic and laparoscopic tubal patency, attempting to set the best therapeutic approach for these patients considering future fertility.

MATERIALS AND METHODS

We conducted a retrospective study, during a 9-year period, between 2016-2024 in two hospitals, "Elena Doamna" Clinical Hospital of Obstetrics and Gynecology and "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology from Iasi, Romania.

The patient's medical data was collected from the medical sheet and reviewed with the medical history of the subjects. The primary selection of cases included 134 patients diagnosed with intrauterine adhesions of which only 102 patients associated infertility and were elected for the study. The study group was later divided in two study cohorts according to the type of infertility, primary or secondary. Other inclusion criteria are: patients that provided an informed consent with age between 18-45 years and hysteroscopic diagnosis of intrauterine adhesion. The exclusion criteria were applicable in cases with unsigned consent, and incomplete follow-up. The study adhered to the principles of the Declaration of Helsinki and received ethical approval from the committees of the participating hospitals, "Elena Doamna" Clinical Hospital of Obstetrics and Gynecology and "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology from Iasi, Romania.

The study was approved by the Hospital Institutional Review Board of both Clinical Hospitals "Elena Doamna" (approval no. 389/13.01.2023) and "Cuza-Vodă" (approval no. 13.881/15.12.2020), as well as from the Ethics Committee of ''Grigore T. Popa'' University of Medicine and Pharmacy Iasi (approval no. 402/15.02.2024). The study's design and data management conformed to both national and international guidelines for human participant research.

In all cases hysteroscopic adhesiolysis was performed to reestablish the anatomy of uterine cavity (13). During the hysteroscopy, a perioperative tubal evaluation was performed, considering the ''air bubble technique'' assessment. The hysteroscopic tubal patency was confirmed by the laparoscopic tubal patency test using blue methylene in almost all cases (5).

The statistical analysis used *SPSS* software, version 18.0 and, at first, data were arranged and summed up for interpretation; after that an analysis was made to deduce

supplementary indicators using comparative statistical approaches. To postulate the statistical results in our study, a representative specimen size of women from Iasi population was elected (n=394,274) and calculated, conceding an estimated sample of 136 persons, with an 8.4% sampling miscalculation. The limits of error arrange nearly with the values found in the literature, and the specimen size selected resembles with the number of patients selected from the two hospitals from Iasi, in 2024.

RESULTS

The distribution of age among included patients is homogeneous, with mean and median values closely aligned (33.14 *vs.* 33 years), and a Skewness test result of 0.039, indicating suitability for applying significance tests to continuous variables (fig. 1).

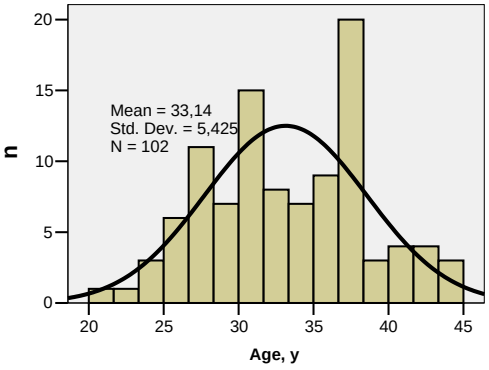

Fig. 1. The histogram of patient's age diagnosed with intrauterine adhesions and infertility

The average age was marginally lower among patients with primary infertility (31.66 compared to 34.13 years; p=0.023), with an age range of 21 to 45 years (Fig. 2). In terms of accessibility within the analyzed group for specialized medical services, a higher rate of 61.8% of the patients originated from urban areas compared with the rural area, but were found no statistically significant differences regarding the infertility type: 56.1% of patients had primary infertility, and 65.6% has secondary infertility (p=0.335).

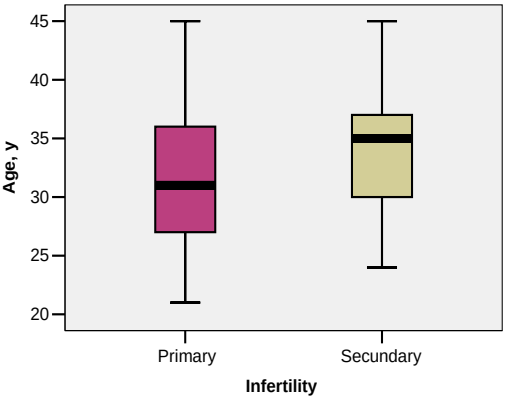

Fig. 2. Comparative mean age across study groups: primary and secondary infertility

Patients with primary infertility were most commonly identified within the 25-34 age group (61%), whereas the ones diagnosed with secondary infertility were mostly encountered among individuals aged over 35 years (50.8%). Preoperative diagnosis supposition according to clinical symptoms was observed in 17.3% of all primary infertility cases and 61% of patients with secondary infertility (p=0.001). Ultrasonographic diagnosis, using transvaginal two-dimensional ultrasonography (2D-US) or three-dimensional ultrasonography (3D-US) was established in 26.9% of patients with primary infertility and 68.3% of those with secondary infertility (14, 15, 16). Among patients diagnosed with intrauterine adhesions and secondary infertility, 32.8% had a history of more than two dilation and curettage (D&C) procedures (p=0.001) (tab. I).

Gynecological history revealed no statistically significant differences in the prevalence of intrauterine adhesions between types of infertility (p>0.05). Still, in patients with IUA and secondary infertility, the D&C (77%, p=0.001) and caesarean section (21.3%; p=0.001) were performed with significantly higher frequency (17) (tabs. I, II).

TABLE I.

The structure of infertility cases based on obstetrical history

Obstetrical history	Primary infertility		Secondary infertility		Chi square test
	No.	%	No.	%	
Intrauterine adhesions					
No. of D&C					
0	41	100	14	23.0	
1	-	-	27	44.3	0.001
2	-	-	15	24.6	
3	-	-	5	8.2	

TABLE II.

The structure of infertility cases based on gynecological cases history

Gynecological history	Primary infertility		Secondary infertility		Chi square test
	n	%	N	%	
Classic myomectomy	0	0.0	2	3.3	0.355
Laparoscopic myomectomy	0	0.0	1	1.6	0.598
Hysteroscopic myomectomy	1	2.4	2	3.3	0.647
Hysteroscopic polypectomy	0	0.0	0	0.0	Nc
Hysteroscopic septoplasty	1	2.4	0	0.0	0.402
Hysteroscopic adhesiolysis	0	0.0	1	1.6	0.598
Uterine arteries embolization for uterine myoma	1	2.4	1	1.6	0.645

Nc= Not computed

The American Fertility Score (AFS) system for intrauterine adhesions was used to correlate the cases of intrauterine adhesion and infertility. A higher number of D&C was significantly associated with AFS stage 2 (tab. III) (17).

TABLE III.

The correlation between the AFS stage and the number of uterine curettages

AFS stage (p=0.001)	Number of D&C			
	0 (n=55)	1 (n=27)	2 (n=15)	3 (n=5)
1	41 (74.5%)	11 (40.7%)	3 (20.0%)	1 (20.0%)
2	13 (23.6%)	14 (51.9%)	11 (73.3%)	4 (80.0%)
3	1 (1.8%)	2 (7.4%)	1 (6.7%)	-

D&C= dilation and curettages

TABLE IV.

Hysteroscopic and laparoscopic tubal patency assessment -intraoperative findings

Intraoperative aspects	Primary infertility (n=41)		Secondary infertility (n=61)		Chi square test
	N	%	N	%	
Laparoscopic tubal patency test	41	100.0	60	98.4	0.598
Laparoscopic Dye test					
Bilateral positive (++)	25	61.0	25	41.0	
Unilateral positive (+)	4	9.8	5	8.2	0.029
Bilateral negative (--)	1	2.4	2	3.3	
Not tested	11	26.8	29	47.5	
Hysteroscopic tubal patency assessment	30	73.2	5	90.2	
Bilateral patent TO Unilateral patent TO	6	14.6	5	8.2	0.038
Bilateral negative patent TO	5	12.2	1	1.6	
History of ectopic pregnancy	0	0.0	4	6.6	0.040

TO= Tubal ostia

The hysteroscopic perioperative tubal assessment indicated that patient's fallopian tubes were largely patent, when using the "air bubble" technique, with tubal ostia showing a predominant patency rate of 73.2% in primary infertility cases compared to 90.2% in secondary infertility cases (p=0.038) (5). The hysteroscopic tubal patency was confirmed by the laparoscopic tubal patency test in most cases, using blue methylene. In all cases of IUAs with primary infertility the tubal patency was evaluated via dye diffusion with methylene blue during the laparoscopic surgery. In comparison, tubal patency assessment was conducted in 98.4% of patients with secondary infertility.

Bilateral positive tubal patency was most frequently observed though the laparoscopic Dye test, with rates of 61% in primary infertility cases compared to 41% in secondary infertility cases (p=0.029). Still, ectopic pregnancy was identified only in 4 cases (6.6%; p=0.04) within patients with secondary infertility (tab. IV).

DISCUSSION

Patients diagnosed with IUAs who undergo hysteroscopic adhesiolysis exhibit an increased risk of infertility and complications during pregnancy. The elevate rate of infertility observed in this population are likely associated with diminished endometrial receptivity. This downturn of endometrial receptivity may result from damage to the basal layer of the endometrium mucosae or from the recurrence of the IUAs following adhesiolysis. However, the precise mechanisms underlying these outcomes remain incompletely understood. The incidence of IUAs varies based on the demographic area studied and the etiology beyond.

Among a cohort of 102 patients with IUA, a significant difference in mean age was identified between those with primary infertility and those with secondary infertility. Specifically, patients with primary infertility had a mean age of 31.66 years, compared to 34.13 years in the secondary infertility group (p=0.023). Compared to existing literature, the mean age in both groups in this study was slightly lower than previously reported averages from other studies (18).

These findings emphasize the evidence that IUA may represent a potential risk factor for infertility and pregnancy complications. The observed age disparity between two groups noticed in our study is particularly noteworthy as may reflect the earlier clinical presentation and diagnosis of IUA with primary infertility (18). Furthermore, the influence of geographic and etiologic factors in the incidence of IUA sustain the IUA pathology. In out study, the higher proportion of patients from urban areas compared to rural areas indicate that better accessibility to healthcare, diagnostic devices and management options in urban regions facilitates earlier detection and treatment options. The lower incidence of our groups compared with other geographic areas with lessen access to medical assistance from the literature can be interpreted as requirement for area-specific prophylactic programs for IUA and infertility (11).

The notable difference in the preoperative diagnosis based on clinical symptoms between patients with primary and secondary infertility (17.3% *vs.* 61%, p=0.001) aligns with the earlier studies that secondary infertility is more likely to involve identifiable risk factors, such as previous intrauterine surgeries, which could prompt clinical suspicion of IUAs (2). Two-dimensional (2D) and three-dimensional (3D) transvaginal ultrasonography (US) aligns with the general understanding, revealing an increased diagnosis rate for IUAs in secondary infertility group (68.3%) compared to primary infertility group (26.9%) (10).

The incidence of dilation and curettage (D&C) confirmed the already known risk factor for IUAs developing. In patients with IUAs and secondary infertility, D&C and caesarean section were frequently performed (77%, p=0.001 as well as 21.3%, p=0.001) which suggests a greater like hood of these procedures for secondary infertility patients and can explain the elevated prevalence of IUAs observed in this group. These results emphasize the literature findings about IUAs development which suggest that a history of uterine trauma through surgical interventions plays a decisive role in the IUAs pathogenesis and impacts reproductive performance. The correlation of AFS system stage 2 and repeated D&C among secondary infertility group reveals how repeated mechanical trauma impacts the IUAs constitution, but also a possible cumulative effect. The repetitive D&C procedures correlate with a severe IUAs or Asherman syndrome as the literature showed already, this article highlights the idea that a prudent and minimal invasive management of intrauterine procedures or surgeries is crucial for to preserve uterine anatomy and function, and further, the fertility potential of the patient (10, 19).

The differences towards tubal patency assessment between primary and secondary patients with IUAs reiterate other studies which suggested that secondary infertility is more frequent correlated with partial or maintained fallopian tubes function even when the IUAs are associated. Our study showed an increase rate of bilateral tubal patency indicating that tubal functionality may be preserved in this group even though IUAs are associated. Moreover, these findings propose that the primary cause of infertility in these patients is rather related to endometrial receptivity disruption than corelated to tubal dysfunctionality. On the other hand, the decrease tubal patency rate found within secondary infertility group can correlate with an increase incidence of anterior uterine interventions or infections that can cause partial tubal occlusion or localized tubal damage. Moreover, the ectopic pregnancies found within the secondary infertility group highlight the feasible impact of IUAs and other prior uterine

interventions or infections which may cause damage of tubal function.

Using hysteroscopy simultaneously, in the same operative time with the laparoscopy for tubal patency appraisal in infertility patients highlights the weight of an inclusive management based on the infertility type but further studies need to investigate the prognostic value of these two methods together for tubal patency diagnostic and their impact on reproductive performance in IUAs patients. Currently, there are new challenges in the treatment of endometrial pathology, involving innovative approaches and opening new perspectives that can impact both the quality of life and patients' response to infertility (20-23).

CONCLUSIONS

Patients with IUAs and primary infertility are more likely to be associated with endometrial or implantation factors, while secondary infertility often involves tubal compromise influenced by prior interventions. The surgical history has a crucial role in the development and progression of IUA, as well as its impact on tubal patency and overall fertility outcomes. The integration of hysteroscopic and laparoscopic evaluations proved essential for accuracy diagnosing IUAs and assessing tubal function helping to individualize the treatment and reducing the risk like ectopic pregnancies, especially in patients with secondary infertility.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest related to the publication of this article.

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INCIDENCE OF BREAST NEOPLASM ASSOCIATED WITH PULMONARY THROMBOEMBOLISM: A RETROSPECTIVE SINGLE CENTER STUDY

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BREAST NEOPLASM IN PATIENTS WITH PULMONARY THROMBOEMBOLISM: A RETROSPECTIVE STUDY (Abstract): This study **aims** to evaluate the incidence of pulmonary embolism among breast cancer patients, emphasizing its correlation with the histological types, cancer staging, and thrombus characteristics **Materials and methods:** We retrospectively analyzed 211 patients diagnosed with pulmonary thromboembolism (PTE) using contrast-enhanced chest computed tomography at "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, from the 1st of January 2023 to 31st of November 2024. Patients with previous diagnosis of breast cancer were identified, and data including demographic details, breast cancer stage, histopathological findings, and thrombus localization were collected. **Results:** The incidence of breast cancer associated with PTE, found in our cohort was 4.73% (n=10), with a majority of postmenopausal women (80%), a median age of 55 ± 6.96 years and an interquartile range (IQR) of 9.25 years. Incidence by cancer stage was as follows: stage I -0.47 % (n=1), stage II - 0.94% (n=2), stage III - 0.94% (n=2), stage IV - 2.36% (n=5). The incidence was higher among patients with stage IV cancer (2.47%) compared to stage I-III (0.5-1%). Histologically, 9 patients (4.26%) were diagnosed with invasive ductal carcinoma (IDC), while only 1 patient (0.47%) had invasive lobular carcinoma (ILC). Studying thrombus localization we found saddle thrombus in one case (0.47 %), lobar artery thrombi in 2 cases (0.94 %) and 7 patients (3.31 %) with segmental and subsegmental arteries thrombosis. None of the patients had PTE preceding breast cancer diagnosis, all PTE occurred after treatment, with acute PTE being found in 2 cases - 0.94%, while the other 8 (3.79%) were diagnosed as chronic thrombosis **Conclusions**: Pulmonary thromboembolism was observed in 4.73% of breast cancer patients, with a majority of postmenopausal women, a higher prevalence in patients with advanced-stage disease, all PTE occurring after treatment, most of them being chronic thrombosis. The study's limitations include a small study group and study duration. **Keywords:** PULMONARY THROMBOEMBOLISM, BREAST CANCER, COMPUTED TOMOGRAPHY, MALIGNANCY-ASSOCIATED THROMBOSIS.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy in women worldwide and the leading cause of cancer-related mortality in females, according to World Health Organization (WHO) (1). Given its high prevalence, the correlation between breast cancer and thromboembolic events, such as PTE, has become a significant clinical concern (2).

PTE is a life-threatening complication and a major cause of morbidity and mortality, with an annual incidence of 39-115 cases per 100,000 individuals (3). PTE is frequently associated with malignancy, particularly due to cancer-induced hypercoagulability and cancer-related treatments, which increase thromboembolic risk by four to seven times compared to the general population (2, 4, 5).

Certain malignancies, such as pancreatic, ovarian, brain, stomach, gynecological, and hematological cancers, carry a high risk for thromboembolism, while lung and colorectal cancers pose an intermediate risk. Breast cancer is traditionally considered to have a lower risk of thromboembolism than these malignancies; however, literature on this topic remains limited (2, 6).

Imaging plays an important role in the evaluation and management of PTE and contrast-enhanced chest computed tomography remains the golden standard for diagnosis (7, 8).

MATERIALS AND METHODS

We conducted a retrospective study using electronic medical records from "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, database analysing patients from the1st of January 2023 to 31st of November 2024, and selected a number of 211 patients diagnosed with PTE. Patients were included in this study if they had histologically confirmed breast cancer and were diagnosed with PTE through contrast enhanced chest computed tomography. SPSS version 29.0 was the software for the statistical analysis. We used descriptive statistics to calculate mean, standard deviation, median, and interquartile range.

Computed tomography pulmonary angiography (CTPA) was performed with a Philips Incisive 64 detector CT with the following protocol: cranio-caudal thoracic scan from the chest aperture to the costophrenic angles, in post inspiratory apnea, beginning with a noncontrast enhanced scan; bolus tracking technique; up to 100 mL (1 mL/kg body weight) iodinated contrast medium (concentration of 370-400 mg iodine/mL) is injected intravenously with an automatic injector, with the speed of 3-4 mL/second (s), followed by 50 mL of saline chaser with the same speed. The delay for the arterial phase scan is 7s after the density in the pulmonary artery reaches 180 HU (Hounsfield Units) and the venous phase is performed at 20 s after the arterial phase (9).

RESULTS

This study aims to provide an in-depth analysis of the correlation between breast cancer and pulmonary thromboembolism through a retrospective observational study. We conducted a retrospective study using electronic medical records from hospital database analyzing patients from the 1st of January 2023 to 31st of November 2024, and selected a number of 211 patients diagnosed with PTE; among them 34 have previously been diagnosed with malignancies, 19 with digestive malignant tumors, 10 with breast cancer and 5 with lung cancer. The incidence of breast cancer associated with PTE found in our study was

4.73% (n=10), less frequently associated with PTE compared to digestive cancers (n=19, 9%) and more frequently compared to lung cancer (2.36%). Among the patients diagnosed with breast cancer, 80% (n=7), were postmenopausal women, while premenopausal women were 20% (n=3). The mean age was 55**,** with standard deviation (SD) 6.96 years and an interquartile range (IQR) of 9.25 years.

The breast cancer stage analysis in our study group was as follows: stage I - 0.47 % (n=1), stage II - 0.94% (n=2), stage III - 0.94% (n=2), stage IV - 2.36% (n=5). This data indicates a higher prevalence of pulmonary embolism among stage IV breast cancer patients (2.36%) compared to those in earlier stages (0.5-1%) (fig. 1).

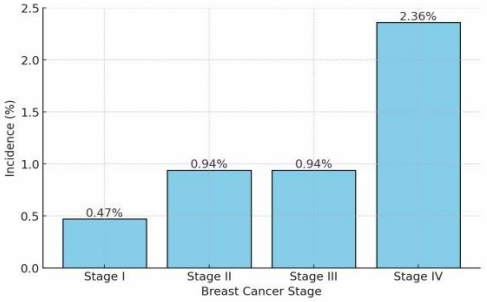

Fig. 1. Breast cancer associated with PTE - stage analysis

Among breast cancer cases, 9 patients (4.26%) were morpho pathologically diagnosed with IDC, while only 1 patient (0.47%) had ILC; these findings are probably related to the frequency of IDC versus ILC, not with the higher tendency of the first type of cancer to be related with PTE (10, 11).

From the perspective of thrombus localization, we found saddle thrombus in one case (0.47 %), lobar artery thrombi in 2 cases (0.94 %) and 7 patients (3.31 %) with segmental and subsegmental artery thrombosis (fig. 2).

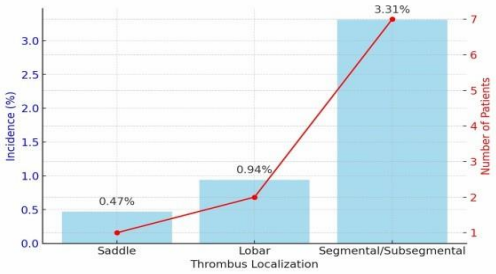

Fig. 2. Breast cancer associated with PTE - thrombus localization

The most important comorbidities in the breast cancer study group were obesity (n=7, 3.31%), heart conditions including ischemic heart disease (n=5, 2.36 %), diabetes (n=2, 0.94%) vasculitis and deep vein thrombosis in the lower limb (n=1, 0.47%) (12, 13).

None of the patients had PTE preceding breast cancer diagnosis, all cases of PTE occurred after treatment, acute PTE was found in 2 cases - 0.94% (the saddle thrombus and one of the lobar artery thrombosis), the other 8 (3.79%) being chronic thrombosis. Considering these two findings we can assume that the thromboembolic risk associated with breast cancer is not necessarily connected to cancer-induced hypercoagulability but rather with cardio-vascular risk factors and cancer-related treatments (14).

DISCUSSION

Our findings align with existing literature indicating that malignancy-associated thrombosis is a well-recognized complication (2, 5, 15). The observed 4.73% incidence of breast cancer patients associated with PTE falls within the range of reported data in the literature for malignancy-associated thrombosis (0.13%-8.65%) which

is higher compared with the incidence of PTE in patients without malignancies (0.11%) (16, 17, 18). PTE incidence that we found is in the middle of the literature range probably because malignancy was found to be associated with PTE particularly in men (4, 19, 20, 21, 22, 23, 24) and our study was exclusively comprised of women.

Moreover, although breast cancer is the number one malignancy in women according to WHO, in our study, digestive cancers (n=19, 9%) were found to be more frequently associated with PTE compared to breast cancers (n=10, 4.73%) This could be attributed to factors such as delayed detection, aggressive tumor type, and distinct thrombotic mechanisms involved in gastrointestinal malignancies.

The most frequent comorbidity in this breast cancer study group was obesity (7 cases) associated in 2 cases with diabetes, both diseases being related to heart conditions and deep vein thrombosis which are associated with a high risk of PTE even without the presence of malignancy and cancer-related treatments (25).

This study is subject to certain limitations, including a relatively small number of breast cancer patients diagnosed with pulmonary embolism, from a single center point of view and a short study duration (two years), which may not fully capture long-term trends and potential biases related to retrospective data collection.

While promising, further validation with a larger cohort and additional predictive variables is required to improve specificity and clinical applicability for PTE in breast cancer patients.

CONCLUSIONS

This retrospective study highlights the notable incidence of 4.73% of PTE in breast cancer patients, with a majority of postmenopausal women, a higher prevalence in patients with advanced-stage disease, all PTE occurring after treatment, most of them being chronic thrombosis. The study's limitations include a small study group and study duration. While breast cancer remains a leading malignancy in women, its association with thromboembolic events needs further research and increased clinical awareness. Future studies with larger sample sizes and multi-institutional data may provide deeper insights into the pathophysiology, and management of breast cancer-associated PTE.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflicts of interest. This research received no external funding.

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MEDICINAL LEECH THERAPY IN DIGITAL REPLANTATION

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MEDICINAL LEECH THERAPY IN DIGITAL REPLANTATION **(**Abstract): Venous congestion represent a complication in reconstructive microsurgery, particularly in digital replantation and revascularization procedures, where achieving venous outflow may be technically impossible. In these cases, medicinal leech therapy (MLT) offers a biologically active method to decompress congested tissues and improve tissue survival. **Material and methods:** The study included a group of 35 patients that was used treated with MLT for venous congestion following digital replantation. Key variables included timing of therapy initiation, treatment duration, leech application frequency, complications, and salvage outcomes**.** Additionally, we conducted a systematic literature search in PubMed, Scopus, and Google Scholar for studies published between January 2014 and March 2024. Following PRISMA guidelines, we included case reports, series, and clinical studies reporting MLT use in digital replantation or revascularization. **Results:** Our cohort achieved an overall tissue salvage rate of 88.6% (31 of 35 cases), with four failures due to arterial injury. From 134 identified records, 10 studies met inclusion criteria. In the literature, MLT was started within 24-48 hours of congestion onset, lasting 4-7 days, with 2-5 leeches applied daily at 2-4-hour intervals. Reported salvage rates ranged from 66% to 85%. Complications included *Aeromonas hydrophila* infections and anemia requiring transfusion. **Conclusions:** MLT remains an effective, evidence-supported adjunct for venous congestion management after digital replantation, particularly when venous anastomoses are not feasible. A standardized protocol based on recent data may improve outcomes and safety in microsurgery. **Keywords:** VENOUS CONGESTION, DIGITAL REPLANTATION, MEDICINAL LEECH THERAPY, MICRO-SURGERY.

INTRODUCTION

Venous congestion is a common and potentially devastating complication in microsurgery and reconstructive surgery. It arises when venous outflow from a transferred or reattached tissue segment is insufficient, leading to blood stasis, tissue edema, increased interstitial pressure, and ultimately ischemic necrosis. This scenario is frequently in digital replantation. While arterial inflow can typically be reestablished through microsurgical techniques, venous outflow remains more technically challenging-especially in cases with small-caliber veins, avulsion or crush injuries, or irradiated recipient beds. Although microsurgical venous anastomoses are the gold standard, they are not always feasible due

to anatomical or intraoperative limitations, such as thrombosis (1). In such high-risk cases, medicinal leech therapy (MLT) has reemerged as a valuable, biologically active adjunct. Reintroduced into modern clinical practice in the 1980s by Foucher *et al.* for digital replantation, MLT has since been used in various reconstructive procedures at risk for venous congestion (2). Leeches (*Hirudo medicinalis*) relieve congestion through both mechanical blood extraction and biochemical effects from their saliva, which contains hirudin, calin, vasodilators, and anti-inflammatory agents (2). These promote sustained bleeding, prevent clotting, and enhance microcirculation. Despite growing evidence of its efficacy-reporting salvage rates of 70% to 85%-MLT remains underutilized, partly due to concerns over infection (notably *Aeromonas hydrophila*), labor-intensive care, and lack of standardized protocols (3-6). This review evaluates clinical evidence from 2014 to 2024 to clarify the current role of MLT in reconstructive surgery, emphasizing indications, outcomes, and practical recommendations for safe implementation.

MATERIALS AND METHODS

To evaluate the clinical impact of medicinal leech therapy (MLT) in modern reconstructive surgery, this study employed a two-part approach.

Retrospective Study. The study included 35 patients who underwent MLT for venous congestion following digital replantation at our center. Informed consent and psychological counseling regarding the use of leeches were obtained prior to treatment. All patients sustained traumatic digital amputations and underwent microsurgical replantation. Data extracted from electronic records included demographics (age, sex), comorbidities (e.g., diabetes, smoking status), injury mechanism, surgical details, timing and characteristics of venous congestion, and MLT specifics (timing, number and frequency of leeches, duration). Outcomes assessed were tissue salvage (defined as survival of the digit without reoperation or amputation), complications (infection, anemia, allergic reactions), and need for further interventions. MLT followed a standardized protocol: clinical diagnosis of congestion (aided by Doppler or oximetry), area cleansing with sterile saline, application of *Hirudo medicinalis* for 20-45 minutes, followed by post-detachment care with heparin-soaked gauze. Leeches were reapplied every 2-4 hours depending on severity, averaging 3 per day, until congestion resolved.

Literature Review. A structured narrative review was conducted following PRISMA guidelines, targeting studies published between January 2014 and March 2024. We searched PubMed, Scopus, and the Cochrane Library using MeSH terms and keywords including “leech therapy”, “plastic surgery”, “reconstructive surgery”, “digital replantation”, “venous congestion”, and “*Aeromonas hydrophila*”. Reference lists were screened manually for additional relevant articles.

Inclusion criteria were: adult human subjects receiving MLT for venous congestion in reconstructive or plastic surgery; clinical outcomes reported (e.g., tissue salvage, complications, transfusions); peer-reviewed English publications; all study types (case reports, series, observational studies). Exclusion criteria included pediatric cases, non-clinical reports, editorials, and non-English articles without accessible

translations. Duplicates and overlapping datasets were excluded.

A structured data extraction framework was used to capture: indications, timing of therapy, duration, frequency and number of leeches, antibiotic use (typically ciprofloxacin or TMP-SMX), anticoagulation, supportive care (e.g., transfusion), and outcome measures. Complications, particularly *Aeromonas hydrophila* infections, were a key focus (7-9). Other issues such as anemia, leech migration, allergic reactions, and psychological intolerance were also noted.

Due to heterogeneity in study design, protocols, and outcome measures, no meta-analysis was performed. Instead, findings were synthesized descriptively, allowing thematic analysis of trends, variations, and practical approaches. Institutional differences in technique-site preparation, leech placement, containment, and post-carewere documented to highlight real-world variability. This methodology reflects clinical reality and aims to guide evidence-informed decision-making in complex reconstructive scenarios. By consolidating a decade of experience, we offer practical insights into the use of MLT in contemporary microsurgery.

RESULTS

Retrospective study. Among the 35 patients, 10 cases (29%) involved amputations at the first phalanx (F1) level. In five of these, both arterial and venous anastomoses were performed; in the other five, only arterial repair was feasible. Venous congestion occurred early in two cases without venous repair and on postoperative day 3 in three cases due to venous thrombosis. Second phalanx (F2) amputations were recorded in 19 patients (54%). Arterial repair was achieved in all cases, but venous anastomosis was feasible in only six. In the remaining cases, venous outflow was compromised due to lack of suitable vessels.

Interrupted sutures were used sparingly to reduce trauma during skin closure. The remaining six cases (17%) involved distal phalanx (F3) amputations, where both arterial and venous repair were technically unfeasible due to small vessel caliber and extensive trauma. Replantation was limited to bony fixation and skin closure.

In all cases, a pulp incision with topical heparin was used to enhance passive venous drainage. Patients received 2-4 leeches daily for 4-7 days, with an average of three per day. Hemoglobin dropped below 9 g/dL in four F1-level cases, but no transfusions were needed. No patients experienced psychological intolerance to leech therapy. Overall, 31 patients (88.6%) achieved complete tissue salvage without necrosis or debridement. The four failures (11.4%) were due to arterial insufficiency or extensive soft tissue damage (e.g., degloving injuries).

In this cohort, the predominant trauma mechanism was clean-cut injury (12 cases), generally associated with sharp objects and favorable outcomes due to limited soft tissue damage. Crush injuries were identified in 10 patients, frequently resulting in significant vascular and soft tissue compromise. Avulsion (4 cases) and degloving injuries (5 cases) presented complex reconstructive challenges due to extensive tissue disruption and impaired venous outflow. Additionally, 4 cases involved saw or machinery-related trauma, typically combining sharp and blunt force components. The

nature of the traumatic insult significantly influenced surgical management and the indication for adjunctive therapies such as medicinal leech application.

Fig. 1. MLT preparation and application

The analysis was conducted to identify factors associated with the success or failure of medicinal leech therapy (MLT) in our cohort (tab. I).

TABLE I.
Analysis of factors affecting leech therapy success

Factor	Odds Ratio (OR)	95% C.I.	p-Value	Significance
Age (per year)	0.98	(0.95 - 1.01)	0.22	No
Diabetes mellitus (yes/no)	0.45	(0.12 - 1.67)	0.24	No
Peripheral vascular disease (yes/no)	0.32	(0.08 - 1.21)	0.09	No (trend)
Smoking history (yes/no)	0.56	(0.19 - 1.63)	0.28	No
Timing of leech initiation (/day delay)	0.78	(0.65 - 0.94)	0.008	Yes

Among all variables assessed, the timing of MLT initiation was the most significant predictor of tissue salvage. Patients who began therapy within 24 hours of venous congestion onset had significantly better outcomes than those with delayed initiation. Each additional day of delay reduced the odds of success (OR 0.78; 95% CI: 0.65-0.94; p = 0.008). This emphasizes the importance of early intervention, as delays may permit irreversible endothelial damage and thrombosis-pathologies less responsive to MLT (11-12). Other patient-specific factors-age, diabetes mellitus, peripheral vascular disease (PVD), and smoking status-were not statistically significant predictors of outcome. However, trends indicated poorer results among patients with PVD and active smokers (success rate 70% *vs.* 89% in non-smokers; p = 0.28), which aligns with known adverse effects of smoking and vascular disease on microcirculation and healing.

Treatment intensity parameters, such as

number of leeches per day and therapy duration, showed no clear association with outcome. These findings suggest that, once initiated appropriately, prolonging treatment or increasing leech quantity does not enhance efficacy. Rather, timely initiation remains the most critical determinant of success, provided therapy is continued until congestion is resolved. OR < 1 indicates a factor protective against failure (i.e., associated with higher success). For timing, OR 0.78 per day means each day's delay significantly lowered odds of success ($p < 0.01$). No other factor reached statistical significance in this sample, though PVD showed a non-significant trend toward worse outcomes.

Timely initiation of medicinal leech therapy (MLT) remains the key determinant of success in managing venous congestion following reconstructive surgery. In our cohort of 35 patients, early application was associated with favorable outcomes across all subgroups, including those with diabetes, peripheral vascular disease (PVD), and smoking history. Although no statistically significant differences were found between these subgroups, the sample size limits definitive conclusions. Complications were consistent with known risks of MLT and were generally manageable. Two patients (6%) developed *Aeromonas* infections, treated effectively with intravenous antibiotics and local care, though one case resulted in partial flap necrosis. These findings highlight the importance of routine prophylactic antibiotics and close monitoring. Anemia occurred in 5 patients (14%) with hemoglobin drops >2 g/dL, managed conservatively without transfusion. Mechanical issues such as leech migration or detachment were rare and easily corrected. Mild psychological discomfort was reported but did not affect compliance; tolerance improved with simple measures like leech concealment or light sedation. No allergic reactions or early terminations were observed. Overall, MLT was safe, effective, and well-tolerated when initiated promptly and managed within a structured protocol, supporting its continued role in microsurgical salvage.

Literature review. The initial literature search across PubMed, Scopus, and Google Scholar (January 2014-March 2024) identified 134 records. After removing 26 duplicates, 108 unique articles underwent title and abstract screening. Of these, 50 were excluded for not meeting inclusion criteria. Common reasons for exclusion included irrelevance to reconstructive surgery, lack of clinical outcomes, non-human or experimental data, and the application of leech therapy in unrelated medical fields. The study selection process is summarized in second figure.

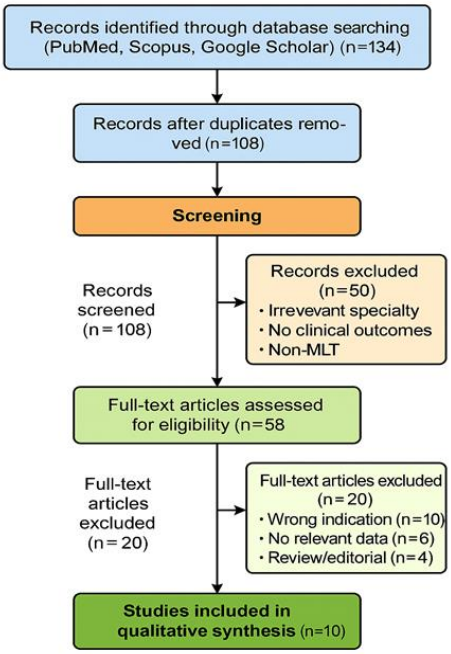

Fig. 2. PRISMA flow chart

Following initial screening, 58 full-text articles were reviewed for eligibility. Of these, 20 were excluded: 10 for incorrect clinical indication, 6 for lacking relevant outcome data, and 4 for being editorials or reviews without original data. Ultimately, 10 studies met all predefined inclusion criteria and were included in the qualitative synthesis. These articles were selected independently by the authors for their focus on the clinical use of medicinal leech therapy in managing venous congestion and for providing detailed information on treatment protocols, outcomes, and complications (tab. II).

TABLE II.

Results of the selected studies

Authors	Clinical indication	Surgical technique	Timing of leech application	Time of therapy	Number of leeches	Application time	Number of sessions
1. Horoz L, Çakmak MF (2023)	Venous congestion after digital replantation or revascularization	Digital arterial-only replantation	Immediately postoperatively if no venous repair is possible or venous congestion is present	5 days, but can extend to 10-12 days for proximal amputations	leeches changed every 2 hours	15 minutes to 2 hours per leech	Applied at 2-6 hour intervals initially, adjusted based on congestion and bleeding (mainte-(maintenance interval 4-8 h); varies by case
2. Pickrel *et al.*, (2020)	Venous congestion post digital replantation/ revascularization	Artery-only or venous anastomosis finger/digit replantation	Prophylactic (immediate) or at first sign of congestion (preferably <24-48 hrs)	5-7 days; weaning after 5 days if stable	Not standardized; 2-hour intervals mentioned; ~2-3/day	30-60 minutes, followed by passive bleeding for up to 6 hours per site	Every 2-3 hours as needed, depending on signs of congestion
3.Z-Hye	Venous	Microsurgical	At clinical		Applied		Every

Authors	Clinical indication	Surgical technique	Timing of leech application	Time of therapy	Number of leeches	Application time	Number of sessions
Lee *et al.,* (2019)	congestion after finger/digit replantation or revascularization	replantation or revascularization of digits	signs of congestion (e.g. dark blood, capillary refill >2s, edema)	Mean of 7 days (range 1-20)	every 1-4 hours; ~2-5 per day (not precisely quantified per patient)	~30-90 minutes per session (implied standard; not directly stated	1-4 hours
4. Arami *et al.,* (2018)	Venous congestion in replanted or revascularized fingers (proximal/middle phalanx level)	Digit replantation and revascularization	Diagnosed late: 24/25 after 48 hours, 1 case at 32 hours after surgery	Mean 2.3 days (range 1-5 days)	Mean 2.88 (range 1-8)	standard detachment assumed (~30-45)	1-3 times per day
5. Cornejo *et al.,* (2017)	Venous congestion after flap surgery or digital replantation	Pedicled and free flaps, digit/appendage replantation	Postoperative, at first signs of venous congestion	Average 4.6 days (range 1-11)	Mean 24.8 per patient (reported only in 14 cases; range 2-77)	20-45 minutes until detachment Followed by passive bleeding for up to 4-6 hours at the site	Not consistently reported; estimated every few hours depending on severity
6. Herlin C *et al.,* (2016)	Venous congestion after finger replantations and flap salvage	Finger replantations and flap salvage	Early application in postoperative hours when signs of venous congestion were noted	6.3 days (range 4-10 days)	Every 2-4 hours initially, then adjusted individually based on congestion	15 minute to 2 hour per leech	Continuous until decongestion stopped progressively between day 5-7
7. Marcia Spear *et al.,* (2016)	Venous congestion in plastic surger - primarily for replants, digit transplants	digital replantation	As soon as venous congestion diagnosed	3-7 days or until angiogenesis reestablished	1-2 per site per session	20-45 minutes per leech session	Every few hours, based on clinical assessment
8. Mumcuoglu *et al.,* (2014)	Venous congestion in reconstructive plastic surgery (flaps, reattached digits, scalp, lips, ears,	Free flaps, pedicled flaps, and digital replantation	Initiated after identification of venous congestion	2-6 days	Typically 1-10 per treatment; some protocols used 3 per hour	30-90 minutes	Every 2 hours initially; 2-3 sessions per day

Authors	Clinical indication	Surgical technique	Timing of leech application	Time of therapy	Number of leeches	Application time	Number of sessions
	nasal tips)						
9.Whitaker *et al.,* (2012)	Venous congestion in reconstructive and microsurgery (replants, flaps, avulsions, etc.)	Variety: replantation (ears, lips, digits, nose, penis), degloving injuries,	When clinical signs of venous congestion appeared	Variable - from a few hours (20 hours) up to 22 days (mean: ~5-7 days)	Ranged from 1/day to 1/hour; in some cases, up to 100 leeches total	Commonly 20-60 min per leech, detachment usually spontaneous	2-8/day depending on severity; most common: every 2-4 hours
10. PA Green *et al.,* (2010)	Venous congestion after microsurgical tissue transfer, digital replantation, free flaps	digital replantation (distal to FDS insertion)	Early application upon signs of venous congestion or prophylactically in venous repair impossibility	~4.2 days average; 7-10 days in case examples	3-6 per application new applie when bleeding stops	Until detachment (~30-90 minutes); 2.45 mL active, 2.5 mL passive blood loss per	Every 2-4 hours initially, based on clinical status

Medicinal leech therapy (MLT) continues to play a valuable role as an adjunctive measure in reconstructive microsurgery, particularly in cases where venous outflow cannot be established through conventional means, such as digital replantation (10-12,13-16). Its ability to provide temporary venous drainage through biologically active decompression makes it a cost-effective and practical alternative when technical limitations or vessel unavailability preclude venous anastomosis (17). In our series, MLT proved to be both effective and well-tolerated across a wide range of patients, including those with comorbidities such as diabetes, peripheral vascular disease, and active smoking history (19-21). Although no statistically significant differences in outcome were noted across these subgroups, observed trends support the established negative influence of vascular comorbidities on healing potential (22).

Complications were consistent with the known risk profile of MLT. Infections occurred in 6% of cases, both due to *Aeromonas* species, and were managed successfully with intravenous antibiotics and wound care (23). These findings reaffirm the necessity of routine prophylactic antibiotic coverage and close clinical monitoring. Anemia was observed in 14% of patients, but all cases were managed conservatively without transfusion. Mechanical issues such as leech detachment or migration were infrequent and easily corrected. Patient tolerance was high, with no therapy discontinued due to psychological distress or adverse reactions (24). A focused comparison between early (< 24 hours) and delayed (> 48 hours) initiation of medicinal leech therapy (MLT) revealed clinically significant differences in therapeutic outcomes and intensity of care. Early MLT was associated with a higher tissue salvage rate (78.14%) compared to 71.00% in the delayed group, reinforcing the importance of timely decompression prior to the onset of irreversible endothelial injury and thrombosis. Moreover, early initiation resulted in longer average treatment duration

(5.29 *vs.* 3.67 days) and more intensive therapy, including a higher number of leeches per day (3.86 *vs.* 3.00) and sessions per day (3.57 *vs.* 2.67). This pattern indicates increased clinical engagement during the critical early postoperative period, allowing more effective venous drainage. The early MLT group also demonstrated a broader therapeutic range, with durations spanning 4-7 days and leech applications ranging from 2 to 6 per day, compared to 2-6 days and 2 to 5 leeches per day in the delayed cohort (25-27). Although the average transfusion rate was slightly higher in the early group (49.29% *vs.* 41.67%), this was likely reflective of more aggressive decompression and prolonged bleeding rather than increased risk, as no rise in major complications was observed (28). These findings suggest that while treatment intensity can be adjusted, delayed MLT initiation compromises outcomes, likely due to progressive micro-vascular damage that becomes non-reversible. The survival benefit with early therapy supports the recommendation that MLT be initiated within 24 hours of congestion onset whenever possible. To ensure both efficacy and safety, structured institutional protocols are essential to guide standardized, timely MLT across surgical teams.

DISCUSSION

Venous congestion remains a critical complication following digital replantation or revascularization, particularly in distal digit injuries where venous outflow is compromised due to avulsed, thrombosed, or non-reconstructible veins. In such cases, medicinal leech therapy (MLT) offers a biologically active adjunct to promote passive drainage until intrinsic venous outflow or neovascularization is established. The study evaluated key clinical variables of MLT-including initiation timing, duration, application frequency, and complications-in a cohort of 35 patients. The observed tissue salvage rate of 88.6% compares favorably with literature-reported rates (66-85%), likely reflecting early therapy initiation (average 1.5 days post-op; most within 24 hours) and adherence to a standardized institutional protocol. Early initiation was statistically associated with improved outcomes ($p = 0.008$). MLT was administered for an average of 5 days, with 2-4 leeches applied daily at 2-4-hour intervals. Feeding durations ranged from 20 to 45 minutes. These parameters align with current best practices. Complication rates were low: *Aeromonas hydrophila* infections occurred in 6% of cases, and 14% developed mild anemia, though no transfusions were required. Minor issues such as leech migration or patient discomfort were infrequent and easily managed. The favorable outcomes observed may be attributed to care according to protocols and consistent use of prophylactic antibiotics-ciprofloxacin-combined with rigorous clinical monitoring. This contrasts with the variability in technique, antibiotic use, and therapy duration noted across many published studies. Salvage rates above 75% are commonly reported when therapy is initiated within 24-48 hours and maintained for at least 4-5 days. Delayed initiation, particularly beyond 48 hours, correlates with decreased viability, despite intensified treatment. Findings from Arami *et al.* and Lee *et al.* support this time-sensitive therapeutic window, with the latter demonstrating improved digital survival when MLT exceeded 4.5 days (29-31). Treatment regimens in published reports generally involve 2-5 leeches per day, applied every 2-4 hours.

Leeches are allowed to feed until detachment, followed by prolonged passive bleeding, which facilitates decompression and enhances perfusion. Adjunctive measures-limb elevation, thermal vasodilation, and heparinized dressings-are frequently used to support MLT efficacy (32). Reported infection rates range from 4% to 20%, most commonly involving *Aeromonas hydrophila*, reinforcing the necessity of antibiotic prophylaxis (33). Anemia, often proportional to therapy duration and leech count, results in transfusion rates up to 60% in some series. In our cohort, careful monitoring and supportive care prevented the need for transfusion (34). Based on this analysis, the following recommendations are proposed for MLT in digital replantation: initiate therapy within 24 hours of venous congestion, apply 2-5 leeches per day, adjusting for severity and surface area, 2-4-hours intervals; allow leeches to feed until detachment, maintain therapy for at least 4 days; taper as clinical improvement is observed, use prophylactic antibiotics (e.g., ciprofloxacin) throughout the treatment period, employ adjuncts such as analgesia, limb elevation, heparin-soaked gauze, and daily hemoglobin monitoring (35).

MLT remains an effective, time-sensitive, and manageable adjunct in the management of venous congestion after digital replantation. When initiated early and guided by protocol, MLT significantly improves tissue salvage in settings where conventional venous repair is not feasible.

CONCLUSIONS

Medicinal leech therapy (MLT) remains a biologically active, evidence-supported, and time-tested adjunct in the management of venous congestion following digital replantation and revascularization. Despite advances in microsurgical techniques, venous congestion continues to pose a significant threat to digit viability, especially in distal replantation where venous outflow cannot be surgically restored. In these high-risk cases, MLT offers a minimally invasive solution that facilitates temporary venous drainage, promotes tissue oxygenation, and allows time for neovascularization.

Although leech therapy remains underutilized in some centers, current evidence from the past decade supports its continued integration into microsurgical practice, particularly when initiated promptly and guided by structured protocols.

CONFLICTS OF INTEREST AND FUNDING

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COMMUNICATION BETWEEN MEDICAL LABORATORY AND CLINICAL DEPARTMENTS - INSIGHTS FROM A SURVEY AMONG ROMANIAN HEALTHCARE PROFESSIONALS

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COMMUNICATION BETWEEN MEDICAL LABORATORY AND CLINICAL DEPARTMENTS - INSIGHTS FROM A SURVEY AMONG ROMANIAN HEALTHCARE PROFESSIONALS (Abstract): Effective patient management can be achieved through extensive collaboration and synchronized action between specialists in paraclinical investigations and attending physicians. However, in practice, proper communication is often difficult to establish and maintain. This study **aims** to assess the communication between laboratory staff and clinicians among Romanian healthcare professionals. **Materials and methods:** an online survey consisting of questions with pre-defined answers was conducted to gain insights into the experiences and opinions of Romanian healthcare professionals regarding communication between clinical departments and the medical laboratory. Results: the survey was completed by 176 respondents, covering all categories of healthcare personnel from both medical laboratories and clinical departments. Overall, laboratory staff reported that they most often communicate promptly about test availability, while respondents from clinical departments felt they were informed about these aspects less frequently. The results also highlight a potential need among medical laboratory staff to be more involved in case discussions and to be more respected as healthcare professionals. **Conclusions:** this study provides relevant insights that can inform future research and initiatives aimed at improving collaboration between clinical and paraclinical departments, with the potential to significantly enhance patient management. **Keywords:** PATIENT MANAGEMENT, MEDICAL LABORATORY, COMMUNICATION.

INTRODUCTION

Paraclinical investigations are an essential part of the diagnosis and treatment process nowadays, with the medical laboratory playing a significant role in patient management (1). Inaccurate or delayed results, as well as suboptimal laboratory infrastructure, might substantially influence the outcomes. However, besides the technical aspects of the laboratory work, there are a few more factors contributing to effective and optimal results. The lack of necessary clinical data submitted with the test order, as well as the failure to promptly contact the clinical team when necessary could result into missing important and vital information regarding the patient (2). The tendency of clinicians to require many unnecessary tests

is common and leads to increased healthcare costs, as well as additional pain and discomfort (3). Even more, the increasing number of unjustified required tests leads to more false positive results, which will require more testing (4). Reasons include the misunderstanding of benefit/harm ratio, the lack of knowledge regarding the costs of tests, as well as the fear of misdiagnosing (5). An improved interaction between clinical and laboratory teams might address these issues. Therefore, a better communication between laboratory and clinical staff might contribute to an increased general satisfaction, reduced healthcare costs and improved patient outcome. The purpose of this study is to highlight new insights on the communication between medical laboratories and clinical departments in Romanian healthcare institutions.

MATERIALS AND METHODS

An online questionnaire (Google Forms) with pre-defined answer options was used as the data collection instrument. The form was distributed among healthcare professionals and was open to participation regardless of their role or professional title. The questionnaire was structured into separate sections tailored for two distinct groups: healthcare professionals working in clinical laboratories and those working in clinical departments. The questions in both sections were designed to address related themes, enabling the comparison of perspectives between laboratory staff and clinicians on shared topics. Survey participation was entirely voluntary. The form was publicly accessible, and completion of the questionnaire was considered as implied consent. Introductory information outlining the study's purpose was provided at the beginning of the form. Only respondents who agreed to participate in the study could proceed to the sections with the questions.

Data collection relied on a general-purpose instrument rather than a validated questionnaire, with the goal of exploring broad perspectives and identifying emerging topics of interest, rather than producing generalizable or statistically rigorous findings. Responses were compiled and processed using the built-in features of Google Forms, along with *Microsoft Excel*. Descriptive analysis was performed, with results expressed as relative frequencies and illustrated through tables and graphical representations.

RESULTS

The survey was filled in by 176 respondents, 53.4% who work in clinical departments, while 46.6% work in clinical laboratories. From the respondents working in clinical laboratories, 53.8% were physicians, 39% were clinical biologist/ biochemist/ chemist, and 6.1% were nurses, as detailed in figure 1. 18.3% of the respondents who work in clinical laboratories reported being head of laboratory, and among this group of respondents, 47.6% work in the public healthcare system, 41.5% in private laboratories and 11% in both.

Among the respondents who work in clinical departments, 14.9% do not work in a healthcare facility that has a clinical laboratory, therefore only 80 out of 94 respondents who work in clinical departments were able to answer the questions about the communication with the clinical laboratory. Of them, 95% were physicians and the others were nurses, and 3.8% of the respondents in this group were head of department. Among respondents who work in clinical departments, 81.3% work in public healthcare facilities, 7.5% in private healthcare facilities and 11.3% in both (fig. 1).

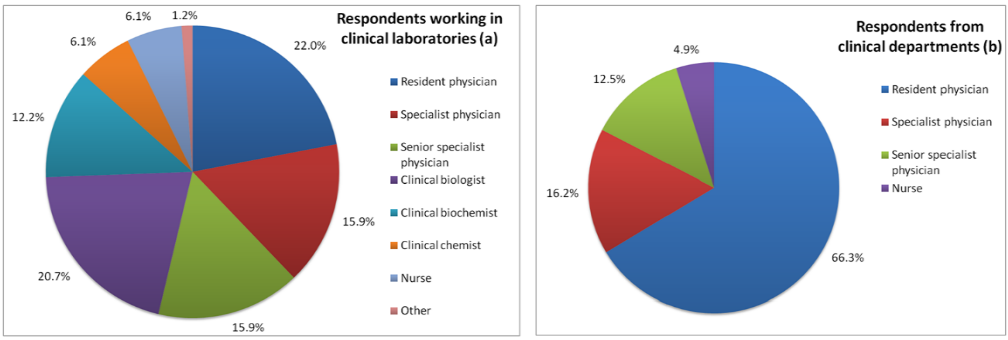

Fig. 1. Professionals from laboratories (a), and clinical (b) departments among respondents

Table I presents, as relative frequencies, the responses to the questions regarding communication between clinical laboratory staff and clinical department personnel, highlighting both perspectives. Figure 2 illustrates the level of satisfaction reported by laboratory staff regarding their collaboration with clinicians and vice-versa, based on several aspects of interdepartmental collaboration and workflow. Respondents rated their satisfaction on a scale ranging from 'Not at all satisfied' to 'Very satisfied'.

TABLE I.

Medical laboratory and clinical department communication – perspectives from both sides

Respondents	Medical laboratory personnel	Clinical department personnel
Frequency of communication between medical laboratory and clinical department		
Never	9.8%	10%
Several times a day	26.8%	12.5%
Daily	20.7%	18.8%
A few times a week	23.2%	28.7%
A few times a month	11%	18.8%
A few times a year	6.1%	11.3%
Other	2.4%	0%
Who initiates direct laboratory-clinical communication?		
Us	31.7%	50%
The others	12.2%	3.7%
Both	51.2%	35%
Not applicable	4.9%	11.3%
What are the topics of discussion?		
Interpretation of laboratory test results for specific patients and cases	89%	77.5%
Available methods, equipment and tests	31.7%	31.3%
Intention to implement new laboratory tests	30.5%	8.8%
Lack of reagents	26.8%	30%

Respondents	Medical laboratory personnel	Clinical department personnel
When new tests are available in the laboratory, are the specialists from clinical departments promptly and in detail informed about the possibility of requesting them?		
Always	57.3%	13.8%
Most of the time	23.2%	27.5%
Sometimes yes, sometimes no	9.8%	18.8%
Most of the time no	1.2%	15%
Never	0%	8.8%
Not applicable	8.5%	16.2%
Do you consider it necessary to involve the medical laboratory staff in discussions with the clinic specialists about the results obtained and their interpretation?		
Always	51.2%	12.5%
Most of the time	32.9%	20%
Sometimes yes, sometimes no	13.4%	55%
Most of the time no	2.4%	10%
Never	0%	2.5%
What do you consider to be most important to communicate between the medical laboratory and the clinical departments?		
Case discussions and interpretation of results in both clinical and technical contexts	26.8%	27.5%
Discussions about tests that are available or unavailable over time	2.4%	10%
Dynamic variation of waiting times, related to the availability and management of medical laboratory resources	6.1%	17.5%
All these are equally important	64.6%	45%
When laboratory tests cannot be performed due to problems related to reagent management and procurement or technical issues, are clinic specialists promptly informed of this?		
Always	48.8%	13.7%
Most of the time	17.1%	17.5%
Sometimes yes, sometimes no	8.5%	20%
Most of the time no	6.1%	25%
Never	2.4%	12.5%
Not applicable	17.1%	11.3%
Is there a procedure that refers to communication between laboratory and clinical department in your institution?		
Yes, but I don't know its contents.	11%	6.3%
Yes, but its content is not followed	6.1%	1.2%
Yes, and its content is followed most of the time	43.9%	10%
There is no procedure	12.2%	17.5%
I do not know if the procedure exists	25.6%	65%
Other	1.2%	0%

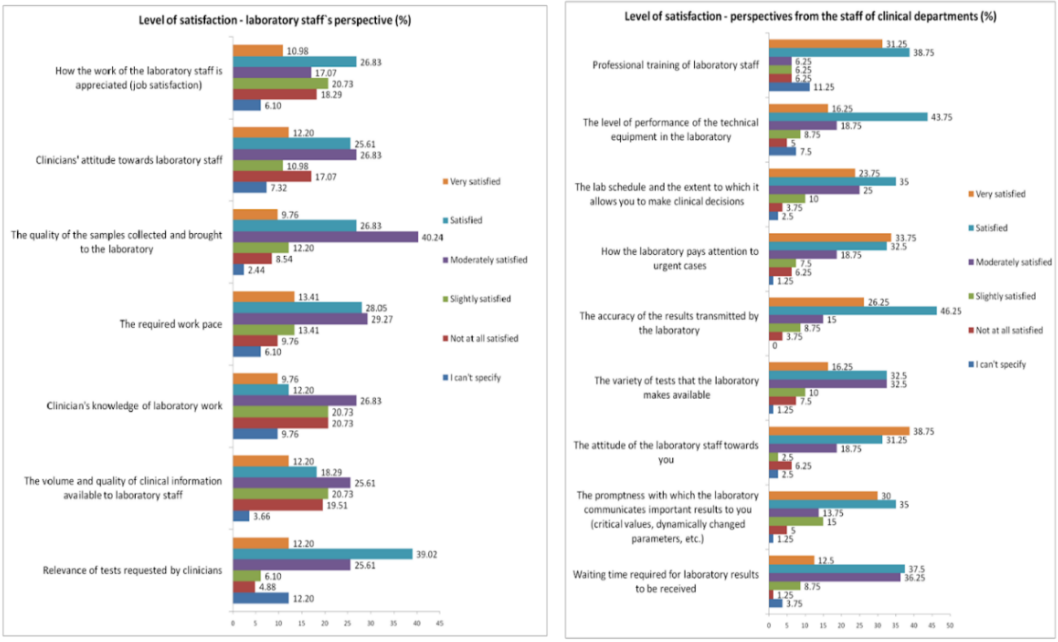

Fig. 2. Satisfaction level reported respondents concerning the laboratory - clinical department collaboration

From all the above, the respondents who work in clinical laboratories chose as the main aspect they appreciate in their collaboration with clinicians both the relevance of tests requested by clinicians (22%) and the volume and quality of clinical information available to laboratory staff (23%), followed by how the work of the laboratory staff is appreciated - job satisfaction (20.7%), the quality of the samples collected and brought to the laboratory (13.4%), clinicians' attitude towards laboratory staff (8.5%), clinician's knowledge of laboratory work (7.3%), and the required work pace (2.4%).

From all the above, the respondents who work in clinical departments chose as the main aspect they appreciate in their collaboration with laboratory personnel the promptness with which the laboratory communicates important results to them - e.g. critical values, dynamically changed parameters etc. (33.8%), followed by the attitude of the laboratory staff towards them (16.3%), the variety of tests that the laboratory makes available (13.8%), waiting time required for laboratory results to be received (11%) and the accuracy of the results transmitted by the laboratory (11%), how the laboratory pays attention to urgent cases (6.3%), the lab schedule and the extent to which it allows them to make clinical decisions (5%), the professional training of laboratory staff (5%) and the level of performance of the technical equipment in the laboratory (1.2%).

Table II presents the perspectives of both the medical laboratory personnel and clinical department staff on the main challenges in their communication.

TABLE II.
Challenges for the communication between medical laboratories and clinical departments

Opinions on the main problem in communication			
Laboratory staff		**Clinicians**	
Lack of formal communication means	35.4%	Lack of formal communication means	60%
Lack of openness on the part of clinicians to communicate	9.8%	Lack of openness on the part of laboratory staff to communicate	10%
Clinicians' attitude towards specialists working in the laboratory	48.8%	The attitude of laboratory staff towards specialists working in the clinical departments	7.5%
Others	6%	Others	22.5%

DISCUSSION

Given the critical role of laboratory test results in clinical decision-making, medical laboratories are a key component of collaborative healthcare, where the patient is increasingly seen as an active participant alongside healthcare professionals (6). Considering the continuous increase in the complexity of technological advances in laboratory medicine, collaboration between the medical laboratory and clinicians has been highlighted since the late 20th century as a critical issue requiring innovation (7). Furthermore, the interpretation of laboratory test results goes beyond simply classifying values as within or outside the biological reference range or considering only the analytical performance (8).

While the importance of communicating critical and alert values of laboratory tests has been widely recognized (9-19) and usually there are procedures implemented for this purpose (11), the need to integrate laboratory specialists into case-related decisions has gained more and more interest, for example, in the field of medical microbiology (12), a subspecialty of laboratory medicine (13). Collaboration with laboratories covers all sectors of healthcare, from primary care to precision medicine (14-16)

Effective laboratory-clinical department communication must be bidirectional, should involve all categories of healthcare personnel, can use all means of communication and should be facilitated by the management (17). Laboratory-clinician communication improvement can be achieved through standardized, clear reporting (18). The collaboration between clinical and paraclinical departments is influenced by organizational and managerial factors within institutions and departments, as well as by socioeconomic, cultural, and educational factors, alongside the individual knowledge and skills of each healthcare professional (19,20). The present study identified both similarities and differences in the experiences and perceptions regarding collaboration between clinical laboratories and clinical departments in Romania. While some aspects, such as the frequency of communication, were perceived relatively similarly by both groups of respondents, other perceptions varied significantly. For example, in relation to the implementation of new tests or testing methods, 80.5% of respondents working in

medical laboratories stated that clinicians are always or almost always informed in a timely manner about the availability of these investigations, whereas only 41.3% of clinicians shared this view. This discrepancy was also reflected in the responses to the question concerning the topics of communication between laboratory and clinical staff. Specifically, the intention to implement new tests was mentioned as a topic of communication by 30.5% of laboratory staff, but by only 8.8% of clinical department respondents.

Respondents working in medical laboratories expressed a strong interest in being involved in discussions regarding test results and their interpretation, with 84.1% believing that such discussions should occur always or almost always. Clinicians, however, were more reserved about the necessity of this type of communication: 55% considered it necessary only sometimes, and just 32.5% opted for always or most of the time.

Another notable difference between the two groups emerged in relation to communicating the inability to perform certain tests due to issues such as reagent supply or technical problems. While 65.9% of laboratory staff stated that this is always or most of the time communicated to clinicians, only 31.2% of clinical department respondents reported the same.

Regarding the regulation of communication between the laboratory and clinicians within institutions, 61% of respondents working in medical laboratories reported the existence of a formal procedure, with 43.9% stating that its content is routinely followed. In contrast, 65% of respondents from clinical departments were unaware of whether such a procedure exists in their institution. This is particularly notable given that 60% of respondents from clinical departments also identified the lack of formal communication channels as a main issue in laboratory-clinician collaboration. Meanwhile, among respondents working in medical laboratories, the most frequently reported problem was the clinicians' attitude towards laboratory specialists (48.8%).

Overall, most respondents from clinical departments reported being satisfied or very satisfied with the various aspects of their collaboration with the medical laboratory, as evaluated in this survey. This can vary from one healthcare system to another, for example Fondoh *et al*. reported a high level of dissatisfaction among clinicians about their collaboration with medical laboratories (21). A larger study conducted in the United States concluded that the clinicians from emergency departments are generally dissatisfied regarding laboratory services that was reduced after corrective actions (22) A survey conducted at the beginning of 2000s in Finland aimed to identify topics of dissatisfaction among clinicians about laboratory investigations (23). After corrective measures were implemented, no improvement in the level of dissatisfaction was identified, suggesting that achieving optimal collaboration between clinical and paraclinical departments is a major challenge.

The current study offers valuable insights into various aspects of communication between laboratory staff and clinicians, highlighting potential trends among healthcare professionals and guiding future research aimed at improving collaboration between clinical and paraclinical departments. However, the results are limited by

the subjectivity of the responses, the modest number of participants, and the use of a general data collection tool rather than a validated questionnaire. Nevertheless, this exploratory study design was appropriate for rapidly identifying key issues that warrant further investigation.

CONCLUSIONS

There is a clear communication gap between laboratory and clinical staff, suggesting that targeted strategies to improve timely and effective information exchange are essential for optimal patient care.

A need for greater recognition and inclusion of medical laboratory professionals in interdisciplinary case discussions was identified, promoting mutual respect and collaborative decision-making.

Discrepancies between clinical and laboratory staff perceptions of communication frequency highlight the importance of establishing standardized communication protocols.

Structured and regular interactions between paraclinical and clinical departments could help align expectations and foster trust, thereby improving healthcare delivery.

The insights gained call for further investigation into the root causes of communication challenges and the development of institutional policies that promote integrated teamwork across all levels of healthcare provision.

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ANALYSIS OF POSTOPERATIVE PAIN SCORES AND CLINICAL CHARACTERISTICS IN PATIENTS ASSOCIATING OBSTETRICAL AND GYNECOLOGICAL INTERVENTIONS IN NORTH-EASTERN ROMANIA

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ANALYSIS OF POSTOPERATIVE PAIN SCORES AND CLINICAL CHARACTERISTICS IN PATIENTS ASSOCIATING OBSTETRICAL AND GYNECOLOGICAL INTERVENTIONS IN NORTH-EASTERN ROMANIA (Abstract): Pain is a vital function of the nervous system, providing the body with a warning of potential physical or psychological injury. Nociceptors are specialized sensory structures involved in the detection of potentially harmful stimuli. They transform noxious stimuli - whether mechanical, thermal or chemical - into electrical signals that are transmitted to the central nervous system via primary afferent fibers of the Aδ and C types. **Materials and methods**: We describe the analysis of a representative cohort of 111 patients, which we analyzed for our study characteristics: age, environment of origin, type of intervention performed, length of hospitalization, pain score, recovery period in hospital. We obtained a study group formed by gynecological and pregnant patients who presented for surgical interventions at the "Cuza-Vodă" Obstetrics and Gynecology Clinical Hospital in Iași between 01.12.2024 and 01.12.2025, that were included in the analysis. **Results:** A wide number of clinical data were analyzed starting with demographic characteristics, type of surgery, number of days of hospitalization, neonatology results. The main statistically significant correlation was a reduced Apgar score at gestational age less than 38 weeks**.** A complex interplay between the various biological responses of the immune system, the autonomic nervous system, vascular regulation, and the central and peripheral nervous systems in response to tissue injury, pathogens, and irritants comprises the sensation of pain by the body. Pain can then play a vital protective role for an organism, as is the case with acute inflammation that results in the perception of pain, leading to avoidance of noxious stimuli and encouraging healing of damaged tissue. **Conclusions:** Adequate management of post-cesarean pain remains a challenge in the hospital. Better interprofessional collaboration, self-administered analgesia, scheduled medical prescriptions and increased availability of analgesic medications may contribute to improving postoperative pain management, with better pain scores. **Keywords:** ANESTHESIA, PAIN, SURGERY, PATIENTS, HOSPITALISATION DAYS.

INTRODUCTION

Postoperative pain can negatively affect both the general physical and psychological condition of a patient, also suppressing their motivation to recover and mobilize as quickly as possible postoperatively, as pain is associated with a reduced ability to accept the doctor's advice, increased psychological stress and a general repression of resuming daily activities.

In contrast, effective pain management promotes the motivation for early patient

mobilization, allowing for improved recovery, facilitating necessary movements and deep breathing, and reducing the negative physiological and psychological impact of pain (1).

Key factors in addressing this problem include comprehensive patient education about pain management options, shared decision-making with healthcare providers, and implementation of specialized and individualized patient-based services and therapeutic regimens for acute postoperative pain (2). Due to the global increase in surgical interventions in the gynecological sphere, along with the global increase in the incidence of cesarean sections (in some cities in Italy or Brazil the incidence reaches 90%), there is currently a greater emphasis on increasing the quality of the surgical act as a whole, including postoperative care and pain management in the anesthesia and intensive care unit.

Pain is a vital function of the nervous system, providing the body with a warning of potential physical or psychological injury. It is both a sensory and emotional experience, influenced by psychological factors such as past experiences, beliefs about pain, fear, or anxiety (3). Nociceptors are specialized sensory structures involved in the detection of potentially harmful stimuli (4). They transform noxious stimuli - whether mechanical, thermal or chemical - into electrical signals that are transmitted to the central nervous system via primary afferent fibers of the Aδ and C types, whose free nerve endings are spread throughout the entire structure of the organism: skin, viscera, muscles, joints and meningeal structures (5).

Pain is, by its nature, a complex and multifactorial phenomenon, difficult not only to treat but also to fully understand. It involves interactions between sensory, affective, and cognitive components, leading to recognition, interpretation, and appropriate emotional response (6-11). From the perspective of the anesthesiologist and the intensive care specialist, understanding and managing pain is a major area of interest, as peri- and postoperative pain is a common reality in clinical practice, requiring effective and personalized therapeutic approaches (7). In addition, growing concerns about the excessive use of opioids in the treatment of acute and chronic pain have generated an urgent need to develop alternative and multimodal pain control strategies (8). In this context, anesthesiologists are increasingly exploring the combined use of pharmacological therapies, such as nonsteroidal anti-inflammatory drugs (NSAIDs), antidepressants, corticosteroids, alpha-2 receptor agonists, ketamine, local anesthetics, magnesium, gabapentinoids, and acetaminophen (9, 10).

MATERIALS AND METHODS

We describe the analysis of a representative cohort of 111 patients, which we analyzed for our study characteristics: age, environment of origin, type of intervention performed, length of hospitalization, pain score, recovery period in hospital. We searched for correlations between patient characteristics, surgery and the registered VAS score associated with the surgery, and recovery period. The motivation of this study was given by monitoring the general well-being of patients in the immediate postoperative period, with the interest of reducing the adverse effects of the types of anesthesia performed - locoregional or general, such as: pain, nausea, chills. Also, a low pain score is associated in specialized articles with better postoperative recovery, faster resumption of intestinal peristalsis, and a decrease in the number of days of hospitalization.

Study group. Gynecological and preg-

nant patients who presented for surgical interventions at the Cuza-Vodă Obstetrics and Gynecology Clinical Hospital in Iași, between 01.12.2024 and 01.12.2025, were included in the analysis.

Inclusion criteria were formed by: age above 18 years and associated obstetrical or gynecological surgical intervention.

Exclusion criteria were patients under 18 years of age; persons with suspected sepsis or associated severe medical pathologies.

General objectives were to evaluate the effectiveness of the treatment of postoperative pain noted as mild, moderate to severe pain in the group of patients studied, in order to differentiate between the importance of major obstetric or gynecological associated risk factors and the extent of the surgical intervention. The study was descriptive, prospective and retrospective, starting from the surgical intervention performed, analyzing clinically and paraclinically, the importance of the relationships between demographic characteristics, associated medical pathology, physiological and pathological medical history and the pain score represented on the VAS scale, respectively the correlations between demographic characteristics, surgical intervention and associated postoperative pain score.

Statistical analysis. Both descriptive and analytical methods were used in the statistical analysis. After collecting the data, in an accessible form to ensure their informational character, their processing was carried out.

The processing actions included the stage of ordering, systematization, centralization and reduction of the volume of information collected, through successive operations of grouping, sorting, coding and synthesizing. The data were expressed in a form that allows them to be classified into certain categories; a variable will not be recorded in more than one form; the data will be grouped by variable categories. The data were centralized in *Microsoft Excel* and *SPSS* databases and processed with the statistical functions to which they are suitable. In the presentation of the data, confidence intervals at the 95% significance threshold were used, and the χ^2 and t-Student tests were used to test the differences.

RESULTS

With the increase in the number of surgical interventions worldwide, but the decrease in the global birth rate, the medicalization of births has occurred and has become an increasingly common practice, therefore we present our patients characteristics and VAS scores according to the surgery they had performed.

Demographic characteristics

Age group distribution. The series of values for age has a normal distribution: variations in the range of 20-79 years; group mean 37.17 years ± 11.40; median 36 years; Kurtosis test result 1.644 and p>0.05 for the Kolmogorov-Smirnov test, which suggests that statistical significance tests can be applied for continuous variables. Based on these data, the age of 36 years (50th percentile) is chosen as the threshold for subsequent correlations, 50.5% of the patients were over 36 years old.

Distribution by area of origin. Out of the total of 111 study group patients, 77 patients were of urban origin, of which 54.5% were over 36 years old, while in the patients of rural origin the age was predominantly under 69 years old (58.8%) (p=0.137).

Clinical characteristics.

Surgical interventions

Out of the total study group (n=111), 52.3% of the analyzed patients underwent cesarean sections (n=54 CST), respectively 14.4% underwent interventions such as total hysterectomy with bilateral adnex-

ectomy (n=16HT+AB) and 9% underwent biopsy curettage (n=10).

Cesarean section surgery. Of the 58 patients with cesarean section, 79.3% were under 36 years of age, while 83% of patients with other types of surgery were over 36 years of age (p=0.001). The structure of the group of patients with cesarean section was predominantly urban (63.8% *vs.* 75.5%; p = 0.130).

Patient gestation. In patients with cesarean section, the series of gestation values varied in the range 1-7, with a mean value of 2.07±1.13 close to the median value of 2, but it does not seem to be normally distributed because the Kurtosis test values> 2 and the Kolmogorov-Smirnov test values < 0.05, which suggests that statistical significance tests cannot be applied for continuous variables. Of the total group of patients with cesarean section, 22.2% had over 3 gestations.

Among patients with gestation under 3 pregnancies (n=42), 83.3% were under 36 years of age, and 71.4% came from urban areas. Among the patients with more than 3 pregnancies (n=12), 75% were under 36 years of age (p=0.389), and 58.3% came from rural areas (p=0.05).

Parity of the analyzed patients. Parity varies in the range 1-4; group mean 1.72 ± 0.76; median 2; Kurtosis test result 1.249 and p>0.05 for the Kolmogorov-Smirnov test, which suggests that statistical significance tests can be applied for continuous variables.

In the case study, the Pearson test revealed that parity was not correlated with age (r= +0.079; p=0.570). In patients from rural areas, parity was higher than in those from urban areas (2.0 *vs.* 1.57; p=0.048)

Number of weeks of pregnancy at which delivery occurred by cesarean section. In patients with cesarean section, the series of values for the number of weeks of pregnancy varies in the range of 31-40; group average 37.94 weeks ± 1.56; median 38 weeks; Kurtosis test result 7.951 and p<0.05 in the Kolmogorov-Smirnov test, which suggests that statistical significance tests cannot be applied for continuous variables. Among patients with cesarean section, 25.9% had a gestational age of less than 38 weeks. Of patients with less than 38 weeks gestational age (n=14), 85.7% were under 36 years of age, and 50% came from urban areas. Of patients with more than 38 weeks of pregnancy (n=40), 80% were under 36 years of age (p=0.488), and 70% came from urban areas (p=0.153). Gestational age 1-2 pregnancies were recorded by 57.1% of patients with cesarean section at gestational age below 38 weeks and 85% of those with gestational age above 38 weeks (p=0.041). In the case series studied, parity was indirectly correlated, reduced in intensity, with gestational age, but statistically insignificant (r = -0.188 and p = 0.174).

Newborn weight at birth. In patients with cesarean section, the series of values of the child's weight at birth varies in the range of 1,330-4,530 g; group average 3,237 g ± 581.33; median 3,320 g; the result of the Kurtosis test is 2.311 and p<0.05 for the Kolmogorov-Smirnov test, which suggests that statistical significance tests cannot be applied for continuous variables. Among the patients with cesarean section, 50% gave birth to children weighing less than 3320 grams (50th percentile). Birth weight below 3320 g was evident in 50% of the patients with cesarean section (n=27), of which 88.9% were under 36 years of age, 59.3% came from urban areas and 76.9% had less than 3 pregnancies.

Among patients with newborns with a birth weight of over 3,320 g (n=27), 77.8% were under 36 years of age (p=0.234), 70.4% came from urban areas (p=0.285) and 77.8% had less than 3 pregnancies

(p=0.599). Gestational age below 38 weeks was recorded in 42.3% of patients with newborns weighing less than 3320 g and 88.9% of those with newborns weighing more than 3320 grams (p=0.011).The Apgar score was slightly lower in patients under 36 years of age (8.33 *vs.* 8.67; p=0.346), from rural areas (8.16 *vs.* 8.51; p=0.195), with 3 or more pregnancies (8.08 *vs.* 8.46; p=0.234), in mothers with newborns weighing less than 3320 g (8.30 *vs.* 8.48; p=0.484).

The only statistically significant correlation was a reduced Apgar score at gestational age less than 38 weeks of gestation (7.71 *vs.* 8.62; p=0.002)

Total number of days of hospitalization, preoperative and postoperative. In patients with surgical intervention such as cesarean section, the series of values for the number of days of hospitalization varies in the range of 1-22 days; group average 5.75 days ± 3.19; median 5 days; Kurtosis test result 12.289 and p<0.05 for the Kolmogorov-Smirnov test, which suggests that statistical significance tests for continuous variables cannot be applied. 64.4% of the patients in the present study group were hospitalized for less than 5 days, and 5.8% of them for more than 10 days, including both preoperative and postoperative days. Associated medical pathology. Among the associated pathologies, associated fetal risk factors such as umbilical cord circulation of the fetus, presented an estimated 2-fold increased risk of cesarean section (RR=2.0; CI 95%: 1.65-2.42; p=0.036). Relatively higher risk for cesarean section was the presence of diabetes mellitus (RR=1.46; 95% CI: 0.81-2.84; p=0.344), hereditary thrombophilia diagnosed antenatally (RR=1.40; 95% CI: 0.85-2.32; p=0.258), or assisted human reproduction procedures IVF (RR=1.29; 95% CI: 0.57-2.92; p=0.534), but the results cannot be extrapolated to the general population (p>0.05).

Pain study using the VAS scale

VAS score. In the study group, the series of VAS score values vary in the range 1-6; group mean 2.42 ± 1.28; median 2; the result of the Kurtosis test -0.505 and p>0.05 in the Kolmogorov-Smirnov test, which suggests that statistical significance tests can be applied for continuous variables. The VAS score was directly correlated, moderately in intensity, with the patient's age, statistically significant (r= +0.353; p=0.001), which suggests that in 35.3% of patients, higher VAS values were found at older ages. The average VAS values were slightly higher in patients from urban areas (2.49 *vs. 2*.26; p=0.389). The lowest average VAS value was recorded in patients with predominantly classical uterine myomectomy, but also laparoscopic (1.50±0.71; p=0.001) and in the intervention type cesarean section (1.88±1.09; p=0.001), and the highest VAS score in patients with Madden radical mastectomy (3.50±0.71; p=0.001) and HT+AB (3.25±1.39; p=0.001)

DISCUSSION

With the increase in the number of surgical interventions worldwide, but the decrease in the global birth rate, the medicalization of births has occurred and has become an increasingly frequently used practice. Reducing pain during natural births is possible with locoregional anesthesia, with epidural anesthesia in over 60% of births in countries such as Ireland and France, and reducing the risks associated with natural birth by performing scheduled cesarean-type interventions at 38-39 weeks of gestation (12). Pain reduction during natural births is possible with locoregional anesthesia, with epidural anesthesia in over 60% of births in countries such as Ireland and France (13), and the reduction of risks associated with natural births by perform-

ing scheduled cesarean sections at 38-39 weeks of gestation (14).

In a study with data from the Nation Perinatal Survey (*Enquête Nationale Périnatale*), a cross-sectional, nationwide, population-based study conducted in France in March 2021, the frequency and risk factors for acute pain during vaginal birth with epidural analgesia were analyzed. Women who gave birth vaginally with epidural analgesia were included. The intensity of acute pain at the time of delivery was assessed using a numerical pain rating scale from 0 (no pain) to 10 (unbearable pain) and classified into 3 groups: no pain/mild pain (0-3), moderate pain (4-6), or severe pain (7-10). Of these patients, 16.9% experienced moderate pain and 31.4% severe pain at the time of delivery. Independent risk factors for severe pain were maternal age < 25 years, overweight / obesity, prolonged pushing efforts, first or second-degree tears, short or long duration of epidural analgesia, self-reported partially effective or ineffective epidural analgesia, and failure to attend obstetric care or consult a temporary anesthetist. Severe pain was more likely to be experienced in nulliparous women with operative delivery compared with nulliparous women with spontaneous delivery. Maternal age ≥ 35 years, education ≥ 5 years after graduation, and use of PCEA were protective factors for severe pain. Therefore, the conclusions show that one in six women reported moderate pain, and the risk factors were the same as for severe pain, with the exception of obesity, first or second-degree tears, operative birth in nulliparous women, and temporary anesthetists.

The pain perception mechanisms are formed by complex interactions occurring in the dorsal horn between afferent neurons, interneurons, and descending modulatory pathways. These interactions determine the activity of secondary afferent neurons. Glycine and gamma-aminobutyric acid (GABA) are important neurotransmitters that act on inhibitory interneurons (15).

Inhibition of pain transmission says that there are mechanisms that act to inhibit pain transmission at the spinal cord level and by descending inhibition from higher centers. Pain control theory (Melzack, Wall, 1965) describes a process of inhibitory modulation of pain at the spinal cord level (14). It helps explain why when we hit our head, we feel better when we rub it. By activating Aβ fibers with non-noxious tactile stimuli, inhibitory interneurons in the dorsal horn are activated, leading to inhibition of pain signals transmitted via C fibers. Transcutaneous electrical nerve stimulation (TENS) is based on the gate control theory. Stimulation of large-diameter afferent nerve fibers by cutaneous electrodes activates inhibitory processes in the dorsal horn and inhibits pain transmission via C fibers.

The Role of Inflammation in Pain.

A complex interplay between the various biological responses of the immune system, the autonomic nervous system, vascular regulation, and the central and peripheral nervous systems in response to tissue injury, pathogens, and irritants comprises the sensation of pain by the body. Pain can then play a vital protective role for an organism, as is the case with acute inflammation that results in the perception of pain, leading to avoidance of noxious stimuli and encouraging healing of damaged tissue (16).

Inflammatory mediators, produced during inflammation, evoke pain by directly activating and sensitizing nociceptors. Inflammation sensitizes nociceptors (pain-sensitive neurons), leading to increased pain perception. Inflammatory mediators, such as prostaglandins and cytokines, di-

rectly activate nociceptors (7). Pain during caesarean section has potential for later consequences. In the UK, pain during caesarean section was the most common cause of litigation related to obstetric anesthesia care. Pain during caesarean section was described by an obstetric anesthetist from the perspective of a patient who experienced pain and offered ideas for improving practice (17). Significant pain during caesarean section can affect the patient's experience and has been associated as an independent risk factor for postpartum posttraumatic stress disorder (PTSD). According to the American Society of Anesthesiologists (ASA), neuraxial anesthesia for cesarean delivery is generally considered the preferred method of anesthesia, being used for over 95% of elective and 80% of emergency cesarean deliveries in the United States (18). Although highly reliable, neuraxial techniques may be inadequate or may not provide complete surgical anesthesia. Pain during cesarean delivery can be managed by supplementing neuraxial anesthesia, intravenous or inhalational analgesia/anesthesia, or conversion to general anesthesia (19, 20).

CONCLUSIONS

Overall, the analgesic protocol we followed had a satisfactory impact on pain management.

Some discrepancies between patient satisfaction by VAS score assessment and patient perception by qualitative assessment suggest the need for an in-depth qualitative assessment for better pain control. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Therefore, it can be expected that assessing postoperative pain using negatively valanced communications will negatively affect the patient's perceptions of her postoperative experience. Postoperative pain management requires accurate and reliable assessment methods, performed regularly and continuously. Although more outcome measures are needed to adequately capture the complexity of the pain experience, in clinical practice, the use of pain assessment techniques shows that simple scales such as the visual analog scale (VAS) score or the verbal numeric rating score (VNRS) are useful for current practice.

In the postoperative context, the patient's functional capacity can also be assessed using the VAS for pain at rest (static) and with movement (dynamic). The lower the score, the faster the immediate recovery, the earlier the patient's mobilization, in the case of cesarean section, the greater the possibility of caring for the newborn, the establishment of lactation, and for gynecological interventions, the reduced incidence of postoperative complications, namely the resumption of vital organ function, intestinal peristalsis, and the decrease in postoperative headache. There is no "gold standard" for postoperative pain management. Choices are determined in part by drug availability, individual preferences, resource limitations, and financial considerations, and are largely based on opioids, supplemented with anti-inflammatory analgesics, nerve blocks, or other adjunctive techniques. Post-cesarean section pain, resulting from surgical tissue damage, is a major cause of patient dissatisfaction and must be addressed seriously to enable mothers to recover rapidly from a functional standpoint and optimize the early stages of mother-infant interaction. Acute post-cesarean section pain, if not adequately controlled, is associated with prolonged hospitalization, increased costs, and the incidence of chronic pain. Ade-

quate management of post-cesarean pain remains a challenge in the hospital. Better interprofessional collaboration, self-administered analgesia, scheduled medical prescriptions and increased availability of analgesic medications may contribute to improving postoperative pain management, with better pain scores.

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MULTIFACTORIAL ANALYSIS OF MALIGNANT ASCITES: ETIOLOGICAL FACTORS AND PROGNOSTIC DETERMINANTS IN A LARGE HOSPITAL COHORT

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MULTIFACTORIAL ANALYSIS OF MALIGNANT ASCITES: ETIOLOGICAL FACTORS AND PROGNOSTIC DETERMINANTS IN A LARGE HOSPITAL COHORT (Abstract): Carcinomatous ascites is a manifestation of advanced-stage disease that can arise from various malignancies, most commonly digestive cancers. This study analyzes the impact of ascites development on disease progression, survival outcomes and several prognostic factors. **Materials and methods**: This is an observational and retrospective study of 1,470 patients diagnosed with ascites, irrespective of etiology, admitted to the Gastroenterology and Internal Medicine Clinic of Sf. Ioan Clinical and Emergency Hospital Bucharest, a Romanian Tertiary Hospital, during 2015-2023. This study aimed to provide a comprehensive statistical analysis of patient characteristics and etiological factors associated with ascites, focusing on malignant ascites. **Results:** Malignant ascites was identified in approximately 10% of patients with ascites and was significantly more frequent in association with digestive malignancies compared with extradigestive tumors. Tumors of digestive origin-particularly hepatic cancers-was markedly more often associated with ascites, while extradigestive neoplasms showed a substantially lower incidence. The presence of ascites was strongly associated with malignancy, patients with ascites having a significantly higher prevalence of cancer compared with those without ascites. **Conclusions:** The findings regarding patients with malignant ascites were contextualized considering local patterns of disease etiology, which may influence prognosis and clinical outcomes. **Keywords:** CARCINOMATOUS ASCITES, DIGESTIVE MALIGNANCY, EXTRADIGESTIVE NEOPLASIA.

INTRODUCTION

Ascites is the pathological accumulation of peritoneal fluid, most commonly occurring as a consequence of hepatic cirrhosis complicated with portal hypertension (1, 2). The onset of ascites in chronic liver disease reflects disease progression and is associated with unfavorable outcomes, increased mortality, and the development of major complications with a negative impact on survival, including hepatorenal syndrome, spontaneous bacterial peritonitis, and hydrothorax (3, 4, 5). Approximately 5-10% patients with compensated liver cirrhosis develop ascites per year, that reduces the overall 5-year survival from 80% to 30% (6). In liver cirrhosis, multiple mechanisms are responsible for ascites

development, most important being portal hypertension with splanchnic arterial vasodilation and lymph hyperproduction (7, 8, 9).

Advanced liver disease is often accompanied by severe cytopenia especially thrombocytopenia due to portal hypertension. A minimally invasive technique, that is partial splenic embolization has been designed in order to reverse this issue. A rare complication of this procedure is spleno-portal axis thrombosis with ascites development (10).

Malignant ascites is defined by accumulation of peritoneal fluid with atypical cells and represents a complex entity that encompasses several illnesses, both digestive and extradigestive neoplasms. Different mechanisms have been proposed regarding the ascites that accompanies abdominal tumors. Approximately 10% of patients exhibit characteristics of malignant ascites, with peritoneal metastasis due to an underlying cancer, usually ovarian or breast cancer, as well as neoplastic disease of digestive origin - gastric, colonic, appendix, biliary tract or pancreas (1). The peritoneal fluid accumulation is caused by obstruction of lymphatic vessels and enhanced vascular permeability due to inflammation which is typical for a neoplastic process (11, 12). Lymphatic obstruction was recognized as a major factor in malignant ascites pathogenesis since 1953 when Holm-Nelson injected India Ink into the peritoneal cavity of mice with malignant ascites and observed that the substance remained into the peritoneal cavity (13, 14).

It is well known that abdominal tumors induce an inflammatory response in the abdominal cavity that facilitates adhesion of malignant cells to peritoneum and carcinomatosis development (12, 15). Malignant ascitic fluid shows high protein level, elevated LDH and low SAAG. The cytological examination of ascitic fluid is not always diagnostic; a negative result does not exclude a neoplastic etiology (11).

A totally different mechanism is seen in ascites that accompanies abdominal tumors. Abdominal neoplasia can lead to the development of ascites through direct tumor invasion of the portal venous system, portal vein thrombosis, or mass effect from tumor bulk, even when the ascitic fluid does not contain malignant cells (11, 16).

Regarding peritoneal carcinomatosis due to intraabdominal tumors, the most frequent neoplasia associated with peritoneal carcinomatosis is ovarian cancer.

The process of carcinomatosis development is initiated by cell exfoliation from primary tumor, followed by their migration into the peritoneal cavity and adhesion of epithelial ovarian cells to mesothelial peritoneum cells and underlying stroma (17, 18, 19). Ascites formation along with carcinomatosis carries the worst prognostic in terms of disease progressions, recurrence and survival (20, 21). Ovarian cancer is the six most common cause of female cancer, the second most frequent gynecological malignancy in Western countries and the most common cancer related death in women, with a low survival rate of 39% at 5 years (18, 22, 23). Almost 70% percent of patients have advanced disease at diagnosis, that is stage III-IV and exhibit peritoneal dissemination (23, 24). Peritoneal metastasis requires a thorough assessment, using cutting-edge imaging methods FDG PET /CT, CT and PET MRI, that allow to establish the disease extent and guide the subsequent therapeutic management (25). In this particular condition, glycoprotein

CA 125 which is widely recognized as a tumoral marker for ovarian cancer, is also synthesized by peritoneum mesothelial cells, independent of malignant etiology, due to the pressure of ascitic fluid exerted on the mesothelial lining (26).

Another malignancy with well-known peritoneal involvement is gastric cancer. Peritoneal carcinomatosis is associated with poor prognosis and a significant decrease in survival rate, the median survival ranging from 2 to 9 months (27, 28).

Malignant ascites is found in 18 % of patients with gastric cancer and rarely represents the presenting symptom at the time of diagnosis (29). Risk factors for peritoneal involvement in gastric cancer are related to cancer stage, age, invasion depth and tumor location, cardia and gastric corpus tumors having high risk of peritoneal dissemination (30). Generally, malignant ascites develops in the advanced stages of gastric tumor and is typically seen as a late manifestation of disease, accompanied by an altered clinical status (31).

Colorectal cancer remains a major public health concern despite the significant progress achieved in early detection through screening programmes. It accounts for aproximately10% of worldwide neoplasms, with 25% of cases being discovered only at metastatic stage (32).

Among these, colorectal tumor with peritoneal metastases represents an advanced and challenging condition associated with a poor prognosis. The most frequent histological subtypes are mucinous adenocarcinoma and signet ring cell carcinoma, both characterized by aggressive behavior and low survival rates compared to non-mucinous forms. An essential diagnostic tool in the evaluation of these tumors is immunohistochemistry, which allows an accurate tumor classification and guides the therapeutical management (33). Its utility has been well established over the time, demonstrating its ability to identify neoplasms with high potential for dissemination and unfavorable clinical outcomes, including peritoneal metastases (34, 35).

Carcinomatous ascites is commonly encountered in elderly patients, who often present with multiple underlying pathologies, including various malignancies, abdominal metastatic disease or peritoneal carcinomatosis from un unknown primary neoplasm (37, 38). In clinical settings, a common scenario consists of substantial abdominal fluid accumulation, reflecting tumor dissemination and peritoneal involvement. A comprehensive diagnostic work-up is essential to evaluate the extent of neoplastic spread and to determine the primary tumor origin, most commonly within the gastrointestinal or gynecologic tract (39).

MATERIALS AND METHODS

This retrospective study analyzed 13,264 patients from consecutive admissions to the Gastroenterology and Internal Medicine Clinic of Sf. Ioan Emergency Hospital over an eight-year period, from 2015 to 2023.

Out of this study population of 13,264 hospitalizations, 1,470 patients (11.1%) were diagnosed with ascites and constituted *the primary study cohort.* Our unit can provide several diagnostic and interventional procedures, such as emergency endoscopy and partial splenic embolization for hypersplenism secondary to liver cirrhosis. Gastroenterology and Internal Medicine wards were analyzed together due to overlapping clinical conditions. The purpose of this study was to systematically

evaluate the demographic and clinical characteristics of patients with ascites and to analyze the underlying etiological factors, with particular emphasis on the patterns and features associated with malignant ascites.

RESULTS

The results are organized into two main parts. The first provides a general overview of the entire study population, based on all hospital admissions (n = 13,264), which serves as the reference population. The second part presents a more detailed analysis focusing specifically on patients diagnosed with ascites (n = 1,470).

Baseline ward population. It is important to note that all age decades were represented in the study population, ranging from adolescents to very elderly patients, including individuals over 100 years of age.

TABLE I.

Distribution of ascites according to age decade and gender

Age group	Female			Male			Overview 2015-2023		
	Ascites	Non-Ascites	Total Female	Ascites	Non-Ascites	Total Male	Ascites	Non-Ascites	Total
10-19 years	0	8	8	0	16	16	0	24	24
20-29 years	2	129	131	4	122	126	6	251	257
30-39 years	6	182	188	43	246	289	49	428	477
40-49 years	47	444	491	140	602	742	187	1.046	1.233
50-59 years	77	769	846	263	895	1.158	340	1.664	2.004
60-69 years	172	1.474	1.646	349	1.629	1.978	521	3.103	3.624
70-79 years	105	1.563	1.668	148	1.272	1.420	253	2.835	3.088
80-89 years	73	1.264	1.337	31	844	875	104	2.108	2.212
90-99 years	5	203	208	5	128	133	10	331	341
Over 100 years	0	1	1	0	3	3	0	4	4
Total	**487**	**6.037**	**6.524**	**983**	**5.757**	**6.740**	**1.470**	**11.794**	**13.264**

Characteristics of patients with ascites

Age comparison. Regarding the study cohort of 1,470 diagnosed with ascites, the prevalence with ascites is not uniformly distributed across age groups; the curve demonstrates a more pronounced occurrence in midlife. Ascites showed a clear age-related distribution. No cases were identified among adolescents aged 10-19 years, suggesting that ascites is exceedingly rare in this age range in the present cohort, while only 2.3% of patients in the 20-29 age group presented with ascitic fluid. In contrast, a sharp increase was observed beginning with the 30-39 age group, in which the prevalence rose more than fourfold to 10.3%. This upward trend continued into the subsequent decades, with the highest prevalence observed in patients aged 50-59 years, at approximately 17%, indicating that nearly one

in six hospitalized individuals in this age range had ascites. Beyond the age of 60 years, the prevalence declined gradually, decreasing to 14% in the 60-69 age group, 8.2% in patients aged 70-79 years, and falling below 5% in the subsequent decades, with values of 4.7% in patients aged 80-89 years and 2.9% in those aged 90-99 years, reflecting a clear reduction in ascites prevalence at advanced ages.

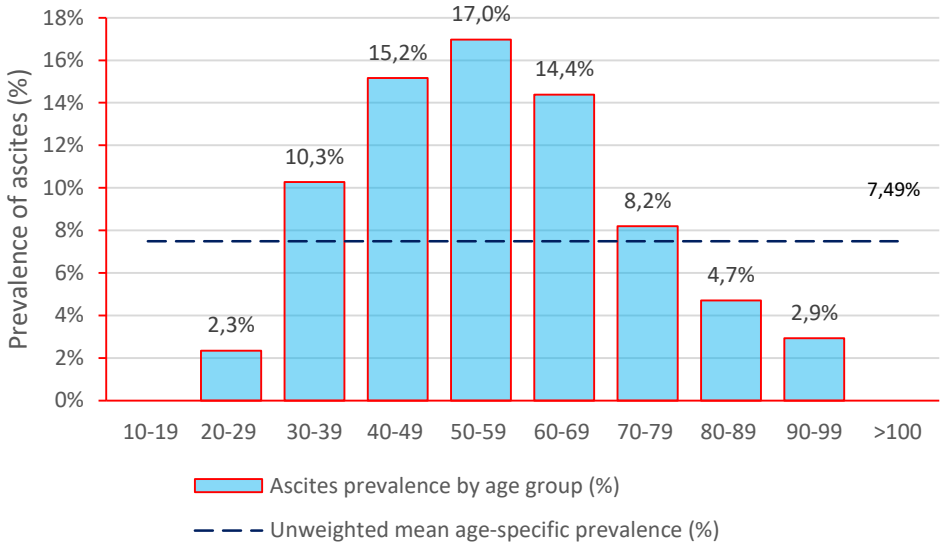

Fig 1. Prevalence of ascites by age groups

Considering all ten age groups as a whole, the mean prevalence is 7.5%, while the dispersion is higher (variation coefficient 85%), confirming that the phenomenon is concentrated around the middle age decade. The chart demonstrates that ascites is not uniformly distributed across age groups but is predominantly a condition affecting middle-aged adults.

Gender comparison. Out of a total of 13,264 hospital admissions, 1,470 patients were diagnosed with ascites. The graphical analysis revealed distinct age- and gender-related patterns in ascites prevalence.

*Male patients***.** From the earliest observable values (approximately 3% in men aged 20-29 years), prevalence increased markedly to 15% in men aged 30-39 years, reaching an absolute peak in the 50-59 decade at approximately 23%, corresponding to nearly one in four male patients.

Overall, men accounted for nearly two-thirds of all ascites cases in the database (983 out of 1,470; 66.8%).

*Female patients***.** In women, prevalence was approximately 2% in the 20-29 age group and increased to around 9-10% across the 40-69 age decades, without exceeding the overall threshold of 11%. The female peak occurred in the 60-69 age group (approximately 10%), remaining about seven percentage points lower than that observed in men of the same age. After the age of 70 years, prevalence in both genders declined and the gap narrowed. In the 70-79 age group, prevalence decreased

to approximately 10% in men and 6% in women. In the 80-89-decade, prevalence among men fell below 4%, while women maintained a prevalence of around 5-6%. In patients aged over 90 years, ascites prevalence dropped below 4% in both genders.

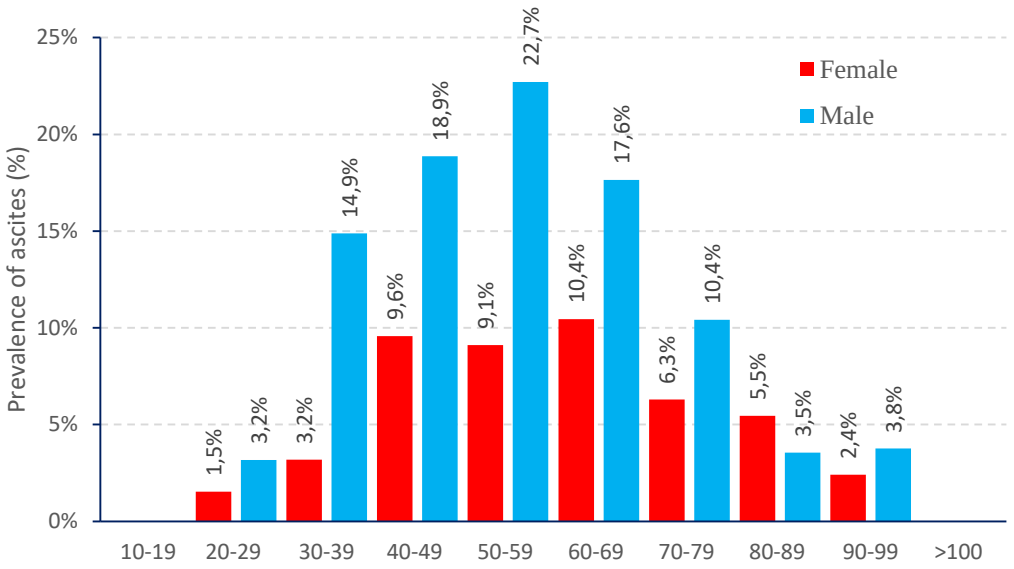

Fig. 2. Prevalence of ascites by gender and age groups

At first glance, the data suggest that men develop ascites more frequently than women: despite a nearly equal number of hospital admissions for each sex, the number of male ascites cases is approximately double from that observed in females. However, to formally confirm this observation, a chi-square (χ^2) statistical test is required, as it assesses the association between the two categorical variables-gender and the presence of ascites.

The following hypotheses were formulated: the null hypothesis (H_0) states that the gender distribution is the same among patients with ascites and those without ascites, whereas the alternative hypothesis (H_1) assumes that the gender distribution differs between the two groups. If the statistical test rejects the hypothesis that gender and ascites are independent, this would indicate that gender should be considered a relevant factor in the clinical interpretation of ascites.

TABLE II.
Gender-specific distribution of ascites and non-ascites cases

Gender	Ascites	No Ascites	Total
Female	487	6.037	6.524
Male	983	5.757	6.740
Total	**1.470**	**11.794**	**13.264**

The chi-square test of independence showed a statistically significant association between gender and the presence of ascites (χ^2 (1) = 169,81, p ≈ 8,14 × 10^{-39}).

Since the p-value is below the conventional significance threshold of 0.05, the null hypothesis is rejected, indicating a statistically significant difference in gender distribution between patients with ascites and those without ascites.

The hospital admitted nearly equal numbers of men and women (6,740 and 6,524, respectively). However, among patients with ascites, the distribution was markedly skewed toward men, with 983 male cases compared with 487 female cases. When expressed as proportions, 14.6% of men developed ascites, compared with 7.5% of women, corresponding to an almost 2: 1 ratio.

The statistical analysis confirms this observation: male gender is strongly associated with the presence of ascites, indicating that the observed difference is not due to random variation but reflects a genuine risk factor.

TABLE III.
In-hospital outcomes by ascites status

Ascites / Non-Ascites	Discharged	Deceased	Transferred	Total
Patients with Ascites	912	489	69	1.470
Patients without Ascites	9.582	1.871	341	11.794
Total	**10.494**	**2.360**	**410**	**13.264**

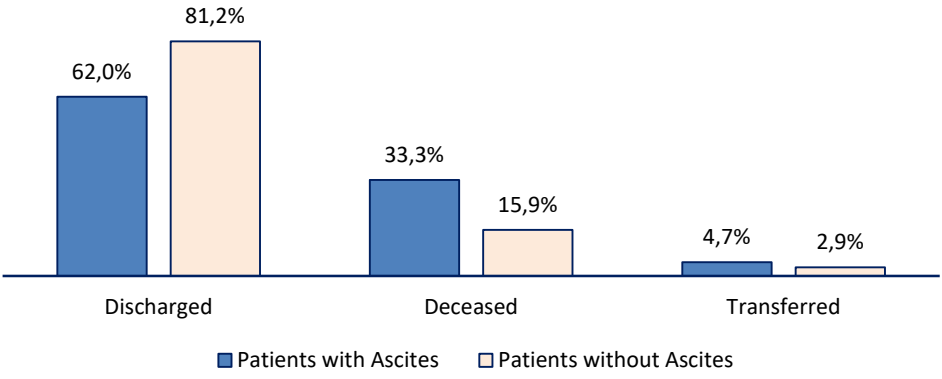

Fig. 3. In-hospital outcomes by ascites status (%)

The outcome profile differs markedly between patients with and without ascites. As illustrated in the chart, the discharge rate was substantially lower among patients with ascites (62%) compared with those without ascites (81.2%). Conversely, in-hospital mortality was notably higher in the ascites group (33.3%) than in the non-ascites group (15.9%), indicating a less favorable clinical course for patients presenting with ascites.

Transfers were uncommon in both groups but occurred slightly more frequently among patients with ascites (4.7% *vs.* 2.9%).

Overall, these findings suggest that ascites is associated with worse short-term hospital outcomes, characterized by a lower likelihood of discharge and a higher risk of in-hospital death. This pattern is consistent with ascites being a marker of more advanced disease and greater clinical severity; however, these observations are descriptive and do not establish causality or account for potential differences in baseline risk between the two groups.

Statistical analysis of patients with ascites and underlying etiologies. This analysis evaluates the association between the presence of ascites and the existence of a malignant tumor. Ascites may have multiple causes; however, in an oncological context, it often represents a secondary manifestation of tumor dissemination or a consequence of tumor-related pathophysiological processes. Identifying the frequency of malignant tumors in patients with ascites contributes to understanding the significance of ascites as a clinical indicator in the differential diagnosis.

TABLE IV.
Association between ascites and tumor status

Ascites status	No malignancy	Malignancy present	Total
YES	1.154	316	1.470
No	10.329	1.465	11.794
Total	**11.483**	**1.781**	**13.264**

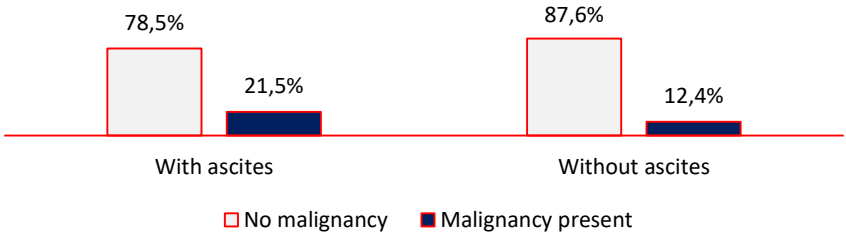

Fig. 4. Malignancy status according to ascites presence

In the analyzed cohort (n = 13,264 patients), ascites was present in 1,470 patients (11.1%). Among these, 316 patients (21.5%), had a prior diagnosis of a malignant tumor, compared to only 12.4% of patients without ascites who had a known neoplasia. The results indicated a significant statistical association between the presence of ascites and tumor status.

Patients with ascites had a prevalence of malignant tumors of 21.5%, compared to 12.4% among those without ascites. This difference of 9.1 percentage points is supported by high values of risk indicators:

- Relative risk (RR = 1.73): patients with ascites have a 73% higher risk of having a malignant tumor.
- Odds ratio (OR = 1.93): the likelihood

of an association between ascites and cancer is nearly doubled.

• Chi-square test (χ^2, 1 df = 92.6; $p < 0.0001$): confirms the statistical significance of this relationship.

These results support the hypothesis that ascites represents an important clinical sign suggestive of an underlying malignancy, particularly in the absence of obvious hepatic causes. Therefore, patients presenting with ascites of unclear etiology should undergo further oncologic and imaging investigations.

TABLE V.

Estimation of the Risk of Malignancy Associated with the Presence of Ascites

Indicator	Value	Confidence Interval 95%
Proportion of cancer patients with ascites	21.50%	19.4% - 23.6%
Proportion of cancer patients without ascites	12.40%	11.8% - 13.0%
Absolute difference	+9.1 pp	—
Relative risk (RR)	1.73	1.55 - 1.93
Odds ratio (OR)	1.93	1.69 - 2.21
Chi-square test (χ^2, one degree of freedom)	≈ 92.6	$p < 0.0001$

Comparison between digestive and extradigestive malignant tumors associated with ascites

The present analysis included a total of 13,264 clinical cases, of which 1,470 patients presented with ascites and 11,794 did not exhibit this feature at the time of evaluation. Within this cohort, 28 distinct types of malignant tumors were identified and classified as digestive or extradigestive malignancies according to anatomical and functional criteria, based on the World Health Organization (WHO) and European Society for Medical Oncology (ESMO) guidelines from the 2020-2025 period. Among the analyzed cases, 2,823 records corresponded to patients with confirmed malignant tumors, forming the database used for the detailed analysis of the relationship between tumor localization and the presence of ascites.

Of these, 1,105 were classified as digestive malignancies and 1,718 as extradigestive malignancies, according to the classifications mentioned above.

This classification includes neoplasms of the digestive tract proper (esophagus, stomach, colon, rectum) as well as those arising from other digestive structures (liver, biliary tract, pancreas, peritoneum), in contrast to tumors originating from other systems - lymphatic, respiratory, urinary, endocrine, nervous, or reproductive. Within these two categories, each of the 28 tumor types was evaluated according to the presence or absence of ascites to determine the etiology of malignant ascites. Tumors of digestive origin (blue) were much more frequently associated with ascites, whereas extradigestive tumors (red) exhibited a substantially lower incidence (fig. 5). The highest proportions of ascites were observed in hepatic cancer (52.0%) and peritoneal carcinomatosis, a condition characterized by the dissemination of tumor cells on the peritoneal surfaces (44,4%), with lower rates in pancreatic cancer (29.1%) and liver metastases (19.3%), these conditions representing the main causes of malignant ascites in the studied cohort.

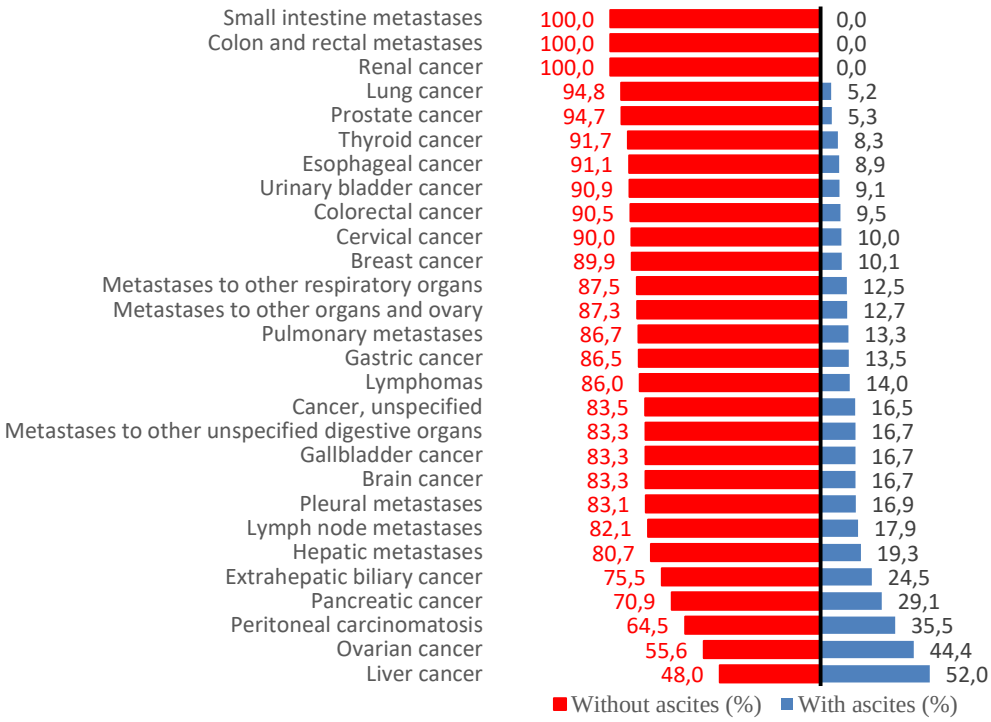

Fig. 5. Distribution of patients without ascites according to the type of malignant tumor

In comparison, extradigestive tumors, such as lung cancer, breast cancer, lymphomas, or ovarian metastases, had a minor involvement (<10%), confirming that malignant ascites is predominantly associated with abdominal neoplasms and only occasionally with systemic or extradigestive tumors.

The differences observed between the two groups (digestive *vs.* extradigestive) reflect distinct pathophysiological mechanisms. In digestive tumors, ascites typically develops as a result of portal obstruction, hepatic invasion, or peritoneal dissemination-mechanisms that promote the accumulation of fluid in the abdominal cavity. In contrast, in extradigestive tumors, ascites usually occurs secondarily and is associated with extensive metastases or widespread lymphatic involvement.

This initial visual and descriptive analysis provided a clear framework for testing statistical hypotheses regarding the association between tumor type and the presence of ascites, which will be explored in detail in the subsequent section. To evaluate the relationship between malignant tumor localization and the development of ascites, a comparative analysis was performed between the two main groups: digestive and extradigestive malignancies.

The difference between the initially reported total of 2,823 oncological records and the 2,261 records included in the final statistical analysis (tab. VI) resulted from a rigorous data selection process. To ensure the validity of statistical testing (including the chi-square test and the calculation of

relative risk) and to minimize potential confounding, the following criteria were applied:

- *Inclusion criteria*: only records with a single, clearly defined tumor localization-either strictly digestive or strictly extradigestive-were retained.
- *Exclusion criteria*: a total of 562 records with concomitant tumor localizations (both digestive and extradigestive) were excluded.

This approach was necessary because the simultaneous presence of both tumor types would have precluded the precise attribution of ascites to a specific tumor category. Consequently, the analysis was performed at the level of oncological records, ensuring statistical independence between the two comparison groups and allowing for reliable estimation of risk indicators, including relative risk (RR) and odds ratio (OR).

For this purpose, 2,261 oncological records corresponding to patients diagnosed with digestive and extradigestive malignant tumors were included. As some patients presented simultaneously with tumors of both types, the analysis was conducted at the level of tumor records rather than individual patients, in order to accurately capture the distribution according to tumor localization.

TABLE VI.

Distribution of Malignant Tumor Records by Tumor Type and Ascites Status

Tumor Type	With ascites	Without ascites	Total
Digestive	267	837	1.104
Extradigestive	150	1.007	1.157
Total	**417**	**1.844**	**2.261**

The results show that ascites was present in 24.2% of patients with digestive malignancies, compared with 13.0% of those with extradigestive tumors. This statistically significant difference (χ^2 = 36.8; $p < 0.001$) indicates a clear association between digestive tumor origin and the development of ascites.

To quantify this risk, the following measures were calculated:

- Relative risk (RR = 1.86): patients with digestive malignancies have an almost twofold higher risk of developing ascites compared with those with extradigestive tumors.
- Odds ratio (OR = 2.14): confirming a significantly increased likelihood of ascites in digestive neoplasms.

These findings support the hypothesis that pathogenetic mechanisms involved in digestive tumors-such as portal venous obstruction, hepatic infiltration, portal hypertension, and peritoneal carcinomatosis-promote peritoneal fluid accumulation to a significantly greater extent than in extradigestive malignancies. In contrast, in extradigestive tumors (e.g., lung, breast, lymphatic, or urogenital malignancies), ascites occurs less frequently and generally in advanced stages of disease dissemination, typically through secondary mechanisms such as peritoneal metastases or lymphatic obstruction.

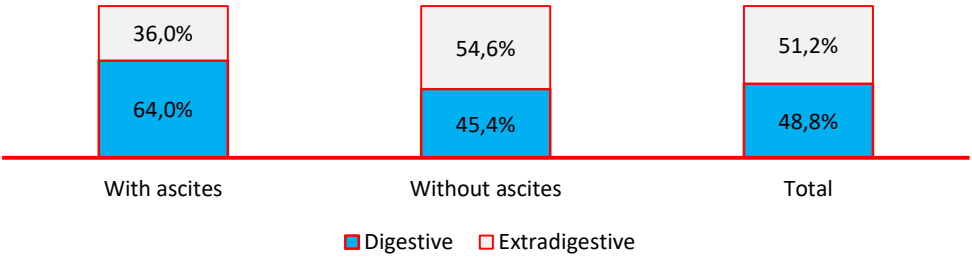

Fig. 6. Percentage Distribution of Digestive *vs.* Extradigestive Malignancies by Ascites Status

Figure 6 highlights the proportional differences between digestive and extradigestive malignant tumors according to the presence of ascites. Of the 417 tumor records associated with ascites, 64.0% corresponded to tumors of digestive origin, whereas only 36.0% were extradigestive. In contrast, among the 1,844 cases without ascites, the distribution was reversed: 54.6% of tumors were extradigestive and 45.4% were digestive. Overall, within the entire dataset of 2,261 tumor records, the distribution was relatively balanced, with 48.8% digestive and 51.2% extradigestive malignancies. However, this balance changed markedly when ascites status was considered, indicating a clear association between digestive tumor localization and the presence of ascites. This observation is consistent with the results of the statistical analysis ($\chi^2 = 36.8$; $p < 0.001$), confirming that malignant ascites occurs significantly more frequently in tumors of digestive origin.

DISCUSSION

Malignant ascites is defined as the accumulation of peritoneal fluid containing malignant or atypical cells and represents a complex clinical entity associated with a wide spectrum of underlying diseases. It occurs in the context of both digestive and extra-digestive malignancies and is typically indicative of advanced-stage disease. The development of malignant ascites reflects multifactorial pathophysiological mechanisms, including peritoneal carcinomatosis, increased vascular permeability, lymphatic obstruction, and tumor-related inflammatory processes (11, 17, 18). Its presence is often associated with significant morbidity and carries important diagnostic, therapeutic, and prognostic implications (23, 24). Benign ascites typically develops in patients with non-malignant conditions such as liver cirrhosis with portal hypertension and is not associated with the presence of cancer or malignant cells in the ascitic fluid. A new clinical entity has been described, referring to the development of ascites in patients with neoplastic disease in the absence of detectable tumor cells in the peritoneal fluid, termed para malignant ascites (11). The clinical presentation of ascites also varies according to patient demographics and underlying disease. Significant differences between genders were observed among patients with ascites. Liver cirrhosis and its main complication, hepatocarcinoma, occur predominantly in men, reflecting the higher prevalence of hepatic risk factors in the male

population. Patient age varies according to the underlying disease: cirrhotic ascites typically occurs in patients around 60 years of age, whereas malignant ascites occurs in older patients (approximately 70 years). Generally, most patients with ascites have it secondary to chronic liver disease, while malignant ascites accounts for nearly 10% of cases, consistent with previously published data (8, 41, 42, 43). Ascites was significantly more frequent in tumors of digestive origin, particularly hepatic cancers and peritoneal carcinomatosis, whereas tumors of extradigestive origin were rarely associated with ascites. On the other hand, abdominal metastatic disease-particularly liver metastases of various origins-showed a lower incidence of peritoneal fluid accumulation. Some authors have reported ovarian cancer as the most frequent cause of malignant ascites development (16). This finding, however, is not consistent with our data, which may reflect the specific patient population of our clinic, where digestive diseases predominate. It is important to consider the limitations of our study when interpreting these results. In particular, the specificity of our department does not encompass the full spectrum of malignancies that can cause ascites, and therefore our findings may not provide a comprehensive perspective on all ascitic etiologies and may not be generalizable to all patient populations.The predominance of abdominal and digestive tumors in our clinic likely influenced the observed distribution of ascites, potentially underrepresenting conditions, such as ovarian or systemic cancers, which are reported elsewhere as common causes of malignant ascites. Recognizing this context is essential for accurately interpreting our data and for comparing it with findings from other institutions.

CONCLUSIONS

Ascites is a clinically important manifestation, reflecting significant underlying disease and carrying diagnostic, therapeutic, and prognostic implications. In our cohort, ascites was more frequently associated with digestive malignancies, particularly hepatic cancers and peritoneal carcinomatosis, whereas extradigestive tumors rarely resulted in fluid accumulation. These findings should be interpreted in the context of our clinic's patient population, which predominantly manages digestive diseases; this specificity likely influenced the observed distribution and may underrepresent other malignancies such as ovarian or systemic cancers. Overall, the presence of ascites, whether benign or malignant, serves as an important clinical indicator warranting careful evaluation and targeted management.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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ETHICAL AND DEONTOLOGICAL ASPECTS OF PALLIATIVE CARE IN GERIATRIC PATIENTS IN ROMANIA. CASE REPORT

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ETHICAL AND DEONTOLOGICAL ASPECTS OF PALLIATIVE CARE IN GERIATRIC PATIENTS IN ROMANIA. CASE REPORT (Abstract): We present the case of an 82-year-old female patient with a personal history of hypertension and diabetes, who presented to the emergency room for marked physical asthenia, loss of appetite and diffuse abdominal pain with onset about a week earlier. Recent history showed that she suffered a same-level fall in the past 24 hours, and was unable to get up from the floor where she had laid for several hours. On admission, the patient complained of severe diffuse osteoarticular pain that kept her in bed; she also complained of lower abdominal pain on superficial and deep palpation that proved to be a urinary tract infection. She was dehydrated and anemic (Hb=7.3 g/dL); she was depressed and very anxious to get home as quickly as possible. The therapeutic approach was focused on the prompt correction and the thorough investigation of the severe anemia syndrome, the complex therapy of osteoarticular pain, and prevention of the complications of immobilization syndrome. Despite patient's wish that multiple and complex investigations not to be conducted and to be discharged from the hospital and go home, the family strongly insisted to continue searching, no matter what, for the causes of anemia with the purpose of prolonging her life, but with very poor outcome on patient's quality of life. With no specific laws concerning the respect for patient's wishes, the medical personnel had very little to say in this matter, even if we watched the decline in her quality of life with every day she spent away from home. **Keywords:** PALLIATIVE CARE, ELDERLY AUTONOMY, FAMILY SUPPORT.

In recent years, the dramatic fall in birth rate, along with the aging of the population and the increasing number of chronic patients has led to an increase in the needs for specific care.

In Romania, isolated initiatives draw attention to the continuous increase in the needs for special care to be provided to elderly patients with severe chronic diseases or disabilities who can no longer take care of themselves (1).

While performing the medical act, the medical team must meet the patients' needs to be informed, to be understood, to be respected, to be helped to reduce anxiety, to feel safe, to be independent, to preserve their dignity and finally to help relieve their pain (2).

Autonomy is a person's right to self-determination, independence and freedom. It is not just the right to self-determination and treatment of the body; it also implies the right to receive any information regarding himself/herself, his/her way of life and health, the right to decide to whom some of his/her personal, sacred data can be entrusted; these are an integral part of the meaning of self and identity. Knowing personal information about someone can play an important role in procuring the sense of security, freedom and self-esteem (3).

When the patient refuses treatment or any investigation, it is considered that his will prevails over his health.

The legal framework in Romania (Law No. 46/2003 on the Patient's Right, updated in 2017, Article 13) grants the patients the right to refuse or discontinue medical treatment, assuming responsibility for their decision, in writing, as long as they are well aware and show no loss of judgement (4). In practice, the conduct in such cases is slightly different. Often the caregivers are the ones informed and involved in treatment decisions making in an attempt of either to protect the patient emotionally or due to the pressure (often becoming threats) exerted by caregivers.

CASE REPORT

We present the case of an 82-year-old female patient, living in an urban area with her husband whom she cared for as he was suffering from a major cognitive impairment (vascular dementia), she herself finding it difficult to move around and receiving help from a nurse with some daily activities. She was known to have a history of cardiovascular (hypertension, angina, and degenerative aortic stenosis) and metabolic (type 2 diabetes treated with medication and complicated by peripheral sensory polyneuropathy) diseases, and recurrent urinary tract infections.

She was hospitalized for significant physical asthenia, loss of appetite, diffuse abdominal pain going on for about a week and severe polyarticular pain which did not allow her to move. From her family's (her sister) statements it came out that the patient had suffered a same-level fall 24 hours previously, without the possibility to get up from the floor, where she was found the next day by her caregiver.

At the time of admission, the patient was in altered general condition, conscious, cooperative, with difficulty in moving around due to the post-traumatic pain, dehydrated, with pale skin and multiple bruises on the upper extremities. Cardiovascular examination revealed rhythmic heart sounds, systolic murmur in the aortic outbreak radiating to the carotids, normal blood pressure. Also noted was the abdomen increased in volume due to *panniculus adiposus*, with lower abdominal pain on superficial and deep palpation.

Biochemical findings showed multiple concomitant medical conditions:

a. *Severe anemia syndrome*, normochromic, normocytic (Hb = 7.3 g/dL), non-regenerative (reticulocytes = 11,377/mm^3), with low serum iron and increased ferritin levels. Considering that in evolution we recorded a drop-in hemoglobin of 1.0 g and the presence of blood in her stools, we interpreted it as an episode of melena, possibly due to a digestive cancer. We explained to the patient and her family that an endoscopy (upper and lower) would be required, investigation refused by the patient, but her family strongly pressed for it. Given the mixed nature of the anemia

(malnutrition + inflammatory syndrome), the therapeutic management was complex, targeting the correction of Hb by administering blood transfusion, iron based medication and dietary supplements.

b. *Urinary tract infection* with *Klebsiella pneumoniae*, *Candida spp.*, with multi-resistant germs and unfavorable progression towards sepsis despite the complex antibiotic treatment. During hospital stay, probably due to bed immobilization and diabetic neuropathy, she developed acute urinary retention which required urinary catheterization and highly contributed to the negative development of sepsis and decline in her quality of life and self-esteem.

c. *Fluctuating blood glucose levels* that required the introduction of low doses of insulin with daily adjustments depending on the glucose profile; the inflammatory syndrome and the depressive syndrome of the patient made her refuse food.

d. *Geriatric evaluation* showed that she was at risk of malnutrition (MNA= 23.5/30), with a minor cognitive impairment (MMSE= 25/30), depressed (GDS= 6/16) and highly dependent on the help of a caregiver.

During hospital stay, she remained confined to bed, resisting all attempts to mobilization, even for personal hygiene. However, with patience and persuasion, we managed to prevent the complications of bed immobilization (daily tapotement, permanent linen change, feeding and hydration with the help of medical staff, regular changes of patient position in bed, etc.); however, due to the increased resistance of the patient to any medical care, after approximately two weeks her general condition deteriorated, she became hemodynamically unstable and was admitted to the ICU department, where she required IOT and mechanical ventilation with positive inotropic support. The invasive maneuvers for endotracheal intubation and urethro-vesical catheterization led to sepsis with multiple starting points (urinary tract, lungs, skin) and with multi-resistant bacteria. At that point, the medical geriatric team considered that it was time to consider palliation versus extensive medication. The possibility to choose home care, in her familiar environment might have improved communication and patient compliance with the hygienic, dietary and drug treatment (5). However, due to lack of qualified personnel for home care in Romania and patient's financial limitations, this approach was not an option.

DISCUSSION

Given that autonomy is one of the ethical principles involving the respect for the right of every individual to make their own decisions, the patients' wishes must be complied with if they are of sound mind and they can decide perfectly regarding their last wishes (6).

Thus, the medical staff had to provide the patient with all necessary information for her to make the right decision, to help her understand and apply the decision, in this case being necessary to stop the aggressive investigations and to conduct invasive treatments only with the patient's approval. In our case, the patient had given her consent to continue invasive investigations because of the pressure exerted by her family, but despite her own wishes. The creed of the medical profession is to ensure the highest possible level of quality of life, especially in the case of terminal patients for which medical interventions have no longer a curative purpose.

The decision made freely by the patient should be checked to determine whether it was imposed by another person, especially by the family, who, besides being a support, must also respect his/her wishes.

In the here reported case, the family permanently refused the patient to be discharged from hospital and cared at home and requested, even claimed sometimes, the continuation of all investigations (including CT with contrast substance) and maneuvers (including admission to the intensive care unit where she was intubated and mechanically ventilated), thus negatively influencing her evolution. In addition, the stress placed on the medical team was significant, caused by confrontations with the patient's family on the provision of complex care, without respecting her wish and her privacy (7, 8).

Finally, respecting the patient's autonomy goes hand in hand with human dignity. The law and the society are *pro* patient autonomy as a human right and as a basic principle in the doctor-patient relationship.

CONCLUSIONS

Regardless of the medical decision, the last word belongs to the patient. In our case, despite all efforts to investigate and treat her, the patient wanted to go home, in her familiar environment, to be with her loved ones in the last moments of her life.

The lack of methodology for the enforcement of the laws on patient rights, but also not knowing the components of such laws, makes such situations difficult to manage by the medical staff, situations in which the emotional elements dominate the legal framework, generating the overstepping of attributions and the involuntary exit from this framework.

The fight is between the slogan: *"We did everything humanly possible*", a slogan the family relies on, and the *motto "Respect the wishes of the loved one and who is ready to leave from amongst us*".

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CLINICAL CHANGES AND BIOMETRIC DIFFERENCES IN PATIENTS WITH PRIMARY OPEN ANGLE GLAUCOMA AND DIABETES

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CLINICAL CHANGES AND BIOMETRIC DIFFERENCES IN PATIENTS WITH OPEN ANGLE GLAUCOMA AND DIABETES (Abstract). **Aim of study** was to emphasize the clinical changes and biometric alterations that appear in primary open angle glaucoma (POAG) patients if diabetes was associated. **Material and methods:** The study was designed in a cross-sectional manner, between 2015-2016 in the Ophthalmology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi. We included 87 eyes, from 87 patients, distributed in 4 groups: 24 patients - the control group (cataract), 26 patients - the type II diabetes group, 16 patients – POAG group, 21 patients - the combined disease group (POAG + diabetes). We recorded data related to the age of the patients (years), best corrected visual acuity (BCVA), intraocular pressure (IOP-mmHg), perimetry parameters (MD, PSD-dB), biometric parameters (axial lengths, anterior chamber depth, CDE - Cumulative Dissipated Energy, lens thickness). The duration of glaucoma/diabetes, the type and number of glaucoma medications were noted. Similarly, the duration of diabetes mellitus, glycemic value and glycosylated hemoglobin were recorded. **Results:** Mean age calculated for the control group was 72.33+/-11.26 years, 69.04+/-9.46 years for the DM group, 75.69+/-5.54 years for the POAG group and 59.95+/-3.89 years for the combined pathology. Secondly, we found the highest IOP in the POAG+DM group, compared with the rest of the study categories (20.33+/-2.3 mmHg in group IV *vs.* 18.19+/-4.3 mmHg in group III *vs.* 15.50+/1.9 mmHg *vs.* 14.21+/-2.68 mmHg in group I), $p<0.05$. We found statistically significant differences between the four groups in respect of lens size and the CDE mean values. Our data revealed that the smallest energy was used in group IV (CDE=8.5+/-1.77), compared to all the other categories: I (CDE=17.79+/-7.36, p = 0.000), II (CDE=18.15+/-11.74, p = 0.000), III (CDE=13.90+/-8.11, p = 0.000). Estimated lens volume in group IV was 4.85+/-0.17mm in POAG+DM patients, significantly larger than the rest of the groups ($p<0.05$) **Conclusions**: younger age, higher IOP, larger lens volume and lower CDE mean values were the parameters significantly different in POAG patients, if diabetes was associated. **Keywords**: PRIMARY OPEN ANGLE GLAUCOMA, BIOMETRY, DIABETES

Glaucoma and diabetes (DM) represent one of the most frequent causes of blindness worldwide. In USA by 2020, 3.3 million patients will develop glaucoma and up to 45% of diabetic patients will develop a form of retinopathy (1). Globally, WHO

estimates a total of 360 million cases of diabetes by 2030, whereas the incidence of glaucoma will also increase. Different sources point out that in 2013 there will be about 64.3 million glaucoma patients (2), increasing to 76 million in 2020 and to 111.8 million in 2040 (3). In addition, ocular damage to diabetes is the main cause of blindness in the active population (30-69 years). Recent epidemiological studies have shown that between 1990 and 2012 there was a 27% increase in the proportion of diabetes mellitus and over 64% in diabetic retinopathy cases (4). If the diagnostic features for the primary open angle glaucoma (POAG) definition are rather clear, the pathogenesis is still incompletely elucidated.

Several risk factors involved in the development and progression of POAG have been described, but the precise contribution of each one cannot be established. The general clinical context integrates glaucomatous illness into a systemic disease category (same as diabetes), based on the principle of "a suffering eye in a suffering body" (5).

Multiple risk factors impacting over DM also influence POAG. For example, lack of exercise in aerobic conditions or obesity may influence the evolution of diabetes or IOP, hence the risk of POAG (6, 7). Theoretically, between glaucoma and diabetes, there are several common pathogenic pathways related to pressure disorders (intraocular pressure, ocular perfusion pressure), microvascular disorders and neuroinflammation. From the clinical point of view, the relationship between POAG and DM triggers 2 coordinates as following: first, presence of diabetes mellitus increases the risk of POAG development and second, in a patient with POAG, the presence of DM enhances early changes with potential neurodegenerative effect.

As such the aim of our study was to emphasize the clinical changes and biometric alterations at baseline in primary open angle glaucoma (POAG) patients, if diabetes was associated.

MATERIAL AND METHODS

The study was designed in a cross-sectional manner, between 2015-2016 in the Ophthalmology Clinic of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi. We included 87 eyes, from 87 patients with indication for cataract surgery, distributed in 4 groups: 24 patients - the control group (cataract), 26 patients - the type II diabetes group, 16 patients - POAG group, 21 patients - the combined disease group (POAG +diabetes). We recorded data related to the age of the patients (years), best corrected visual acuity (BCVA), intraocular pressure (IOP-mmHg), MD (dB) and PSD (dB) in automated perimetry (24-4 program, Sita Fast, Humphrey® Visual Field Analyzer, Carl Zeiss, Meditech, CA), biometric parameters: axial lengths, anterior chamber depth, lens thickness and CDE (Cumulative Dissipated Energy) during phacoemulsification. The duration of glaucoma/diabetes, the type and number of glaucoma medications were noted. Similarly, the duration of diabetes mellitus, glycemic value and glycosylated hemoglobin were recorded.

In the study, we only included cases of uncomplicated cataract surgery. Diagnosis of POAG was based on the EGS criteria, described somewhere else (8), whereas the diagnosis of diabetes was based on patients charts and previous documents. Patients with significant general or ophthalmological disorders (e.g. age-related macular

degeneration, retinal vascular occlusions) were excluded, as well as all the cases with previous ocular surgeries.

Cataract surgery was indicated by the ophthalmologist at a time when there was significant lens opacification or when the patient requested it due to significant alterations in the quality of life and vision. Biometric parameters were recorded via immersion A-scan technique.

Ethics. All patients enrolled in the study included in the doctoral research expressed verbally and in writing an agreement for participation in the study, by signing an informed consent. The study protocol and the informed consent were approved by the Ethics Commission of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, as well as by the similar department attached to the "Sf. Spiridon" County Clinical Emergency Hospital Iasi.

Statistical analysis. Data analysis was performed using the *SPSS 20.0* software. The demographic data of the four groups were analyzed. Descriptive statistics elements were used to calculate the averages and median of all numerical parameters entered in the database (age, IOP, biometry parameters, duration of glaucoma/diabetes, number of topical medications in glaucoma, MD, PSD, CDE); $p < 0.05$ was considered statistically significant. The correlations between the variables were performed by Pearson correlation test. The correlation coefficient (r) was declared significant for a $p < 0.05$ value.

RESULTS

In the current study we evaluated data from 87 patients: 44 males (50.5%) and 43 females (49.5%). Yet the gender distribution within the study groups showed that in glaucoma patients there were more males (group III and IV), compared to healthy controls or diabetes group where females were more numerous (fig. 1).

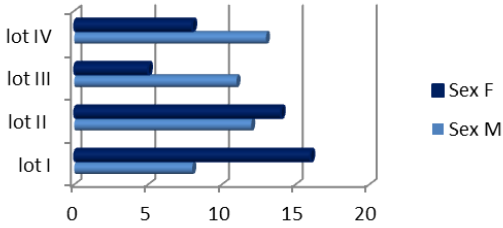

Fig. 1. Sex distribution between study groups

Mean age of the subjects was 72.33 +/- 11.26 year in the control group, 69.04+/- 9.46 years for the DM group, 75.69+/-5.54 years for the POAG group and 59.95+/- 3.89 years in the combined pathology (fig. 2). Absolute values show that the oldest patients belong to the POAG group, whereas the youngest age was calculated for the subjects with combined pathology (POAG + diabetes). Differences were statistically significant when compared DM *vs.* POAG group ($p=0.015$), Control *vs.* POAG+DM group ($p=0.000$), DM *vs.* POAG+DM group ($p=0.000$), POAG *vs.* POAG+DM group ($p=0.000$).

The IOP dynamics between the four groups followed an opposite profile compared to age distribution (fig. 2). Thus, we found the highest IOP in the POAG+DM group, compared with the rest of the study categories (20.33+/-2.3 mmHg in group IV *vs.* 18.19+/-4.3 mmHg in group III *vs.* 15.50+/-1.9 mmHg in group II *vs.* 14.21+/-2.68 mmHg in group I), $p<0.05$.

The number of substances required for IOP control in the two groups with glaucoma did not show significant differences: 3+/-0.87 *vs.* 2.85+/-1.02 drugs (POAG *vs.*

POAG+DM, p=0.489). Yet the clinical impact of this finding might be different: if diabetes was associated with POAG, the patients had significantly higher IOPs despite similar, maximal treatment.

Escalation of treatment (cataract surgery) in these glaucoma patients was necessary, at a younger age and longer life span compared to subjects with glaucoma, but without diabetes.

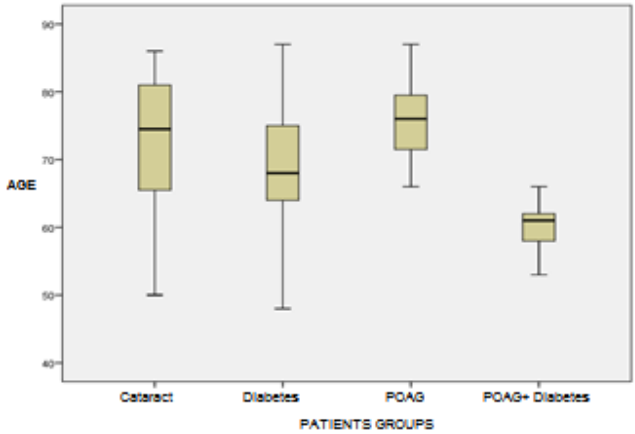

Fig. 1. Age of subjects compared between groups

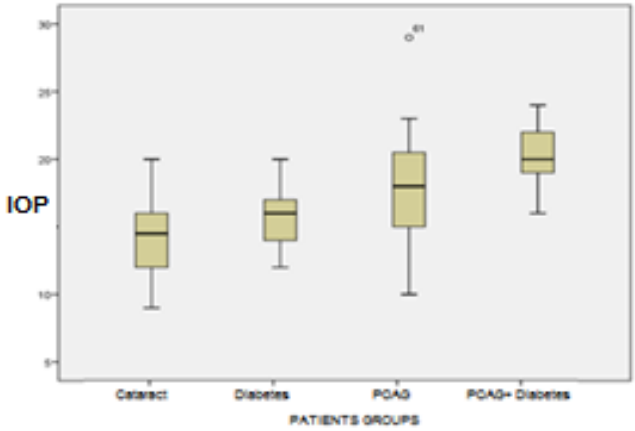

Fig. 2. IOP means compared between groups

Biometric data was summarized in Table I. There were significant differences in these parameters regarding lens size and CDE mean value. Thus, the smallest amount of ultrasound energy was necessary in the POAG+DM group, when compared to the other study group categories. Differences in CDE values for the compared groups were: I *vs.* IV (p=0.000), II *vs.* IV (p=0.000), III *vs.* IV (p=0.000). Also, the largest lens was found in group IV, if diabetes was present in glaucoma patients (p<0.05 compared to healthy controls or POAG patients). No differences were present between the four groups regarding axial lengths (mm) or ACD (mm).

TABLE I

Comparison of means in biometric data between study groups.

Parameter	Control group	Diabetes mellitus group	POAG group	POAG+DM group
AL (mm)	22.87+/-0.64	22.97+/-0.40	22.74+/-0.66	22.87+/-0.65
ACD (mm)	2.96+/-0.41	2.81+/-0.31	2.80+/-0.54	2.74+/0.31
L (mm)	2.36+/-0.35	4.12+/-0.26	3.79+/-0.63	4.85+/-0.17
CDE (mm)	17.79+/-7.36	18.15+/-11.74	13.90+/-8.11	8.5+/-1.77

AL – axial lengths; ACD - anterior chamber depth, L – lens, CDE – Cumulative Dissipated Energy

Clinical implication of this result proves that the structure of the lens in the glaucoma patients is different if diabetes is present; moreover, cataract surgery becomes necessary in younger ages and dictated also by a significantly higher IOP under maximally topical tolerated medication and increased lens size.

From functional deficit point of view, in this study the MD values were similar between groups and helped us categorize our glaucoma patients as having an "advanced disease", according to the EGS criteria (tab. II). However, it should also be considered that the MD recordings might have been influenced by the degree of lens opacification (cataract), mostly since there was no concordance with the degree of localized glaucoma damage (PSD).

Separate analysis of PSD in our glaucoma patients showed that in the combined pathology (POAG+DM) group, the localized deficit was less prominent in comparison with the POAG group. Age has been shown to be a risk factor in POAG, therefore the significantly younger age of the POAG+DM group compared to POAG group might be an actual explanation for this finding. Duration of glaucoma between groups III and IV was similar (45.63 +/- 26.48 months *vs.* 36.86+/- 15.86 months, p>0.05).

TABLE II

Functional perimetric parameters compared between glaucoma groups in the study

Group	POAG group	POAG+DM group	p<0.05
MD (dB)	-13.59+/-9.35	-12.13+/-8.9	p=0.683
PSD (dB)	4.25+/-4.22	2.05+/-0.90	p=0.026

From the metabolic point of view, both groups of diabetic patients exhibited similar values of the blood sugar level: 171 mg/dL in group II *vs.* 155.05 mg/dL in group IV, p> 0.05. Yet a significant longer duration of diabetes was found in group II compared to group IV (133 +/- 77.82 *vs*. 115 +/- 81.24 months, p = 0.004), combined also with a poorer long term metabolic control. HbA1c mean values (%): 8.12 +/- 1.81 (group II) *vs.* 7.12 +/- 1.35 (group IV), p = 0.036.

In POAG and diabetes patients, clinical correlations relevant for this study were depicted in Table III.

PSD is correlated negatively with the age, therefore an earlier glaucoma deficit in younger patients, if diabetes is also present in POAG patients. An increase in medication was correlated with a decrease of the MD levels, stating that we treated more aggressively the patients once we observed a decrease in the functional perimetric parameters

levels. No correlation was detected between the perimetric indices, as they might have not necessarily reflected the reality in terms of glaucoma severity, but lens opacification degree (MD is influenced by the opacities in the lens, as opposed to PSD).

Mean IOP is correlated with the intensity of treatment (number of topical medications) and the blood sugar level. Therefore, if diabetes is associated with POAG, it induces an increase in IOP, increasing the demand for an aggressive treatment.

In POAG, clinical correlations relevant for this study were depicted in Table IV.

TABLE III

Correlations between clinical parameters in POAG+DM patients

Pearson test (r) p<0.05	Age	IOP	Number of medications	MD	PSD	Blood sugar	HbA1c
Age	1	-0.148 0.521	0.09 0.681	0.322 0.207	-0.461 0.039	-0.195 0.396	-0.112 0.630
IOP	-0.148 0.521	1	0.529 0.14	0.307 0.231	0.287 0.208	0.520 0.016	0.140 0.546
Number of medications	0.95 0.681	0.529 0.014	1	-0.545 0.024	-0.021 0.929	0.135 0.561	0.428 0.053
MD	0.322 0.207	0.307 0.231	-0.545 0.024	1	-0.350 0.168	-0.076 0.773	0.047 0.859
PSD	-0.461 0.039	0.287 0.208	-0.021 0.929	-0.350 0.168	1	0.195 0.396	0.355 0.114
Blood sugar	-0.195 0.396	0.520 0.016	0.135 0.561	-0.076 0.773	0.195 0.396	1	0.557 0.009
HbA1c	-0.112 0.630	0.140 0.546	0.428 0.053	0.047 0.859	0.355 0.114	0.557 0.009	1

TABLE IV

Correlations between clinical parameters in POAG patients

Pearson test (r) p<0.05	Age	IOP	Number of medications	MD	PSD	Blood sugar
Age	1	0.154 0.569	0.338 0.889	-0.533 0.091	-0.01 0.970	-0.133 0.624
IOP	0.154 0.569	1	0.790 0.042	0.083 0.807	0.513 0.042	0.543 0.030
Number of medications	0.038 0.889	0.790 0.042	1	0.503 0.115	0.311 0.024	0.521 0.039
MD	-0.533 0.091	0.083 0.807	0.503 0.115	1	-0.649 0.031	0.427 0.191
PSD	-0.01 0.970	0.513 0.042	0.311 0.024	-0.649 0.031	1	-0.447 0.082
Blood sugar	-0.133 0.624	0.543 0.030	0.521 0.039	0.427 0.191	-0.447 0.082	1

IOP levels in these patients correlates positively with the functional localized deficit (PSD) and with the increase of blood sugar levels (although we were not analyzing patients with diabetes). Of course, the higher the IOP became in these

patients, the more aggressive the treatment became. In this study category, we obtained a correlation between the perimetric indices (MD/ PSD) which might indirectly state that based on older age and lack of a metabolic disorder such as diabetes, the MD was less influenced by the lens compound contributing to the general reduced retinal sensitivity (cataract formation) and the glaucoma disease evolved in its specific correlated pattern.

DISCUSSION

In this study, the presence of diabetes modified the clinical and functional parameters, all together with some phacoemulsification parameters (CDE) in glaucoma patients. Biometric measurements were similar between groups, except for the mean lens thickness (mm) that was increased in the combined pathology group, compared to the other categories of interest (controls or glaucoma patients). This only proves that the presence of diabetes induces an early and excessive hydration of the collagen lamellas, based on some very low levels of sorbitol in adult diabetic lenses. The altered osmotic mechanism increases the risk of cataract even more, altogether with glycation and oxidative stress (11). The relationship between the polyol pathway and sugar cataracts has been studied extensively using streptozotocin-induced diabetic rats and galactose fed rats as animal models for insulin-dependent diabetes mellitus (IDDM). In these models, sugar cataracts progress (12).

Our study proves that the metabolic disorders are more frequent in younger female patients, while in older male patients' glaucoma is more frequent. A higher mean IOP at presentation, combined with younger age and increased lens volume required prompt cataract surgery intervention in the combined group. Aim of our approach was to decrease IOP and reduce the number of topical medications, therefore an improvement quality of life in our POAG patients. It is true that in both glaucoma groups, the number of topical substances was maximal and comparable, yet the mean IOP was significantly higher in the POAG+diabetes group. Therefore, a supplementary metabolic disorder may alter the overall status in glaucoma patients.

An IOP in its high teens (18 mmHg, as we calculated in our study) in a moderate-advanced glaucoma patient increases the risk of POAG progression; therefore, a surgical intervention was discussed. In the context of glaucoma patients, cataract extraction decreases IOP and/or lowers the number of substances used to control IOP (13). Moreover, cataract extraction before trabeculectomy offers a safer approach both for the patient and the surgeon if performed first (13). As such we preferred this approach in our study. Adding a fourth substance would have been an option if the stage of the glaucoma was different (early) and if the quality of life in our patients had remained the same.

Using the lowest ultrasound energy in the combined pathology group pleads only for the above-mentioned changes induced by metabolic imbalances in the lens, if diabetes is discussed. Therefore, a different structure and density is met in the diabetic lens compared to the normal involutional changes induced by age alone. The lens capsule is impermeable, therefore an increase in the oncotic pressure within the lens accelerates cataract formation. Intense hydration of collagen lamellas produces soft intumescence of the lens in diabetic cataract compared to sclerotic changes in

age related cataract.

Functional parameters in glaucoma patients are also influenced by diabetes. Based on the Hodapp classification, our POAG cases are categorized as moderate to advanced disease, but formation of cataract can artificially decrease MD values and influence glaucoma severity scale. In this respect, by inspecting the PSD maps the clinician might interpret the parameters in a different way as it was the case in our study. The discrepancy between the MD/PSD maps pleaded also for a cataract influence over the parameters than only glaucoma damage. An interesting aspect that we found in our study was that the mean PSD values in the combined pathology (POAG+diabetes) group were decreased compared to the POAG group. A possible explanation for less localized damage in POAG+diabetes category could be the age aspect: age glaucoma damage increases with age; therefore, an older age might have more pronounced damage (POAG group, mean age=75 years old) compared to younger age (POAG+diabetes group, mean age = 59 years-old). Still it is difficult to establish the exact glaucoma extension of damage in both groups, since patients from both categories were evaluated before cataract surgery, for which there was a strong recommendation.

Study limitations were the small number of patients and the echo-biometric methods for the anterior segment measurements.

CONCLUSIONS

In our study we found several clinical changes that "modulated" the decision about the best treatment options in primary open angle glaucoma patients, especially if they associated a general risk factor such as diabetes. Further studies and more data, especially using recent advances in ocular imaging such as AS-OCT technology, are needed to provide a complete overview of anterior segment measurements, so as the best management is offered to diabetic glaucomatous patients.

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NEWS

THE BENEFITS OF SODIUM-GLUCOSE COTRANSPORTER 2 INHIBITORS ON METABOLIC PARAMETERS

Sodium-glucose cotransporter 2 inhibitor (SGLT2i) blocks reabsorption of glucose by inhibiting SGLT2 in kidney, promotes the renal excretion of glucose and improves blood glucose control without requiring insulin secretion. A recent study retrospectively picked up patients with type 2 diabetes who had been continuously prescribed SGLT2i for 3 months or more (26 patients treated with tofogliflozin, 34 patients with canagliflozin, 27 patients with empagliflozin, 23 patients with ipragliflozin, 68 patients with dapagliflozin and 71 patients with luseogliflozin) and compared metabolic parameters including coronary risk factors before the SGLT2i treatment with the data at 3 and 6 months after the SGLT2i treatment started. Each SGLT2i ameliorated metabolic parameters, in different patterns. SGLT2is reduced body weight, systolic and diastolic blood pressures, plasma glucose, hemoglobin A1c, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, uric acid, triglyceride and non-high-density lipoprotein-cholesterol (HDL-C), and elevated HDL-C; however, they did not affect LDL cholesterol levels. Change in each metabolic parameter was significantly correlated with each metabolic parameter at baseline (Yanai H, Hakoshima M, Adachi H, Kawaguchi A, Waragai Y, Harigae T, Masui Y, Kakuta K, Hamasaki H, Katsuyama H, Kaga T, Sako A, Effects of Six Kinds of Sodium-Glucose Cotransporter 2 Inhibitors on Metabolic Parameters, and Summarized Effect and Its Correlations with Baseline Data. *J Clin Med Res* 2017; 9(7): 605-612. doi: 10.14740/jocmr3046w. Epub 2017 May 22).

Dan Lupaşcu

PARTICULAR ASPECTS IN A WILLIAMS - BEUREN SYNDROME CASE OF A 15-YEARS-OLD TEENAGER - CASE REPORT

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PARTICULAR ASPECTS IN A WILLIAMS - BEUREN SYNDROME CASE OF A 15-YEARS- OLD TEENAGER (Abstract): Williams-Beuren Syndrome is a rare genetic disease determined by a submicroscopic deletion located on the long arm of chromosome 7. This disease was first described by Doctor J.C.P. Williams in 1961 and was later updated by Beuren. This disease occurs at a ratio of between 1/20,000 and 1/7,500. It is a congenital disease, affecting all ethnic groups and all populations world-wide, independent of sex. In most cases, those affected, are considered isolated occurrences especially because the parents of these patients are clinically healthy. In rare cases, this disease can be inherited from one parent, meaning, the inheritance pattern is autosomal dominant. Parents cannot benefit from prenatal diagnostic testing except in the case in which another family member has already been diagnosed with this disease. The only diagnostic test for this syndrome is through FISH which can stipulate the deleted DNA segment on the 7 q 11.23 chromosome. **Keywords**: WILLIAMS-BEUREN SYNDROME, KARYOTYPE, FISH TECHNIQUE, AORTIC STENOSIS.

The parents asked for genetic testing to be done since their daughter was suspected for having Williams-Beuren Syndrome. The general clinical examination showed: fragile phenotype, pale skin, light hyperelasticity, cephalic extremity: bilateral epicanthus, retraction of the maxillary floor, short, flat nose, symmetric and hypertrophic nasal alae, stellar iris, macrostomy and micro retrognathia (fig. 1). The skeletal system: pectus carinatum, kyphotic posture, narrow thorax, fallen shoulders (fig. 2), bilateral pes plantus, bilateral femoral ante version, long fingers and toes. Cardiovascular system: hypoplasia of the abdominal aorta and the presence of mitral valve prolapse. Digestive system: irritable bowel syndrome, constipation, nonseptic chronic colitis. Normal urinary system. Genital system: echographic vascular uterine dila-

tations. Endocrine system: hypocalcemic spasmophilia, hypothyroidism. Nervous system: OTR present and hyper-sensitive, sociable, friendly, difficulty in learning and concentration; IQ=56. Paraclinical investigations: normal complete blood count and normal renal and hepatic functions. Hypercholesterolemia and low ionic calcium are also present.

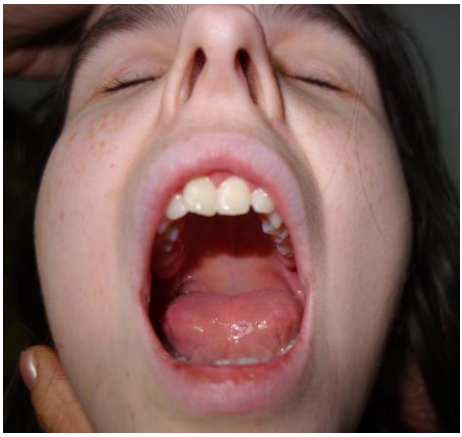

Fig.1. Macrostomy with full lips, macroglossia

Fig. 2. Kyphotic and scoliosis posture fallen shoulders

Genetic exam: The patient is the second child of three children. The couple is not of consanguineous origin. The pregnancy progressed abnormally with episodes of imminent abortion, treated at the moment of occurrence. The baby was born naturally at full term with a cranial presentation. The baby's weight: 2,950 grams. APGAR 8. The baby was breastfed. According to the mother's declaration, the baby's psychomotor development was normal. In 2003, the patient was admitted to the Pediatric Hospital in Iasi, where she underwent a clinical examination, resulting in the presumption of Williams Syndrome. A microculture from a peripheric blood sample yielded a karyotype through PHA stimulation with and without G markings with a chromosomic formula of 46, XX, which is normal. In 2014, the MEDSANA genetics laboratory in Bucharest used a MLPA (Multiplex ligation-dependent probe amplification) technique and the karyotype had deleted DNA sequences in the 7 q 11.23 region which were deleted hemizygous DNA sequences specific for patients diagnosed with Williams Syndrome. Thus, in the present case, the patient has been positively diagnosed with Williams-Beuren Syndrome. The patient's siblings of 17 and 15 years of age, respectively, have a longiline phenotype, proportional body structure, normal age-oriented intellectual development, renal abnormality, hernia, deglutition difficulty and hypercalcemia.

Diagnostic: Williams-Beuren Syndrome, hypoplasia of the abdominal aorta, hypothyroidism, spasmophilia with hypocalcemia and normal magnesium values.

DISCUSSION

The clinical diagnosis of Williams-Beuren Syndrome can only be obtained

through team work between geneticists, cardiologists, pediatrists, ophthalmologists, otorhinolaringologists, neurologists, psychologists and other specialists. The diagnosis can only be confirmed by studying the karyotype through the MLPA technique of the critical zone on 7 q 11.23 where the genes are located (ELN-LINK1) (2). Common signs of the syndrome are: specific facial features, vascular abnormalities of the heart (3), hypercalcemia, low birth weight, stunted growth, low muscle tone, deglutition difficulty, irritability, colics in the first year of life, abnormal dentition, abnormal kidneys, hernia, hyperacusis (4), friendly character with an interest in adults (5), stunted development, problems with concentration, intellectual handicap of varying degrees, underdeveloped fine motor skills, they are bothered by loud sounds such as clapping, loud laughs, other loud sounds, dogs barking (6). The patient in this case showed 14 out of the 20 possible signs of those missing are: normal birth weight, stunted growth in the first years of life, abnormal kidneys, hernia, difficulty in deglutition and hypercalcemia (1).

Treatment and follow-up

Currently, there is no specific treatment. The only treatment available is symptomatic. Calcium and vitamin D supplements are not administered. It is important that the patient refrains from sun exposure. Logopedic therapy is necessary for language rehabilitation, vocabulary improvement, receptive and expressive language development. Behavioral therapy is the basis for learning for these children, which can take years, based on the child's learning capacity. Kinesiotherapy is also an important aspect. Cardiovascular monitoring is necessary to track renal and aortic stenosis's that can form. The blood pressure of the patient should be monitored in both arms. Psychotropic drugs are necessary in treating attention deficit. Periodic endocrine check-ups are needed to diagnose type 2 diabetes and hypothyroidism in the early stages (7). Ophthalmic, hearing, urinary and genital assessments are performed. Calcemia, calciuria and cholesterol levels are also monitored. Psychologic evaluations and psychiatric treatments are necessary to monitor cognitive deficiencies and behavior (8).

Evolution and Prognosis: Facial signs become more prominent with time. Vascular stenosis varies in several degrees of severity. Reactions to stimuli are accentuated. The patient may suffer from spatial-visual and coordination problems. They do not have true spatial perception, suffering from a fear of heights and uneven surfaces. Moreover, they are socially adapted. However, they will need constant adult supervision for their entire lives, only able to perform easy tasks.

Genetic advice: Prenatal diagnosis, through echography or other tests, is not possible. The only investigations permitted is an invasive technique through chorionic villus sampling and using the FISH (Fluorence In Situ Hybridization) technique thereafter in the 12th week of gestation; also, amniocentesis can be performed in weeks 16-18 (9). There are currently no methods of prevention. The recurrence risk in the future children of parents with this syndrome is less than 5%. The recurrence risk of the patient's siblings of having this syndrome is less than 1%. The recurrence risk that the patient will have children with this disease is 50%.

CONCLUSIONS

In our vision, we found the particularities of this case: cardiovascular abnormali-

ties such as abdominal aortic hyperplasia, the absence of hypercalcemia and present of hyper-cholesterolaemia. Caring for a child with Williams Syndrome can be difficult and could benefit from familial support. All family members should try to maintain a friendly behavior, a calm demeanor and understanding. The family will need to work in close contact with the child's family doctor as well as other specialists to maintain a certain degree of normality in every stage of the child's life.

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LIMITATION OF THE FETAL ULTRASOUND IN DETERMINING THE FETAL GENDER

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LIMITATION OF THE FETAL ULTRASOUND IN DETERMINING THE FETAL GENDER (Abstract): Genital abnormalities are a rare finding on prenatal ultrasound but can be seen when detailed examination is performed following detection of another structural abnormality or, and more commonly, when the genitalia are examined because of parental curiosity. The etiology of these unexpected genital anomalies is broad and includes an isolated anomaly, an underlying genetic syndrome, Intra-Uterine Fetal Growth Restriction (IUGFR), chromosomal abnormalities and, although rare, anomalies of steroid biosynthesis or androgen insensitivity. We report the case of a 31-years-old patient that followed the prenatal consultation and the fetus was diagnosed after being born with a rare severe case of perineal hypospadias. **Keywords**: HYPOSPADIAS, INTERSEXUALLITY DISORDER, ULTRASOUND.

Ultrasound is principal method in most parts of the world for prenatal sex determination. Indication for prenatal sex determination can be medical or nonmedical. The medical indication is: X-linked disorders, testicular feminization syndrome, pseudohermaphroditism, genital anomalies, ambiguous genitalia, and determination of zygosity in multiple pregnancy (1, 2, 3, 4, 5). The nonmedical reasons are: preparturition shopping, curiosity, husband/relatives' request (4).

CASE REPORT

We report the case of a pregnant woman aged 31-years-old, routinely ultrasound examined initially at 6 weeks of gestation, when a unique embryo with no abnormal findings was identified.

All ultrasound examinations were performed transabdominally using a Voluson E8 Expert machine (BT10 software) with a 4e8-MHz curved array three-dimensional transducer.

Subsequent examinations revealed the diagnosis of Trombophilia at 10 weeks of gestation treated with Enoxaparinum 4,000 UI anti-XA and Gestational diabetes mellitus type A1 treated by diet modification.

Subsequent examinations at 18, 22, 28 weeks of gestation revealed a normal morphological female fetus (fig. 1, 2).

At 32 weeks of gestation, during the

third trimester morphology, the ultrasonic aspect of the gender modified- instead of labia we saw scrotal folds, which include both testes and no penis (fig. 2). The fetal biometric measurements correlated with his estimated gestational age showed no fetal anatomic abnormality except in the external genitalia.

Both parents were informed of the development and to establish a diagnosis they were advised for a diagnostic amniocentesis or a noninvasive blood test from maternal serum to determine the fetal gender. They refused.

Fig. 1. Ultrasound showing attempts of viewing female genitalia (22 weeks)

Fig. 2. Ultrasound showing female genitalia (22 weeks)

Fig. 3. Ultrasound showing male genitalia (32 weeks)

After 39 weeks of gestation, a baby presenting a severe birth defect of the external genitalia was born through C-section for the diagnosis of acute fetal distress.

The aspect of external genitalia was ambiguous, with two separated, non-fused labioscrotal folds, a relatively well represented and differentiated phallus in the midline, covered by excessive skin on the dorsal aspect; a single orifice, the urethral meatus was opening below the phallus, in the perineum skin (fig. 4). At palpation, gonads were present in each of the labioscrotal folds and rectal examination did not revealed any abnormal structures. Because of the findings at birth, the diagnostic

in this case is severe perineal hypospadias with incomplete penoscrotal transposition; male sex was also confirmed genetically, the karyotype being a normal 46 XY; no associated malformations of urinary tract were identified. The boy was scheduled for hypospadias repair.

Fig. 3. Severe perineal hypospadias with incomplete penoscrotal transposition

DISCUSSION

We present a case of severe hypospadias where the prenatal ultrasound allowed us to ascertain its severity and the perineal anatomical relationships, to advise parents on the expected surgical outcomes.

Hypospadias is one of the most common congenital anomalies, with an estimated prevalence between 0.2 and 4.1 per 1,000 live births of both sexes (6). It is defined by a failed development of the spongy urethra and ventral foreskin (7). The incomplete fusion of the urethral folds' results in anomalous location of the meatus at some point of the ventral side of the penis, instead of the tip. The foreskin does not fuse on the ventral side, because of nonunion of ectodermal folds, being redundant dorsally. It is often associated with a ventral curvature of the penis (also known as chordae), caused by atresia of the corpus spongiosum distal to the hypospadias urethral meatus.

The severity of hypospadias is graded upon the position of the urinary meatus and the extent of ventral penile angulation. Severity rises as the distance of the displaced urethral opening increases from the normal position at the tip of the glans and with increasing penile curvature. Hypospadias is classified into three grades, depending on the location of the meatus: first degree/mild (glandular or coronal urethral opening), second degree/moderate (in the penile shaft) and third-degree/severe (urethral opening within the scrotum or perineum).

The exact etiology of the disorder remains unknown. It seems that the origin of hypospadias is multifactorial; several risk factors have been identified such as genetic predisposition, placental insufficiency, and substances that interfere with natural hormones, endocrine disruptors (7, 8, 9, 10).

Hypospadias usually is not a single anomaly, up to 40% of fetuses have associated upper urinary tract anomalies. Cryptorchidism and inguinal hernias are found in 7-10% of all hypospadias cases and are considered the most common extragenital anomalies (11).

Other extragenital malformations (5-10%) are heart defects, cleft palate, neural tube defects, and anorectal malformations. It has also been described in the context of various genetic syndromes, such as XXY, XXXXY, trisomies 13 and 18, Fraser syndrome, the Smith-Lemli-Opitz, Opitz-Frias Wolf-Hirschhorn (9).

Attention should be paid in detecting abnormalities in the external genitalia dur-

ing second trimester ultrasound. Conventional sonographic criteria for the diagnosis of hypospadias are a small penis, with a blunted bulbous tip and two parallel echogenic lines at the top, representing the lateral folds of the foreskin. This was called by Meizner (12) the "tulip sign" for the diagnosis of severe hypospadias. This sign could not be seen in our case (fig. 3).

However, in certain cases, especially when the testicles are undescended, it is difficult to distinguish between external female genitalia and severe hypospadias. So, ambiguous genitalia would be included as main differential diagnosis, congenital adrenal hyperplasia as primary cause. The sonographic findings in this case are: clitoromegaly, which can mimic a penis, and enlarged adrenal glands bilaterally with discoid morphology (13). This diagnosis can be ruled out by obtaining male karyotype, but in this cases the parents refused both attempts in doing so.

Although 2D ultrasound is still the gold standard for diagnosing fetal genital abnormalities, prenatal diagnosis using 3D has been reported previously (14, 15).

By 3D representation, the external genitalia are shown with a great clarity of structures and their anatomical relation which confirm the diagnosis of third-degree hypospadias. In this case, even though severe hypospadias was supposed, the position of the fetus and the quantity of amniotic fluid were not favorable for 3D imaging when it was available (fig. 1). In conclusion, the diagnosis is not supported by 3D imaging, therefore, the multiplanar representation of the genital area that would allow us to obtain a better understanding of hypospadias and get proper parental counseling from pediatric surgeons, with the possibility to report the prognosis and future treatment was not available until birth.

CONCLUSIONS

If in most cases, normal 2D ultrasound along with 3D imaging is more than enough in establishing fetal gender, we conclude that in selected cases fetal karyotype obtained by amniocentesis is needed to make a positive diagnosis of gender and distinguish between a single anatomical perineal malformation and a genetic syndrome.

Also, we argue that 3D imaging when possible is an important tool in assessing the severity and the prognosis of the congenital perineal malformation.

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NEWS

MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY IN CUTANEOUS MELANOMA: AN UPDATED REVIEW

The molecular pathogenesis of cutaneous melanoma is strongly correlated with a constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway. The most frequently affected genes are v-raf murine sarcoma viral oncogene homolog B (BRAF) and neuroblastoma Ras viral oncogene homolog (NRAS), which show mutations in up to 40% to 50% and 15% to 20% of melanomas, respectively. The development of targeted therapy for metastatic melanomas that harbor a specific subset of BRAF mutations has led to the combined approval of 3 drugs (vemurafenib, dabrafenib, and trametinib) and their companion diagnostic molecular tests. These tests are real-time polymerase chain reaction–based assays designed to detect missense mutations at nucleotide position 1799 of BRAF, which result in the substitution of valine at amino acid position 600 of the BRAF protein (BRAF V600 mutations). Recently, the widespread availability of gene sequencing has led to the identification of multiple additional BRAF mutations of uncertain pathogenic and therapeutic significance. Also, gene sequencing has revealed that melanomas can have mutations in many other genes of the MAPK signaling pathway, some of which might be clinically relevant. In this paper, the authors provide a general overview of the role of the MAPK pathway in the pathogenesis of cutaneous melanoma, focusing on BRAF mutations and their therapeutic implications (Acosta AM and Kadkol SS. Mitogen-Activated Protein Kinase Signaling Pathway in Cutaneous Melanoma: An Updated Review. *Archives of Pathology & Laboratory Medicine* 2016; 140(11): 1290-1296).

Doina Butcovan

COMPLEMENTARY MULTIDISCIPLINARY ELDER ABUSE SERVICE IN A GERIATRIC CLINIC

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COMPLEMENTARY MULTIDISCIPLINARY ELDER ABUSE SERVICE IN a GERIATRIC CLINIC (Abstract) **Aim:** To describe a pilot, innovative intervention project combining the adoption and adaptation for hospital use of a screening instrument designed for use in primary health care settings in Canada (and translated into 6 other languages) and a dialogical narrative therapeutic approach. The development of a complementary multidisciplinary elder abuse service (CMEAS) in a private-public partnership. **Material and methods:** Between June 2015 – March 2016 elderly hospitalized in Iasi town, Geriatric Clinic and suspected of being abused had the benefit of a complementary multidisciplinary elder abuse service (CMEAS) after being screened for abuse, neglect or abandonment experiences. **Results:** A total of 450 patients admitted to the Geriatric Clinic were invited for the study and 152 raised suspicion of abuse experiences and were screened with EASI. Of these patients, 132 where found positive and were invited to participate in CMEAS. **Conclusions:** Such a multidisciplinary service requires the collaboration between the geriatric team (medical service), psychologist, social worker, legal advisor, and psychiatrist, referral of cases to relevant public and private community services and their monitoring after hospital discharge throughout project duration. **Keywords**: COMPLEMENTARY SERVICES, MULTIDISCIPLINARY APPROACH, ABUSE, ELDERLY.

„Elder abuse is a global public health and human rights problem that crosses sociodemographic and socioeconomic strata" (1). The presence of abuse in geriatric care complicates health care exponentially. Elder abuse is associated with a long series of health problems, a high level of emergency service utilization, including readmissions within less than one month, and financial burden for the family and community public services (1).

Healthcare practitioners play a key role in the identification of elder abuse, quite often medical checkups being the only regular interaction that older people have outside their home. Nowadays, elder abuse is encountered routinely in medical practice (2).

In Romania, elder abuse has not been systematically identified in public hospital or primary medicine settings. Elder abuse very recently became a health policy topic as the Romanian government adopted in

July 2015*" The National Strategy for the Promotion of Active Aging and the Protection of Elderly 2015-2020".* The pilot experimentation of a CMEAS in an Iasi geriatric clinic was done in the context of the lack of psychological, social, legal and psychiatric services in the public hospital and the lack of referral of potentially identifiable cases to the relevant existing community services.

In this descriptive study of the CMEAS a public-private partnership was created between a geriatric clinic, two universities, and four professional associations (psychologists, family practitioners, nurses, and social workers). The partnership was funded via NGO Fund in Romania, under the project „I Refuse Silent Abuse", initiated by the Psiterra Association based in Iasi.

MATERIAL AND METHODS

The medico-psycho-social-legal model used for the identification of abuse in the hospitalized elderly relies on hospital-based medical geriatric care and MCEAS and referral and monitoring after discharge in the community. This model was developed in a 13-month project and was implemented during the last 10 months of the project at a geriatric clinic. It had the following stages: 1) geriatric assessment; 2) obtaining informed consent; 3) use of Elderly Abuse Suspicion Index (EASI) as a screening instrument; 4) initial psychological assessment; 5) social inquiry report; 6) CMEAS team meeting with the patient - using both dialogical and narrative principles; 7) implementation of the personalized intervention plan; 8) patient's discharge from hospital, monitoring, and the case closure.

A total of 450 patients admitted to the Geriatric Clinic were invited for the study and 152 raised suspicion of abuse experiences and were screened with EASI. Of these patients, 132 where found positive and were invited to participate in CMEAS.

The assessment interview was in fact a narrative conversation (6) facilitated by a psychologist previously trained in narrative therapy by courses accredited by the *Romanian College of Psychologists* organized by Psiterra Association in Iaşi, Romania.

If abuse was suspected but the patient was denying it or was reluctant to disclose information, the psychologist left his/her contact information, the description of the CMEAS and of the project, letting the patient know that he/she could contact the team any time he/she is ready or becomes preoccupied with the abuse and its effects. If the patient accepted the CMEAS, or CMEAS and referrals, the social worker initiated the social inquiry. At this first meeting the psychologist's goal was to get patient's agreement for an open dialogue meeting (with the doctor, psychologist and social worker, at least) (7).

The information collected in this study was evaluated using clinical and epidemiological methods, statistical and mathematical techniques and procedures, descriptive statistics were performed using software *SPSS 18.0.*

RESULTS

During the 10 months of CMEAS implementation of the Psiterra narrative and dialogical model of care 152 elderly patients of the total of 450 eligible admitted elders were screened using EASI. They were all referred to CMEAS and following psychological assessment the presence of abuse was substantiated in 132 (29.3%) of the referred patients.

Working narratively and offering the patient the opportunity to decide on any aspect of the intervention plan, a total of 61 patients (46.2%) accepted to receive com-

plementary services during hospital stay and accepted to be referred to community public and private services, including the services offered by the religious organizations (parishes they belonged to). Other 41 patients (31.1%) accepted only CMEAS services during their hospital stay, but no referrals. That is, a total of 102 elderly patients with confirmed suspicion of abuse received services during their hospitalization. Only 30 elderly (22.7%) identified with abuse refused to receive services either from CMEAS or to be referred to existing community services.

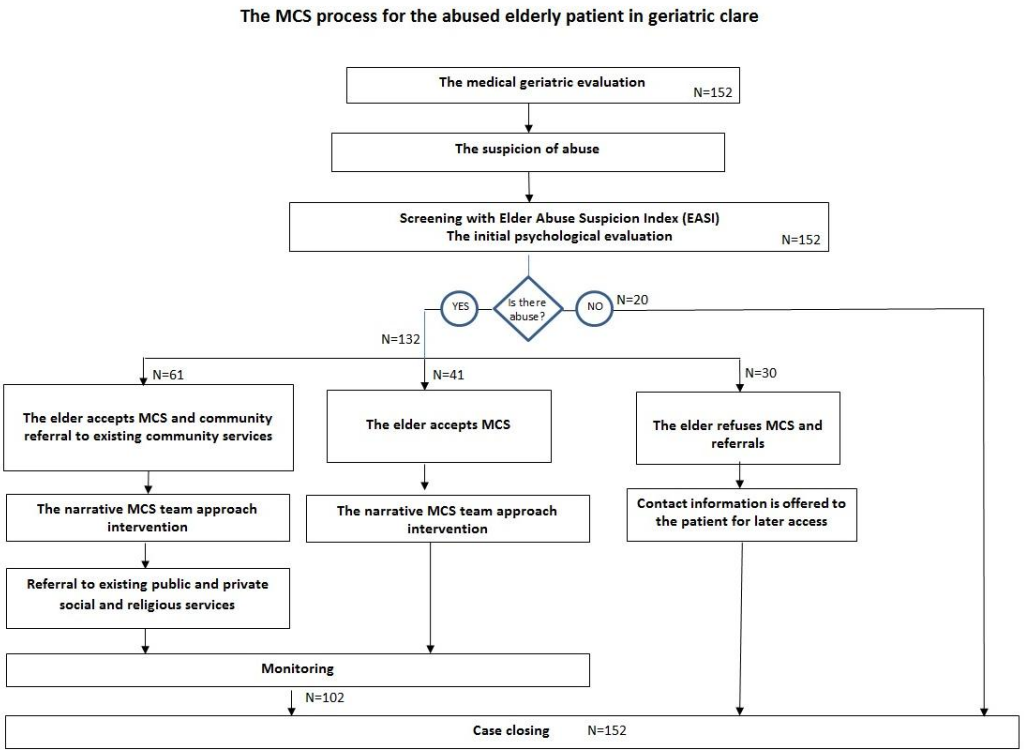

Fig. 1. MCS process diagram

DISCUSSION

The demonstration project for the implementation of CMEAS based on dialogical and narrative principles proved influential on three levels:

1) clinical (micro), improving the well-being of the elderly patient (both during hospital stay and after discharge, the patients being monitored until the end of the project);

2) organizational (mezzo), by providing complementary services only to the geriatric ones, through training seminars on the screening methods for elder abuse using the EASI scale, training seminars accredited by CMR on recognizing elder abuse and hospital-based multidisciplinary intervention;

3) by integrating medical, psychological and social services and promoting the awareness of elder abuse; and sectorial (macro) by the promotion of public awareness about elder abuse and by launching a

dissemination campaign in professional media and to major public stakeholders in healthcare and psycho-social care in Romania.

As to professional training and development, as other reports suggest (3), the healthcare professionals are in an ideal position to identify, support and refer the persons who experience abuse while aging. The lack of specialized training to deal adequately with cases of suspected abuse can be a possible explanation for the fact that many cases of elder abuse go unreported.

The innovative intervention piloted the development of multidisciplinary complementary services in a public hospital in line with the new strategic directions within *"The National Health Strategy and for the Promotion of Active Aging" (*2015-2020). In the complex situation of geriatric patients exposed to abuse, neglect or abandonment it is even more relevant to read Rita Charon (Columbia University, New York) saying that *„We are all united in the efforts to treat patients, no matter of our specific skills and responsibilities. We together have the capacity to confer dignity on the lives of the sick, and, one may argue, we require that all of us join in the commitment to dignified care"* (8).

Making central the voice of the abused elderly and giving priority to his/her voice permits „the facilitation of the freedom to choose among alternative behaviors, all the areas of functionality of the assisted person, contributes to his or her development, to his or her plenary experience of freedom and of meaningfulness. This axiological experience is inducing a modification of what we may describe as internal motivation and a reorientation in relation to change, initially having an ambivalent position towards change" (9).

Identifying the traumatic experiences that affect compliance and patient adaptation is crucial in the case of elderly patients; the rapid identification of abuse has also an effect on changing the perception of health care personnel regarding the quality of health care provided to the patients (10).

CONCLUSIONS

In our study, of the 450 patients admitted to the geriatric clinic during the 10-monthscreening, one third (33.3%) were identified as having been exposed to various types of abuse, neglect or abandonment and were referred to CMEAS by the medical staff. The 20 false positive responses (13% of the total 152 patients screened for abuse with EASI) underscore the importance of professional training in the use of screening instruments as well as the need for immediate referral to psychological and social services able to substantiate abuse experience and to support the access to existing resources in the family and community.

Our study underscores the high sensitivity of the assisted elderly in the communication with the medical staff, increased trust in participating in medical care activities, improvement of symptoms, and a shorter hospital stay; on the medical staff side, there was a greater sensitization towards the elder patient's needs and a more open personal expression in relation to the patient.

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NEWS

CARDIOVASCULAR SAFETY OF CELECOXIB, NAPROXEN, OR IBUPROFEN FOR ARTHRITIS

In the PRECISION trial (Prospective Randomized Evaluation of Celecoxib Integrated Safety versus Ibuprofen or Naproxen) the goal was to asses cardiovascular, gastrointestinal, renal, and other outcomes with celecoxib compared with two nonselective nonsteroidal antiinflamatory drugs (naproxen or ibuprofen). Patients who required NSAIDs were randomly assigned to receive celecoxib (100 mg twice a day), ibuprofen (600 mg three times a day), or naproxen (375 mg twice a day). For patients with rheumatoid arthritis investigators could increase the dose of celecoxib (200 mg twice a day), the dose of ibuprofen (800 mg three times a day) or the dose of naproxen (500 mg twice a day). Also for patients with osteoarthritis, increases of the doses of naproxen or ibuprofen were permitted. A total of 24,081 patients were assigned to the celecoxib group, the naproxen group, or the ibuprofen group for a mean treatment duration of 20.3±16.0 months and a mean follow-up period of 34±13.4 months. 68.8% of the patients stopped taking the study drug, and 27.4% discontinued follow-up. The results showed that the risk of gastrointestinal events was significantly lower with celecoxib than with naproxen or ibuprofen. Also the risk of renal events was significantly lower with celecoxib than with ibuprofen but was not significantly lower with celecoxib than with naproxen. The study concluded that at moderate doses, celecoxib was noninferior to ibuprofen or naproxen with regard to the primary composite outcome of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke (Nissen S, Yeomans N, Soloman D et al. Cardiovascular Safety of Celecoxib, Naproxen, or Ibuprofen for Arthritis. *New England Journal of Medicine* 2016, DOI:10.1056/NEJMoa1611593).

F. Mitu

BOWEL HABITS OF HEALTHY INDIAN CHILDREN LESS THAN TWO YEARS OF AGE

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BOWEL HABITS OF HEALTHY INDIAN CHILDREN LESS THAN TWO YEARS OF AGE (Abstract): **Objectives**: To define the normal frequency and consistency of stools of healthy Indian children between 0-24 months of age. **Material and Methods**: Parents of children aged up to 24 months were asked on a three-day recall basis about their child's usual bowel habits and dietary history. Bowel habit was recorded in terms of number of stools the child passes per day, stool consistency, the age at which night bowel movements stopped, and age of regular bowel movements. Feeding type was recorded as exclusive breast feed, mixed milk feeds or on solid feeds. The bowel habits were correlated with the age of the child and type of feeding. **Results**: Children in first six months of life had stools which were predominantly "pasty" and "runny like cream" with high and variable frequency. Beyond six months of age they had "solid" and "pasty" stools with a frequency of one to two per day. On analyzing the combined effect of type of milk feed and age on bowel frequency and consistency children beyond one month of age either on exclusive breast feed or on mixed milk feed had similar frequency and consistency of stools. By one year of age more than 90% of children had attained regular stool pattern and no night time bowel movements. **Conclusions**: First of its kind study from India which describes the stool pattern of normal healthy children less than two years of age. **Keywords**: BOWEL HABITS, HEALTHY CHILDREN, INDIAN

Bowel habits have been defined previously in literature in terms of frequency and consistency of stools. Many researchers in the past across different regions of the world have studied the bowel habits of children and their correlation with age and type of feed (1-5). Bowel habits of children less than two years are quite varied and there is no definite hard data on stool pattern of Indian children particularly less than two years of age. Parents are unaware of what the normal frequency of stool per day and consistency of stool should be and when should either of them be called as abnormal. Thus the aim of our cross sectional study was to define the bowel habits of healthy Indian children between 0-24 months of age.

MATERIAL AND METHODS

1,196 children aged up to 24 months were recruited from the postnatal wards and good baby clinic of our tertiary care hospital from April 2011 to December 2014. Children were recruited in the following predefined age groups of: 0-14 days, 15-28 days, 1-3 months, 4-6 months, 7 -12 months and 13-24 months.

The following children were excluded from the study group

1. Those suffering from fever, dysentery or any concurrent illness during the time of study.

2. Children who have any gastro intestinal illness or any severe illness requiring medication in last one month.

3. Those with past history of significant chronic medical illness or have underwent any surgery.

4. Children who were born preterm i.e. <37 completed weeks of gestation.

5. Those having weight and height less than the third percentile for the corresponding age.

Informed consent was obtained from the parents. After recording the socio demographic data and anthropometry of the children, parents were asked on a three-day recall basis about their child's usual bowel habits and dietary history. The consistency of stools was divided into1) solid, 2) pasty/soft like peanut paste, 3) runny like cream and 4) liquid like water. The type of diet was divided into three types a) Exclusive breast feed b) Mixed Milk Feeds: Breast feed and top milk feed c) Solid: Children who were started on complementary feed. Before taking opinion of parents they were verbally given the details of each stool consistency item to avoid any misinterpretation. They were also asked about the age at which night bowel movements stopped, and age of regular bowel movements. Interpretation of regular bowel movements was that bowel movements were nearly at same time of day and mothers could predict timing of bowel passage.

RESULTS

A total of 1196 children were studied, out of which 580 (48.5%) were males. Number of children recruited in each age group were 198(0-14 days), 200 (15-28 days), 208 (1-3 months), 200(4-6 months), 200(7 -12 months) and 192(13-24 months). Majority of the parents were from urban setup and had free access to toilet. Primary caregiver was mother to 80% of children. 95% children were born in a health care setup and had received pediatric care at birth.

Dietary History

The dietary history revealed that mixed milk (breast milk + top milk) type of feeding was the predominant feeding in children less than six months of age while solid feeding was the predominant type beyond that age (tab. I).

TABLE I

Feeding pattern of children less than 2 years of age

Age Group		Feeding type		
		Exclusive	Mixed	Solid
1	0-14	184	14	0
2	15-28	88	112	0
3	1-3 months	52	156	0
4	4-6 months	28	128	44
5	7-12 months	8	24	168
6	>13-24 months	0	8	184

Bowel Frequency with age

The mean frequency of stool in each group is given in figure 1. The mean frequency was high and variable in first six months of life and subsequently became constant after six months of age. Only 17% of children less than one month passed fewer than 2 stools per day. Interestingly there were nearly 15-34 % of children beyond one month and less than one year of age who did not pass stools daily.

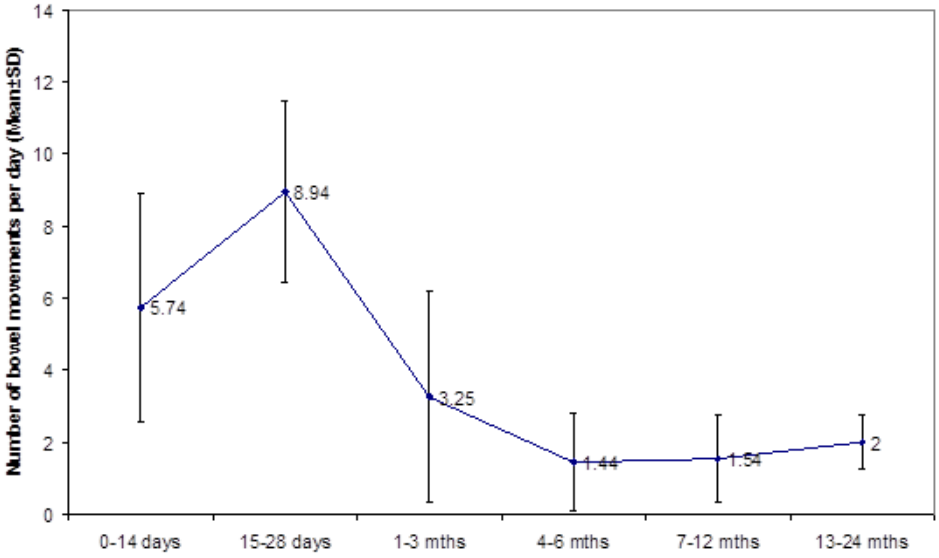

Fig. 1. Mean frequency of Bowell movements in each age group

Bowel Frequency
with various types of diets

It was seen that children who were on exclusive breast feed had highest frequency and variation of stools per day with a mean of 5.56 ± 3.94 (range0-20/day). The mean number of stools of children who were on mixed milk feed was 4.32 ± 3.55 stools per day (range0-12/day) and the children who were on solid feeds had mean stool frequency of 1.64 ± 0.9 stools per day (range 0-4/day).

Bowel Consistency with age

"Pasty" and "runny like cream" stools were the predominant types till six months of age. Beyond six months of age most children passed "solid" and "pasty" stools.

Bowel Consistency
with various types of diets

Children who were exclusively breast fed and on mixed milk feed had "pasty" or "runny like cream" stools while children on solid feeds had predominantly "solid" stools

Combined effect of type of milk feed and age on bowel frequency and consistency

This was assessed in children up to six months of age. In initial few days of life(<15days) children on breast feeding had higher stool frequency but after first month of life children with mixed milk feed and exclusive breast feed had similar frequency of stools per day.

The consistency of stool was similar in both the type of feed pattern. In first month of life it was predominantly "runny like cream" and beyond that mainly "pasty" up to six months of life

Regular bowel movements
and stopping of night movements

Half of children had regular bowel habits by the completed age of six months and almost all (92%) had a regular habit by one year. At age of six and twelve months only 40% and 5% of children respectively had persistence of night bowel movements (fig. 2).

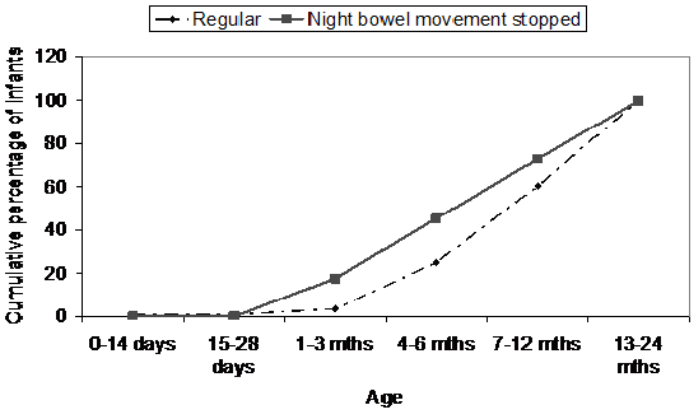

Fig. 2 Pattern of regular Bowel movements and stopping of night movements

DISCUSSION

To our knowledge the bowel habits of healthy Indian children have not been described till now and this study tries to provide an over view of the stool pattern of young children less than two years of age and also correlation with the type of feeds they consume. Studies in past from various countries across globe have been published and on comparison with them we had some interesting observations (1-5).

Maximum numbers of children were on mixed feed pattern in children less than six months. Exclusive breastfed were only 34% which less than the pan India percentage of 46% (6). This is itself a cause of concern and an urgent need for launching special program to promote exclusive breast feeding in the study region

Our study has showed that maximum stool frequency was in early part of life and as the child grew the frequency decreased and almost became static by 12 months of age to 1-2 stools per day. Along with age, type of feed given also did effect the stool frequency of children. Children on exclusive breast feeding had highest frequency and variation of stools per day an observation shared by studies in past (1, 4-5). One interesting observation we found that nearly 15-34% of children between ages of 1-12 months did not pass stools every day. This is deviation from the Australian data which says that only <5% of children did not pass stools every day (5). The reason for this deviation could be because of less percentage of exclusive breast fed infants in our study group. Human milk oligosaccharides which are prebiotic oligosaccharides have shown to have positive effects on stool consistency and stool frequency (1).

Bowel consistency also varied with age. “Pasty” and “runny like cream” stools were the predominant types till six months of age. After that “solid” and “pasty stools” were the predominant stool consistencies. These findings are similar to many studies from the past reflecting the normal physiology of gut development which hasn’t changed over the years (1, 3, 5).

The bowel consistency is also affected by child’s diet. Children who were exclusively breast fed and on mixed milk feed had “pasty” or “runny like cream” stools while children on solid feeds had predominantly “solid” stools. The possible explanations given for this are that gastric emptying time for breast milk is faster than that

of formula milk, difference in intestinal bacterial flora of children on different feeds and levels of circulating peptides which influence the bowel habit are different in infants on different feeds (7-9).

Studies by Stien et al, Jaffe et al and Thompson et al 1998 have reported that intestine of infants continues to develop after birth and bowel movements decrease during sleep. Hence it's believed that bowel movements become regular as the age of children increase. In our set of children similar pattern was seen and 92% had a regular bowel habit and 95% had stoppage of night bowel movements by12 months of age (10-12).

Our study gives an overview of stool pattern of children less than two years. However, India being ethnically diverse country with different child rearing practices a large multicenter pan India will give a better understand of stool pattern of normal children of same age group.

CONCLUSIONS

To conclude our study tries to define the stool pattern of normal healthy children less than years of age in terms of stool frequency, consistency and its association with feeds. This study will help all pediatricians to be aware of what normal stool habits is will in turn help in better management of children and counselling of parents in their day to day practice.

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ANATOMICAL AND CLINICAL ORBSERVATIONS ON STRUCTURAL CHANGES OF THE HIP JOINT

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ANATOMICAL AND CLINICAL OBSERVATIONS ON STRUCTURAL CHANGES OF THE HIP JOINT (**Abstract):** The hip, the second largest joint of the human body, with its primary contribution to locomotion, is exposed to numerous traumatic or non-traumatic risks. Regardless of the initial pathology, there is always almost the same result: diminution of range of motion, onset of pain and functional impotence, as well as change of biomechanics of walking. Through its high frequency, morpho-functional imbalance (clinically expressed both imagistic and biologically, in one or several joints), osteoarthritis is a disease with a multifactorial etiology and a complex pathogeny. **Material and method**: The study included 244 patients aged between 18-85 years, clinically and paraclinically investigated, especially for the osteoarthritis of the hip, admitted to Rheumatology Clinic I, Rehabilitation Hospital in Iasi, from January 2012 to December 2014. **Results and Discussion**. The high prevalence of degenerative diseases of the joints in old age is analyzed in accordance with the results of the estimations, which showed that, in fact, most of the patients remain undetected, undiagnosed and untreated. Main symptoms are pain in the coxofemoral joint, radiating or not on the lateral or anterior face of the thigh down to the knee, morning stiffness after a long rest, limping or walking with small steps, and muscle atrophy of the group of muscles that are responsible for the stability of the joint. **Conclusions**: A strict discipline is needed from the patient's side, in order to keep and apply the doctor's indications in a chronic disease that requires a long therapy, on one hand; on the other hand, a close cooperation between various experts is needed, in order to customize and apply the most effective program, at the right time. **Keywords**: OSTEOARTHRITIS, DEGENERATION OF CARTILAGE, BONE RECONDITIONING, FRACTURE.

The human locomotor system contains structures of the body that, through complex interactions between components and between components and the environment, generate support and movement of the body. Anatomically, a joint is a system made of passive and active components (1).

The hip joint (coxofemoral joint) is a synovial joint that connects the femur bone (femoral head) and the basin of the skeleton (acetabular cavity). It has the form of a typical spheroidal joint, a triaxial enarthrosis with a ligament transfer found at the area between the trunk and the free lower limb, with three axis of movement. When in orthostatic position and resting on both pelvic limbs, the femoral head is loaded under compression (2). As it rests on both

femoral heads, the basin needs only very small muscle forces for frontal stabilization. The body weight is directly and evenly distributed on both lower limbs. The following movements are made at the hip joint: flexion - extension, abduction - adduction and internal rotation - external rotation (3).

Friction and / or wear may occur as a result of the relative movement between the joint parts. An increase in wear or deterioration may also be due to changes in the synovial fluid or cartilage (4).

Hip osteoarthritis is joint damage caused by an interdependence between the morphological and functional imbalance. It is the most common hip disorder, a painful chronic arthropathy, a progressive and highly disabling disorder, with higher prevalence in males, that limits the static and dynamic status of a person as well as the self-care routine activities.

The common goal of all forms of treatment is to break the progressive pathogenic chain of the disease in order to create conditions for regeneration of the joints.

The purpose of this paper is to evaluate the possibilities for diagnosis and treatment, considering the fact that the radiological examination is essential during diagnosis, monitoring, progress, and the options for therapeutic indications.

We used ANOVA test for descriptive statistical analysis (*t*-Student) quantitative significance to measure variability and the weight of the observations. The data base was managed in Microsoft Office Excel, while the statistical analysis was made in MedCalc and Epi Info 2000.

MATERIAL AND METHODS

The study group was randomly chosen; the data were collected from the patient charts, and the patients were interviewed through a questionnaire.

The study was conducted on a group of 244 patients aged between 18 and 85 years, assisted in Rehabilitation Hospital in Iasi, Rheumatology Clinic I, from January 2012 to 31 December 2014. The selection of the group was based on inclusion and exclusion criteria. The method consisted of statistical analysis of results obtained by analyzing the patient charts, considering: background, age, gender, onset symptoms, risk factors, radiological manifestations, type of recommended treatment, response to therapy, treatment – induced toxicity, as well as the quality of life.

We included the measured height and weight, abdominal perimeter (normal if less than 94 cm in men and 88 cm in women), calculation of body mass index, reading of blood pressure and pulse. The investigations also included EKG, paraclinical tests (level of serum glucose, cholesterol and HDL – cholesterol, triglycerides, highlighting the inflammatory syndrome – ESR and CRP). The anthropometric measurements revealed obesity in 179 cases of women (73.36%) and 51 (48.2%) cases of men.

Concerning the climatic factors, 74 (30.32%) cases complained of the aggravation of symptoms due to environmental changes. Occupational risk activities were revealed in 8.60% of patients operating with vibrating instruments or performing running or horse riding.

RESULTS

The hip pain is a chapter in pathology with great significance for medical rehabilitation, since its prevalence is higher and higher, asking for expert's involvement in a complex and effective treatment.

Hip osteoarthritis met in adults over fif-

ty years old and in seniors, with an equal prevalence in both genders, is the main cause of disability and prosthetics in elderly (5,6,12). The patients benefit from a complex and long lasting therapeutic scheme, in two stages: a medical one, conservative, combining hygiene of the joints, drugs and maintenance or functional reeducation physical therapy, and a surgical stage (6,7).

Most often, degenerative pathology of the hip shows in 57-79 age group. The age, with morphological and functional changes it induces in this structure, has a major role in setting of the degenerative process. 187 patients (76.63%) came from urban settings and 57 patients (23.57%) from rural setting, predominantly from urban setting, with a high statistical difference ($p < 0.005$).

The major symptom of the disease was pain, felt in the inguinal area, frequently radiating on the inner side of the thigh, more intense around the knee, on the lateral or posterior side of the lower limb, without extending the lower third; to a great extent, the pain was felt almost continuously with nocturnal recrudescence and morning stiffness.

174 (71.31%) cases were males, while 7 cases (28.69%) were females; the difference is statistically significant ($p < 0.005$).

There were cardiovascular comorbidities in 49 patients (20.08%), respiratory - 11 patients (4.52%), gastrointestinal - 24 patients (9.83%), osteoarticular - 51 patients (20.90%), neurological - 14 patients (5.73%) and other comorbidities - 95 (38.94 %).

In some forms of osteoarthritis, the genetic blueprint may be considered, which in our case is present in 71% of the patients, who reported a degenerative articular disease in first degree relatives.

Pathological personal antecedents of the cases are: fractures - 74 cases (30.32%), luxations - 34 cases (13.93%), meniscal injuries - 13 cases (5.34%), hemarthrosis - 17 cases (6,98%), synovitis - 21 cases (8,60%), metabolic and endocrine diseases - 18 cases (7.39%), rheumatoid arthritis -19 cases (7.78%), neurological diseases - 9 cases (3.68%), circulatory disorders - 39 cases (15.48%) (table I).

TABLE I

Presence of pathological antecedents in patients enrolled in the study

Antecedents	No.	%
Fractures	74	30.32
Circulatory disorders	39	15.48
Luxation	34	13.93
Synovitis	21	8.60
Rheumatoid arthritis	19	7.78
Metabolic and endocrine diseases	18	7.39
Hem arthrosis	17	6.98
Meniscal injuries	13	5.34
Neurological diseases	9	3.68

As found in other authors (9,12), we discovered that fractures were the most frequently involved in etiopathology of osteoarthritis (30.32%), followed by peripheral circulation disorders (15.48%) and luxation (13.93%).

In most of the cases, the diagnosis of hip osteoarthritis is facilitated by a careful anamnesis, which points out to the onset of the pain, location, radiating pain and by the physical examination of the patient. The onset of the disease is insidious; for many patients it starts as fatigue of the coxofemoral joint after a long static or dynamic activity. Pain is the main symptom in all primary or secondary forms of disease; patients experience the symptom spontaneously and in motion, in groin, retrotro-

hanterian or gluteal area. Some patients report pain on the knee ("the cry of the hip"), or, as *Lequesne* notices, knee pain might be the only expression of hip involvement (5).

The pain is mechanical, the patient presenting an antalgic gait, shortening the duration of leaning against the affected part. The limping appears, caused by muscle insufficiency of the middle gluteal muscle, while in advanced phases, the patient displays an obvious vicious attitude. The most damaged muscle groups are the extensors and the abductors of the thigh due to the biomechanical changes during gait and to the decrease of their physical demands. Muscular analysis of thigh was required by major hypotonic in thigh and especially in the *gluteus medius* muscle, known as the muscle which is mostly prone to hypotrophy in case of inactivity. This results in gait waddling on one side due to the disturbance of the muscular balance of the basin, changing the parameters of gait.

Physical examination, performed in orthostatic, in static and dynamic position and in decubitus, shows at inspection, touch, percussion and examination of mobility the following: vicious position of the lower limb and trunk, amyotrophia, muscle contraction of abductor muscles, pain during direct percussion of the great trochanter, shortening of the pelvic limb, disorders of the statics of the spinal cord.

During physical examination of the hip, we noticed the predominance of objective signs that concerned synovia, in spurt flares and pain during mobilization of the joints. The walking perimeter varies a lot among patients; 132 cases (54.09%) still have a walking perimeter over 500 m.

Special tests of assessing the degree of affecting the articular mobility were made in 137cases (56.14%). Positivity of special tests on hip mobility were: Patrick test – 59 (43.06%) cases, Trendelenburg test - 41 patients (29.92%) cases, Thomas test - 21 (15.32%) cases, Ober test - 16 (11,67%) cases (fig. 1).

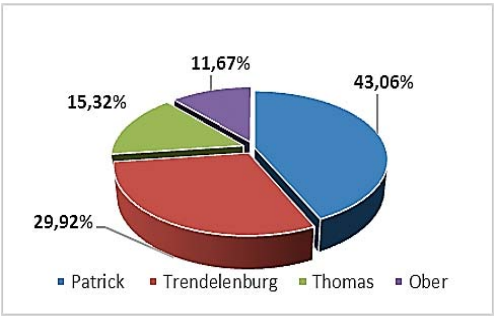

Fig. 1. Positivity of special tests on hip mobility

Non-specific inflammatory tests (ESR, CRP etc.) were positive in 41 (16,80%) cases.

In case of our research, similar to other authors' research (9,11), the early diagnosis of hip osteoarthritis was made through special investigations: ultrasonography in all 244 cases, CT in 71 (29.09%) cases, IRM in 64 (26.22%) cases, arthroscopy in 29 (11.88%) cases (fig. 2).

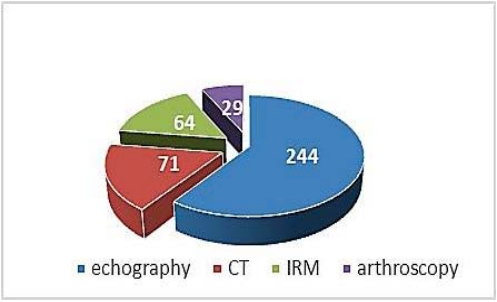

Fig. 2. Special tests were made in early diagnosis of hip osteoarthritis.

IRM imagistics is better than radiography and CT when assessing cartilage integrity and cephalic contour, with indications in early stages of the disease.

Radiography is the first line examination. Anterior-posterior and lateral radiography of the basin in orthostatic position are used to exclude other causes of coxopathy and also to make a coxometric evaluation and to show the degenerative changes in the hip joint and in the proximal joints, in the lower lumbar spine and in sacroiliac joints (7).

The radiological semiology of hip osteoarthritis was characterized by the following changes in the studied cases: narrowing of the joint interline (which seems to be the most accurate assessment tool of the stages of hip osteoarthritis, showing the progressive destruction of joint cartilage) (8). Radiologic changes were present almost constantly; reduction and even disappearance of joint space -159 (65,16%), sclerosis of subchondral bone in 48 cases (19.67%), osteophytes - 8 (3.27%), geodes -2 (0.81%), subluxations -12 (4,91%), erosive phenomena - 11(4.50%), ankylosis – 4 (1.63%) cases.

In 1982, *Baird* et al. (see reference 9) confirmed the value of CT after hip luxation, detecting the intraarticular fractures.

Ultrasonography brings guidance, since joint is difficult to approach and it is used as a special means, "the extension of the clinic exam", but the hip joint is deep and difficult to access.

The intensity of the primary degenerative process was: first degree - dubitable osteoarthritis, 12 (4.93%), second degree - minimal, 136 (55.73%), third degree - mild, 74 (30,32%), fourth degree - severe, 22 cases (9.02%) (fig. 3).

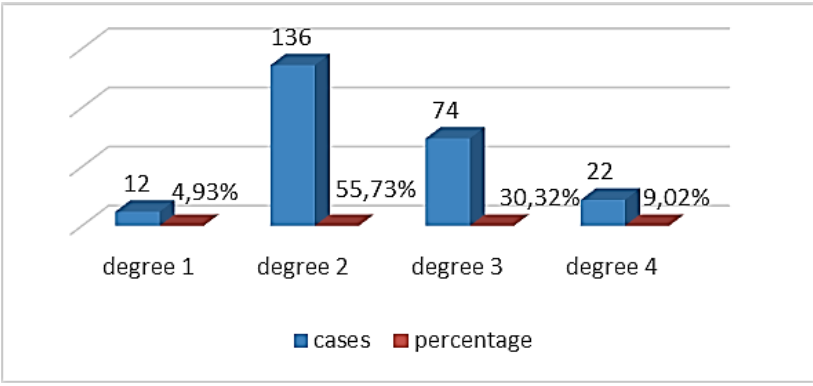

Fig. 3. Evaluating the degree of the primary degenerative process

Treatment: The patient usually follows two therapeutic stages. The first stage is the conservative treatment that delays the development of the disease and maintain an acceptable functional status by using a multidisciplinary approach: drug therapy, physiotherapy and orthopedic rehabilitation. In the second stage, the morphological and functional damage leads to surgery mostly in the form of joint prosthetics. At this stage, is estimated that the optimal age for prosthetic rehabilitation is after the age of 65.

Conservative treatment aims to relieve

pain, to improve the functional deficit and to slow the progression of the disease. The conservative therapeutic methods are pharmacological and non-pharmacological. Pain and inflammation were relieved with antalgic, anti-inflammatory and muscle relaxing drugs. Pharmacotherapy was used in 139 (56.96%) cases, and 74 (30.32%) subjects had a pluridisciplinary treatment.

The initial antalgic treatment included acetaminophen 0.5-1 g every 6-8 hours, without exceeding the maximum doze of 3 g per day, while AINS were used as anti-inflammatory drugs (diclofenac, piroxicam or meloxicam).

In hip osteoarthritis, the local infiltrations were avoided because corticosteroids have a transient effectiveness, increasing the degradation rate of the articular cartilage.

Electrotherapy and physical therapy were used as non-pharmacological therapy.

The pharmacological treatment was accompanied by adverse reactions, such as: gastric pain – 56 (22.95%) cases, nausea – 72 (29.50%) cases, vomiting – 12 (4.91%) cases, constipation/ diarrhea – 7 (2.86%) cases, headache, vertigo – 9 (3.68%) cases, allergic reactions – 10 (4.09%) cases, anemia/ leucopenia/ thrombocytopenia – 18 (7.37%) cases, increase of blood pressure – 13 (5.32%) cases and no side effects – 47 (19.26%) cases.

The non-pharmacological methods of rehabilitation are electrotherapy, massage, thermotherapy, hydrotherapy, balneotherapy and physiotherapy.

The rehabilitation program included electrotherapy that has an analgesic and excitomotor effect and stimulates local circulation in 15 cases (6.14%). The massage was performed in 45 patients (18.42%) through analgesic decontractive applications used to stimulate local circulation and tone the deficient muscle groups, providing a real benefit to the patient.

The design of a physical therapy program for hip rehabilitation considered that the joint provides the monopod and bipod support joint in statics and dynamics forming with the pelvis a functional unit. This is possible due to a particular architecture and to an effective muscle apparatus for achieving the pelvic-femoral balance. The physical therapy program was made according to the clinical, radiological and functional evaluation and it was adjusted depending on comorbidities.

Physical therapy was performed in 84 (34.42%) cases, involving various procedures for muscle strengthening, torn muscle relief, asuplisation of capsular ligaments, and exercises for increasing the joint amplitude.

The physiotherapy program used in the study group provided an effective relief of pain, changes of vicious attitude of flexion, adduction and external rotation, the increase of joint range of movement and muscle strength, the rehabilitation of dynamic muscle control in walking, the ability to perform certain daily activities, so that the patient's quality of life is highly impacted.

The outcome of the rehabilitation programs, namely the improvement muscle strength in the basin area, led to a limitation of tilting movements during walking. This limitation was functionally shown by the relief of limping, even if it was not permanently removed.

The surgical path is used for inserting hip prostheses through minimally invasive methods and it has been extensively studied over the past decades. The minimally invasive methods have a less impact on the peri

- and intra-articular ligamentous structures. Minimally invasive surgery is less aggressive and highly recommended in old patients with chronic diseases, as it allows a much faster functional recovery (11). Limiting surgical and anesthetic risks is very important, especially as the age of patients undergoing such interventions has continuously dropped in recent decades (12). Hip replacement or, in medical terms, total hip arthroplasty, is a successful story in orthopedics as it provides a solution for millions of people with irreparable damage of this joint.

DISCUSSION

The structural changes of the hip and particularly the functional changes, such as the onset of pain, muscle contractures and / or retractions contribute to the limitation in the mobility of the coxofemoral joint.

In the study group, the onset of pain in the affected hip led to the development of poor habits and bad static and dynamic postures. Body weight was automatically distributed on the healthy lower limb and the healthy joint was overloaded. This habit represents a bad motor engram that changes the normal gait. The gait becomes limping and support appears and a change in step times. The deviation of the center of gravity to the front is significantly higher in patients experiencing pain in the coxofemoral joint than in patients free of these disorders.

A rigorous and comprehensive biomechanical analysis of the hip joint can provide important additional information for the complex preoperative assessment, leading to a future therapeutic approach.

The study group consisted of 244 patients, most of them aged over 65 years, mostly originating from urban settings (76.63%). During physical examination of the hip, there were objective signs that concerned the synovial part and mobility pain.

Wright (11) describes a syndrome that destroys the femoral nucleus, related to poor blood supply of the upper femoral epiphysis. *Jacques Calve* (12) published in "Surgery Journal" a monography, entitled "On a particular form of pseudo-coxalgia associated with a characteristic deformity of the upper end of the femur". The author describes the disease clinically and radiologically, excluding the traumatic and infectious etiology.

The importance of the hip "discharge" increased gradually due to research conducted by *Waldenstrom* and *Danforth* (12). *Catterall*'s radiological research is remarkable (17). The external epiphyseal fracture was described by Fergusson, consisting of separation of an external fragment from the epiphyseal nucleus, a consequence of the external epiphyseal surplus (19).

Lawrence (11) showed that in subjects between 15-24 years old, the degenerative lesions pointed out by X-rays are present in 10% of the cases, compared to 80% in age groups over 55 years.

Compared to our study, the regularity of the femoral head is appreciated radiologically by concentric circles, placed at 2 mm distance, conceived by *Mose* (11); articular surface index, first used by Meyer, offers information about the morphology of the head; it is the ratio between the height of the head and its diameter (18).

Trueta (9) believes that osteoarthritis is secondary to high blood pressure in the hip joint, influencing this way the nutrition of the cartilage. Intraarticular forces will cause loss of elasticity connective tissue,

degenerative and necrotic phenomena in the areas where they act.

De Sèze and *Duriève* (12) believe that some vascular processes that influence the trophicity and metabolism of joint structures may cause hip osteoarthritis. *Rutishauser et al*. suggest that venous stasis has an important role in the development of osteoarthritis.

Axaser suggests that the chondrolysis process can occur due to damaged blood circulation in the subchondral area (9). *Pommer and Lang* show that this disease is the result of a dynamic imbalance between cartilage resistance and loading forces. This leads to severe changes in the physiological properties of the cartilage. This mechanical functional theory explains the presence of marginal osteophytes, bone products caused by improper mechanics (11).

Trueta explains the predominance of the fractures in 56 male patients (22.95%) (14) by traumatic factors that act in this period. *Chung* (11) explains this predominance in males by a particular vascular structure, unproven, but experimental.

Worldwide, osteoarthritis is the most frequent muscular-skeletal disease, affecting population over 55 years of age *Hinman, Hezwood* (11), while future forecasts predict a significant increase of incidence and severity, with particular implications for the health of the population.

The mechanical theory of *Freeman* (12) attributes the degenerative process to fracturing of collagen fibers.

Ficat (11) divides the disease into mechanical, structural, inflammatory and infectious chondroses, according to the condition and etiology of the destructive processes.

CONCLUSIONS

Our observations revealed the high frequency of fractures – 74 (30.32%) cases after 60 years of age, proving the fact that degenerative osteopathy is an old age disease; most of the subjects came from urban settings – 187 (76.63%) cases, with an easier access to health care services.

At study enrollment there were the following comorbidities: 49 (20.8%), respiratory -11(4.52%), gastrointestinal - 24 (9.83%), osteoarticular - 51(20.90%), neurological - 14 (5,73%), other - 95(38, 94%) cases.

We were able to identify at least one risk factor that could be incriminated in the etiology of the degenerative disease in all patients: fractures -74 (30.32%), luxations - 34 (13.93%), meniscal lesions -13 (5.34%), hemarthrosis – 17 (6.98%), synovitis – 21 (8.60%), endocrine and metabolic disease – 18 (7.39%), rheumatoid arthritis - 19 (7.78%), neurologic diseases - 9 (3.68%), circulatory disorders - 39 (15.48%) cases.

As for radiological investigation, we concluded that it has an important role, facilitating the diagnosis, without being indispensable.

Although not lacking side effects, the pharmacological treatment contributed to pain relief in patients with degenerative hip pathology who face an important degree of disability (pain, vicious position, walking perimeter). The rehabilitation of joint mobility must be associated with the rehabilitation of strength in muscles that perform these movements.

It became obvious the fact that a therapeutic, educational, medicinal and physical combination is able to control the patients' sufferance from the onset of the disease, in an effective patient-physician cooperation.

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THE MALFUNCTIONS OF A NATIONAL TRANSPLANTATION SYSTEM: MULTI-LAYERED EXPLANATIONS FROM WITHIN

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THE MALFUNCTIONS OF A NATIONAL TRANSPLANTATION SYSTEM: MULTI-LAYERED EXPLANATIONS FROM WITHIN (Abstract): Romania has one of the lowest posthumous organ donation and transplantation rates in Europe. **Aim**: To explore the perspectives on the national transplantation system deficits shared by the physicians responsible for the identification and referral of potential posthumous organ donors. **Material and methods**: The study consists of semi-structured interviews with the key informants (ICU physicians, neurologists and neurosurgeons) in county hospitals in Moldavia. **Results:** The explanations for the current Romanian organ transplantation rate, as emerged from the participants' answers, are multi-layered. Overall, the national transplantation system is accused of being plagued with serious problems: it is underfunded, understaffed, its resources have been distributed unevenly among medical centers, the communication between its elements is disrupted, leaving the majority of the transplant professionals practically detached from its upper layer and affecting their motivation to perform their transplantation-related tasks. Furthermore, some of the participants point to the strong reluctance of the population to donate their deceased relatives' organs. **Conclusions**: The results suggest a set of possible multi-level interventions that could alleviate these problems. **Keywords**: TRANSPLANTATION, ORGAN SHORTAGE, TRANSPLANT SYSTEM, HEALTH PROFESSIONALS, POPULATION, ROMANIA.

There is a universal shortage of cadaveric organ donors; while the number of patients waiting for transplants continues to increase, organ donation rates remain low. These rates vary widely between countries, Romania ranking near the bottom of the European hierarchy, with 3.6 deceased donors per million of the population in 2009 (1). Only 31% of the Romanians would agree to donate their organs after death and 34% would consent to donate organs of a deceased family member, far below the European means of 55% and 53% respectively (2).

An important factor influencing the posthumous organ donation rates is the refusal of the bereaved families to give consent for donation and the public's reluctance to donate (3). The attitude of the health professionals towards organ dona-

tion and transplantation, as well as their knowledge on these issues, also influence the decision to donate of the family members (4, 5). Their influence is not limited to the conversation with the family members, but it extends to their own involvement in terms of identification and referral of potential organ donors (6, 7).

In addition, the characteristics of the national transplantation system influence the work of the health professionals involved (8, 9) and, consequently, the number of donated organs. Among others, the technological level of the available medical facilities, the adequacy of personnel training or the degree of articulation between the various levels of the medical system can hinder or stimulate the efficiency of the transplantation system.

The aim of our study was to explore the perspectives of the physicians involved in the identification of potential organ donors on the factors influencing the functioning of the Romanian transplantation system and on the available interventions that could limit the negative influences.

MATERIAL AND METHODS

We used a qualitative methodology based on semi-structured interviews with open-ended questions. The participants were physicians of one of the three specializations involved in the diagnosis of brain death (neurology, neurosurgery and ICU) working in hospitals from the Moldavian counties, and responsible for the identification and referral of potential brain death donors. 24 physicians were contacted; 17 agreed to participate (10 men, 7 women): 6 neurologists, 6 neurosurgeons and 5 ICU specialists. The participants' age ranged from 41 to 57 and their work experience from 3 to 19 years. The interviews lasted about 50 minutes each and were all recorded.

The study was approved by the Research Ethics Commission of the University of Medicine and Pharmacy "Grigore T. Popa"- Iaşi. All participants were informed about the aim and procedures of the study and freely consented to be enrolled.

The interviews were transcribed verbatim, and analyzed in a grounded theory approach. The interviews were coded using the county name and the medical specialty abbreviation (N- Neurology; NS- Neurosurgery; ICU- Intensive Care Unit). In order to access and explore the essential meanings of the answers, the constant comparative analytic procedure was employed, by first reducing them to concepts through open coding. Then, we explored the associations between the various categories in different parts of the textual data. Each hypothesis that emerged across this coding process was compared to the new statements in participants' answers, thus assessing its validity. When the meanings of the new statements differed from the previously established relevant hypotheses, the latter were reformulated in order to accommodate them. When the differences were too large, indicating the existence of distinct perspectives on the same issue, the new meanings were theoretically condensed into a new partial hypothesis.

RESULTS AND DISCUSSION

The inner problems of the Romanian transplant system

The participants pointed out several categories of inner problems, distributed on several layers, from the individual one, the human resources employed in the system, to more general, organizational issues. On the first layer, some of the respondents identified the health professionals' low

motivation. On one hand, their involvement in the transplant- related interventions is an additional task, interfering with the already busy daily work schedule:

"It is an extra activity and the not so numerous employees already have to cope with a busy schedule. It is natural for interest in such activity, that we consider collateral, to be reduced" (NS1).

Therefore, the level of effort invested in the organ transplant– related tasks is low:

"I think it is also about our lack of involvement" (NS2).

A possible solution to this motivational issue is, for some participants, the financial retribution of their involvement in the transplant- related tasks.

"Those in charge of transplants should be paid more, no doubt about it!" (ICU5)

On the other hand, the low external rewards of the physicians could be compensated by their internal motivation, grounded in the life-giving character of transplantation. The satisfaction stemming from saving the life of the organ recipient can help them rationalize the efforts they had invested in the lost cause of saving the life of the deceased donor:

"We often struggle for patients who we know to have little chance, and we see no solution, but maybe it is then we get a new one, a new meaning…of all the work invested. Despite the end of that particular life, a new one might continue" (ICU2).

This type of approach involves the health professional's concern for the welfare of the potential organ recipient. Altruism appears to be an important factor for the health professionals' approach on organ donation (10). However although altruism should be at the heart of the medical profession, its current practical relevance is diminished. This issue would require educational interventions directed to physicians, such as development of campaigns aimed at stimulating their altruistic tendencies as motivators for their involvement in the organ transplant- related tasks:

"I say 'education' because we should get involved in this field of activity selflessly, as a result of our belief. In the long run, this is our purpose in medicine, or at least it should be" (ICU1).

Beyond the motivational issues, some physicians point to a systemic dysfunction in the informational circuit between the levels of the Romanian transplant system. Its main consequence is the informational deprivation of the physicians, not only in terms of their scientific update on the relevant medical issues, but also concerning the basic statistics of the outputs of this system:

"Not even us, the doctors, have knowledge of the number of kidney transplants performed in Romania so far, as well as their consequences" (N2).

The limited personal experience of many physicians with organ transplantation- related procedures can influence their attitude towards organ donation and gradually instigate further reticence among this first and essential layer of the Romanian transplant system. Other studies reveal that an important variable in this respect is health professionals' amount of personal experience with managing and referring brain dead patients (11). On the other hand, even when potential donors are identified in these "lower level hospitals", the lack of feedback on the successful transplant procedures that are performed elsewhere further induces a sense of futility regarding their efforts. Providing follow-up information on organ recipients could prove an effective intervention in order to raise the

level of support for and involvement in transplantation- related tasks among these health professionals (12).

An explanation for the scarcity of the information transmitted to the lower levels of the system is the traditional, and still ongoing, status of transplant interventions as high-end procedures, reserved to a select group of professionals:

"The specialized literature has not presented this side of the transplant as a real treatment possibility, but more like some sort of performance...reserved to a limited number of doctors, having experience, conditions and so on...even now this topic is still viewed as high performance or excellence in medical practice" (N3).

At present, this situation is perpetuated by the insufficient funding only covering the organ transplant- related expenses of the large medical centers:

"Large centers alone have this possibility. Funds were granted and still just the first layer benefited from technology, money and specialists" (ICU1).

Another consequence of this lack of articulation of the transplant system is the deficient communication between its various levels on current relevant issues:

"I think the most important problem is the lack of communication between ICU and the donation team" (ICU4).

Given the fast-paced nature of the set of procedures required by organ retrieval, these communication issues hinder their success, even causing failures in potential donation cases:

"There was a case here in which everyone stuttered: who's responsible for transplant, who's the one doing this, who's the one doing that… when things were finally clear, it was too late" (NS 1).

The lack of adequate funding is a serious issue, invoked by all our respondents. It affects the performance of the transplant system in two ways. First, it leads to dramatic shortage in the necessary technical equipment, limiting, in some cases, the available transplant- related procedures:

"There is also a deficit concerning the county hospitals equipment, necessary for brain death diagnosis and for maintaining artificial ventilation. They are very scarce in ICU, where the priority is set on the patients with a higher chance of survival." (NS6).

Second, the insufficient funding creates personnel insufficiency, limiting the tasks that the medical staff can perform and, consequently, their involvement in the transplant- related procedures. Furthermore, these insufficiencies raise ethical challenges regarding organ transplant-related procedures, since they require the exclusive investment of a considerable part of these scarce resources, affecting the medical care offered to the other patients:

"If I have two nurses on my shift, one should see to the special care of the donor, which is highly unlikely, since I wouldn't know what to do with the other severe patients in the department. This would not be fair to the other patients." (ICU5)

Subsequently, the cost – benefit analysis of the current situation questions the overall utility of organ transplantation, especially in the hospitals that do not perform actual transplant interventions, but should merely identify potential donors.

Overall, the current under-financing of the medical system as a whole seriously affects the rationality of the transplantation sub-system. The lack of involvement of hospital professionals in the transplant-related tasks appears as a way of defending the functionality of the rest of the system,

focused on providing medical care to the sick, by not deploying it of the already scarce resources:

"If you don't have the money, you do not perform transplantations. The transplant procedures become possible when the system is fit for it. If you don't even have the foundation, you can't perform transplantations, it's as simple as that" (N2)

Nevertheless, in line with the issue of the uneven distribution of resources, some participants remarked the paradoxical character of the Romanian transplant system, mirroring the entire medical system. This paradox stems from the massive differences not only in funding, but also in specialized education and experience between the two levels of the system: the "closed circle" of the first-layer medical centers and the rest of the hospitals:

"[..] again, this is a reflection of the Romanian medical system, with very competent peaks and the contrasting rest of the medical mass, struggling for survival, due to the lack of personnel, equipment, funds and so on." (N3).

In order to alleviate these structural problems, the Romanian physicians mostly prescribe top-down interventions: higher financial investments at all the levels of the transplant structure and the increased democratization of the system. Generally, the experience of other countries (9) shows that organizational improvements can increase the efficiency of the transplant system and, ultimately, the organ transplantation rate.

The attitudes of the general population toward organ donation

The reluctance of the general population to consent to organ donation from their deceased next of kin is frequently invoked as a major cause of the shortage of available organs for transplantation in many countries (13). Our participants pinpoint several factors contributing to the public's reluctance that have been identified by other investigations as predictors of people's willingness to donate. An important such factor is the lack of information on the topics of brain death and organ transplantation (14), which causes difficulties in explaining the diagnosis of brain death to the bereaved families (15):

"The public's lack of knowledge concerning transplantation is a frequent difficulty in discussing donation with the potential donors' families." (NS5)

The general lack of information is attributed by our respondents to the low involvement of the media in popularizing this topic:

"No kind of lobby for transplants, no mass media popularization, neither scientific, nor in terms of popular medicine have been made." (N2)

Moreover, the rare media reports of transplantation cases led to the perception of this procedure as an exceptional and unavailable medical option:

"People perceive this kind of procedure simply as an accidental performance." (N6)

Thus, a possible way to change people's attitudes toward organ donation is the more frequent media coverage of transplant interventions and organ donation. More extensive media coverage of this topic would alleviate the current "eccentricity" of organ transplantation in the public perception, which, in its turn, would positively influence people's attitudes. This idea is supported by previous results (16) showing that social advertisement of organ donation increases the occurrences of the organ donation topic in the discussions among fami-

ly members.

Another factor of the frequent reluctance of the Romanians to donate the organs of their next of kin, identified by some of the participants, is their low levels of altruism:

"My kin died, let others die, too! This is simply selfish and absurd!' (N1).

While altruism has long been recognized as an important internal motivator in these circumstances (17), it is usually conceived as a global orientation towards other people in general, close to the notion of 'humanitarian impulses' (18). However, the psychological deficit criticized by the health professionals in our sample mainly concerns the Romanians' "*lack of civic sense*" (N2), their lack of interest in the welfare of their peers. This perspective moves the reference point in the definition of altruism from the global level to the strictly national one and suggests that deeper issues in the area of the Romanians' collective identity could function as deterrents of the public's willingness to donate. Some of the participants suggested that media should take on a third mission, besides informing and raising awareness of organ donation, that of stimulating people's general altruism.

"Those more likely to donate have certain pieces of information and an open mind" (ICU2).

Finally, another factor identified by the participants as generating the high organ donation refusal rates in Romanian is the low level of trust that people have in the medical system as a whole. As a result, the relationships between the health professionals and their patients are frequently impaired by the latter's suspicions regarding some hidden personal interests of the former. Consequently, the requests for the donation of their deceased relative's organs are met with reluctance:

"The patient coming to the hospital already feels that everybody is a thief and wishes him no good, and those who trust what we know we must do are fewer each day." (NS5)

Moreover, the physicians working in the smaller hospitals have to deal with yet another type of suspicion, concerning their professional skills. Due to their mistrust in the medical competence of the hospital representatives, many of the relatives of brain dead patients withhold their consent, refusing the disconnection of the artificial life-support systems:

"People don't really trust us around here, at least not enough to consent to disconnect their kin from the life support system." (NS6)

Medical mistrust has been confirmed by previous studies as a significant predictor of attitudes towards organ donation (19). Due to its ubiquitous character highlighted by the physicians confronting it, our results suggest that it could dramatically influence the Romanians' stances on this topic.

CONCLUSIONS

Our study shows that the Romanian transplantation system is plagued with serious problems. It is underfinanced, understaffed, the resources being distributed unevenly among medical centers. The communication between its elements is disrupted, leaving the majority of the physicians practically detached from its upper layer, of those performing actual transplant procedures. Consequently, these physicians' motivation to involve in the tasks that are reserved for them by this undemocratic state of affairs is affected. At the same time, a clear set of possible interven-

tions at various levels can be inferred. First, organizational changes are mandatory, not only larger financial investments, but also stronger articulation of the various levels of the transplantation system. The latter would mainly entail increasing the communication between its two current levels- the medical centers performing transplant surgeries and those responsible only for the identification and referral of potential donors- as well as narrowing of the gap between them in terms of information, resources and, ultimately, surgical procedures performed. Second, media campaigns are called for in order to fill the public's massive knowledge gaps on the organ donation and transplantation and to stimulate people's altruistic perspectives on them. In addition the transplantation system could also benefit from campaigns addressing the health professionals' intrinsic motivations to involve in transplant-related tasks.

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NEWS

PROSPECTIVE ANALYSIS OF 55 CASES OF TUBERCULOSIS MENINGITIS IN NORTH INDIA

Tuberculosis meningitis (TBM) is a serious public health problem in developing countries as it leads to significant mortality and residual neurological sequelae. Despite being an endemic country for TB, data regarding clinical, radiological and laboratory (biochemical and microbiological) parameters and final outcome of adult TBM patients is sparse in India. Analysis of such variables in various countries has shown association of various factors with the prognosis of the disease like age, stage of disease, level of consciousness, presence of extraneural TB, isolation of *Mycobacterium tuberculosis* from CSF, biochemical studies and hydrocephalus. The availability of such data in a high developing country may help in prediction of the prognosis of such patients and help in early intervention of preventive measures to improve the subsequent outcome of the patients. In conclusion, TBM is a serious extrapulmonary form of TB and should arise suspicion in mind of clinician based on clinical, laboratory and radiologic results. The present work was planned to frame a model for prediction of mortality in TBM cases by including variables of age>40 years, past history of tuberculosis (TB), presence of basal exudates and hydrocephalus (Kaur H, Sharma K, Modi M, et al. Prospective Analysis of 55 Cases of Tuberculosis Meningitis in North India. Journal of Clinical and Diagnostic Research. 2015, Vol-9(1): 15-19).

Doina Butcovan

EGFR MUTATIONS IN NON-SMALL CELL LUNG CANCER: LOCAL EPIDEMIOLOGY AND CLINICAL IMPORTANCE

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EGFR MUTATIONS IN NON-SMALL CELL LUNG CANCER: LOCAL EPIDEMIOLOGY AND CLINICAL IMPORTANCE (Abstract): **Introduction:** Lung cancer's dismal prognosis led to new therapeutic approaches among which TKIs being among most promising; **Material and method:** Retrospective study at the Regional Institute of Oncology Iasi of non small cell lung cancer patients which underwent molecular investigations between November 2013 – September 2014. EGFR mutation status (positive, negative, undetermined) was assessed with an Entrogen EGFR kit using DNA extracted from paraffin embedded samples (surgical or endobronchial biopsies) with the Macherey-Nagel "NucleoSpin FFPE DNAkit" and then amplified on a Applied Biosystem 7500 Real Time PCR System. **Results:** There were 63 adenocarcinoma samples (17 females, mean age 60,9+/-9 years): 49 primary lung tumors and 14 secondary lesions (brain, lymph nodes, pleural). There was insufficient bioptic material for three cases. TTF1 status was determined for 46 patients – six were negative. There were twelve mutations identified (7 female subjects, 5 male) - six L858R, five Del 19 and one G719X; ten were TTF1 positive for the remaining two TTF1 status was unknown. Female sex predominance was statistically significant (p=0.02, chi squared). Mean age for mutation positive patients was 64+/-10 years; there were three never smokers, three active smokers and no data on smoking status was available for six subjects. **Conclusion:** Although small dimension of the study group precludes statistical significance EGFR mutations seem to correlate with TTF1 status. **Keywords**: MUTATIONS, LUNG CANCER, EPIDEMIOLOGY.

Lung cancer is a public health problem being the most important neoplastic cause of death – each year 1.3 million deaths are registered (1). Overall the 5 year survival is less than 15% despite therapeutical efforts (surgery, radiotherapy and chemotherapy either used alone or as a combination).

Non small cell lung carcinoma accounts for almost 80% of overall lung cancers and most predominant subtypes are adenocarcinomas, squamos cell carcinomas, adenosquamos carcinomas and large cell carcinomas. Small cell and squamos cell carcinomas incidence has decreased in the last years for both sexes and many countries presumably because of reduction of tobacco consumption. Presently adenocarcinomas are the most prevalent non small cell

carcinoma subtype (almost 50% of all lung cancer cases) (2).

Lung cancer treatment is dictated by disease stage and patients general health status and comorbidities. Stage I, II and IIIa non small cell lung cancer patients are usually offered a surgical solution frequently associated to an adjuvant therapy (chemotherapy and sometimes radiotherapy); this approach has a reasonable chance of achieving cure (3). However 50 to 70% of NSCLC patients either have an advance stage at diagnosis or relapse after a curative-intent treatment. Standard chemotherapy may improve survival, alleviate symptoms and improve quality of life for these patients. EGFR Tyrosine kinase inhibitors may be a therapeutic option as they may significantly increase relapse free survival and possibly global survival (4). Still, using these compounds require identification of some specific genetic abnormalities that drive tumor development.

Current oncogenetics is considered a discrete process characterized by progressive accumulation of genetic abnormalities involving both oncogenes (promoting tumoral development) and suppressor genes (exercising a limiting effect). Suppressor genes may be classified a "caretakers" and "gatekeepers"; both alleles have to be inactivated to allow tumor development. On the other hand single allele oncogene mutations are sufficient to stimulate cell proliferation. Bioinformatics research show an average of 120 genetic anomalies for a cancer cell. Among those a limited number can be considered driver mutations for tumor development; the others are dubbed silent mutations and their involvement is limited (passenger genes) (5, 6).

There is genetic data suggesting frequent involvement of KRAS, EGFR and BRAF mutations in lung cancer. As well as ALK, ROS1, RET and RON translocations and less frequent newly described JAK2 or ErbB4 (7).

EGFR is a transmembrane protein coded on a chromosome 7 gene (short arm) and belonging to the ErbB receptor family whose members are: HER1/ErbB1 (EGFR), HER2/neu/ErbB2, HER3/ErbB3 and HER4/ErbB4. It consists of a single 1186 aminoacid polypeptide chain. EGFR can be found on the surface of almost any normal cell but it is also intensely expressed on skin and digestive tract; this explains the TKI side effects. EGFR is also largely expressed on epithelial bronchial cells and moderately on alveolar epithelial cells. Three regions may be described – an extracellular one involved with ligand interaction; a transmembrane region characterized by hidrophoby and an intracellular region associating tyrosine kinase activity (8).

Inactive EGFR can be found as a monomer on membrane level. Following ligand interaction EGFR will activate and form homodimers. Several ligands have been described: TGF-α, EGF, amphyregulin, heparin-binding EGF-like growth factor, betacellulin and epiregulin (9, 10).

EGFR may also interact with another ErbB family member such as ErbB2/Her2/neu forming an active heterodimer.

EGFR dimerization induces conformational changes thus increasing intrinsic intracellular tyrosine kinase activity. This results in multiple signal pathways activation; best known are: the RAS/RAF/MAPK pathway involved with cell proliferation, tumor invasion and metastasis, PI3K/AKT pathway leading to cell survival by inhibiting apoptosis thru nuclear transcription factors activation and the JAK/STAT

pathway similarly involved in transcription activation (11, 12).

Under normal conditions tyrosine kinase activity is balanced by tyrosine phosphatase; there are various mechanisms leading to tyrosine kinase dysfunctions – mutations are probably the most important. Extracellular domain mutations may induce ligand independent cell proliferation; such is the case for some glioblastomas, ovarian cancers and non small cell lung cancers. Point mutations of the extracellular domain have been described for myeloma and some somatic mutations were associated with cervix and bladder cancers (8, 13, 14).

The vast majority however involve the intracellular tyrosine domain; there have been no reports on transmembrane region mutations involved in ligand independent receptor activation.

Historically all advanced stage NSCLC patients were offered first line therapy – a combination of two agents (one being a platinum compound), docetaxel or permetrexed as second line therapy and erlotinib as a second or third line option.

The development of tyrosine kinase inhibitors (TKI) such as gefitinib and erlotinib has brought some minor survival improvements if whole NSCLC patient population was considered. Later research showed that some characteristics such as race (Asian), histology (adenocarcinoma), sex (female) and smoking status (non smoker) associate a greater response if EGFR TKI are used (7, 15, 16). This data lead the discovery of EGFR activating mutations which are predictive for response to TKI.

Mutation status can be assessed by either direct sequencing or targeted approaches.

Sequencing techniques have the main advantage to identify any mutation (previously known or not); their main drawback is the less sensitivity – samples with tumoral content over 20% are required and the method is work intensive including a PCR amplification stage prior to sequencing(1).

Targeted approaches are mainly used to detect a limited number of high clinical significance mutations – usually therapy response predictors. Sensitivity is higher and are reliably used for small biopsy or cytology samples (with down to 1% tumoral cell content).

All EGFR activating mutations were found on exon 18-21(3). Three mutation classes have been described: class I – exon 19 deletions almost exclusively involving the 747 – 749 aminoacid sequence accounting for around 44% of total EGFR mutations. Class II contains point mutations leading to aminoacid substitutions. Most frequent point mutation involves exon 21 (codon 858) leading to arginine>leucine substitution and accounts for 41% of activating TK changes. Less frequent (around 2%) class II mutations involves exon 18 - the 719 glicine is replaced by serine, alanine or cysteine (G719X) and L861Q for exon 21. Class III mutations are exon 20 duplicates or inserts and account for around 5% of total EGFR mutations (15).

To sum up, exon 19 deletions and exon 21 point mutations account for 90% of total activating EGFR mutations and are dubbed „classic" activating mutations. Their activating effect is achieved by ligand independent tyrosine kinase domain conformational changes. Exon 20 inserts associate with primary TKI resistance and T790M point mutation with secondary resistance. Activating mutations positive status is the best response predictor for TKI therapy;

recent data showed gene amplification and/or hyperexpression alone not to be effective independent predictors. Thus EGFR sequencing is the best approach to predict TKI response and progression free survival (1).

Some pharmacological agents have been developed to block different signaling pathways; almost every single one belong to the tyrosine kinase inhibitor group and EGFR targeted agents are included to every day practice (17-19).

MATERIAL AND METHOD

Retrospective study including IRO Iasi non-small cell lung cancer patients which underwent molecular investigations starting November 2013 untill September 2014.

Main aim was to retrospectively evaluate the EGFR mutations prevalence in non-small cell lung cancer in IRO Iasi patients during November 2013 – September 2014.

Surgical, endobronchial or minimally invasive transthoracic biopsy samples were collected from primary tumors and secondary sites (cerebral, pleural, lymphatic).

Sample processing

Samples were formalin fixed and paraffin embedded; tissue blocks were microtomed to 3-5 μm sections. Diagnosis was reached using classical stains and immunhistochemistry approaches (TTF-1, p63, CK7, CK5/6 were the most frequently used markers).

DNA extraction

The pathologist marked the high tumoral cell percentage regions on hematoxylin eosin stained 3 μm sections – then manual macrodissection on relevant 10 μm sections was performed to reduce non malignant cell content. Paraffin was removed (toluene 5 min, ethanol 3 min, ethanol 2 min) and DNA extraction was performed using Machery-Nagel "NucleoSpin FFPE DNAkit" and then amplified on a Applied Biosystem 7500 Real Time PCR System. The DNA quality (A260/280) and quantity (ng/μl) was evaluated by UV spectrofotometric method on a Eppendorf BiophotometerPlus and a HellmaTrayCell-Light path 1mm and a second measure was performed by fluorimetric method on a Qubit® 2.0 Fluorometer with the Qubit® dsDNA HS Assay Kit. The method that we used for the detection of EGFR mutations requires a 5-10 ng of genomic DNA per reaction (40-90 ng for a sample), with a limit of detection between 0,1-1% diluted in the wild-type genomic DNA. EGFR mutation status (positive, negative, undetermined) was assessed on Entrogen mutation EGFR kit using 10 ng of DNA extracted from paraffin embedded samples (surgical or endobronchial biopsies).

Demographic/clinical data was also collected – age, smoking status

Each patient signed an informed consent form.

RESULTS

There were 63 adenocarcinoma samples (17 females, mean age 60,9+/-9 years): 49 primary lung tumors and 14 secondary lesions (brain, lymph nodes, pleural). There was insufficient bioptic material for three cases (5%). TTF1 status was determined for 46 patients – forty were positive, 6 were negative and 17 had no data provided.

There were twelve mutations identified (7 female subjects, 5 male) - six L858R, five Del 19 and one G719X; ten were TTF1 positive for the remaining two TTF1 status was unknown. Female sex predominance was statistically significant (p=0.02, chi

squared).

Mean age for mutation positive patients was 64+/-10 years; there were three never smokers, three active smokers and no data on smoking status was available for six subjects.

DISCUSSION

Our data reveals 19.04% mutation positive; the majority are point mutations L858R and del19, there was only one G719X case.

From the six L858R cases 4 were females; exon 19 deletions were present for three females and 2 males, the G719X mutation patient was male.

There were more females among mutation positive patients but less than literature data; this may be interpreted as a selection bias and insufficient addressability.

Our study group was characterized by frequent exon 21 mutations (50% of total mutation positive); this may have practical implications given the fact that best TKI response is seen with exon 19 mutations (20). There is available data reporting on worse survival for exon 21 mutation patients as compared to exon 19 mutation subpopulation mainly treated with afaftinib but possibly also with first generation TKI (21, 22).

There are reports correlating TTF-1 expression and mutation status – EGFR mutations seem to be frequent in TTF-1 positive tumors (23, 24). Our data is similar as mutations were found almost exclusively in TTF-1 positive tumors. Published data suggests different mutation incidence – Asians associates greater mutation frequency (35-40%) as compared to Caucasians (11-13%) while black ancestry falls between (25, 26). Smoking status associations could not be assessed because history data was not always available or reliable.

EGFR mutations were identified for 12 out of 63 tested patients; to our knowledge this is the first Romanian study of this type up to date.

CONCLUSION

Although small dimension of the study group precludes statistical significance EGFR mutations seem to correlate with TTF1 status. More data from large patient numbers is necessary to profile EGFR mutation spectrum in local population. Limited data available seems similar to literature data. Local standard of practice could probably benefit from more intensive testing.

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LEFT VERTEBRAL ARTERY HYPOPLASIA IN A TEENAGER WITH VERTIGO AND HYPOTENSION – CASE REPORT

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LEFT VERTEBRAL ARTERY HYPOPLASIA IN A TEENAGER WITH VERTIGO AND HYPOTENSION (Abstract): The vertebral arteries (VA) as a part of the vertebrobasilar cerebral circulation are one of the main branches of the subclavian artery. They have a diameter that varies from 1.5 to 5.0 mm. Congenital vertebral artery (VA) hypoplasia is an uncommon embryonic variation of posterior circulation with a frequency reported to be 2-6% from autopsy and angiograms. Vertigo is an illusion of movement, often horizontal and rotatory. In this article we present the case of a teenager diagnosed with left vertebral artery hypoplasia after episodes of headache, vertigo, drop attack and hypotension. **Keywords**: VERTEBRAL ARTERY, HYPOPLASIA, VERTIGO, DROP-ATTACK.

Vertebral artery hypoplasia was first described in the 19th century. Congenital hypoplasia of vertebral arteries is an uncommon embryonic variation of posterior circulation with a frequency of 2-6%, reported from autopsy and angiograms. It is classified as a vessel with a diameter in the entire course of less than 2mm to 3mm, or with a side difference equal or greater than 1:1.7 (1, 2).

This congenital variation can lead to cerebral hypo perfusion in the vertebrobasilar system supporting the brainstem and the occipital lobes a condition known as vertebrobasilar insufficiency (VBI). Common symptoms of VBI include: loss of vision in one or both eyes, double vision, dizziness (vertigo), numbness or tingling in the hands or feet, nausea and vomiting, slurred speech, changes in mental status, including confusion, loss of balance and coordination difficulty swallowing, generalized weakness in a part of the body (drop attack) (3,4).

Rapid unilateral injury to either peripheral or central vestibular structures produces the acute vestibular syndrome, which consists of severe vertigo, nausea and vomiting, spontaneous nystagmus, and postural instability (5).

It is important to differentiate a peripheral vestibular syndrome from a central one as the central vestibular disorders are caused often by an acute stroke that can rapidly lead to death.

We present the case of a teenager, who

was diagnosed with left vertebral artery hypoplasia after recurrent episodes of vertigo, headache and an episode of drop-attack.

CASE PRESENTATION

A 15 year old male teenager was admitted to our department, at the end of January 2015 for morning headaches, dizziness and chest pain. The patient is non-smoker, without any significant family or personal pathologic history.

Physical examination showed pale skin and mucosa, normal state of nutrition (BMI 21.8 kg/m^2), rhythmic heart sounds, with a hearth rate of 88/min, arterial blood pressure 90/65 mmHg. Clinical examination did not reveal any tenderness of the abdomen, the liver and spleen were not palpable, he had no signs of meningitis.

Laboratory tests at admission showed hypocalcemia and mild iron deficiency.

The electrocardiogram (ECG) revealed normal sinus rhythm (74/min), tall symmetric T waves in all derivation. The ECG Holter and ABPM have no pathological changes.

The echocardiography highlights grade II-III mitral regurgitation and level II tricuspid regurgitation.

Ophthalmologic, neurological, psychiatric consultations and electroencephalography were normal.

He was released with recommendations of symptomatics.

Subsequently, in March 2015 the patient is re-admitted for the evaluation of a physical asthenia (drop-attack episode) after exercise and psychic exertion. Clinical exam, ECG and neurological consult were again normal.

Given the echocardiography findings, the drop-attack episode and the persistent headaches, we performed a contrast MRI that revealed a threadlike vertebral artery (hypoplasia) with no other changes (fig.1, fig. 2).

Throughout the hospitalization he received treatment with Betaserc 24 mg twice per day and Tanakan 40 mg, 3 tablets per day with positive outcome. He was released with the recomandations to continue the treatment for 5 and 3 months respectively.

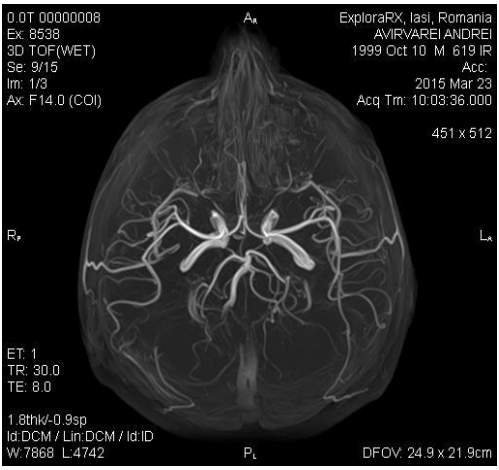

Fig. 1. Left vertebral artery hypoplasia – axial incidence

DISCUSSION

Vertigo is not a single disease entity but the cardinal symptom of different diseases of varying etiology; these may arise from the inner ear, brainstem, or cerebellum or may be of psychic origin (6).

Disequilibrium, which occurs when the brain receives inadequate information about the body's position from the somatosensory, visual, and vestibular systems, may result from peripheral neuropathy, eye disease, or peripheral vestibular disorders (7). Ménière's syndrome appears to be the final common pathway of the mechanisms the inner ear responds to almost any injury (8).

Functional disturbances of the optic nerve generate general physical and pathological reactions and some spontaneous remissions of immediate blindness can be explained. Secondary appearance of impairments or blindness can be explained by brain hematomas, edemas, nervous system disorders (9).

Presyncope is caused by cardiovascular disorders reducing cerebral perfusion (7).

Our patient, a teenager of 15 years, had no prior cardiac or neurological disease but had symptoms of headache, vertigo, chest pain and an episode of drop-attack that can suggest a cardiac cause.

However the physical exam, laboratory and paraclinic explorations found a cerebrovascular cause, more specific a left vertebral artery hypoplasia.

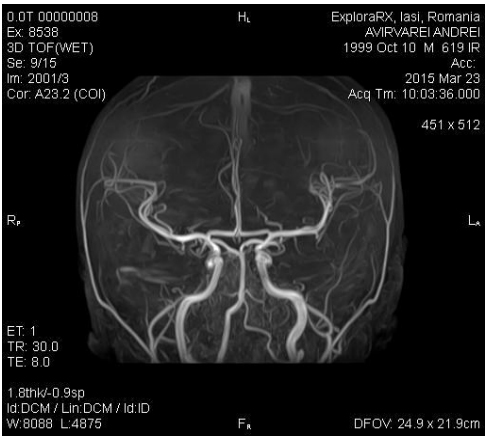

Fig. 2. Left vertebral artery hypoplasia – coronary incidence

Congenital anatomical variations of both vertebral arteries are relatively frequent; left vertebral artery dominance presents in 50% of the population, while similar size vertebral arteries present with an only 25% prevalence (10).

Therefore, because of this high prevalence, hypoplasia of vertebral artery alone is not considered a risk factor for stroke as it is a common variant in up to 75% of the general population (11).

However recent case reports and cohort studies suggest that its occurrence should not be negligible, mostly if it coexists with known risk factors of stroke (age>65, african-american race, female gender, family history and prior stroke, AIT or hearth attack, high blood pressure, cigarette smoking, diabetes mellitus, cardiovascular disease, high blood cholesterol, obesity and physical inactivity) that may increase the negative clinical impact (1, 2, 10, 11, 12).

CONCLUSIONS

Our patient was discovered with mitral and tricuspid regurgitation that are mostly mild and no restrictions or therapy is needed but in time it may lead to complications as abnormal heart rhythms, infections of the heart valve or heart failure. This is why we need to raise awareness on the fact that in a young patient with vertebral artery hypoplasia, without any important risk factor for stroke or cardiac disease, attention should be still high for controlling them and getting a positive outcome.

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NEWS

THE IMPORTANCE OF MOLECULAR BIOLOGY TESTS IN THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTION

C. difficile is an anaerobic bacterium that causes pseudomembranous colitis after antibiotic therapy. Between 3% and 7% of healthy people are colonized with *C. difficile*, but the percentage increases among people hospitalized. *C. difficile* infection diagnosis is based on clinical manifestation and is confirmed by the immunoassay test for *C. difficile* toxins and PCR for bacterial DNA. A recent study showed that the exclusive use of PCR in the diagnosis of *C. difficile* infection without immunological tests for toxins may conduct to over diagnosis and overtreatment. The duration of diarrhea during treatment, rate of complications and mortality rate for patients Tox−/PCR+ were comparable to patients without *C. difficile* (Tox−/PCR−) and lower than Tox+/PCR+ patients. These findings suggest that the treatment is not necessary for patients who were positive by PCR testing but negative by toxin immunoassay (Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. *JAMA Intern Med*. 2015. doi:10.1001/jamainternmed.2015.4114).

Cătălina Luncă

PSYCHOMETRIC HEPATIC ENCEPHALOPATHY SCORE FOR THE DIAGNOSIS OF MINIMAL HEPATIC ENCEPHALOPATHY IN ROMANIAN CIRRHOTIC PATIENTS - PARTIAL RESULTS

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PSYCHOMETRIC HEPATIC ENCEPHALOPATHY SCORES FOR THE DIAGNOSIS OF MINIMAL HEPATIC ENCEPHALOPATHY IN ROMANIAN CIRRHOTIC PATIENTS - PARTIAL RESULTS (Abstract): In Romania, minimal hepatic encephalopathy (MHE) is underdiagnosed and undertreated in patients with liver cirrhosis. The psychometric hepatic encephalopathy score (PHES) is recommended as the first-line tool for the diagnosis of MHE. **Aim:** To use PHES in a group of Romanian cirrhotic patients and highlight the relationships between demographic and biological factors and tests results. **Materials and Methods:** Of the 148 patients with liver cirrhosis admitted to the Iaşi Institute of Gastroenterology and Hepatology between August 2014 and February 2015 only 80 who met the eligibility criteria were enrolled and completed the study. Blood samples for routine tests and serum ammonia were collected from all patients on the day of PHES evaluation. **Results:** In the study group (n = 80) mean age was 57.7 ± 8.5 years, and the average number of years of education was 11.2 ± 2.7. The main causes of cirrhosis were alcohol consumption (31 patients - 38.8%), hepatitis C virus infection (29 patients - 36.3%) and hepatitis B virus infection (17 patients - 21.3%). Age, number of years of education, severity of liver disease (measured by using Child-Pugh and MELD scores) and some biological constants (albumin, bilirubin, International Normalized Ratio- INR) were significantly correlated ($p < 0.05$) with most psychometric tests. **Conclusions**: PHES is a practical, objective and useful method for the diagnosis of MHE. Demographic and biological factors correlated with the results of the psychometric test. The PHES requires standardization before applying it in Romanian cirrhotic patients. **Keywords:** MINIMAL HEPATIC ENCEPHALOPATHY, PSYCHOMETRIC HEPATIC ENCEPHALOPATHY SCORE, LIVER CIRRHOSIS.

Minimal hepatic encephalopathy (MHE) is a brain dysfunction caused by liver insufficiency and/or portosystemic shunting; patients with MHE have a normal neurological and mental status on physical examination but neurocognitive deficits detectable by neuropsychometric or neurophysiological tests (1). MHE has a negative impact on quality of life, daily activities (reading, driving, social interactions, decreased work performance) and is associated with a high risk for progression to overt hepatic encephalopathy (OHE) (2). The diagnosis of MHE is necessary in cirrhotic patients who are at

increased risk for work and automobile accidents, have subjective complaints, or report a decline in work performance (3). Several neurophysiological and neuropsychological tests are used to diagnose MHE, but most of them are expensive and require sophisticated equipment and highly trained personnel (4). According to the recommendations of the International Society for the Study of Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN), the psychometric hepatic encephalopathy score (PHES) is the first-line option in the diagnosis of MHE (5). PHES consists of 5 paper-pencil tests performed at the bedside and involves minimal costs: digit-symbol test (DST), number connection test A and B (NCT-A, NCT-B), serial dotting test (SDT) and line drawing test (LDT) (6). According to previous studies, test results can be influenced by multiple factors (age, occupation, education) and the resulting nomograms differ depending on the tested population (7-11). In this study our objectives were: i) use of PHES in a group of Romanian cirrhotic patients; ii) correlations between demographic and biological factors and psychometric test results.

MATERIAL AND METHODS

148 patients with liver cirrhosis were included in the study. The diagnosis of liver cirrhosis was based on clinical, biological, imaging (ultrasound, endoscopy, and computed tomography) or anatomopathological criteria (liver biopsy). Patients were admitted to the Institute of Gastroenterology and Hepatology – Iasi "St. Spiridon" Hospital between August 1, 2014 and February 28, 2015. After applying the following exclusion criteria, 80 (54%) patients were enrolled and completed the study (7.9):

- OHE (diagnosed according to West Heaven criteria) – at current admission/history;
- Diagnosis of hepatocarcinoma /other malignancies;
- Use of psychotropic drugs or neuropsychiatric disorders (dementia, epilepsy, ethanol withdrawal);
- Use of lactulose and/ or antibiotics;
- Active ethanol consumption ($\geq$ 50 g/day in men and $\geq$ 30 g/day in women);
- Chronic kidney, lung or heart diseases;
- Visual disturbances not corrected by glasses.

On the day of the psychometric tests blood samples were collected for routine tests and venous ammonia. Liver function was assessed by calculating the Child-Pugh and MELD (Model for End Stage Liver Disease) scores. Subjects included in the study were interviewed about education, occupational status, alcohol consumption, knowledge of Romanian alphabet and numbers. After a preliminary demonstration, all patients completed the 5 tests that make up PHES (DST, NCT-A, NCT-B, SDT, LTT) as instructed by the physician investigator. The results of DST test were measured as points and those of NCT-A, NCT-B and SDT tests as seconds. The results of the LTT were measured as both the time needed to complete the test (LTTt, seconds) and as the error score (LTTe). Tests were conducted between 10 am and 3 pm (in a well-lit, quiet room), being supervised and interpreted by a single investigator. PHES forms and the directions for administration were made available to our team by the courtesy of Prof. Dr. Karin Wissenborn (Hannover - Germany).

Written informed consent was obtained from all patients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the ethics committees of the Iasi "Grigo-

re T. Popa" University of Medicine and Pharmacy and "St. Spiridon" Hospital.

For statistical analysis the Statistical Package for Social Sciences (SPSS version 19.0, Chicago, IL, USA) was used. Quantitative data were expressed as means ± standard deviation or as number of subjects percentages (n, %). To measure the relationship between two variables the Pearson correlation coefficients were used. A *p* value of less then 0.05 was considered statistically significant.

RESULTS

For the patients enrolled in the study (n = 80) mean age was 57.7 ± 8.5 years, and the average number of education years was 11.2 ± 2.7. Distribution of patients by sex, age and years of education is shown in fig. 1 (A and B).

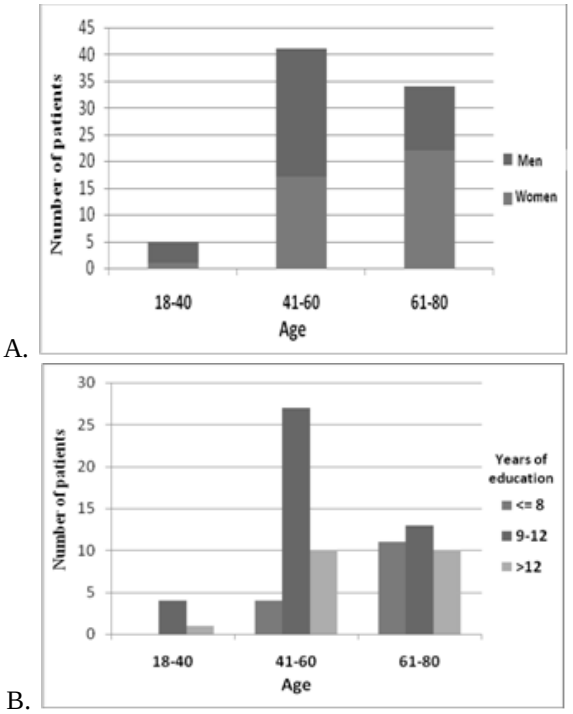

Fig. 1. Distribution of patients by sex, age and years of education

The main cause of liver disease was alcohol consumption - 31 (38.8%) patients, followed by hepatitis C virus infection - 29 (36.3%), hepatitis B virus infection - 17 (21.3%) and autoimmune diseases - 3 (3.6%). Distribution of patients according to Child-Pugh classification was as follows: Class A - 38 (47.5%) patients, class B - 32 (40%), class C - 10 (12.5%). The complex demographic data, biological parameters, causes and severity of liver disease (Child-Pugh and MELD scores) are shown in tab. I.

The results of DST, NCT-A, NCT-B, SDT, LTT-t LTT-e tests (mean ± SD) were: 30.1 ± 9.3 points, 57.4 ± 24.8 s, 149.6 ± 56.3 s, 71.7 ± 15.1 s, 105.3 ± 33.6 s and 47.6 ± 29.5 points, respectively. Age, number of years of education and severity of

liver disease (measured by Child-Pugh and MELD scores) were significantly correlated ($p < 0.05$) with the results of the majority of psychometric tests. Alcohol consumption, environment (rural/urban), gender (male/female) did not influence the final results (tab. II).

The following serum biological parameters correlated statistically significant ($p < 0.05$) with most of psychometric tests: INR (International Normalized Ratio), serum albumin and bilirubin (tab. III).

TABLE I

Demographic and biochemical data of patients with liver cirrhosis;

Demographic and biochemical data	*Patients with cirrhosis (n=80)*
Age (years, mean ± SD)	57.7±8.5
Sex (male/female)	40 (50%)/40(50%)
Education (years, mean ± SD)	11.2 ±2.7
Ethanol consumption (YES/NO)	22(27.5%)/58(72.5%)
Environment (rural/urban)	39 (48.8%)/41(51.2%)
Etiology of cirrhosis	
Toxic	31(38.8%)
Hepatitis B virus	17(21.3%)
Hepatitis C virus	29(36.3%)
Autoimmune	3 (3.6%)
Child-Pugh class	
A	38 (47.5%)
B	32 (40%)
C	10 (12.5%)
MELD score (mean ± SD)	11.8±4.6
Na (mEq/l,mean ± SD)	137.1 ± 3.5
K (mEq/l, mean ± SD)	4.2 ± 0.6
Creatinine (mg/dl, mean ± SD)	0.8 ± 0.3
INR (mean ± SD)	1.2 ± 0.3
Albumin (g/l, mean ± SD)	3.7 ± 0.7
Bilirubin (mg/dl, mean ± SD)	2.3 ± 2.3
Venous ammonia (µg/dl, mean ± SD)	81.8 ± 34.6

Abbreviations: MELD (Model for End Stage Liver Disease); INR (International Normalized Ratio

TABLE II

Correlations between psychometric tests and age, years of education, sex, alcohol consumption, environment, Child-Pugh and MELD scores (* $p<0.05$).

	Age	Years of education	Sex	Alcohol consumption	Rural/urban area	MELD	CHILD
DST	-0.26364910*	0.48018815*	-0.11736210	-0.1700156	0.17134152	-0.3234033*	-0.3693688*
NCT-A	0.26766790*	-0.39211942*	-0.13674703	-0.1033609	-0.11707596	0.18080225	0.1200654
NCT-B	0.27141097*	-0.44046582*	-0.09071686	0.07273519	-0.19221237	0.32029709*	0.26329258*
SDT	0.08546600	-0.15259250	0.17765616	0.20666285	-0.09305067	0.43673586*	0.36023270*
LTT-t	0.28679874*	-0.35411326*	-0.09058447	0.14695623	-0.17481955	0.37394262*	0.09654697
LTT-e	0.32089561*	0.33878592*	0.22874515	0.05153683	0.01508878	0.17508411	0.31959084*

Abbreviations: DST (Digit symbol test); NCT-A (Number connection test A); NCT-B (Number connection test B); SDT (Serial dotting test); LTT-t (time needed for LTT completion); LTT-e (LTT error score; MELD (Model for End Stage Liver Disease)

TABLE III

Correlations between psychometric tests and biological markers (* p<0.05).

	Ammonia	Sodium	Potassium	Creatinine	INR	Albumin	Bilirubin
DST	-0.19096095	0.173910981	-0.118279441	-0.027122314	-0.393310966*	0.318942804*	-0.25499673*
NCT-A	0.14583293	-0.062347228	-0.042255051	-0.09419454	0.312294237*	-0.055879366	0.116113029
NCT-B	0.10417198	-0.221240205	0.072077943	0.043443918	0.410377888*	-0.34655810*	0.310698541*
SDT	0.2033036	-0.17078756	0.125162098	0.215927252	0.381825385*	-0.31481725*	0.331568395*
LTT-t	0.12917021	-0.066871712	-0.102456334	-0.136267769	0.286184224*	-0.008166931	0.264441637*
LTT-e	-0.01882208	0.006143077	0.0476194	0.150228774	0.110697533	-0.33875244*	0.161874506

Abbreviations: DST (Digit symbol test); NCT-A (Number connection test A); NCT-B (Number connection test B); SDT (Serial dotting test); LTT-t (time needed for LTT completion); LTT-e (LTT error score); INR (International Normalized Ratio).

DISCUSSION

PHES is a useful tool for the diagnosis of minimal hepatic encephalopathy and for the study of neuropsychological disorders associated to this pathology (visual-spatial orientation, psychomotor speed, visual perception, attention, concentration and working speed) (12). Psychometric tests were initially studied by Schomerus et al. and standardized by Weissenborn et al. in the German population (6). The tests were later adopted and validated in other countries - Spain, Italy, Mexico, India, Korea and Poland (7-11).

Our study presents the partial results of the PHES standardization for the diagnosis of MHE in Romanian cirrhotic patients. PHES was applied to 80 patients diagnosed with cirrhosis, equally distributed by gender, from both rural and urban areas. In agreement with data in the literature (7-11) in our group of patients the severity of liver disease (Child-Pugh and MELD scores), demographic factors (age and years of education) and biological parameters (bilirubin, INR, albumin) correlated with the results of psychometric tests. In our study, sex, alcohol consumption and environment (rural/urban) did not influence the final results of the tests, the data being consistent with studies conducted in Italy or Spain (10, 11).

Some studies for PHES standardization, such as those in Poland and Korea, showed a statistically significant correlation between ammonia and psychometric tests (7.9). In our study patients ammonia level did not affect the test results, possibly because we used venous and not arterial blood sampling. The statistically significant correlations between Child-Pugh and MELD scores found in our study have been also reported by other studies (7 to 9,11).

In our group of patients with liver cirrhosis we have demonstrated a significant association between biological parameters (serum albumin, INR, serum bilirubin) and most of the psychometric tests. In the Polish study PHES correlated with serum albumin, while in the Korean study there were no statistically significant associations between serum parameters and neuropsychological tests (7, 9).

The significant correlations between psychometric tests and demographic factors certify the need for PHES standardization in the respective country population prior to its use for the diagnosis of MHE. The use of psychometric tests in a sample of healthy individuals and subsequently the provision of norm data represents the next step for the validation of PHES in the Romanian population.

CONCLUSIONS

PHES is a useful, objective, inexpensive and simple method for the diagnosis of MHE in patients with liver cirrhosis. In our study, psychometric tests correlated with the severity of liver disease (Child-Pugh and MELD scores), demographic factors (age and years of education) and serum parameters (bilirubin, INR, albumin). In order to apply the PHES to Romanian patients, the psychometric tests require standardization based on specific demographic factors (age and years of education).

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DIAGNOSTIC AND PROGNOSTIC VALUE OF PRESEPSIN IN THE EMERGENCY DEPARTMENT

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DIAGNOSTIC AND PROGNOSTIC VALUE OF PRESEPSIN IN THE EMERGENCY DEPARTMENT (Abstract): Sepsis syndrome is a common situation and has devastating implications on health care systems worldwide. Biomarkers may have an important role to highlight the presence, absence or severity of sepsis. **Material and methods**: Retrospective study is conducted on a group of 95 suspected sepsis patient (0.16%) out of a total of 56,996 patients that were presented in the Emergency Department / Emergency County Hospital "St. Spiridon" from Iaşi during 01.01.2012-01.12.2012. The study aims to establish the following: the incidence of sepsis diagnosis, analysis of prognostic factors, relationship between the presepsin value and clinical outcome. **Results**: In men, there was a distribution of cases between the age of 22 years up to 89 years with an average confidence interval between 59.32 and 67.23 years, and the distribution of cases varies among women from age of 40 years up to 93 years, with an average confidence interval between 65.53 and 75.47 years The most frequent primary source of infection is localized at the pulmonary level, in a total of 21.1%, followed by abdominal location occurring in a total 14.7%. Area under the curve in ROC curve analysis regarding mortality showed significant values of area for presepsin (0.859), leukocytes (0.790), traumatic injuries association (0.761) and the presence of gastrointestinal history. **Conclusions**: Presepsin has diagnostic value, early prognostic value and is an early marker of mortality in septic patients. Trauma associated with severe systemic infection leads to an increase in mortality. **Keywords**: SEPSIS, BIOMARKER, PRESEPSIN, MORTALITY.

The progress made over the last two centuries in fighting against infectious pathology may suggest at first glance an optimistic vision of this type of pathology compared to the early era of antibiotics. Sepsis is defined as an infectious disease, caused by bacterial microorganism, evolving as a serious systemic infection and will progress to severe sepsis (acute organ dysfunction caused by an infection or suspected to be caused by an infection) and to septic shock (severe sepsis associated with hypotension that does not respond to fluid resuscitation therapy), installed as a result of discharging in the blood flow of pathogens, toxins and cellular disintegration products. Sepsis and its clinical forms must be regarded as stages of the same disease (1).

Sepsis syndrome is a common in Intensive Care Units and has devastating implications on health care systems worldwide.

Estimated average annual cost for care of patients with sepsis was calculated to be worth $ 16.7 billion in 2008 (2).

Severe systemic infection and sepsis are challenging overcrowded emergency services, and in literature is described various causes of severe *sepsis*, even after intrauterine devices insertion (3). Other issue is the fact that septic infections were characterized by etiologic polymorphism and resistance to antibiotics (4). Failure to recognize these entities at the appropriate time, followed by early initiation of therapy and supportive etiological treatment can lead to degradation of vital parameters or even death in these patients. Hence the need for the use of laboratory diagnostic methods easy to use with high sensitivity and specificity to allow an early diagnosis prior installation of septic shock phase.

Biomarkers may have an important role to highlight the presence, absence or severity of sepsis, and can differentiate a bacterial infection of viral or fungal, in addition helps the differential diagnosis of sepsis (systemic infection) with a local infection.

Presepsin (sCD14-ST) - CD 14, is a glycoprotein expressed on the cell membrane surface of monocytes and macrophages present in macrophages, monocytes and granulocytes, is responsible for intracellular signal transmission triggered by the presence of endotoxins. Soluble fraction was called soluble CD14 subtype or presepsin has increased plasma levels in infections.

Cut-off value of presepsin allowing systemic inflammatory response syndrome differentiation within bacterial infectious diseases and nonbacterial was determined to be 600 pg/ml, the clinical sensitivity and specificity were 87.8% and 81.4% (5).

MATERIAL AND METHODS

This study is a retrospective study conducted on a group of 95 (0.16%) patients with suspected sepsis out of a total of 56,996 patients that were presented in the Emergency Unit - Emergency County Hospital “St. Spiridon” from Iaşi during 01.01.2012 -01.12.2012.

The study aims to establish the following:

- Incidence of sepsis diagnosis presented in the Emergency Unit - Emergency County Hospital "St. Spiridon "- Iasi;
- Clinical-tracking according to the precocity of when the patients are diagnosed and how long the therapy is initiated;
- Analysis of prognostic factors;
- Analyzing the relationship between trauma and sepsis on survival;
- The relationship between the obtained presepsin value and clinical outcome (on early diagnosed and properly treated patients).

The study protocol included the following data taken from the group of patients: epidemiological, clinical and laboratory variables, medical treatment administered in the Emergency Department (ED) - Hospital “St. Spiridon” from Iaşi, evolution.

Inclusion criteria: patients over 18 years; patients with clinical signs of infection accompanied by the presence of at least two of the following criteria: temperature > 38°C or < 36°C, heart rate > 90 / min, respiratory rate > 20 /min and leukocytosis (>12,000 mm^3) or leukopenia (<4,000 mm^3).

Exclusion criteria: patients under age of 18 years; patients who are pregnant or lactating.

The diagnosis of sepsis was made based on the clinical signs (specific and usually signs) in conjunction with other investiga-

tions, respecting the diagnostic criteria from Surviving Sepsis Campaign statements (5).

For each patient with suspected sepsis in the ED, approach was standardized based on specific protocols existing on emergency medicine specialties. The patient was clinically evaluated, vital signs was monitored and when clinical suspicion of infection arise the clinicians started to complete a case sheets including clinical and laboratory variables listed according with the definition of sepsis.

For patients who experienced associated traumatic injuries, protocol was applied for diagnosis and treatment of trauma patients according to existing guidelines and protocols.

Data collected from records of patients meeting inclusion criteria were processed statistically using statistical analysis software IBM-*SPSS 22.0.*

RESULTS

Structurally, the study group included 61 men, corresponding to a percentage of 64.21% and 34 women, representing a rate of 35.79%. Statistical analysis highlights an average age of 65.86 years in study participants, the mean age being 63.28 on men and 70.5 years on women (tab. I).

TABLE I

Table comparision mean age male/female

Sex related distribution	Mean	N	Std. Deviation
Male	63.28	61	15.443
Female	70.50	34	14.254
Total	65.86	95	15.351

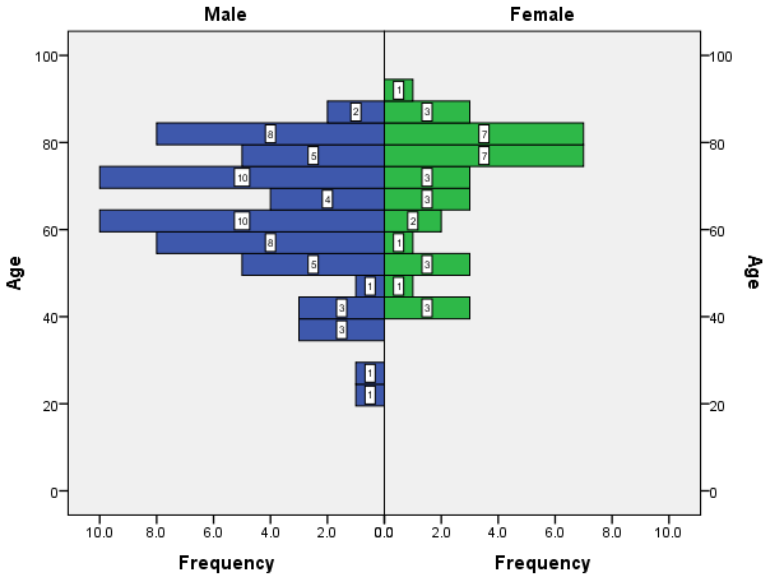

Fig. 1 Distribution of patients according to sex and age group

In men, there was a distribution of cases between the age of 22 years up to 89 years with a average confidence interval between 59.32 and 67.23 years, and for women the distribution of cases varies by age in a range from 40 years up to 93 years, with an average confidence interval between 65.53 and 75.47 years. The highest frequency recorded in men's was in 60-80 years age range and for women the most frequent cases were recorded in the range 70-80 years (fig. 1).

The most frequent primary source of infection is localized in the lung, 21.1%, followed by abdominal location occurring in 14.7%. In a percentage of 45.3% we could not detect during their presence in the E. D."St.Spiridon" the primary source of infection (tab. II).

TABLE.II

Primary source of infection

Primary source		Frequency	Percent	Valid percent	Total percent
Territory	Unspecified	43	45.3	45.3	45.3
	Pulmonary	20	21.1	21.1	66.3
	Cutanat	7	7.4	7.4	73.7
	Genito-Urinary	7	7.4	7.4	81.1
	Abdominal	14	14.7	14.7	95.8
	Central Nervous System	3	3.2	3.2	98.9
	Cardiac	1	1.1	1.1	100.0

The distribution of the starting point of systemic infection coupled with a mean age of patients shows a high average age for starting point located on pulmonary territory (71.5 years), followed by abdominal starting point (70.64 years), with the lowest average age if systemic infection was with cardiac starting point (22 years) (tab.III).

TABLE III

Epidemiological characteristics by age and primary source of infection

Diagnostic	Mean	N	Std. Deviation	Range	Minimum	Maximum
Unspecified	65.26	43	14.825	68	25	93
Pulmonary	71.50	20	13.690	51	38	89
Cutaneous	59.71	7	14.557	39	40	79
Genito-Urinary	61.86	7	13.434	33	51	84
Abdominal	70.64	14	13.765	45	44	89
Central Nervous System	53.00	3	19.672	39	35	74
Cardiac	22.00	1	.	0	22	22
Total	65.86	95	15.351	71	22	93

In addition to the usual biological constants laboratory determining, presepsin values determination was made in all patients included in the study, we observed an average value of its 2,519.25 pg / ml (tab. IV).

TABLE IV
Biological variables values

	N	Minimum	Maximum	Mean	Std. Deviation
Leucocytes	95	880	66,100	17,778.42	11,012.784
Thrombocytes	95	52,900	60,2000	253,029.47	133,950.395
INR	95	0	2	0.54	0.727
Glycemia	95	46	460	155.55	77.027
pH	95	6.80	7.58	7.3501	0.17002
RA	95	4.9	39.0	21.088	6.9188
Presepsin	95	145	20000	2519.25	3,169.939

We analyzed using Receiver Operating Characteristic curve (ROC) the correlation between the presepsin value and mortality in the group of patients, along with other variables but statistical significance values were obtained only between the value of leukocytes, history of gastrointestinal disease and trauma association in patients with sepsis (tab.V, fig.2). Area under the curve (AUC) showed significant values for presepsin (AUC=0.859), leukocytes (AUC=0,790), traumatic injuries association (AUC=0.761) and the presence of gastrointestinal history.

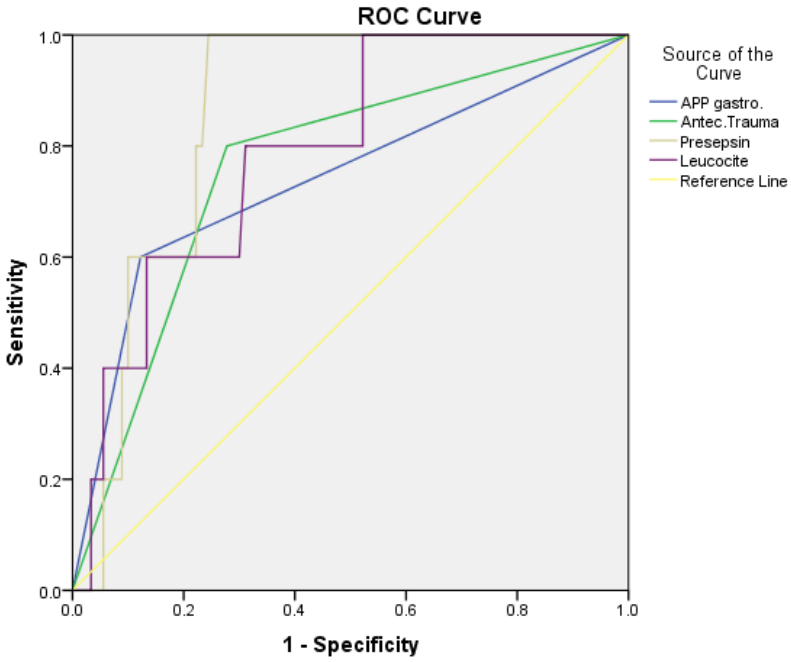

Fig. 2 ROC curve mortality-presepsin, leucocytes, trauma, and history of gastrointestinal disease

TABLE V

ROC Curve Mortality

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Gastro-intestinal history	.739	.134	.073	.477	1.000
Trauma history	.761	.108	.050	.550	.973
Presepsin	.859	.046	.007	.769	.949
Leucocytes	.790	.087	.030	.619	.961

The analysis of correlations between variables was made using Pearson method, significant correlations were detected between traumatic injuries and need for orotracheal intubation maneuver, mortality, low Glasgow coma score (GCS less than 9 points) and admission on intensive care unit. The analysis of presepsin show statistically significant correlations between this values and the presence of septic shock, need for vasopressor medication and need for advanced management on airways orotracheal intubation (OTI) (tab. VI, VII, VIII).

TABLE.VI

Corelation presepsin-TAS

		Presepsin	TAS
Presepsin	**Pearson Correlation**	1	-.308**
	Sig. (2-tailed)		.002
	N	95	95

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE VII

Corelation presepsin-vasopressor

		Presepsin	Vasopressor
Presepsin	**Pearson Correlation**	1	.315**
	Sig. (2-tailed)		.002
	N	95	95

**Correlation is significant at the 0.01 level (2-tailed).

TABLE VIII

Corelation Traum –OTI, GCS, Mortalitate

Trauma asociated		OTI	GCS	Mortality
Trauma	**Pearson Correlation**	.395**	-.251*	.254*
	Sig. (2-tailed)	.000	.014	.013
	N	95	95	95

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The sepsis average age described in the literature has increased over time, from an average of 68.2 years up to 64.1 years, the average age obtained in the present study were of 65.86 years (7). Men have more susceptibility than women to develop sepsis and severe sepsis, the data of the present study is consistent with international reporting (8).

Most commonly the initial source of infection that developed sepsis is described in the literature to be on pulmonary level, followed by 2nd primary location on gastrointestinal level, the values obtained by us located the same frequency in the top, and also in literature a significant proportion of sepsis cases described the primary infection location as uncertain or not identified (8, 9).

Presepsin is described as the only independent variable that can be associated with survival at 28 days or hospitalization in the intensive care ward and prognostic accuracy is increased compared with procalcitonin and other biomarkers (10, 11, 12). The area under the curve obtained in our study show values comparable to those described in the few studies published up to present date, the value obtained in this study were AUC=0.859, compared with other studies that showed values between AUC=0.640 to AUC= 0.790 (11, 12).

The correlation between the occurrence of septic shock, presepsin value and need for vasopressor therapy was recently described in the literature (13).

The combination of traumatic injuries and further development of sepsis and severe sepsis is described in the literature, with a predominance of this pathology in males, with a mortality still high, ranging from 19.5-23% of cases (14, 15) . Due to the increased severity of this association is explained correlation with the need for advanced airway management and low Glasgow coma score on this category of patients.

CONCLUSIONS

Presepsin early diagnostic value may also have prognostic value over the subsequent evolution of the patient.

Presepsin is an early marker of mortality in septic patients.

Trauma associated with severe systemic infection leads to an increase in mortality compared with patients in the group that did not have a diagnosis of trauma associated (p=0.013).

Endotracheal intubation maneuver needs to be considered in patients with the diagnosis of sepsis associated with trauma (p=0.00076).

Combination of multiple trauma injury and sepsis will lead to a fast deterioration in neurological status of the patient, the Glasgow coma score fell below 9 points in these patients (p = 0.014).

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NEWS

INTERACTION BETWEEN EBOLA VIRUS AND THE EXTRACELLULAR MATRIX

Ebola virus disease (EVD) represents a global public health threat concern. Limited therapeutic and prophylactic options are available for patients suffering from this disease. The EV glycoprotein (GP) is the main determinant causing structural damage of endothelial cells which triggers the hemorrhagic diathesis. However, the molecular mechanisms of this phenomenon remains elusive. In a study by Veljkovic et al. the interaction of GP with endothelial extracellular matrix (ECM) was investigated using the informational spectrum method (ISM), a virtual spectroscopy method for analysis of the protein-protein interactions. The results of the study suggest that Elastin Microfibril Interface Located Proteins (EMILINs) are involved in interaction between GP and ECM. The study concluded that this finding leads to a better understanding of EV/endothelium interaction and its role in pathogenesis, prevention and therapy of EVD (Veljkovic V1, Glisic S1, Muller CP2, Scotch M3, Branch DR4, Perovic VR1, et al. In silico analysis suggests interaction between Ebola virus and the extracellular matrix. Front Microbiol. 2015; 6: 135. doi: 10.3389/fmicb.2015.00135. eCollection 2015).

Teodora Vremera

PARTICULARITIES OF THE ETIOLOGY OF HYPONATEREMIA IN THE FRAIL ELDERLY PATIENT

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PARTICULARITIES OF THE ETIOLOGY OF HYPONATEREMIA IN THE FRAIL ELEDERLY PATIENT (Abstract). Hyponatremia is the most common electrolyte disorder in medical practice, and its prevalence is increasing in frail elderly patients. There are many reasons behind this phenomenon: the changes induced by aging itself, the presence of comorbidities and polypharmacy, the increased risk of dehydration, increased frequency of iatrogenesis and therapeutic noncompliance. Of the iatrogenic causes, we will refer to hyponatremia induced by thiazide-type and thiazide-like diuretics (1) due to 4-fold increase in prevalence in the frail elderly patients. Hyponatremia is a disease that benefits from prompt and appropriate treatment with excellent results, but the key to therapeutic success is the determination of its cause - which can be difficult in the elderly, and of patient's volume status for a correct classification of the disease. Hyponatremia in frail elderly patients may be asymptomatic or mimic other diseases. These are due to the almost constant association of varying degrees of geriatric syndromes, the most often responsible for symptom mystification being the cognitive impairment, the presence of comorbidities that have similar symptoms, the overlapping of acute events - fractures resulting from same-level falls that distort the diagnosis. We report 3 cases of hyponatremia characterized by atypical symptoms that was induced by indapamide treatment. Hospitalization for thiazide-induced hyponatremia is frequent, suggesting suboptimal monitoring, especially during the treatment initiation phase. Literature data suggest that the ionogram should be obtained 7 to 14 days after treatment initiation to detect the early onset of hyponatremia. There are reports of hyponatremia occurring after years of treatment, suggesting the need for constant monitoring of a patient treated with long-acting thiazide diuretics. Considering an iatrogenic cause (most commonly induced by thiazide-type or thiazide-like diuretics) should be in the forefront of physician's mind, but it requires a thorough medical history, assessment of patient compliance and cognitive status as well as support from patient's family. Identification of the cause of hyponatremia should prevent recurrences and provide adequate patient education to prevent secondary complications. **Keywords**: HYPONATREMIA, FRAIL ELDERLY PATIENTS, INDAPAMIDE TREATMENT.

Hyponatremia is the most common electrolyte disorder in medical practice, and its prevalence is even greater in frail elderly patients due to the addition of some particularities of this age segment: changes induced by aging per se, presence of comorbidities and polypharmacy, increased risk of dehydration (both by a natural predisposition to low daily fluid intake of the elderly and by the existence of such conditions that favor dehydration as prolonged and repeated hospitalizations, perioperative hydrological

restrictions), increased frequency of iatrogenesis and therapeutic noncompliance. Among the iatrogenic causes, we draw attention to hyponatremia induced by thiazide-type and thiazide-like diuretics (1) due to the up to 4-fold increase in prevalence in the frail elderly patient (2). Most elderly patients have varying degrees of hyponatremia. The severe form (Na < 125 mmol/l) is usually characterized by obvious clinical symptoms: altered general state, marked physical asthenia, seizures, nausea and vomiting culminating with the severe forms: hyponatremic encephalopathy, noncardiogenic pulmonary edema, coma and death (3). Moderate forms of hyponatremia have more attenuated and polymorphic symptoms, and in frail elderly patients they may be asymptomatic or mimic other pathologies. These are due to the almost constant association of varying degrees of geriatric syndromes, the most frequently responsible for symptom mystification being cognitive impairment, the presence of comorbidities with similar symptoms, the overlapping of acute events - fractures resulting from same-level falls that distort the diagnosis (3.4). Hyponatremia is a disorder that benefits from prompt and appropriate treatment (1), but the key to therapeutic success is the determination of its cause - which can be difficult in the elderly, and of patient's volume status for a correct classification of the disease.

To exemplify, we report three cases with various forms of hyponatremia who shared the advanced age, presence of comorbidities and polymedication, the common element being the treatment with thiazide-like diuretic (indapamide).

CASE REPORTS

Case 1: a 68-year-old woman with polymedication due to polypathology: digestive (gastroesophageal reflux disease, chronic gastritis and hepatitis C for 11 years treated with 3 different drugs) and cardiovascular (AHT and heart failure for which she received a 10-day treatment with 4 different drugs - a fixed combination of perindopril 10 mg and indapamide 2.5 mg, trimetazidine, platelet antiaggregant and statin). Five days before the doctor's appointment, the patient became symptomatic, presenting vertigo, headache, nausea and vomiting, and presented to the Gastroenterology department. Routine biological screening revealed severe hyponatremia (Na = 121 mmol/L) and hypokalemia (K = 3.28 mmol/L). A digestive cause was ruled out and she was referred to the Geriatrics Clinic. The thorough history confirmed the compliance with treatment and low-salt diet and a normal neurocognitive status (MMSE = 29/30). The volume status was assessed using echocardiography, which revealed normal diameter and almost complete inspiratory collapse of the inferior vena cava, suggestive of a diagnosis of iatrogenic euvolemic hyponatremia (indapamide). Slow correction of ionic imbalance was initiated by the discontinuation of the thiazide-like diuretic and normal dietary salt intake followed by oral and parenteral hydration with isotonic saline for 8 days. The course was slowly favorable, with the remission of symptoms and correction of ionic imbalance, on discharge the Na level being within normal ranges (143 mmol/L).

Case 2: an 83-year-old male patient with difficult to take history and without medical documents, with cardiovascular and rheumatic polypathology (according to his daughter who also mentioned the home treatment with indapamide) and who had sustained a craniocerebral trauma from same-level fall, for which he was urgently investigated at the Neurosurgery department. Routine biological screening revealed

severe hyponatremia (Na = 118 mmol/L) and hypokalemia (K = 3.2 mmol/L) and he was referred to the Geriatrics Clinic. The assessment of volume, clinical and echocardiographic status oriented the diagnosis towards hypovolemic hyponatremia possibly of multiple etiology: iatrogenic (indapamide) and secondary to dehydration by insufficient fluid intake. Geriatric assessment revealed impaired neurocognitive status (MMSE = 19/30) and malnutrition risk. The conservative correction of hypovolemic hyponatremia was initiated by discontinuing the thiazide-like diuretic and replacing it with a non-dihydropyridine calcium blocker to control blood pressure levels. The patients received parenteral hydration for 5 days with 500 mL of isotonic serum enhanced with 20 mL micro perfusions of 5.85% NaCl with dynamic ionogram monitoring and slow correction of hyponatremia to prevent osmotic demyelination with serious neurological consequences (5). For the correction of hypokalemia, 10 mL intravenous KCI was added to the treatment schedule on admission day, followed by oral administration in doses adapted according to the ionogram.

Case 3: a 69-year-old female patient polymedicated and with polypathology (cardiovascular, receiving treatment with 5 different drugs/day, metabolic, insulin-dependent diabetes mellitus for which she was on treatment with 2 oral antidiabetic drugs and insulin, dyslipidemic syndrome and morbid obesity, and cerebral pathology for which she took 3 other drugs/day); the patient reported the onset of symptoms a few days earlier by progressively increased leg edema, dyspnea at increasingly smaller efforts, physical asthenia and vertigo, for which she presents to the Cardiology Department where, in addition to the existing diagnoses, altered renal function (Cl creatinine = 39 mL/min) and severe hyponatremia (Na = 124 mmol/l) were detected and the patient was referred to the Geriatrics Clinic. Clinical assessment (leg edema, moderate dyspnea) and echocardiography (slight inferior vena cava inspiratory collapse) suggest hypervolemic hyponatremia of multiple etiology, the main causes being the lack of therapeutic compliance (chaotic treatment, especially in case of indapamide) and diet with inadequate fluid intake (polydipsia). Geriatric evaluation revealed mild cognitive impairment (MMSE = 25/30) and depressed status (GDS = 8/15). Correction of electrolyte and volume disturbances was initiated: discontinuation of indapamide and replacing it with intravenous furosemide, water restriction (500 mL/day), correction of diabetes. The course was slowly favorable at the same time with the correction of hyponatremia being obtained the correction of renal dysfunction (CI creatinine at discharge = 76 mL / min). In all cases, the Schellong test was negative, ruling out orthostatic hypotension.

DISCUSSION

Hyponatremia usually occurs within the first few days of treatment with thiazide diuretics but may also occur after months of treatment (6). Although thiazide-type and thiazide-like diuretics differ in chemical structure, they share the same mechanism of action, namely the inhibition of the Na-Cl co-transporter in the distal convoluted tubule (7). Currently, thiazide diuretics are a class of first-line drugs in the treatment of high blood pressure in the elderly.

Hospital admission for hyponatremia induced using thiazide diuretics is frequent, suggesting suboptimal monitoring, especially at the time of treatment initiation. According to the literature the ionogram should be obtained 7 to 14 days after thiazide initiation to detect the early onset of hypo-

natremia. There are case reports of hyponatremia occurring after years of treatment, suggesting the need for constant monitoring of patients on long-term treatment with thiazide diuretics (7). Our three cases exemplify these situations - early or late occurrence of hyponatremia after indapamide treatment. Previous values for electrolytes were not available until hospital admission.

There are numerous studies aimed at identifying the causes of hyponatremia in the elderly. The most common cause is the syndrome of inappropriate antidiuretic hormone (SIADH) (8, 9), closely followed by the treatment with thiazide-type and thiazide-like diuretics (7, 10). However, the accurate determination of the etiology of hyponatremia in a frail elderly patient should also consider other causes more rarely encountered in other age groups.

The presence of comorbidities may play an independent role in triggering hyponatremia, with attention being paid to neoplasia. There are studies that associate the presence of hyponatremia (especially the euvolemic and hypovolemic forms) with a significantly increased mortality among patients diagnosed with lymphoma, breast cancer, colorectal and pancreatic cancer, bronchopulmonary cancer, and hepatocellular carcinoma (11, 12, 13, 14).

The presence of comorbidities is always associated with polymedication (> 5 different drugs per day) (3, 15), which is a known risk factor for iatrogenic hyponatremia, the most commonly incriminated being the thiazide-type and thiazide-like diuretics. Angiotensin converting enzyme inhibitors are among the most prescribed drugs together with thiazide diuretics (2), followed by sartans, non-steroidal anti-inflammatory drugs and antidepressants (which affect water homeostasis), raising the hypothesis of drug interaction as a possible cause of hyponatremia. It should be noted that the presence of hypokalemia is frequent in patients with thiazide-induced hyponatremia (1).

Older age (1), female gender and low body mass index (10) are other risk factors for hyponatremia in patients treated with thiazide diuretics (6); to these must be added the non-compliance with drug therapy, often associated with varying degrees of cognitive disturbance, and the phenomenon of self-neglect, elements that will interfere with the rate of administration and the doses of recommended drugs (16).

Finally, we should make a special mention of the frail elderly patients requiring surgery, most commonly hemiarthroplasty for hip fracture, who frequently develop moderate hyponatremia perioperatively mainly due to the recommendations for preoperative water restriction (4, 5).

Symptoms of hyponatremia in frail elderly patients are polymorphic, represented by same-level fall preceded or not by vertigo, fatigue, confusion, nausea, vomiting, and neurological symptoms (7). In patients with acute hyponatremia, nausea, vomiting and headache due to cerebral edema (1) are more common, as in our Case 1. Unfortunately, these symptoms are commonly attributed to other concomitant diseases or other overlapping acute events, with a delayed detection of hyponatremia and an often incorrectly identified etiology. Iatrogenic pathology and dehydration should be the main causal lines given the increased thiazide-type and thiazide-like diuretic prescription for in the elderly and the hypovolemic status of the elderly. Thus, in the management of hyponatremia the first gesture is discontinuing the thiazide (if present in the therapeutic regimen), which is both diagnostic and therapeutic test. Attention should be paid to the slow cor-

rection of the imbalance in order not to induce osmotic demyelination, with severe neurological consequences (5).

CONCLUSIONS

With these cases we underlined the fact that treatment with thiazide-type or thiazide-like diuretics in the elderly requires frequent monitoring of electrolytes given the high risk of severe hyponatremia, clinical condition with atypical or less symptomatic clinical picture which delays (sometimes with fatal outcomes) the diagnosis. Considering an iatrogenic cause (most commonly induced by thiazide-type or thiazide-like diuretics) should be in the forefront of physician's mind, but it requires a thorough medical history, assessment of patient compliance and cognitive status as well as support from patient's family. Identification of the cause of hyponatremia should prevent recurrences and provide adequate patient education to prevent secondary, severe complications.

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THERAPEUTIC PARTICULARITIES IN AMIODARONE INDUCED THYROID DISORDER IN PATIENTS WITH UNDERLYING CARDIAC CONDITION

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THERAPEUTIC PARTICULARITIES IN AMIODARONE INDUCED THYROID DISORDER IN PATIENTS WITH UNDERLYING CARDIAC CONDITION (Abstract). **Aim:** the analysis of therapeutic approach in patients with basic heart condition and amiodarone induced thyroid dysfunction – correlations with the evolution and prognosis. **Material and methods:** The study included 215 patients, 90 men and 125 women aged between 35 and 87, with different cardiac pathologies hospitalized in the Cardiology Clinic between 2004 -2014, who received amiodarone treatment, in most cases for prophylaxis of various arrhythmias, both supraventricular and ventricular. During the evolution, these patients have developed amiodarone-induced thyroid dysfunction (hypo- or hyper function). **Results and discussion:** The evaluation of thyroid function after starting treatment was performed in 187 patients (86.97%). Diagnosis of amiodarone-induced thyroid dysfunction was based on hormonal dosages of TSH, FT4 and FT3, endocrinological examination and thyroid ultrasound. Thyroid dysfunction treatment was initiated, depending on the situation, in all patients during the hospitalization. Treatment included anti thyroid drugs or hormones substitution and in some cases a minimal dose of prednisone. **Conclusions:** Thyroid dysfunction regardless of the type (with hypo- or hyper function) requires continuous changes of the cardiovascular treatment, and association, where appropriate, with thyroid dysfunction medication. In some cases the latter determines cardiovascular side effects, for instance corticotheraphy may become a factor of imbalance for the hemodynamic status of the patient (by fluid retention, increased blood pressure, hyper glycemia). **Keywords:** AMIODARONE, THYROID HORMONES, ANTI THYROID DRUGS

Amiodarone is a class III anti arrhythmic drug (according to the classification of Vaughan Williams) which is effective in the treatment and prophylaxis of arrhythmias, supra ventricular and ventricular (especially those that are life-threatening) (1). Apparently, it presents structural similarities with the thyroxin hormone produced and released by the thyroid gland (1, 2, 3, 4, 8). Thyroid abnormalities were observed in 14-18% of patients receiving the low dose of amiodarone (1, 2, 3, 8). Thyroid effects are variable: abnormal thyroid function tests detected only by

periodic laboratory control (TSH, FT4, FT3) in the absence of any clinical manifestations or thyroid clinical dysfunction: amiodarone-induced thyrotoxicosis form, or hypothyroidism. Both can occur on a previously normal thyroid gland or in the context of a pre-existing thyroid impairment that may worsen (2, 3, 4, 5, 8, 9).

Mechanism of amiodarone activity on thyroid hormones levels is particularly complex: Amiodarone inhibits 5'-deiodinase and thereby decreases the peripheral conversion of T4 in T3. Their renal elimination also decreases, T4 level increase and T3 decrease by about 25%, T4 and T3 entry in peripheral tissues is also inhibited. T4 levels increase by 40% in the first 1-4 months of treatment with amiodarone (10, 11, 12). An inhibition of the pituitary deiodinase can be seen after 3 months of treatment and leads to higher levels of thyroid stimulating hormone (TSH). Amiodarone, together with its metabolite, also have a direct cytotoxic effect on the thyroid follicular cells, the result being a destructive thyroiditis. It acts as a competitive antagonist of T3 at the cardiac level (3, 4, 6, 7, 8, 9, 13, 14, 15).

Amiodarone-induced thyroid impairment (AIT) can resemble the clinical and biochemical picture of either hypothyroidism or hyperthyroidism. Amiodarone - induced hyperthyroidism may be of two types: Type 1 AIT occurs in patients with an underlying thyroid pathology such as autonomous nodular goiter or Graves' disease - in these patients, there is accelerated thyroid hormone synthesis secondary to the iodide load from the amiodarone therapy (the Jod-Basedow phenomenon) (15); Type 2 - appears as a destructive inflammatory process followed by destruction in patients with a previously normal thyroid. It is a destructive thyroiditis with preformed hormones releasing from the damaged thyroid follicular cells (10, 11, 13, 14, 15). This type of development requires specific therapeutic management that involves the administration of glucocorticoids. Combined forms of thyroid impairment are rare (10, 11, 12, 13, 15). The thyrotoxic phase may last several weeks to several months, and it is often followed by a hypothyroid phase with eventual recovery in most patients. For unclear reasons, the toxic effects of amiodarone may take two to three years to manifest (10, 11).

This study analyzes the therapeutic particularities in amiodarone-induced thyroid dysfunction in patients with severe underlying heart disease in order to improve long-term prognosis.

MATERIAL AND METHODS

The study included 215 patients hospitalized in the Cardiology Clinic of "Sf. Spiridon" Hospital Iasi between 2004-2014, who received amiodarone treatment, mostly for prophylaxis of various arrhythmias, both supraventricular and ventricular. The group included 90 (41.86%) men and 125 (58.13%) women aged between 35 and 87 (fig. 1).

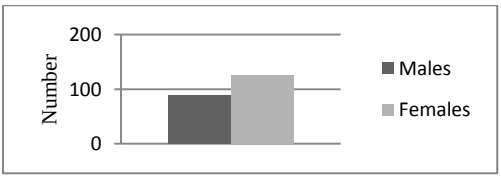

Fig. 1. Gender distribution of patients

Basic cardiac pathology of the study group included: acute viral myocarditis - 2 (0.93%) cases, acute myocardial infarction -16(7.44%) complicated with supraventricular arrhythmias (atrial extra-systoles, paroxysmal atrial fibrillation), systematized ventricular extra-systolas (bi, trigeminy) and ventricular tachycardia, other forms of

ischemic heart disease – 11 (5.11%) cases, ischemic dilated and toxic cardiomyopathy - 49 (22.79%) cases, hypertension - 76 (35.34%) cases complicated with atrial and ventricular arrhythmias (fig 2). In 60 patients (27.9%) it was performed electrical cardio version to convert atrial fibrillation to sinus rhythm and amiodarone was administered pre- and post-cardio version using a standard protocol.

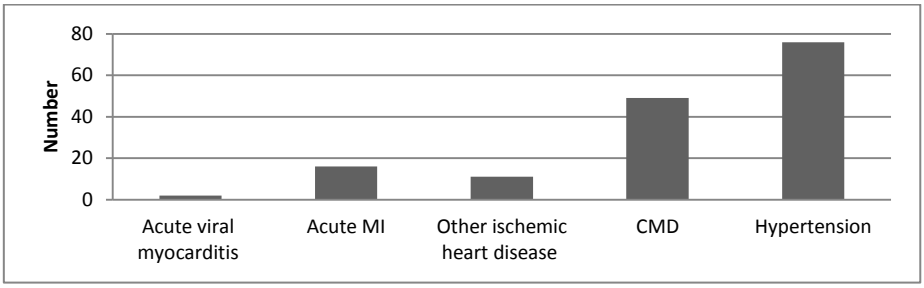

Fig. 2 Basic cardiac pathology of the study group

Patients were assessed clinically (heart auscultation, assessing signs of cardiac decompensation) and laboratory (12-lead electrocardiogram, echocardiography 2D, M mode and Doppler, chest radiography and laboratory data common in patients with heart disease: renal function and electrolytes, liver function, cardiac enzymes). Since patients were going to receive amiodarone in the treatment regimen, thyroid function was investigated for all by dosing routine hormone (TSH, FT4, FT3) prior to treatment.

RESULTS AND DISCUSSION

The administration of amiodarone was mostly in the load form - 800-1200 mg/day for 7-10 days followed by maintenance dose 200 mg (1 tablet/day 5-7 days/week (1 -1.4 g/week). In some patients the initial administration was intravenous, within 24 hours, because a rapid action was required.

Duration of treatment until the time of discovery of thyroid damage was between 6 months and 8 years. In all patients the thyroid function was investigated prior to the administration of amiodarone (TSH, FT4 and FT3 levels that were within normal limits). Evaluation of thyroid function after starting treatment occurred in 187 (86.97%) patients. Diagnosis of amiodarone - induced thyroid dysfunction was based on laboratory data – i.e. dosing TSH, FT4 and FT3.

Amiodarone - induced hypothyroidism has been diagnosed clinically in 39 (20.85%) patients and confirmed by elevated levels of TSH and low levels of FT4. Thyrotoxicosis occurred during Amiodarone treatment in 13 (6.95%) patients (fig 3). In all cases of thyrotoxicosis TSH level was low with FT4 normal or increased. 2 patients presented undetectable TSH values with FT4 and FT3 greatly increased (in one case with FT4 100 IU/ml). In 7 patients with thyrotoxicosis ultrasound showed a hypervascularization of thyroid (thyrotoxicosis type 1) and in 6 cases the vascularization was decreased or absent (thyrotoxicosis type 2). Thyroid ultrasound was abnormal only in 16 patients with amiodarone-induced hypothyroidism: enlargement of the thyroid, hypoechogenic areas. In the remaining patients, the thyroid ultrasound investigation was not performed.

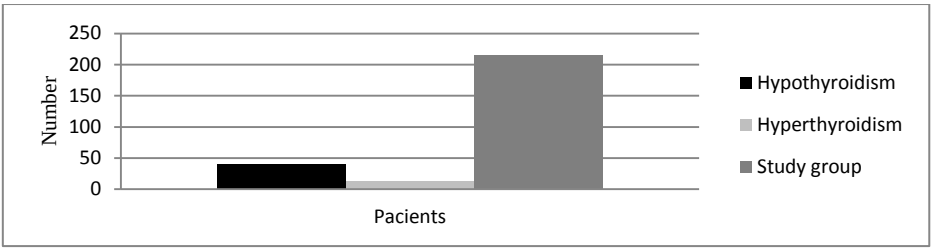

Fig. 3. Patients with amiodarone thyroid dysfunction

All patients with amiodarone-induced thyroid dysfunction were monitored initially in hospital for a period depending on the particularities of each case (between one week and one month). Thyroid dysfunction treatment was initiated, if necessary, in the hospital.

The first evaluation was performed after one month, and at 3, 6 and 12 months afterwards and consisted of clinical examination, electrocardiogram, echocardiogram, TSH and FT4, FT3 determination. In patients receiving Methimazole, monthly blood counts were indicated. Also, in patients receiving prednisone, the blood glucose and lipid profile were monitored. The patients were evaluated than annually.

For patients who confirmed the diagnosis of hypothyroidism we still maintained amiodarone in the treatment plan and we associated Levothyroxine in gradually increasing doses (25 μg/day 7 days, than 50 μg/day, up to one month after the patient was assessed once again). One month evaluation revealed: normalization of TSH and FT4 (all patients) and normalization of the thyroid ultrasound aspect. Evaluation at 3 and 6 months showed normal levels of thyroid hormones; we continued the initial dose of Levothyroxine. All patients maintained normal thyroid function at 12 months under a minimum dose of Levothyroxine. No patient required a dose more than 50 μg/day.

For patients diagnosed with amiodarone - induced thyrotoxicosis it was necessary to replace amiodarone with another anti arrhythmic drug and start treatment with Methimazole (doses between 10-40 mg/ day) or/plus prednisone. The evolution of these patients was unpredictable; in 25 % of cases, after a variable period of time (3 to 6 months) they developed hypothyroidism (TSH greatly increased) which imposed a temporary elimination of anti thyroid drugs. After a short period of hypothyroidism (3 months), 3 patients presented thyrotoxicosis relapse, which led to the resuming of treatment with anti thyroid drugs. Only one patient in this group needed thyroidectomy.

Amiodarone-induced thyrotoxicosis was more common in male patients, which presented an evolution and a very unfavorable prognosis: 2 patients had atrial fibrillation and atrial flutter with rapid ventricular rate (over 150/min) resistant to anti arrhythmic treatment, and another two patients died due to worsening heart failure phenomena evolving to cardiogenic shock and refractory acute renal failure.

Patients diagnosed with amiodarone-induced hypothyroidism had a somewhat favorable evolution after hormone replacement. Levothyroxine therapy improved the clinical picture of hypothyroidism in all patients without requiring discontinuation of amiodarone in any of the cases.

The side effects related to the medication of thyroid dysfunction in the study group were the following:

- 3 patients (one male and two females) had moderate leucopenia (between 2,800-3,400/mm^3) that could be related to the treatment with Methimazole - no therapeutic intervention was required;

- In the group of patients treated with Levothyroxine no major adverse effects were observed, only in 5 cases heart rate increased, unjustified by any other situation;

- In two patients (one male and one female) who received the combination of Methimazole and prednisone high blood glucose levels were observed between 2 weeks and 2 months after the initiation of corticotherapy, but not exceeding 150 mg/dl; one of the patients presented as well high values of blood pressure.

CONCLUSIONS

Amiodarone - induced thyroid dysfunction requires immediate therapy. Our study showed that hypothyroidism amiodarone-induced generally had a better prognosis after hormone replacement therapy and did not require removal of anti arrhythmic regimen, thyroid dysfunction in this case being solved only by the introduction of thyroid hormone replacement therapy.

However, Amiodarone-induced thyrotoxicosis proved to be a severe clinical presentation, worsening heart failure by maintaining a high heart rate, generally resistant to anti arrhythmic drugs; it usually required the removal of amiodarone treatment and anti thyroid drugs; in all cases the evolution was slow and unpredictable, sometimes being fatal. Two patients had atrial fibrillation and atrial flutter with rapid ventricular rate (over 150/min) resistant to anti arrhythmic treatment, and another two patients died due to worsening heart failure. We consider thyroid dysfunction (either hypothyroidism or thyrotoxicosis) a negative element in the evolution of patients with pre-existing heart disease, not only by clinical worsening of the underlying disease, but also by the need to permanently review the cardiovascular treatment and the possible association with appropriate medication to the thyroid dysfunction. It is also necessary to consider the side effects of thyroid dysfunction medication, including corticosteroid treatment.

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NEWS

THE UTILITY OF LIPOPOLYSACCHARIDE-BINDING PROTEIN IN THE DIFFERENTIATION BETWEEN PERIPROSTHETIC JOINT INFECTION AND ASEPTIC LOOSENING?

The pre-operative differentiation between periprosthetic joint infection (PJI) and aseptic loosening after total hip (THA) or knee (TKA) arthroplasty is essential for successful therapy and relies in part on the use of molecular markers. A recent prospective, controlled, clinical trial study conducted by Friedrich MJ and collaborators assessed serum levels of lipopolysaccharide-binding protein (LBP) from one hundred and twenty patients presenting with a painful TKA or TKA with indication for surgical revision. Pre-operative blood and serum samples were collected and analysed also for white blood cell (WBC) count and C-reactive protein (CRP). The definite diagnosis of periprosthetic joint infection was determined on the basis of clinical, microbiological and histopathological examination. LBP showed significantly higher values in PJI compared with aseptic loosening ($p < 0.001$) and control ($p < 0.001$), with a specificity of 66 % and a sensitivity of 71 % at a cutoff value of >7 ng/ml. In combination with CRP, the positive predictive value for PJI was at 0.67; negative predictive value with both negative was at 0.77. The authors concluded that the use of LBP in serum appears not to be a more accurate marker than CRP level in serum for detecting PJ, and on the basis of these results, they cannot recommend the sole use of LBP for differentiating PJI and aseptic loosening following THA and TKA. (Friedrich MJ, Randau TM, Wimmer MD et al. Lipopolysaccharide-binding protein: A valuable biomarker in the differentiation between periprosthetic joint infection and aseptic loosening? *Int Orthop*. 2014 May 15. [Epub ahead of print]. PMID: 24827968).

Simona Tucaliuc

PROGRESSIVE DYSPNEA – COMPREHENSIVE EVALUATION OF A COMMON SYMPTOM

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PROGRESSIVE DYSPNEA- COMPREHENSIVE EVALUATION OF A COMMON SYMPTOM (Abstract): Dyspnea is one of the most common symptoms in the clinical setting, the main causes being represented by respiratory and cardiovascular disorders but sometimes it is difficult to identify and document the underlying mechanism of breathlessness. We present the case of a 75-years-old male patient, former smoker, with progressive worsening dyspnea. He performed a complex imagistic, respiratory, and functional assessment, the results showing severe emphysema with mild obstructive syndrome, in contrast with severe impairment of diffusing capacity of the lung for carbon monoxide (DLCO) due to inhomogeneous distribution of ventilation/perfusion ratio. This case brings to front a certain type of chronic obstructive pulmonary disease (COPD), with suggestive clinical exam but with minimal impairment of spirometry results in the presence of debilitating dyspnea. In order to perform an accurate diagnosis, alternative functional diagnostic measurements, like estimation of DLCO, should be used.
Keywords: DYSPNEA, EMPHYSEMA, COPD, DLCO.

Dyspnea or shortness of breath is one of most common symptom in clinical setting, being defined as"a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity" (1). The main causes are represented by respiratory and cardiovascular disorders but sometimes it is difficult to identify and document the underlying mechanism of breathlessness.

CASE REPORT

We report a case of a 75-years-old man, repeatedly admitted in Pulmonary Rehabilitation Clinic of the Iaşi Rehabilitation Clinic Hospital, in July and September 2013, for inclusion in the pulmonary rehabilitation program. The patient has experienced progressive worsening dyspnea in the last two years. He was a heavy smoker (50 pack-years) but he quitted two years ago. His personal medical history included arterial hypertension grade 2, associated with high cardiovascular risk because of his age, male sex, dyslipidemia and impaired fasting glycemia, and a basal cell carcinoma of the left nasogenian region, surgically removed.

Clinical exam revealed a slight barrel chest, hyperresonant chest wall percussion

and diminished vesicular breath sounds, as well as blood pressure values adequately controlled by amlodipine 10 mg daily.

Peripheral arterial oxygen saturation (S_pO_2) was 88-90% on room air, decreased at 82% during the 6-minute walk test, while lab values showed polycythemia (Hb 18.21 g/dL, Ht 54.34%), most probably as a result of chronic hypoxemia. Radiologic imaging of the lungs (chest X-ray, computer tomography) revealed severe bilateral emphysema (fig. 1).

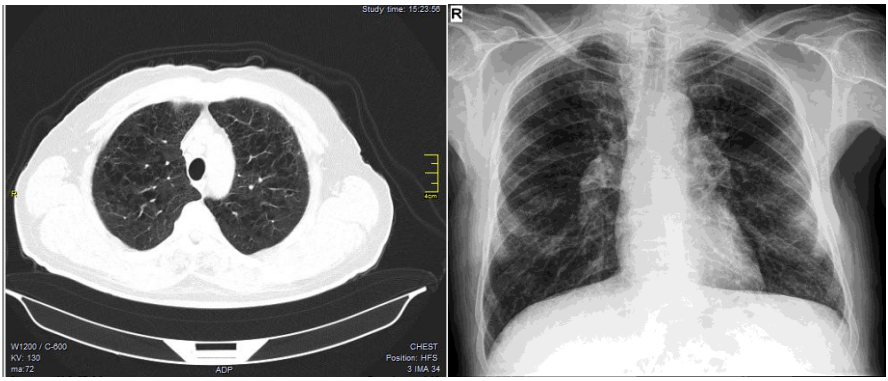


Fig. 1. Radiologic aspect of lungs parenchyma, showing severe bilateral emphysema (thoracic CT–left, chest X-ray-right)

The patient performed a complex respiratory and functional assessment in our clinic, according to the current guidelines (2), and also in other medical services, that included subjective assessment of dyspnea on Borg and modified Medical Research Council Dyspnea (mMRC) scale, the COPD Assessment Test (CAT), 6-minute walk test, spirometry, diffusing capacity of the lung for carbon monoxide (DLCO), body plethysmography, echocardiography.

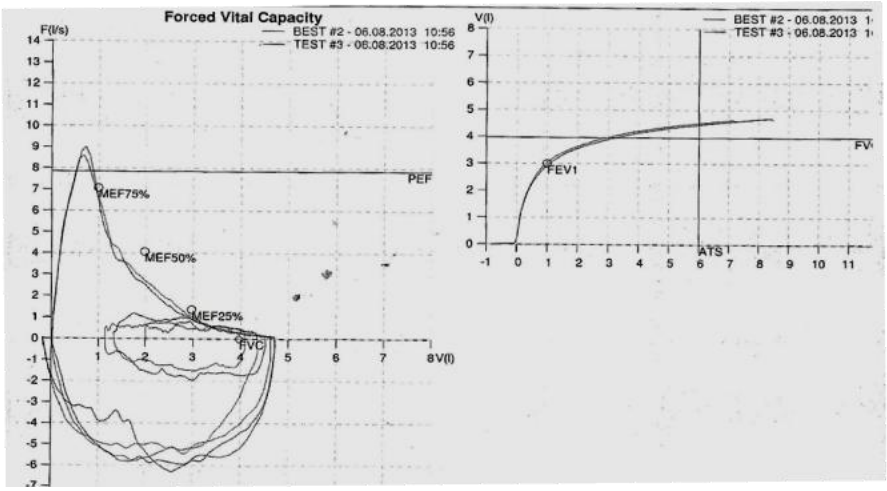
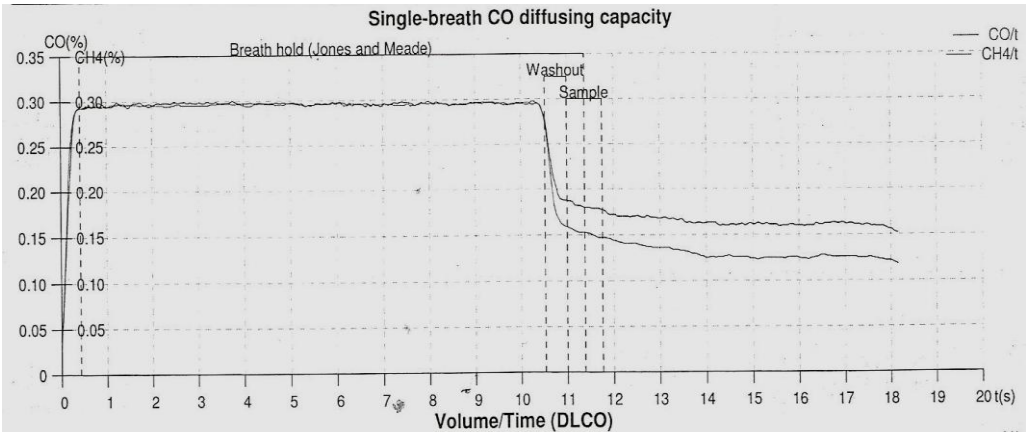


Fig. 2. Spirometry (displaying mild obstruction)

The main findings were a severe perception of dyspnea (Borg scale 7-8, mMRC scale 4) and a very high impact level of disease on daily living activities (CAT 30), a mild obstructive syndrome (FEV_1/FVC 68%, FEV_1 101%), decreased mid-expiratory flows (MEF_{50} 52% from predicted, MEF_{25} 39% from predicted) (fig. 2) and a small increase of residual volume (RV 3.34 L, 121% from predicted), with normal values for central airways resistance (Raw 1.95 cmH2o/l/s) and total lung capacity (TLC 7.87 L, 110% from predicted). There were no signs of pulmonary hypertension or right heart involvement detected by cardiovascular ultrasound.

The most prominent findings came from DLCO, showing a severe decrease of DLCO adjusted for hemoglobin (DLCO 6.37 ml/min/mmHg, 24% from predicted) and DLCO/VA ratio (DLCO/VA 27% from predicted) (fig. 3).



Parameter	UM	Description	Pred.	LLN	TEST#5	%Pred.
ERV	l(btps)	Expiratory Reserve Volume	1.00	0.80	0.06	6
Vt	l(btps)	Tidal Volume			1.35	
FiO2	%	Inspiratory O2 concentration			21.00	
DLCO	ml/min/mmHg	CO Diffusing Capacity	26.29	21.03	6.85	26
DLCO(corr)	ml/min/mmHg	DLCO corrected for Hb, CHb and PB	26.29	21.03	6.37	24
DLCO/VA	ml/min/mmHg/L	DLCO per unit of alveolar volume	3.68	2.94	0.99	27
FiCO	%	Inspired CO concentration			0.297	
FiCH4	%	Inspired CH4 concentration			0.299	
FaCO	%	Alveolar CO concentration			0.153	
FaCH4	%	Alveolar CH4 concentration			0.183	
Breathold	sec	Breathold time			10.96	
VA	l(btps)	Alveolar Volume			6.45	
Hb	g/dL	Hemoglobin concentration			17.50	
Sample Vol	ml	Sample collection volume			1032	
Washout Vol	ml	Washout volume			2174	
IVC(DLCO)	l	Inspiratory Vital Capacity during DLCO	4.11	3.29	4.20	102
FetCO	%	End Tidal CO concentration			0.005	
FetCH4	%	End Tidal CH4 concentration			0.001	
DLCO3eq	ml/min/mmHg	CO Diffusing Capacity (3 equations)	26.29	21.03	3.39	13
DLCOmean	ml/min/mmHg	CO Diffusing Capacity (Mean)	26.29	21.03	6.61	25
DLCO/VAmean	ml/min/mmHg/L	DLCO per unit of alv. vol. (Mean)	3.68	2.94	0.95	26
VAmean	l(btps)	Alveolar Volume (Mean)			6.49	

Fig. 3. Schematic of gas concentrations and lung volume during the single-breath CO diffusing capacity

DISCUSSION

Spirometry is the basic evaluation method of pulmonary function when a history of smoking and dyspnea are present in a male patient. Sometimes, there is an imbalance between spirometry results and clinical picture, like in our case. Despite the severe dyspnea, this former smoker had some clinical signs of pulmonary emphysema at initial evaluation, radiologically confirmed, but only borderline obstructive pattern of spirometry, more accentuated in distal airways, and a small increase of the residual volume on body plethysmography. Also, episodes of exacerbations in this clinical case were scarce. In order to refine the diagnosis it was performed single-breath CO diffusing capacity test that revealed severe impairment of DLCO due to inhomogeneous distribution of ventilation/perfusion ratio. This finding raised the question whether some other concurrent clinical conditions, such as pulmonary hypertension through obliteration of the pulmonary arterioles or the involvement of the pulmonary capillary bed in the setting of a systemic vascular disease were existing (2), but none of these was confirmed.

These data revealed a chronic obstructive pulmonary disease (COPD) and accordingly to revised GOLD guideline the patient was in the GOLD 1 stage, class B of risk. It is well-known that patients with COPD have a large heterogeneity in their clinical presentation, and recently it was proved that it comes from genetic basis. Data from COPD Gene Study showed that there are four subgroups of smokers, associated with clinical symptoms, including exacerbations and dyspnea, and with known COPD-associated genetic variants. These groups are: the relatively resistant smokers, without or with mild emphysema despite heavy smoking, smokers with mild upper zone emphysema-predominant, those with airway disease predominant and the patients with severe emphysema, the latter group being strongly genetically associated with rs8034191 in the chromosome 15q region (3).

Furthermore, emphysema is a term derived from pathology, the modified lungs structure being a result of protease-antiprotease imbalance, followed by the protease-mediated destruction of elastin and the destruction of the gas-exchanging structures. The diagnosis is often used in practice but rarely well-documented. In COPD, the relative contribution of obstructive bronchiolitis and emphysema varies from patient to patient. Pulmonary emphysema is more associated with gas transfer abnormalities than with decreased FEV_1, as in our case, so that the measurement of DLCO provides valuable information on the functional impact of chronic pulmonary disease, being more useful than spirometry in patients with breathlessness and a mild degree of airflow limitation (4).

CONCLUSIONS

This case brings to front a certain type of COPD, with suggestive clinical exam but with minimal impairment of the spirometry values in the presence of debilitating dyspnea. In order to perform an accurate diagnosis, alternative functional diagnostic measurements, like estimation of DLCO, should be used. Additionally, future development of the genetic testing will allow early identifying of the particular subtype of smokers that will develop a pattern of very severe obstructive emphysema, in order to close monitoring and initiating motivational preventive programs to them to determine them to quit smoking, through counseling and medication.

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NEWS

JUVENILE IDIOPATHIC ARTHRITIS AND ANTHROPOMETRIC MEASUREMENTS, BODY COMPOSITION, AND NUTRITIONAL PARAMETERS

A group of researchers from Department of Paediatrics, Turku University Hospital, Turku-Finland conducted a study that assessed the effect of juvenile idiopathic arthritis (JIA), its subtypes and disease activity on anthropometric measurements, body composition, and nutritional parameters. A cross-sectional cohort of 40 JIA patients, aged 3-10 years, was compared with 40 healthy children matched for age and gender. Concentrations of nutritional and inflammatory biomarkers in the blood, anthropometric measures, and clinical status were recorded and the parents filled in a 7-day food diary and completed the Childhood Health Assessment Questionnaire (CHAQ). The JIA patients had low disease activity: 60% had inactive disease, the median CHAQ score was 0.125, and the median erythrocyte sedimentation rate (ESR) was 6 mm/h. Significantly higher values for central and peripheral adiposity were found in JIA patients compared with in healthy controls, and obesity/overweight was more common despite no differences in weight-for-height. The intake of energy (kcal/day) was significantly higher in the JIA patients ($p = 0.036$). The nutritional biomarkers were comparable in both groups. The JIA subtype and disease activity did not affect body composition, energy intake, or the nutritional biomarkers. They concluded that even JIA patients with low disease activity had a higher central and peripheral adiposity and a higher energy intake than their healthy peers and neither disease subtype nor disease activity had any association with changes in body composition.(Grönlund MM, Kaartoaho M, Putto-Laurila A, Laitinen K. Juvenile idiopathic arthritis patients with low inflammatory activity have increased adiposity. *Scand J Rheumatol*. 2014 Sep 2:1-5. [Epub ahead of print]. PMID: 25178152)

Simona Tucaliuc

PAIN MODULATION BY CURCUMIN AND ASCORBIC ACID IN MICE

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PAIN MODULATION BY CURCUMIN AND ASCORBIC ACID IN MICE (Abstract) **Aim:** The present study aims to evaluate whether ascorbic acid (AA) and curcumin, two substances with redox properties, have similar effects on different models of pain in mice. **Materials and methods:** This study included a total of 28 mice that were divided into four groups. One group (AA) received intraperitoneally 500 mg/kg b.w. AA for 21 days and the 2-nd group (curcumin) received 120 mg/kg b.w. curcumin by gastric gavage for two weeks. Other two groups serve as control and received vehicle in a dose – time manner similar to that of the treated groups. The pain models (oro-facial formalin induced pain, paw formalin induced pain and visceral pain) were performed 24 h after the last dose. **Results**: when compared with control groups, curcumin significantly decreases pain perception in oro-facial (p=0.01 1-st phase, p=0.002 2-nd phase) and paw formalin induced pain (p=0.04 1-st and 2-nd phase) while AA stimulates pain perception in acid acetic induced visceral pain (p=0.05) and increases oro-facial inflammatory pain induced by formalin (p=0.02) but demonstrates analgesic effects on paw formalin induced pain (p=0.003 1-st phase, p=0.01 2-nd phase). **Conclusions:** ROS production is important in pain modulation. Structures involved in the process of pain have different antioxidant defense capacities. Curcumin and AA are able to modulate pain perception, but beside their antioxidant capacities, there are other mechanisms involved.
Keywords: CURCUMIN, ASCORBIC ACID, PAIN, ANALGESIA.

According to recent studies, reactive oxygen species (ROS) are reported to be associated with chronic pain, especially neuropathic and inflammatory (1). They are also closely related to central sensitization and the elevated ROS in the spinal cord and colon have proven to be involved in visceral pain (2).

Recent studies suggest that oxidative stress and the balance between oxidants and antioxidants can be directly involved in certain diseases characterized by different types of pain or inflammation (3).

Curcumin, a hydrophobic polyphenol, protects mitochondrial dysfunction and modulates endogenous antioxidant enzymes, scavenges ROS and NO-based radicals, neutralizes ROS and RNS-based radicals and increases the synthesis of glutathione. On the other hand, several studies demonstrate that curcumin has pro-oxidant capacities through its ability to increase oxidized glutathione (GSSG) and to also reduce glutathione (GSH), thus decreasing

the GSH/GSSG ratio (4).

It was demonstrated that ROS are involved in the apoptosis induced by curcumin in A549 lung adenocarcinoma cells, but also that curcumin prevents the mitochondrial dysfunction and suppresses the ROS increase (5).

Curcumin seems to have analgesic proprieties, but the mechanism by which it induces antinociception is not clear. Activation of K (ATP) channels (6) or transient receptor potential vanilloid 1 (TRPV1) activation have been proposed (7). Ascorbic acid (AA) is an important antioxidant known to quench ROS, and its oxidase form dehydroascorbic acid (DHA), enters mitochondria via facilitative glucose transporter 1 (Glut1) and accumulates in the mitochondria as ascorbic acid (mtA). The accumulation of AA in the mitochondria inhibits the oxidative mitochondrial DNA damage and thus AA may be useful in generating therapies by antagonizing the effects of ROS (8).

Although AA has a controversial role in pain therapy, it has been shown that a combined antioxidant therapy can be safe and efficient in pain relief for patients with chronic diseases (9).

The purpose of our study was to evaluate if, by modulating ROS production through two antioxidant substances (AA and curcumin), similar effects will be obtained on different models of pain (orofacial formalin induced pain, paw formalin induced pain or the acetic acid induced visceral pain).

MATERIAL AND METHODS

Animals. The experiments were conducted using male BALB/c mice (28-34 g), housed at 21 ± 2 °C under a 12-h light/dark cycle, with access to food and water ad libitum. All animals were habituated to the testing laboratory for at least five days. The experimental protocols and procedures described in this article complied with the European Communities Council Directive 86/609/EEC and followed the International Association for the Study of Pain's (10) and the University of Medicine and Pharmacy "Grigore T. Popa" ethical guidelines for investigations of experimental pain in conscious animals.

Drugs. The drugs used in these experiments were: vitamin C intravenous solution 750mg/5 ml (Arena, Romania), 37% formaldehyde (Fluka, Germany), acetic acid (Sigma, Germany) and Curcumin (Turmeric powder, Sigma, Germany). AA was freshly diluted in distilled water to avoid precipitation and was disposed after first use to avoid oxidation. Curcumin was freshly prepared in olive oil.

The acetic acid induced visceral pain. Writhing Test (WT test) was performed as described by Koster *et al*. (11). After acclimatization in an acrylic observation chamber for at least 20 minutes, the mice received 0.1 ml /10 mg kg b.w. i.p. injections with 1.0% (v/v) acetic acid (12). A writhe was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body.

The paw formalin test (PFT). The mice were injected with 20 μL of 5% formalin (from 37% formaldehyde) subcutaneously into the plantar surface of the right hind paw using a microsyringe. Pain and inflammatory response was measured by the total time (in seconds) spent by mice licking and/or biting the injected paw during periods of 0–9 min (first / neurogenic phase) and 10–40 min (second / inflammatory phase) (13).

The orofacial formalin test (OFT). Nociception induced by formalin was performed as described by Luccarini *et al.* (14). Animals received 20 µL of 5% formalin into the right whisker pad. The nociceptive score was determined by recording the number of seconds the animals spent grooming the injected area with the ipsilateral hind paw or forepaw, often accompanied by the contralateral forepaw, for the first 6 min (first / neurogenic phase) and from 7 to 40 min (second / inflammatory phase).(15)

Study design. The study used a total of 28 mice that were divided into four groups: group AA mice receiving intraperitoneal 500 mg/kg b.w. AA for 21 days. Group Curcumin receiving two weeks curcumin in combination with oil by intragastric gavage 120 mg/kg b.w. The other two groups that served as control received an equal volume of distilled water (AA Control), olive oil (Curcumin control) respectively in the same time manner. All tests were performed 24 h after the last dose.

Statistical analysis. The data obtained were expressed as the mean value ± SEM. Statistical evaluations were performed using *SPSS 20.0* software One-way analysis of variance (ANOVA) and Student's t-test for independent variable were used to assess within and between groups variability. The significance level of the results was set at $p<0.05$.

RESULTS

The effects on paw formalin induced pain. Curcumin has shown an analgesic effect on both phases on the formalin induced pain (phase 1 p=0.04 respectively phase 2 p=0.04) when compared with control group (fig. 1). AA has also demonstrated an analgesic effect on both phases (p=0.01) as compared with control group (fig. 2). No significant differences were observed between curcumin and AA groups.

The effects on orofacial formalin induced pain. The analgesic effect of curcumin was statistically significant in both phases (phase 1 p=0.01, phase 2 p=0.01 respectively) when compared with control group (fig. 1).

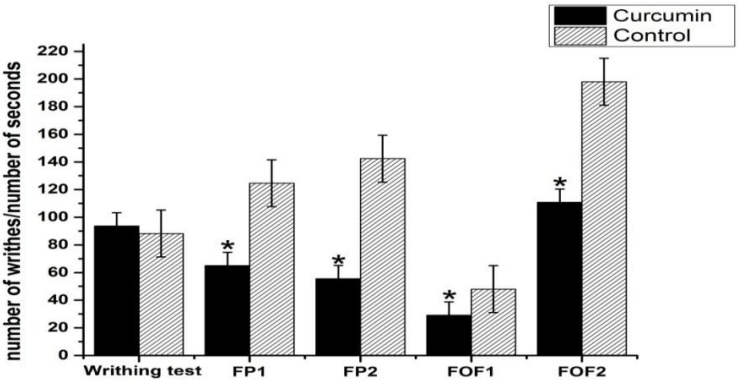

Fig.1. Effects of 14 days treatment with curcumin on the writhing test and phases 1&2 of the orofacial (FOF) and paw (FP) formalin tests. The results are expressed as mean± S.E.M. * $p < 0.05$ as compared with control group

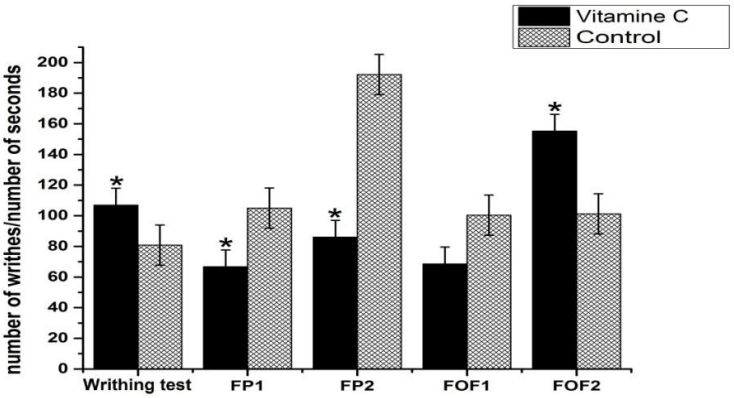

Fig. 2. Effects of 21 days treatment with AA on the writhing test and phases 1&2 of the orofacial (FOF) and paw (FP) formalin tests. The results are expressed as mean± S.E.M.* $p < 0.05$ as compared with control group

By contrary, AA has no effect on the first phase, increases pain perception on the second phase (p=0.01), as compared to the distilled water group. Significant differences were observed between curcumin and AA groups in the first phase. (p =0.007).

The effects on visceral pain. Although curcumin has shown potential on orofacial and paw formalin induced pain, the effect on visceral pain had no statistical significance (fig. 1). However, AA stimulates pain perception compared to the control group (p=0.05) (fig. 2).

DISCUSSION

In our study, we demonstrated that long term administration of curcumin and AA modulate pain perception in formalin and acetic acid induced pain. Thus, curcumin significantly decreases pain perception in oro-facial and paw formalin induced pain, while AA has an analgesic effect on paw formalin induced pain. On the contrary, AA increases the oro-facial inflammatory pain induced by formalin and visceral pain.

Under physiological conditions, AA mainly exists in its reduced form and in trace quantities in the oxidized form, dehydroascorbic acid (DHA). Beside its antioxidant capacities, AA seems to down-modulate NF-KB signaling (15), as well as norepinephrine production (16).

Chen and collaborators have shown that AA might generate H_2O_2-dependent cytotoxicity toward a variety of cancer cells in vitro and in vivo, without adversely affecting normal cells (17), while Martinovich *et al* suggested that Ca (2+) release in HEp-2 cancer cell line is the mechanism through which AA increases mitochondrial ROS production (18). Thus there are enough evidences that AA has both pro and anti-oxidant capacities.

At the dose used in our study, AA seems to exert proprieties, having an anti-oxidant effect paw formalin induced pain and acting as a pro-oxidant on the inflammatory phase in orofacial formalin induced pain and the acetic acid visceral induced pain. Besides redox proprieties, we cannot exclude, that changes in epinephrine levels secondary to AA administration might be

involved in pain modulation. Indeed, it is well-documented that norepinephrine induces both antinociception by activation of the NO/cGMP/KATP pathway (19) and nociception by enhancing pain facilitation from the brain (20).

Our results suggest that AA has different effects on pain at the same dose. These effects depend probably on cells resistance to ROS production and on pain structures sensitivity to epinephrine stimulation.

Similar to vitamin C, curcumin, a mitochondrial modulator, has both pro- and antioxidant activities. The underlay mechanisms of curcumin effects are not clarified, but regulation of various molecular targets, such as nuclear factor-κB, inflammatory cytokines and other enzymes are implied (21).

In our study, curcumin had a significant antinociceptive effect on both phases of formalin induced pain, with no effect on visceral pain. Indeed, peripheral and central pathways for visceral pain are unique and some receptors such as acid sensing ion channels, sodium channels, calcium channels, kappa opioid receptor, appear to be particularly important to modulating pain. In addition to the acetic acid induced pain, nociceptive messages from the inflammated peritoneum involve neurokinin A rather than substance P as a mediator. By contrast, inflammatory phase in formalin induced pain is mainly mediated by substance P(22). Thus, the curcumin antinociceptive effects are not only a consequence of its antioxidant activity; other mechanism seems to be also involved. Considering everything, we have demonstrated that: ROS production might have a place in pain modulation; neuroanatomical structures involved in pain modulation have different antioxidant defense capacities and curcumin and AA are able to modulate pain perception, but there are other mechanisms involved, beside their antioxidant capacities.

CONCLUSIONS

By using two ROS modulators (ascorbic acid and curcumin), we demonstrated that oxidative stress might have an important place in pain pathophysiology. Further studies are however, however necessary to further explain the involvement of curcumin and ascorbic acid in pain modulation.

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ORTHOSTATIC INTOLERANCE – AN EXPRESSION OF AUTONOMIC DISFUNCTION IN PARKINSON'S DISEASE

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ORTHOSTATIC INTOLERANCE – AN EXPRESSION OF AUTONOMIC DYSFUNCTION IN PARKINSON'S DISEASE (Abstract): Orthostatic intolerance syndrome is an important issue, having multiple etiologies and being the result of an inadequate response to changing in body position, namely the transition from supine to standing, a process that causes a series of responses regulated by the autonomic nervous system. **Aim:** The aim of this study was to create a profile of patients with Parkinson's disease and orthostatic hypotension, a form of orthostatic intolerance, identifying the risk factors for this condition. **Material and methods:** We conducted a study on 41 patients with Parkinson's disease selected from 200 consecutive patients with orthostatic hypotension, hospitalized in Cardiology Clinic of Colentina Clinic Hospital which met the diagnosis criteria for orthostatic hypotension. In order to meet the inclusion criteria, all patients were evaluated according to a preset protocol: medical history, clinical and paraclinical examination, including neuro-psychiatric assessment and the orthostatic tolerance test. **Results and discussion:** The decline in standing blood pressure was related to the presence of hypotensive neurological drugs, especially Levodopa, in chronic treatment, but it was not influenced by age or cardiovascular co morbidities. There is an improvement in blood pressure both in supine and upright position at discharge when compared to admission values. **Conclusion:** The degree of decrease in the standing blood pressure values was correlated with the presence of potentially hypotensive neurological drugs, particularly Levodopa treatment, with a favorable effect of administration of non-pharmacological treatment and changes made in chronic neurological treatment. **Keywords:** ORTHOSTATIC HYPOTENSION, ORTHOSTATIC TOLERANCE TEST, PARKINSON'S DISEASE.

Orthostatic intolerance syndrome is an important issue, having multiple etiologies and being located on the border of several medical specialties, as diagnosis and treatment. After essential arterial hypertension, orthostatic hypotension is the most common disorder of blood pressure regulation in adults aged over 65 years, with prevalence between 5 and 30%, depending on the studied population and the used definition.

Prospective studies have shown that orthostatic hypotension is a predictive factor for ischemic stroke and all-cause mortality in elderly people (1-3).

This condition is one of the least understood manifestations of autonomic dysfunction, being the result of an inadequate response to changing in body position, namely the transition from supine to standing, a process that causes a series of responses regulated by the autonomic nervous system. The term"autonomic dysfunction" refers to any dysfunction of the autonomic nervous system - central, peripheral or secondary to other conditions (1,2).

There are two types of autonomic dysfunction- acute and chronic form. Chronic form can be divided into primary and secondary, the last one being more common. However, when failure dominates the clinical picture and a clear cause is not obvious, it is called primary dysautonomia. Primary dysautonomia, in its turn, can be divided into four syndromes: isolated pure dysautonomia, systemic nerve atrophy, Parkinson's disease with autonomic dysfunction (3) and Lewy body dementia, while secondary autonomic dysfunction, involving autonomous damage and disease makes its debut in the context of diabetes mellitus, amyloidosis or various polyneuropathies (4-6). While primary and secondary autonomic dysfunctions are caused by structural damages of the autonomic nervous system (either central or peripheral), drug-induced orthostatic hypotension is about functional impairment.

The clinical aspects that suggest the diagnosis at initial assessment are represented by the onset of the symptoms (dizziness, syncope) after switching from supine to standing, possibly after prolonged immobilization and the existence of a temporal relationship between the onset and a change in dosage of potentially hypotensive drugs, parkinsonism, presence of autonomic neuropathy or hypovolemia.

Effective treatment of orthostatic intolerance can be a challenge. The main therapeutic target consists of improving the symptoms in the transition from supine to standing position and functional status of the patient while reducing the risk of falls and syncope, being less oriented to strictly normalize the blood pressure levels.

MATERIAL AND METHODS

We conducted a study on 200 consecutive patients hospitalized in Cardiology Clinic of Colentina Clinic Hospital which met the diagnosis criteria for orthostatic hypotension as mentioned in the syncope guide of the European Society of Cardiology: a symptomatic reduction of the systolic blood pressure from baseline values with more than or equal to 20 mmHg or/and of the diastolic blood pressure with more than or equal to 10 mmHg, or a systolic pressure-lowering below 90 mmHg (3,5). To determine the blood pressure we used the active method (active standing), the orthostatic tolerance test being performed both on admission and at discharge, by measuring blood pressure and heart rate initially supine, then standing at 1, 3 and 5 minutes after changing position (4).

In order to meet the inclusion criteria, all patients were evaluated according to a preset protocol: medical history, clinical and paraclinical examination, including neuro-psychiatric assessment. All the patients with Parkinson's disease were further selected in order to create a profile of these patients and to identify the risk factors for orthostatic hypotension. These neurological patients were investigated before and after non-pharmacological treatment, assessment and adjustment of the current drugs.

Statistical analyze was done using the Microsoft Excel 2007 and *SPSS* for Windows programs.

RESULTS

The larger selection group diagnosed with orthostatic intolerance (n= 200 patients) consists of 107 women and 93 men. The Parkinson's disease study group included 41 patients, the gender distribution showing a slightly higher percentage of male patients (18 men vs 13 women), representing 19.35% of total male patients and 12.15% of females (fig. 1). The mean age of neurological patients was 76.41 years (64 - 86 years).

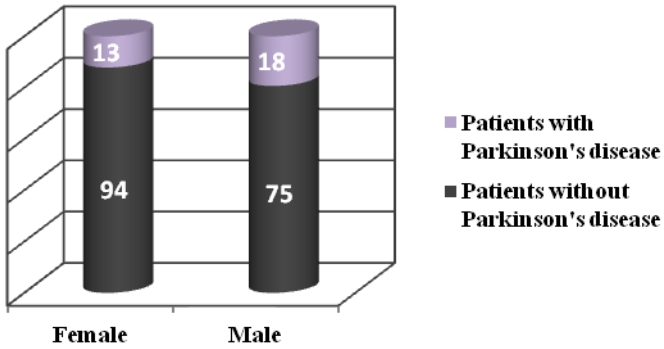

Fig. 1. The frequency of patients depending on the presence of Parkinson's disease

All the 41 Parkinson's disease patients were treated with neurological drugs, ten patients receiving also psychiatric medication, Levodopa being the most used agent in combined therapy (tab. I).

Average supine blood pressure on admission was 129/75 mmHg versus 127/72 mmHg at discharge (p=0.265) (fig. 2), while the lowest of standing blood pressure values at admission was 75/40 mmHg and at discharge was reaching 90/60 mmHg (p<0.001) (fig. 3).

TABLE I

The distribution of cases according to the neurologicaltreatment

Medication	Number of cases
Seligelinum	1
Amantadinum	1
Biperiden	1
Acidumvalproicum	1
Carbamazepinum	1
Rasagiline&Ropinirole	1
Amantadinum&Seligelinum	2
Fenobarbitalum&Carbamazepinum	1
Levodopa+benserazide	18

Medication	Number of cases
Seligelinum&Levodopa+benserazide	4
Ropinirole&Levodopa+benserazide	1
Rasagiline&Levodopa+benserazide	1
Pramipexole&Levodopa+benserazide	1
Amantadinum&Levodopa+benserazide	1
Ropinirole& Levodopa &Carbidopa	1
Donepezile&Acidumvalproicum& Memantine&Levodopa+benserazide	1
Amantadinum&Levodopa&Carbidopa	1
Levodopa&Carbidopa&Amantadinum&Pramipexole	1
Levodopa&Carbidopa&Memantine&Pramipexole	1
Levodopa&Carbidopa&Entacapone&Amantadinum&Pramipexole	1
Total	41

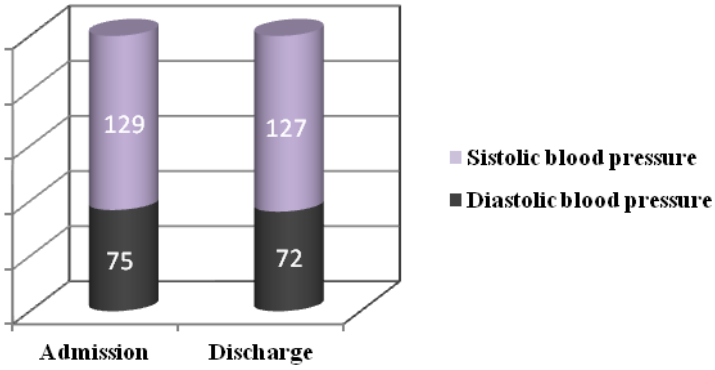

Fig. 2. Average supine blood pressure values

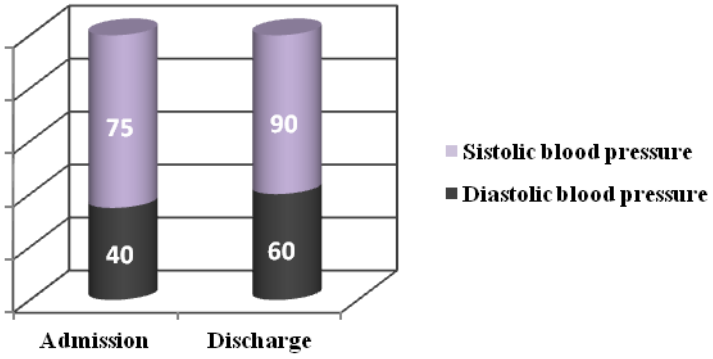

Fig. 3. Minimum of standing blood pressure values

Orthostatic tolerance test on admission revealed an average of 109/65 mmHg compared to 122/69 mmHg at discharge (p=0.007) (fig. 4).

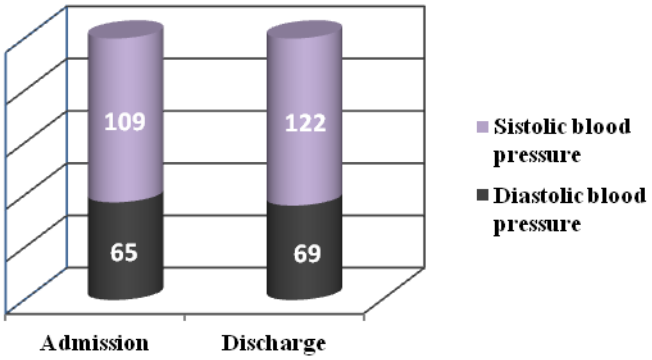

Fig. 4. Average of standing blood pressure values

There was no significant difference between the mean age of patients with orthostatic hypotension and those with negative tolerance test at the time of discharge (p= 0.929). Patients with persistent orthostatic hypotension at discharge had a prevalent cardiovascular history, compared to their counterparts without orthostatic hypotension, but the difference did not reach the statistical significance (p= 0.789).

All the patients received non-pharmacological treatment and 10% were treated with vasopressor agent Midodrine. Neurological medication is influencing the maintenance of orthostatic hypotension at discharge ($p < 0.05$), Levodopa having the most significant hypotensive effect ($p < 0.001$).

DISCUSSION

We used the analysis of postural changes in blood pressure values in order to find responsible modifiable factors that influence this type of pathology and to determine the frequencies for the studied group.

It is known that in Parkinson's disease, the prevalence of orthostatic hypotension is between 43 % and 58 % and may reach up to 60 % in some studies (7). Two factors could explain this high prevalence: first, dopaminergic drugs that may induce or aggravate orthostatic hypotension, and, second, Parkinson's disease dysautonomia (8, 9).

Our Parkinson's disease study group had a relatively homogenous gender distribution and a high mean value of age (76.41 years) which probably contribute to orthostatic hypotension. Also, the degree of decrease in the standing blood pressure values was correlated with the presence of potentially hypotensive neurological drugs, Levodopa being the most powerful contributor to this phenomenon, these findings being supported by several previous studies (7).

Age and cardiovascular co morbidities were not influential over the presence of orthostatic intolerance, as we would have expected, but we had not perform a detailed analyze of these cardiovascular diseases and their hemodynamic effects.

Despite these facts, the results showed a significant improvement of blood pressure values in both supine and upright position

at discharge, compared to values determined on admission, for these neurological patients. This favorable outcome is even more relevant as it could not be explained by the use of vasopressors, as long as only 10% of patients were receiving Midodrine.

The reasonable explanation for the improvements in blood pressure values both in supine and upright position at discharge, when compared to the values measured on admission, remains the adjustment of concomitant hypotensive medication and the association of non-pharmacological treatment, which were performed in all patients.

CONCLUSIONS

The results indicate that the degree of decrease in the standing blood pressure values was correlated with the presence of potentially hypotensive neurological drugs, particularly Levodopa-associating treatment. Our research also revealed that the administration of non-pharmacological treatment and changes made in chronic neurological treatment explain the favorable response to orthostatic tolerance test at discharge when compared with admission.

We suggest that he management of orthostatic hypotension in patients with Parkinson's disease should always start with patient education, identification and, if possible, a change of the potential hypotensive neurologic therapy.

ACKNOWLEDGEMENTS

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UNMASKING A YOUNG ADULT MIGRATORY DEEP VENOUS THROMBOSIS – CASE REPORT

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UNMASKING A YOUNG ADULT MIGRATORY DEEP VENOUS THROMBOSIS – CASE REPORT (Abstract): Venous thromboembolism is a known complication of cancer which impacts on patient mortality and quality of life. The primary site of cancer is an important risk factor, with highest rates observed in patients with brain, pancreas, gastric, kidney, ovary and lung cancers. The extent of metastatic spread further adds to the risk. In this article, we present the case of a young patient who was diagnosed with an aggressive form of pancreatic neoplasm with secondary determinations, without any previous digestive symptoms, with the occasion of a recurrent and migratory deep venous thrombosis (DVT). **Keywords**: CANCER, PANCREAS, DEEP VENOUS THROMBOSIS, RECURRENT.

Deep venous thrombosis (DVT) and pulmonary embolism (PE) are major causes of morbidity and mortality (1). Several patient's characteristics are associated with a higher incidence of venous thromboembolism (VTE) and include, among others (2), age greater than 65 years, female gender, obesity, immobility, major surgery and infection (3,4). The risk of VTE may also substantially be increased by heritable prothrombotic states such as factor V Leiden or prothrombin gene mutation or acquired conditions (e.g. antiphospholipid antibodies), in addition to a prior VTE. Cancer patients are known to be at increased risk of VTE. The primary site of cancer is an important risk factor for VTE, with highest rates observed in patients with brain, pancreas, gastric, kidney, ovary and lung cancers. The extent of metastatic spread further adds to the risk (5, 6).

We present the case of a young patient with recurrent and migratory DVT, who was diagnosed with an aggressive form of pancreatic neoplasm with secondary determinations, without any previous digestive symptoms.

CASE PRESENTATION

A 42 year old man, from an urban area, was admitted to our department, in October 2012 for swelling of legs, local pain, heat

and redness, irritability, fatigue. The patient was a smoker for twenty years ago, and denied alcohol consumption. In August 2012, he was diagnosed with DVT of the left leg, and with two weeks before current admission in the hospital he was diagnosed with DVT on the right femuro–popliteal axis and superficial phlebitis of the right leg, being under treatment with Acenocumarol 4 mg per day, Diosminum 500 mg 2 tablets per day and Sulodexidum 250 ULS 2 tablets per day. The patient declared loss in body weight, approximately 4 kg in the last two months.

Physical examination showed pale skins and mucosa, normal state of nutrition (BMI 23.6 kg/m^2), arterial blood pressure 125/80 mmHg, heart rate 69/min, respiratory frequency 16/min. Clinical examination did not reveal any tenderness of the abdomen, liver and spleen were not palpable. Local clinical exam revealed edema, redness and heat of both legs.

Laboratory tests at admission showed leucocytosis (12,700/mm^3) and neutrophilia (66.5% - 9,500/mm^3), mild normochromic, normocytic anemia (Hb 12.9g/dl), dyslipoproteinemia, very increased C reactive protein (12.71 mg/dl), hyperglycemia (187 mg/dl), therapeutic hypocoagulability (INR 2.39), absence of fibrin degradation products (FDP) and increased D-dymers (21.7 μg/ml). Liver function tests revealed mild hepatocytolysis (ASAT 47 U/l, ALAT 74 U/l), cholestasis (total bilirubin 1.43 mg/dl, conjugated bilirubin 0.73 mg/dl, alkaline phosphatase 617 IU/l, GGT 751 IU/l), and normal total serum proteins. The platelet count, blood urea, creatinine, sodium and potassium, alkaline reserve, uric acid level, urinary examinations were within normal range.

The abdominal ultrasound examination revealed increased volume of liver, nodular structure, unhomogeneously overall, and presence of a small quantity of right pleural fluid, which was also confirmed radiological. The cardiac ultrasound exam evidenced normal systolic and diastolic function of left ventricle, atherosclerotic ascending aorta.

Doppler venous ultrasound revealed, on the right leg, an 8.5 mm thickness thrombus in the deep femoral vein at third inferior thigh and subcutaneous edema, absence of Doppler signal in the popliteal and posterior tibial veins, peroneal and saphenous vein until middle third of the thigh. On the left leg, in the third inferior thigh, there was detected a thrombus of 8.4 mm thickness, with partial recanalization, incompressibility and absence of Doppler signal on the posterior tibial vein, saphenous vein and veins from the posterior side of the calf.

Considering the recurrent and migratory character of DVT in a young patient, we examined the thrombophilic state, by testing possible deficiences of antithrombin III, protein C and S, factor V Leiden and prothrombin gene mutations, lupic anticoagulant, antiphospholipid antibodies and MTHFR genes (mutations C677T and A1298C). All of these tests were within the normal range. Also for thrombotic state under chronic oral anticoagulation (with therapeutic INR), taking in consideration the young age, absence of risk factors (obesity, hip or lower limb fractures, immobility, major surgery or trauma, infection), we determined the tumor markers, which revealed increased carcinoembryonic antigen (CEA - 37.7 ng/ml) and very increased level of tumoral antigen CA 19-9 (>1000 IU/ml), with normal levels of prostate specific antigen and alpha-feto-protein.

In this stage, regarding the markedly changes in tumor markers, inflammatory tests and the ultrasound aspect of the liver, we considered compulsory an abdominal computer tomography (CT) examination, which revealed a solid and expansive tumor of pancreatic body and tail, imprecise delimited, size of 46/57/45 mm, with decreased density compared to the rest of pancreatic parenchyma in arterial and venous time (fig.1), secondary liver determinations (fig.2), thrombosis of the left portal vein, splenic artery and vein (fig.3,4), probability of splenic infarctions. There was also detected tumoral lymphadenopathy placed around the pancreatic tumor, in mesenteric and celiac area, in the splenic hilum and on the small gastric curvature.

During hospitalization, which involved primary therapy with subcutaneously anticoagulant (sodium enoxaparine), then oral anticoagulant (acenocumarol), under INR control, diosminum and sulodexidum, analgesic and anxiolytic treatment, clinical and paraclinical evolution was slowly favorable, with reduction of the legs thrombotic signs (redness, pain and swelling) and without any gastrointestinal signs or symptoms.

Fig.1. The pancreatic tumor does not have demarcation limit by left adrenal gland (body) and by first ileal loop

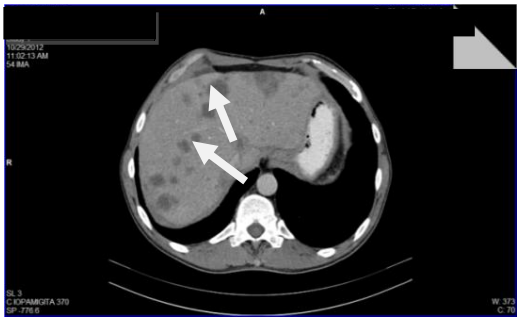

Fig. 2. Multiple hepatic solid lesions, with reduced vascularity, some with tendency to confluence, disseminated in both liver lobes

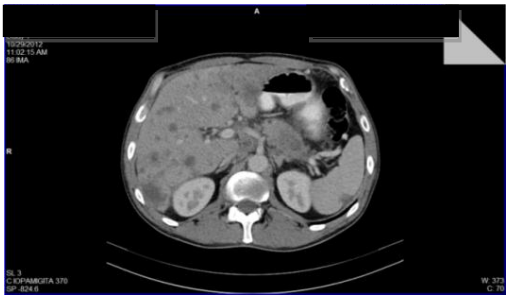

Fig.3. The splenic vene cannot be seen (completely obstruction)

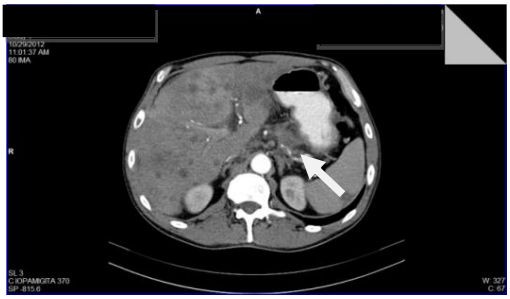

Fig.4. Spleen artery is permeable, with present blood flow on the first 6.5 mm, being obstructed at the level of the tumor

The patient was directed to the surgical department, where, because of the advanced stage of the neoplasm, was not operated, but received recommendations to

address to oncologic department. In oncology, the patient was subjected to two chemotherapy cures, with an unfavorable evolution, the death occurring at the end of November 2012.

We consider this is an interesting medical case, in which a migratory and recurrent DVT in a young patient discovered an aggressive form of pancreatic neoplasm, with hepatic secondary determinations, without any previously digestive symptoms and with a very fast unfavorable evolution.

DISCUSSION

It has been recognized that venous thromboembolic events are common in patients with cancer. Patients with cancer represent approximately 15-20% of all new cases of venous thromboembolism (4). Cancer is associated with about a 3-fold increased risk of recurrent VTE both during and after anticoagulant therapy, and among patients with cancer, the risk of recurrence is about 3-fold higher in those with metastatic disease (7).

Our patient, a relatively young man, developed recurrent and migratory DVT, without any digestive symptom, abdominal pains, loss of appetite, or disorders of bowel transit, but only with about 4 kg loss in body weight in the last two months. Because of recurrent and migratory character of DVT, taking in consideration that the patient didn't have any thrombotic risk factors (normal thrombophilic state, normal state of nutrition, absence of immobility, major surgery, fracture or infection), under chronic oral anticoagulation (with therapeutic INR), our thought was to investigate a possible malignant cause of thrombosis, starting with tumoral markers, which had significant changes (especially CEA and CA19-9) and abdominal CT. Abdominal CT revealed an advanced stage of pancreatic neoplasm (T4N1M1), with secondary liver determinations, disseminated tumoral lymphadenopathies and invasion of portal and splenic veins. The diagnostic of secondary diabetes mellitus by hyperglycemic level is related to the location of the tumor into the pancreatic body and tail. Also, inflammatory state (leukocytosis and increased CRP) are related to the tumor presence, and hepato-cytolysis and cholestasis are due to secondary liver determinations and invasion of portal vein.

Recent studies showed that VTE tends to be associated with advanced and aggressive forms of cancers, while average life expectancy is poor in this group of patients (40% mortality at 6 months after diagnosis of VTE) (8). While the risk of VTE by tumor type remains uncertain for the majority of cancers, the risk appears to be highest for patients with malignant brain tumors and cancer of the ovary, pancreas, and lung (6). Multiple and interdependent mechanisms are responsible for the hypercoagulability state in patients with cancer. Tumor procoagulant activity, host inflammatory responses and extrinsic factors, which are frequently iatrogenic, are involved. Furthermore, recent evidence has shown that tumor-induced coagulation activation is intrinsically involved in tumor cell growth, angiogenesis and metastasis (2).

In conclusion, admitting that there are cases of cancer associated with DVT, by this report we want to draw attention on the fact that, in a young patient with recurrent DVT, without any apparent risk factor for thrombosis, attention should be higher to check for a thrombophylic state or for an active malignancy, even in the absence of signs or symptoms of organ sufferance.

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NEWS

PERIODONTAL STATUS OF PATIENT'S UNDERWENT HEMODIALYSIS THERAPY

The patients with chronic renal failure undergoing hemodialysis are susceptible to periodontal diseases due to systemic complications of the disease and using different drugs. A group of researchers from the Department of Periodontics of Babol University of Medical Sciences, Iran investigated the periodontal status of patient's who underwent hemodialysis. The study realized in Shahid Beheshti Hospital in Babol, Iran included one-hundred-fifteen hemodialysis patients (63 males and 52 females) with the mean age of 47.9±15.3 years. Periodontal parameters (plaque index, gingival index, clinical attachment level and probing pocket depth) were measured. The data were registered and analyzed. The plaque index, gingival index, clinical attachment level and probing pocket depth were 2.37±0.55, 2.36±0.63, 3.98±1.61 and 4.41±1.4, respectively. Significant positive correlations were found between the participants' age and plaque index ($p<0.024$) and $p<0.001$, respectively. Clinical attachment level was significantly higher in males (4.39±1.57) than females (3.53±1.56), $p<0.02$. The obtained data revealed that, especially in males, a longer duration of hemodialysis is associated with severe periodontal diseases. (Jenabian N, Ghazi Mirsaeed AM, Ehsani H, Kiakojori A. Periodontal status of patient's underwent hemodialysis therapy. *Caspian J Intern Med*, 2013; 4 (2):658-661).

Irina Grădinaru

NUTRITION IN INFLAMMATORY BOWEL DISEASES

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NUTRITION IN INFLAMMATORY BOWEL DISEASES (Abstract): The relationship between nutrition and inflammatory bowel disease (IBD) is a complex one, the evaluation and correction of nutritional deficits being integral part of therapy in these patients. The diet that consists in excessive consumption of meat, sugar, fats (with a higher ω 6 /ω 3 polyunsaturated fatty acids ratio) are factors involved in the epidemiology and pathogenesis of IBD. Malnutrition is present among most IBD patients, being the result of multiple mechanisms from digestive symptoms to inflammatory process, intestinal resection and administration of medications. Oral diet is high-calorie, high protein at correcting the vitamin and mineral deficiencies. Enteral diet has both an adjuvant role and that of inducing and maintaining remission as single treatment, particularly in children. Parenteral nutrition is reserved for patients with obstruction, fistula, toxic megacolon, short bowel syndrome, severe malabsorption, and other conditions that make enteral nutrition impossible or inefficient. **Keywords**: NUTRITION, INFLAMMATORY BOWEL DISEASE, ENTERAL DIET.

Role of nutrition in IBD pathogenesis. The incidence of inflammatory bowel disease (IBD) has increased over the last decades, particularly in developed countries, countries that have adopted habits specific to other geographical areas (Japan, India), or in people that migrated to developed countries and took over new alimentary habits. This epidemiological trend is primarily accounted for by dietary change (1).

Increased consumption of sugars, fats, low-fiber or high raw diets may cause by the high content in titanium oxide, silica, etc. resistance to degradation, or by incorporation in Peyer plaques macrophages, exacerbation of inflammation in Crohn disease (CD) in genetically predisposed individuals. Excessive consumption of meat and alcohol favors relapse in ulcerative hemorrhagic recto colitis (UC) (the sulfur compounds in these foods intensifies the inflammatory process) (2).

Fats with higher ω 6/ω 3 polyunsaturated fatty acids (PUFA) ratio increase the incidence of CD. European studies have shown that higher linoleic acid levels are associated with increased risk for UC. If ω-3 PUFA have anti-inflammatory properties (fish oil rich in ω-3 PUFA decreases the production of inflammatory mediators interferon γ:, PGE 2), ω 6 PUFA (arachidonic acid, linoleic acid) have proinflammato-

ry properties (3, 4).

Prevalence of nutritional deficits in IBD. Malnutrition is represented by the discrepancy between body needs and food intake and its components, from macronutrients - carbohydrates, lipids, proteins - to micronutrients-vitamins, minerals.

Malnutrition is present in 85% of IBD patients, and weight loss in 80% of CD and 18-62% of UC cases. Growth deficiency, especially in CD, is not only due to inadequate calorie intake but rather to the inhibition of the growth factor due to elevated leves of proinflammatory cytokines. Deficiency in vitamins A, D, K, E promotes inflammation which is found in 40 to 90% of IBD patients, commonly without clinical signs and without any decrease in the nutritional intake (5, 6).

Nutritional status. A single indicator is not enough to determine the nutritional status, its assessment requiring nutritional history, physical examination and laboratory data (5, 6). Subjective global assessment, originally used in oncology, could be a tool for the screening of IBD patients (using the following parameters: history of weight loss, food intake, gastrointestinal symptoms, functional capacity) (7) (tab I).

TABLE I.
Subjective global assessment in IBD patients

Criteria	Adequate nutrition	Moderate malnutrition	Ssevere malnutrition
Unintentional weight loss (last 6 months)	loss < 5%	5 - 10%	> 10 %
Diet	to the needs	70 - 90 % of the needs	< 70 %
Gastrointestinal symptoms (anorexia, nausea, vomiting, shortness of breath, change in taste)	absent	intermittent	daily
Functional capacity	normal	reduced	
Disease type	remission	moderate	severe

Serum levels of albumin may be used as a marker of nutritional status. Hypoalbuminemia occurs in 25-80% of CD patients and 25-50% of those with UC and reflects the metabolic response to stress, so that in IBD its level can be normalized by proper nutrition and by the correction of inflammatory process (8).

Etiopathogenesis of malnutrition in IBD. There are multiple causes and mechanisms that explain the occurrence of malnutrition in IBD. Decreased food intake is due to anorexia, nausea, vomiting, abdominal pain and diarrhea, as well as to restrictive diets. In explaining anorexia in IBD, an important role is played by pro-inflammatory cytokines: TNF-alpha, IL 1β, IL -6, leptin, produced by the activation of monocytes, macrophages, lymphocytes to various stimuli that cause disturbances in the use and absorption of proteins, carbo-

hydrates, lipids (9).

Maldigestion occurs in (10):

- sclerosing cholangitis which is characterized by the decrease of bile secretion decreases with slow digestion and absorption of fats and fat-soluble vitamins;
- blind loop syndrome (small bowel bacterial overgrowth) causes fat maldigestion as a result of bile salts deconjugation by bacteria;
- overgrowth of small bowel intestinal flora causes progressive mucosal alteration together with the destruction of mucosal disaccharides by inflammation, leading to carbohydrate maldigestion, osmotic diarrhea and flatulence.

Malabsorption is secondary to extensive intestinal inflammatory process, intestinal resections that cause a decrease in absorption surface, microbial overpopulation (blind loop syndrome) that causes bile acid and vitamin B_{12} malabsorption (11).

Fever and infection (sometimes septic) increase the caloric needs, resulting in weight loss.

Another mechanism is the increased losses as a result of inflammation and proteins and fluids transudation through altered mucosa: mucosal bleeding often causes anemia; fistula result in loss of Zinc, electrolytes, fluids, non-absorbable fats bind luminal Calcium (Ca) and Magnesium (Mg) and causing decreases in Ca and Mg levels (12).

Medications may also represent potential sources of malnutrition (13): Sulfasalazine decreases the absorption of folic acid and dihydrofolate reductase activity; Corticosteroids inhibit Ca absorption and increase urinary Mg level; Antibiotics induce the decrease of vitamin K; Cholestyramine binds bile acids and affects the metabolism of fat soluble vitamins.

Nutrition in the treatment of IBD. It is a supportive therapy by correcting specific nutritional deficits determining the induction and maintaining remission.

Oral nutrition. Its particularities will be established following a dietitian advice for a healthy, individualized diet with an elementary, fractioned intake with liquid oral supplements. Compliance with elemental or semi-elementale diets in CD and acute UC episode is poor due to their cost, taste, smell, texture (14, 15).

Resting energy requirements are not increased in CD patients and BMI can be used to assess the caloric needs. The energy requirements of IBD patients are strictly dependant of the BMI (13) (tab. II).

TABLE II.

Energy requirements of IBD patients according to BMI

BMI (kg/m^2)	Energy requirement (kcal/kg body weight)
< 15	36 - 45
15 - 19	31 - 35
20 - 29	26 - 30
> 30	15 - 25

Proteins. Patients with IBD have increased protein needs due to intestinal losses and increased catabolism (infection/abscess/required surgery). Protein needs are assessed by body weight and disease status. The recommended daily protein intake for IBD patients without kidney disease is 1-1.5 g/kg, being reduced to 0.8 g / kg body weight for the patients with renal failure not receiving dialysis (15).

Vitamins and minerals. (10) (tab. III).

TABLE III
Vitamin and mineral requirements and treatment of deficiencies in IBD patients

Nutrient	Recommended daily requirements	Signs and symptoms of deficiency	Recommended replacement oral dose
Zinc	15 mg	Dry, flaky skin, palm peeling, diarrhea, mental status changes	50 mg elemental/day
Iron	10 -15 mg	Microcytic anemia, fatigue	300 mg 1-3/day
B_{12} (cobalamin)	3 mcg	Megaloblastic anemia, parenthesis, ataxia, diarrhea, mental status changes	1000 mcg/day
Folate	400 mcg	Sore mouth, glossitis, diarrhea, inattentiveness, megaloblastic anemia	1 mg/day
Calcium	800 - 1500 mg	Osteopenia, osteoporosis, tetany	1500 - 2000 mg/day
Magnezium	400 mg	Nausea, muscle weakness, arrhythmia, confusion, convulsions	150 mg elemental Mg 4x/day
Vitamin D	400 UI	Rickets, osteomalacia, bone pains, muscle weakness, tetany	variable

Vitamin B_{12} deficiency is found in distal ileal CD or in case of gastric resection (intrinsic factor deficiency) or terminal ileum (absorption deficit). Due to these aspects serum methylmalonic acid (S-MMA) concentration is elevated, vitamin B_{12} concentration is decreased, and homocysteine is elevated (influenced by vitamin B_6 and folate status). B_{12} deficiency stages are (16): I, II – plasma and brain stores are depleted; III – elevated levels of S-MMA and homocysteine, with clinical signs starting to occur; IV - macrocytosis with increased mean corpuscular volume (MCV).

The treatment consists in the administration of vitamin B12: i.m ampoule = 1000 μg 1 ampoule/month, intranasal 500μg x 4/month. p.o cp = 1000 μg, 30 cp/month.

Folic acid deficiency occurs either through increased requirements (methotrexate is a folic acid antagonist and sulfasalazine blocks the absorption of folic acid) or by decreased intake (the main sources of folic acid - fruits, vegetables - are difficult to tolerate for IBD patients). Supplementation with folic acid 1 mg/day corrects the deficiency and protects against the risk for colorectal cancer. A special mention must be made on increased homocysteine levels that occur in vitamin B_{12} and folate deficiency, with increased risk for thrombosis, atherosclerosis, cardiovascular diseases, stroke (17).

Calcium and vitamin D deficiency. Bone metabolism disorders increase by 40% the risk of fractures in IBD. DEXA scan (dual energy X-ray absorptiometry) evaluates osteoporosis diagnosis and the therapeutical programme. The prevalence of osteoporosis is evaluated at 3-58% in UC and 4-50% in CD, due to deficiencies in proteins, vitamin D, and Ca intake, presence

of inflammatory cytokines and corticosteroids. A major role is represented by old age, weight loss, disease status, and treatment with corticosteroids. Supplementation with Ca + vitamin D preparations (1000-15000 mg Ca + vitamin D 400-600 IU / day) is recommended and taking into consideration the fact that Prednisone can induce vitamin D resistance by increasing parathyroid hormone level and calcium losses. In patients with severe malabsorption 2,000-4,000 IU of vitamin D/day need to be administered parenterally, and in osteoporosis, bisphosphonates are preferred (18, 19).

Mineral deficiency. *Zinc deficiency* occurs in fistulas and short bowel syndrome. The recommended dose for substitution is 15 µg/ kg/day. *Iron deficiency* secondary to hemorrhage occurs in 80% of UC patients and in 40% of those with colonic CD. In iron deficiency anemia injectable iron (100-200 mg ferrous sulfate) is preferred because iron administered p.o is accompanied by gastrointestinal intolerance in 21% of cases, and intestinal inflammation and increased risk for colorectal cancer. *Magnesium deficiency* occurs in case of fistulas and colectomies, and short bowel syndrome and parenteral administration is preferred: 5% magnesium gluconate which has a high solubility (10).

Enteral nutrition (EN) in IBD, used since the 70's and 80's, may be used as therapy or as adjuvant to drug therapy. Studies on EN showed several characteristic aspects for patients with CD (20.21): corticosteroid therapy is more effective in obtaining remission compared to EN in adults; EN in children, particularly ileum located, is superior to corticosteroid therapy, being the choice of first-line treatment for reducing the negative effects of corticosteroids on growth and bone metabolism; No difference was observed between the three enteral nutrition formulas: elemental (nitrogen in the form of free amino acids); oligomeric (nitrogen in the form of peptides or protein hydrolyzates); polymeric (nitrogen in the form of whole proteins).

The studies on EN in UC have shown inconsistent results, most of them not recommending it either in inducing or maintaining remission.

Enteral therapy is not a standardized therapy throughout the world with differences in Europe, America, Canada, and Japan. According to the ESPEN Guidelines on Enteral Nutrition (2006), the general goals of enteral nutrition are: prevention and treatment of subnutrition, improvement of weight growth and development in children and adolescents, and a better quality of life. For adults with active forms of disease EN is indicated as sole therapy if the patient refuses or has contraindications to corticosteroids (22).

Combined therapy is indicated perioperatively in malnourished patients with intestinal stenosis (weight loss > 10%, BMI <18.5, and albumin below 30 g/l). The response to enteral nutrition is assessed after 3 to 6 weeks (23).

EN diminishes inflammation and induces remission without the side effects of 5-amino-salicilyc derivates (5 ASA), steroids, or Azathioprine (AZA). It also has a role in maintaining remission, especially in patients with EN-induced remission. It also can cause "mucosal healing" through its local immunstimulatory action (24).

The disadvantages of EN are related to its high costs, not covered by insurance, lack of compliance to prolonged administration (4-8 weeks), absence of generally accepted protocols, medical skepticism, and last but

not least the administration route.

Enteral nutrition is administered orally, by nasogastric or nasoenteric tubes. If EN should be administered for more than 4 weeks, a percutaneous gastrostomy or jejunostomy is required (in CD patients this procedure increases the risk for fistulas). The main complications are: aspiration, hemorrhage, perforation, nose bleeding, pneumothorax, tube obstruction, intestinal ischemia and in stoma there is a high risk of infections, fissures or ulcerations (22).

Parenteral nutrition. Parenteral nutrition (PN) is indicated in the following situations: obstruction, fistula, toxic megacolon, short bowel syndrome, severe malabsorption, loss of fluids and electrolytes, other conditions that make EN impossible/ineffective. Crohn's disease is one of the main indications for PN at home (20% of patients on PN have CD). The most common complications of PN can be: liver, biliary, catheter sepsis, thrombosis, decreased quality of life (25).

Nutrition in IBD complications

Fistulas in CD are due to Zinc, electrolyte and fluid losses that require nutritional correction. PN is used, or when fistula flow is less than 1500 ml/24 hours, EN can be used. There are studies in the literature on small series of adult patients showing that PN as sole therapy may delay or avoid surgery. The data available so far do not support the possibility of fistula closure solely by digestive rest.

In ***stenoses*** nutrition depends on stenosis length and type (inflammatory *vs.* fibrous). When oral nutrition is possible the diet should be low in dietary fibers and hard to chew foods to prevent obstruction. Tight, fibrous stenoses may require EN or PN prior to surgery.

In ***toxic megacolon*** EN or PN is an adjuvant to drug therapy. A randomized study on 42 patients with UC - fulminant form – which received corticosteroid therapy associated with EN *vs*. PN - demonstrated the superiority of EN (the number of colectomies was identical for arms, but the side effects, postoperative complications, and albumin levels were in favor of EN).

In IBD patients requiring ***surgery***, PN is administered perioperatively. A preoperatively adequate nutritional status decreases the risk of postoperative complications and IBD recurrence. Nutritional therapy is indicated in patients who lost more than 10% of their weight in the last 3-6 months, have a body mass index <18.5 kg/m^2 or hypoalbuminemia (albumin <30 g/l). NP should be administered postoperatively until patients resume enteral or oral nutrition as in the case of the other abdominal surgeries (26).

Short bowel syndrome (SBS) is a malabsorption disorder secondary to extended bowel resection (in adults less than 200 cm remaining intestine). There are three stages of disease progression (27): Phase 1, lasting up to several weeks, is characterized by massive loss of fluids and electrolytes; Phase 2, adaptation phase (lasting approximately 2 years) is characterized by anatomical changes (increase in villus height and enterocyte number) and functional changes (changes in permeabiity and transmembrane transporters); Phase 3 is that of maintenance.

Long term PN is indicated in all cases with jejunocolic anastomosis and less than 50 cm jejunum remaining and in those with colectomy and less than 100 cm residual small bowel. Given the complica-

tions and difficulties of long-term PN, the return to oral or enteral nutrition is attempted through: improve oral and liquid diets, micronutrient supplementation, use of trophic factors, antisecretory and antidiarrheal drugs, and antibiotics (to treat bacterial overpopulation syndrome). In case of failure, small bowel transplantation should be considered.

Oral rehydration solutions should contain glucose and electrolytes (Na>90 mmol/l). Hyperosmolar solutions (e.g. fruit juices) will not be administered. The diet aims to increase energy intake by 50% (by repeated small meals) in association or not with EN (preferably a polymeric or semi-elemental formula administered through nocturnal gastric tube). It should consists of: 50-60% complex carbohydrates (which reduce osmotic load), 20-30% fat (long- and medium-chain triglycerides), and 20-30% protein (meat, fish). Supplementation with soluble fibers delays gastric emptying and increase intestinal adaptation. Oxalate-rich foods (cola, tea, coffee, draft beer, cherry, orange, lemon, red cabbage, saffron, French fries, etc.) should be restricted due to the increased risk for oxalate nephropathy (which occurs in 25% of SBS patients due to the inability to eliminate oxalates because fatty acids bind calcium) (28).

As to micronutrient supplementation the following should be administered: vitamin A (5,000 to 30,000 IU/day i.m. or p.o.); vitamin B_{12} (300-1.000 mg/month s.c. or i.m.); vitamin C (250-500 mg/day p.o.); vitamin D (800–1.600 IU/day p.o. or i.m.); vitamin E (400 U 3x/day p.o.); folate (1 mg /day p.o.); iron (325 mg x 3/day p.o.); zinc (50 mg elemental Zn - 220 mg tablet, 1-2 x/day); selenium (100 mg/day p.o.); calcium (1500-2000 mg/day p.o.); potassium (20 - 80 mEq/day); magnesium (magnesium oxide 12-24 mEq/day - will be given parenterally or nocturnally not to aggravate diarrhea (29).

Encouraging results in studies on animal models were obtained with intestinal mucosal growth factors: growth hormone (GH) (approved by the Food and Drug Administration-FDA in the treatment of short bowel syndrome), glucagon-like peptide 2 (GLP-2), glutamine, peptide YY analog. In humans, however, the results are controversial, so these factors cannot yet be recommended in clinical practice (27).

In **conclusion,** nutrition in IBD is a complex problem that requires a good nutritionist - gastroenterologist collaboration and patients with IBD will benefit by the assessment and correction of existing deficits. Enteral nutrition is an adjuvant therapy in inducing and maintaining clinical remission and in mucosal healing.

Parenteral nutrition is reserved for severe cases with complications.

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PERIPARTUM CARDIOMYOPATHY: A CHALLENGE FOR CARDIOLOGIST

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PERIPARTUM CARDIOMYOPATHY: A CHALLENGE FOR CARDIOLOGIST (Abstract): Peripartum cardiomyopathy (PPCM) is a rare but potentially life-threatening condition that occurs in previously healthy women during the last month of pregnancy and up to 5-6 months postpartum. The etiology and pathophysiology remain uncertain, although recent observations strongly suggest the specific role of prolactin cleavage secondary to unbalanced peri/postpartum oxidative stress. PPCM is a diagnosis of exclusion, as it shares many clinical characteristics with other forms of systolic heart failure secondary to cardiomyopathy. The management of heart failure requires a multidisciplinary approach during pregnancy, considering the possible adverse effects on the fetus. After delivery, the treatment is in accordance with the current guidelines for heart failure. Some novel therapies, such as prolactin blockade, are proposed to either prevent or treat the patients with PPCM. A critical individual counseling concerning the risks of subsequent pregnancy must be considered. Because of its rare incidence, geographical differences, and heterogeneous presentation, PPCM continues to be incompletely characterized and understood. For all these reasons, PPCM remains a challenge in clinical practice, so future epidemiological trials and national registries are needed to learn more about the disease. **Keywords**: PERIPARTUM CARDIOMYOPATHY, PROLACTIN, ECHOCARDIOGRAPHY, BROMOCRIPTINE, RIK OF RELAPSE.

DEFINITION, EPIDEMIOLOGY

In 1849, Ritchie was the first to establish a relationship between heart failure and puerperium. However, peripartum cardiomyopathy (PPCM) was recognized as a distinctive form of cardiomyopathy in 1937, when Gouley (cit. 1) described the clinical and pathological features of a severe heart failure associated with a dilated cardiomyopathy in the later months of pregnancy persisting after delivery. In 1965, Walsh (cit. 1) was the first to propose the specific period for the diagnosis, and highlighted that other conditions must be excluded. In 1971, Demakis et al. (2) defined the condition PPCM. The investigators established the original diagnostic criteria, which were subsequently confirmed by the National Heart Lung and Blood Institute [NHLBI] and the Office of Rare Diseases of the National Institutes of Health [NIH] Workshop, and completed with an echocardiographic criterion (3) (tab. I).

In 2010, Heart Failure Association of the ESC Working Group on PPCM proposed a new definition: "an idiopathic car-

diomyopathy presenting with heart failure secondary to LV systolic dysfunction towards the end of pregnancy or in the months following delivery, where no other cause of heart failure is found. It is a diagnosis of exclusion. The LV may not be dilated but the ejection fraction is nearly always reduced below 45%“ (1).

TABLE I

Original definition of peripartum cardiomyopathy

Classic criteria (Demakis et al.) (2)
1. The development of heart failure in the last month of pregnancy or within the first 5 months postpartum
2. The absence of an identifiable cause for heart failure
3. The absence of recognizable heart disease prior to the last month of pregnancy
Additional criterion (NHLBI & the Office of Rare Disease of NIH) (3)
4. Left ventricular systolic dysfunction demonstrated by classic echocardiographic criteria (depressed ejection fraction or shortening fraction)

Although the condition has been defined by several confusing names, PPCM is the preferred term because it highlights the overall chronological spectrum of the disease (4). Another accepted term is pregnancy-associated cardiomyopathy or early peripartum cardiomyopathy, used for those females with cardiomyopathy developing heart failure before the last month of pregnancy or at least five months after delivery (5).

Good data about incidence are unavailable because so few population-based registries exist. Until recently, only small prospective studies were available to estimate the incidence of the disease (5-8). Only two large retrospective population-based studies have been conducted in USA with a reported incidence ranging between 1:3189 live births and 1:4025 live births (9, 10). The estimates are almost similar for Japan and Australia. PPCM is sporadic in Europe in the white women (11,12). The more recent data from the United States suggest the highest incidence in African-Americans (10). Outside the United States, the most comprehensive data came from the Peripartum Cardiomyopathy Project in Haiti, which estimated the incidence of PPCM as high as 1case/299 live births (7). The data have been confirmed by Gentry (13), who noted an incidence of 1 case/1000 live births in South Africa. Although not clearly delineated, there are several suggested risk factors for the development and recurrence of PPCM (2, 3, 11, 14-19):

- african race appears to be the strongest risk factor;
- advanced maternal age – the disease generally occurs over the age of 30 years;
- multiparity – 71% of cases occur after ≥ 3 pregnancies compared with 8% in primigravidas;
- twin pregnancies in 8-13% of cases compared with 1-2% rate noted among healthy women;
- gestational hypertension in 43% of cases, substantially higher than the 8% to 10% incidence in the overall pregnant population;
- the unique association with prolonged use of tocolytics for a period of at least four weeks;

- certain cultural practices such as consuming lake salt or rock salt known as "kanwa" or heating of the body on a clay bed with a fire beneath to keep warm;

- socio-economic level summarized in a stereotyped profile: "poor African female, with malnutrition and multiparity, making strenuous and sustained physical effort during pregnancy".

ETIOLOGY AND PATHOGENESIS

Despite extensive research into its underlying etiology and pathogenesis, it is not clear exactly how PPCM occurs (17). The pathogenetic mechanisms of PPCM have been difficult to study as its incidence is too low to allow meaningful evaluations, and the suitable animal models to study the disease are rare. Several putative hypotheses, such as abnormal immune response to pregnancy, citokine-mediated inflammation, increased myocyte apoptosis, abnormal response to hemodynamic stress, genetic susceptibility, malnutrition, increased adrenergic or vascular disease have been proposed, but, at the present time, two hypotheses are foremost: pregnancy associated hormonal changes, specifically the role of prolactin, and viral infection.

Pregnancy is a physiological state associated with enhanced oxidative stress. In order to protect the heart, an efficient antioxidant defense mechanism counteracts the oxidative stress. The total antioxidant capacity increases in the last trimester with a peak in early postpartum (20). STAT3 is a cardiac tissue-specific DNA-binding protein which protects the heart from pregnancy-induced oxidative stress (21). The protein pathway is activated by full-length 23-kDa prolactin, a hormone with no adverse effects on the heart. Reduced levels of STAT3 lead to an unbalanced peri/postpartum oxidative stress, a potent stimulus for the activation of prolactin-cleaving protease catehpsin D in cardiomyocytes. The result is cleavage of the nursing hormone prolactin into an antiangiogenic, proapoptotic and proinflammatory 16-kDa subfragment, which also promotes ventricular dilatation and reducing of cardiac function. The same fragment inhibits vascular endothelial growth factor-induced proliferation of endothelial cells and migration, induces apoptosis, dissociation of capillary structures, impairs nitric oxide-mediated vasorelaxation, and cardiomyocyte function (22,23). In explanted terminally failing hearts from PPCM patients, low STAT3 protein levels are displayed, suggesting the role of this signaling pathway in the pathogenesis (22). Prolactin production is not limited to pituitary gland, various other cell types, such as fibroblasts, being able to produce it. PPCM is often associated with a high degree of cardiac fibrosis mediated by locally produced prolactin, which enhances the circulating pituitary 16-kDa prolactin damaging cardiac effects. There is more evidence linking findings from experimental models to human PPCM. Patients with acute PPCM have increased serum levels of oxidized low-density lipoprotein and significantly elevated pro-apoptotic serum markers (e.g. soluble death receptor sFas/Apo-1), indicative for enhanced oxidative stress, activated cathepsin D, and 16-kDa prolactin compared with pregnancy matched healthy controls (24). Consistent with the idea of prolactin involvement, blockade by bromocriptine, a dopamine D2 receptor agonist, was tested. Bromocriptine eliminates the substrate for the generation of 16-kDa prolactin, and prevents the onset of disease in the mouse model of PPCM (22) (fig. 1).

Several reports suggest that bromocriptine may have beneficial effects when added to the standard therapy of heart failure in women with acute onset of PPCM (25,26).

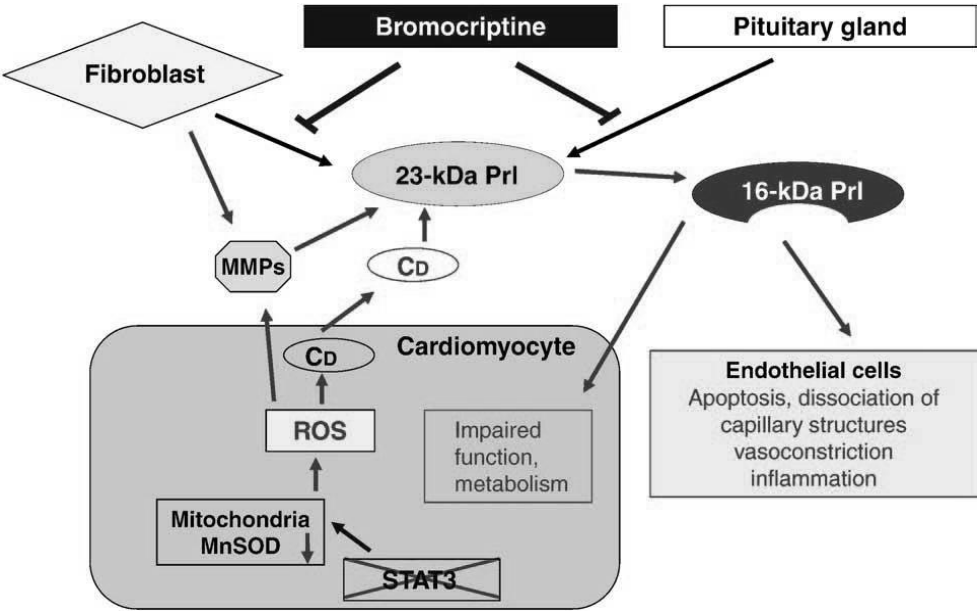

Fig. 1. Schematic mechanism for the development of PPCM (adapted from 24)

Myocarditis as a cause of PPCM in humans was first suggested by Gouley (cit. 1) in 1937. Since then, several investigators have supported this hypotheses. On the other hand, the prevalence of viruses detected in endomyocardial biopsies ranges from less than 10% to 78%, with a similar incidence in controls, suggesting no specific role for viral infection. It is worth noting that the molecular pathological study of endomyocardial biopsies found a high prevalence of viral genomes (parvovirus B19, human cytomegalovirus and herpes virus 6, Epstein-Barr virus) as well as inflammatory changes consistent with myocarditis (30.7%) (28). Another investigation suggests that viral infection increases the severity of myocardial damage in postpartum mice in comparison with nonpregnant control subjects (12). It is possible that the postviral immune response to be directed inappropriately against native cardiac tissue proteins leading to LV systolic dysfunction in the presence of the characteristic hemodynamic changes during pregnancy. Given the imunosuppressed state of pregnancy, it is logical that pregnant women are more susceptible to infection or viral reactivation (3). At the present time, no convincing data exist that myocarditis is the primary etiology of PPCM. Further studies using newer technologies such as PCR are needed to confirm a pathogenic role (17).

DIAGNOSIS OF PERIPARTUM CARDIOMYOPATHY

Patients with PPCM present with classical signs and symptoms of systolic heart failure due to other cardiomyopathies (28).

NYHA class III or IV functional status seems to be the most common initial presentation (6). Some case series describe unusual presentations such as acute cyanosis, multiple thromboembolic events or liver failure (16,28). A latent form of PPCM without overt clinical symptoms has been reported (29). The clinical diagnosis still represents a challenge because symptoms of early heart failure can appear in normal late pregnancy and after delivery. The diagnosis should be considered whenever women experience unexplained heart failure during the last month of pregnancy or within 5 months following delivery. It is important to note that 78% of PPCM cases develop heart failure symptoms in the first 4 months after delivery (30). Interestingly, Fett (31) reported clinically normal postpartum in women with asymptomatic echocardiographic systolic dysfunction, who either developed dilated cardiomyopathy or completely recovered LV function. These cases may represent a latent phase of PPCM before the development of dilated cardiomyopathy later in life or subclinical dilated cardiomyopathy presenting in early pregnancy or a viral myocarditis, distinct conditions from true PPCM. The rapid onset of heart failure symptoms in the peripartum period is another clue for diagnosis. Very recently, Fett (32) proposed a screening tool for early diagnosis (tab. II). A score ≥ 5 has always been associated with LV systolic dysfunction. A score > 4 suggests the need for further investigation, such as BNP and echocardiography. If the score is < 4 the patient should be monitored for BNP and C-reactive protein levels. If increased levels, echocardiography should be performed.

TABLE II

Self-test for early diagnosis of heart failure in PPCM

Signs/Symptoms	**Characteristics**	**Scoring**
Orthopnea (difficulty breathing when lying flat)	None Need to elevate head Need to elevate $\geq 45^0$	0 1 2
Dyspnea (shortness of breath on exertion)	None Climbing 8 or more steps Walking on level	0 1 2
Unexplained cough	None At night Day and night	0 1 2
Swelling lower extremities	None Below knee Above and below knee	0 1 2
Excessive weight gain (during last month of pregnancy)	< 2 pounds/week 2-4 pounds/week > 4 pounds per week	0 1 2
Palpitations (sensation of irregular heart beats)	None When lying down at night Day and night, any position	0 1 2

The diagnostic work-up should focus on precise echocardiographic identification of new LV systolic dysfunction, peptide natriuretic measurement (3, 15), and ruling out other causes of heart failure. Additional investigations should be based on clinical suspicion. Blood tests, electrocardiogram, chest X-ray should be done in all patients, although none of these can help in screening or positive diagnosis. Echocardiography is the most widely used imaging method, which provides valuable, reproducible diagnostic and prognostic information. The technique is important to exclude other causes of heart failure and to monitor the evolution under treatment (1). In PPCM, cardiac MRI can be helpful at initial presentation to conduct further etiologic investigations (34). The interest for the technique is suggested by the ability to differentiate PPCM from other forms of cardiomyopathy, like Tako-Tsubo or ischemic cardiomyopathy. The technique might be a useful method for guiding biopsy to the abnormal area (35) and for prognostic stratification (36). Fett (37) supports cardiac MRI for guiding the immunosuppressive therapy in the inflammatory forms of PPCM, as this option of treatment has successfully been tested in "myocarditis-like" PPCM. The Heart Failure Association of the ESSC Working Group on PPCM (1) recommends cardiac MRI to be performed at 6 and 12 months for a better assessment of cardiac functional changes. PPCM remains a diagnosis of exclusion. Early detection is critically important to the patient with PPCM, because delayed diagnosis may be associated with increased morbidity and mortality (18, 33).

MANAGEMENT OF PERIPARTUM CARDIOMYOPATHY

When considering treatment during the peripartum period, a multidisciplinary approach is needed. Involvement of a cardiologist, obstetrician, anesthetist, intensivist, and neonatologist is imperative as earliest as possible after the diagnosis. The medical treatment is generally similar to that for other forms of non-ischemic dilated cardiomyopathy, with some general exceptions because of the risks of certain drugs on the fetus and newborn. The aims of medical treatment should be to reduce cardiac afterload and preload, while increasing myocardial contractility, to prevent complications, particularly thromboembolism, cardiac arrhythmia, progressive heart failure, and to improve long-term prognosis. Current therapeutic options consist of conventional supportive treatment for acute and chronic heart failure (38). Novel therapies are emerging, but the available data are inconsistent and limited. Some studies suggested that immunosuppressive drugs might be helpful in patients with active biopsy proven lymphocytic myocarditis, only after active viral infection is excluded (1). At present time, the role of immunosuppressive therapy in women with negative biopsies remains unknown. It is important to note that current therapies with ACEI, ARB and β blockers may have an additional effect on controlling the overactive immune system in PPCM. Also, immunomodulatory therapy acting on inflammatory cytokine TNF-α may be beneficial. Pentoxifyline, a xanthine agent known to inhibit the production of TNF-α and to prevent apoptosis, has been studied in PPCM. Sliwa et al. (39) reported a significant improvement of LV function > 10%, and end-diastolic dimensions, a reduction in mortality rate, and greater increase in functional status. Considering the observations that strongly suggest prolactin

cleavage as a specific mechanism for the development of PPCM, specific inhibition of its secretion with bromocriptine is promising. Several case reports demonstrated recovery of LV function after treatment with bromocriptine (25,40,41). Very recently, Sliwa (42) reported the results of a prospective, single-center, randomized, proof-of-concept pilot study of women with newly diagnosed PPCM receiving standard therapy with or without bromocriptine for 8 weeks. The addition of bromocriptine appeared to significantly improve LV function (27% at baseline, to 58% at 6 months). Analyzing these data together, Fett (43) remarked that important details of studies design must be corrected for appropriate results. Also, Fett suggests that bromocriptine treatment should be limited to those patients with LV ejection fraction < 35%, because of poor prognosis with standard therapy in this category. At the present time, a large prospective, multicenter, randomized trial is needed to allow bromocriptine extensive use. Such a trial is on-going in Haiti and South Africa (5).

In addition to the treatment of heart failure, an obstetrical plan for close monitoring must be developed when PPCM is diagnosed during pregnancy. A collaborative approach is essential to optimal care. Serial clinical assessments should be scheduled during late pregnancy. Antenatal testing, such as non-stress test and amniotic fluid index, or biophysical profile, baseline ultrasound scan for fetus is also recommended (44). Spontaneous vaginal delivery is preferred in stable women with healthy fetus. For patients with newly diagnosed PPCM before delivery, labor should be induced, or a cesarean section must be planned if mothers are critically ill (15). Breastfeeding should be avoided in PCCM, although several drugs have been tested and are safe.

PROGNOSIS AND PREGNANCY OUTCOME

Very few studies have been done to assess the long-term survival and recovery outcomes in PPCM. Although PPCM is a form of dilated cardiomyopathy, a characteristic feature is that a higher rate of spontaneous recovery of LV function occurs. However, a subset of women, despite using an optimal medical treatment, follows a rapid and irreversible course, associated with persistent LV dysfunction, severe heart failure, or premature death. Analyzing the long-term prognosis, Duran (45) concluded that NYHA functional class, QRS duration, and LV parameters at the time of diagnosis were important predictors. Initial cut-off values of $\leq$ 5.5 cm for LV end-systolic diameter, and > 27% for LV ejection fraction were identified to predict complete recovery of LV function, while QRS duration $\geq$ 120 ms was a predictor for mortality. Recently, Baruteau (34) discussed the potential significance of cardiac MRI in prognostic stratification. Elevated C-reactive protein and Fas/APO-1 were also reported to be related to decreased LV function and mortality (42). These perspectives remain to be evaluated by further studies.

Follow-up of patients with PPCM is similar with that for other forms of cardiomyopathy and LV systolic dysfunction. Considering that the recovery interval is not restricted to the first 6-12 months postpartum, it is strongly recommended to continue treatment and follow-up for a long period of time to achieve best results (37). An echocardiogram should be repeated at about every 6-12 months until recovery is

confirmed, or a plateau is reached (1). Dobutamine stress echocardiography may be performed to assess the potential for LV function recovery, by measuring the inotropic contractile reserve (46). The technique is useful especially when LV systolic function is normal, and the contractile reserve remains decreased.

One of the most important issues in PPCM is the safety of subsequent pregnancies. Even after the full recovery of LV function, the risk of relapse might be present. LV systolic function seems to be the key prognostic factor when counseling women with PPCM about the further risks. Individual planning might be done after an echocardiogram was performed:

- if LV ejection fraction is < 25% at diagnosis or incompletely recovered, the advice should be against further pregnancy (1);

- even if LV function is normal, the patients ought to have stress-echocardiography. Women with an abnormal LV inotropic response to dobutamine have a moderate risk of relapse and pregnancy is not recommended. Women with complete recovery on both echocardiography and dobutamine stress test can be informed about the low rate of complications. In this group, pregnancy can be completed in almost all cases (5).

In postpartum period, it is imperative to give contraceptive counseling and educate the patients about the existing alternatives. The combined oral contraceptives, containing estrogens and progestins are contraindicated, as estrogens increase the thromboembolic risk. Intrauterine systems are the most efficient and safe methods of contraception. Sterilization methods, including vasectomy, tubal ligation, and insertion of intratubal stents may be considered (1).

At the present time, no protocols for decision-making when counseling women with PPCM about risks of subsequent pregnancies are established. For this reason, it is advisable that every women who experienced PPCM to be considered at risk, and to be closely monitored by the medical team, in a high-risk obstetrical center.

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ASPECTS OF THE MOLECULAR-GENETIC PROFILE IN PATIENTS WITH ISCHEMIC HEART DISEASE

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ASPECTS OF THE MOLECULAR-GENETIC PROFILE IN PATIENTS WITH ISCHEMIC HEART DISEASE (Abstract): **Aim:** To assess the clinical and molecular-genetic aspects in patients with various forms of ischemic heart disease (IHD). **Material and Methods**: This case-control study was conducted in the interval 2007-2011 and included 405 patients with acute coronary episodes admitted to the Chisinau „Sfanta Treime" Municipal Hospital and Institute of Cardiology. Polymorphism of candidate genes angiotensin converting enzyme (ACE), angiotensin II type 1 receptors (AT1R), endothelial nitric oxide synthase (eNOS), and platelet GPIIb/IIIa receptors (PlA1/PlA2) were identified by amplified fragment length (AFLP) and restriction fragment length polymorphism (RFLP). **Results:** There were significant differences in the frequency of D risk alleles in coronary patients compared with controls (78.65% *vs.* 61.24%, p <0.05). The distribution of angiotensin II type 1 receptor A1166C gene polymorphism in the group of patients with IHD was: AA genotype - 72 (25.74%), CC genotype - 47 (16.78%), CA genotype - 161 (59.28%) patients. **Conclusions:** GPIIb/IIIa A2A2 gene haplotype is associated with susceptibility to IHD and increased prevalence of dyslipidemia, particularly in smokers. The carrier state of DD genotype and D allele of ACE gene can be used as markers of increased risk for IHD and myocardial infarction (MI) and is associated with increased blood pressure. **Key words:** ISCHEMIC HEART DISEASE, GENE POLYMORPHISM, ARTERIAL HYPERTENSION

Early multipopulational studies - ECTIM in Europe and Framingham in the USA - have demonstrated that IHD occurs as a result of the interaction between two groups of factors - genetic and environmental. Studies aimed at identifying the genes responsible for the heritability of IHD and MI have uncovered several candidate genes with different roles in vascular biology that are believed to be involved in the pathogenesis of IHD; of these more important are the genes encoding ACE, AGT1R, eNOS, and platelet GPIIIa receptors (PlA1/PlA2 polymorphism). They were investigated separately or associated in the European population, but the genetic complexity of this disease was not foreseen, new approaches being needed (1-4). Currently there is no accurate data on the clinical and evolutionary aspects in patients with ischemic heart disease in relation with the polymorphic markers of candidate genes associated with susceptibility to MI. These shortcomings determined us to study the clinical and genetic features in patients with various forms of ischemic heart disease.

MATERIAL AND METHODS

This study included 405 patients admitted in the interval 2007-2011 to the Chişinău „Sfanta Treime" Municipal Hospital and Institute of Cardiology for an acute coronary event with or without ST segment elevation. The control group consisted of 290 matched persons without IHD (data used for matching were age, gender, residence and professional activity). Sex-distribution in the study groups was uniform, male/female ratio being 2:1, that is 2 times more males ($p < 0.001$). Mean age was 57.93 ± 0.34 years, with insignificant variation in the control group ($p > 0.05$).

The study was polycentric, case-control, approved by the National Ethics Committee for Clinical Trials and Drug Development of the Ministry of Health of the Republic of Moldova (no. 331, 03.06.2010). All subjects were native-born citizens and residents in the Republic of Moldova of comparable socio-economic status and ethnically matched. Patients were included in the study in order of their hospital admission, after clinical and enzyme stabilization and obtaining informed consent, this type of patient selection ensuring randomness of the study group. Criteria for inclusion in the study were the clinical diagnosis of acute Q wave and non-Q-wave myocardial infarction, unstable or exercise angina pectoris, in agreement with the recommendations of the European Society of Cardiology (5).

Exclusion criteria: hypercholesterolemia (total cholesterol ≥ 8 mmol/ l) and secondary hypertriglyceridemia, pacemaker implant with evidence of ventricular pre-excitation, atrioventricular conduction blocks (2nd or 3rd degree sinoatrial or atrioventricular block), active liver disease, acute gastrointestinal diseases, severe kidney disease, and associated diseases that influence life expectancy.

Standard questionnaires were used to collect data on past and current medical history, examination results, and also personal and demographic data, cardiovascular risk factors, family history of coronary artery disease, hemodynamic data; lipidogram, blood glucose level, cardiac enzymes, instrumental investigations - ECG, echocardiography, coronaro-angiography.

Polymorphism of candidate genes angiotensin converting enzyme (ACE), angiotensin II type 1 receptors (AT1R), endothelial nitric oxide synthase (eNOS) and platelet GPIIb/IIIa receptors were identified by amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism, RFLP) (6-9).

Data were computer processed by variation, correlation, and descriptive analysis methods. The relationships between the studied phenomena were determined by using simple linear regression, quantitatively expressed by the correlation coefficient rxy. For estimating genetic frequencies we used POPULATION GENETIC ANALYSIS by Nei Masatoshi (1987), Molecular Evolutionary Genetics, Diploid Data Set at the Genetics Center, New York University, Langone. Frequency of studied genes loci was calculated with the help of Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Association of ACE insertion/deletion polymorphism (287 bp) with conventional risk factors and cardiovascular complications

Distribution of coronary patients according to ACE insertion/ deletion (I/D) polymorphism confirmed the prevalence of homozygous individuals with risk DD genotype as compared with the controls (19.64%

vs.. 11.03% ($\chi 2$ = 8.77, p <0.05), while genotype II was prevalent in the control group (33.11% *vs.*. 19.64%, $\chi 2$ 13.31, p <0.01). There were no significant differences in the number of heterozygous (ID) in both groups (60.72% *vs.* 55.86%, p> 0.05). ACE I/D polymorphism genotyping and the estimation of allele frequency revealed significant differences in the presence of risk D allele in patients with coronary heart disease compared with the controls (78.65% *vs.* 61.24%, OR = 1.29, $\chi 2$ = 8.77, p <0.05). Compared with those in which this was not present (II), the analysis of the risk factors and clinical manifestations revealed that ACE DD homozygous or ACE ID heterozygous genotypes in patients with IHD is associated with increased prevalence of hypertension (90.91% and 88.24% *vs.* 78.18%), SBP (155.32 ± 1.46 mmHg and 140.5 ± 1.31 mmHg vs.. 125.42 ± 1.36 mmHg), DBP (95.42 ± 1.35 mmHg and 90.6 ± 1.28 mmHg *vs.* 80.5 ± 1.84 mmHg) and recurrent angina (40.00% *vs.* 34.11% *vs.* 23.64%, respectively, p <0.01).

The differences between study groups reached statistical significance (p <0.05). No statistically significant differences were found between carriers of genotypes II, DD or ID in terms of degrees of hypertension. Considering the spectrum of risk factors and the clinical presentation according to ACE gene polymorphism recorded in this study, it appears that the presence of D allele and, in particular, homozygous DD state are associated with blood pressure values exceeding the optimal level (rxy = 0.81, p (DD-II) <0.01). D allele carrier state and heterozygous ID state are associated with a significantly higher risk of cardiovascular death (rxy = 0.27, p (ID-II) <0.05) and recurrence of angina symptoms (rxy = 0.42, p (ID-II) <0.05).

Association of AT1R A1166C polymorphism with conventional risk factors and cardiovascular complications

Genotype frequencies of AT1R A1166C polymorphism in the group of patients with IHD were: AA genotype was detected in 72 (25.74%) of the IHD patients, CC in 47 (16.78%) and AC in 161 (59.28%). In the control group genotype frequencies were: 31 CC carriers (10.69%), 162 AC carriers (55.86%), and 97 AA carriers (33. 40%). No significant differences in the presence of the studied genotypes were found (p> 0.05).

Genotyping of AT1R A/C polymorphism showed no conclusive differences between the presence of risk allele C in patients with coronary heart disease (72.83% *vs.* 70.71%, p> 0.05), or non-risk allele A frequency (27.17% *vs.* 29.29%, p> 0.05), compared with controls.

Comparative analysis of the characteristics of IHD patients grouped according to cytosine or adenine variants (A/C) of AT1R gene, revealed the association of homozygous CC state or heterozygous AC state with increased prevalence of hypertension (95.49% and 89.44% *vs.* 68.33%, p <0.05).

Estimation of the correlation between clinical determinants and AT1R A/C gene polymorphism showed that the presence of the risk CC genotype in the coronary patients is associated with increased prevalence of hypertension (p <0.01) compared with homozygote AA genotype (p <0.05).

Association of gene polymorphism Asp298Glu NOS with conventional risk factors and cardiovascular complications

The distribution of Asp298Glu eNOS gene polymorphism genotype frequencies in IHD patients showed no differences between IHD patients and controls in terms of frequency of AG genotype (53.21% *vs.* 57.93%, p> 0.05) and risk allele AA frequency (63% *vs.* 79%, p> 0.05). No signifi-

cant age-related differences were found, but there was a tendency towards accumulation in women (37.84% *vs.* 24.27%, p = 0.06).

Comparative analysis of characteristics of IHD patients, grouped according to Asp298Glu eNOS gene polymorphism revealed that homozygous state with risk genotype AA or heterozygous (AG) state are associated with increased prevalence of hypertension (96.00% and 87.91% *vs.* 69.64%, p <0.05), with no clear difference in terms of obesity (57.33% and 44.96% *vs.* 37.50%, p> 0.05). Analysis of clinical manifestations shows that almost 89.33% of the AA genotype carriers had hypertension grade II-III, while such levels of hypertension were found in only 69.64% of the GG carriers and 85.23% of AG carriers.

Analysis of echocardiographic findings showed reduced ejection fraction <50% in more than half of AG genotype carriers (57.05%), the same being also found in patients with genotypes GG and AA (42.86% *vs.* 46.67%, respectively) .

Estimation of the correlation between clinical determinants and eNOS gene A/G polymorphism has shown that as compared with non-carrier individuals (GG), homozygous AA state and heterozygous carriers of eNOS A/G polymorphism in coronary patients are associated with increasing prevalence of arterial hypertension (rxy = 0.84, p (AA-GG) <0.01).

Association of PlA1/PlA2 GPIIb/IIIa gene polymorphism with conventional risk factors and cardiovascular complications

Analyzing the frequencies of PlA1/PlA2 genotypes according to the polymorphism detected by Msp-I enzyme digestion we found that risk haplotype A2A2 was detected in 63 (22.50%) of the patients and 28 (09.66%) controls, the difference being statistically significant ($\chi 2$ = 16.28, p <0.001). Significant age-group differences were not found, but a trend of male prevalence (53.39% *vs.* 43.24%, p = 0.06) was noticed.

Analysis of A1A2 GPIIb/IIIa gene polymorphism genotyping revealed that mutant A2 allele tents to be more common in the IHD patients compared with controls (72.85% *vs.* 70.71%, p = 0.06). At the same time, the frequency of recessive A1 alleles in the coronary patients was lower than in the controls.

Platelet membrane glycoproteins play an important role in platelet adhesion and aggregation. The allelic variants for GPIIb/IIIa bind to fibrinogen being the key reaction in the process of platelet aggregation. The presence of P1A2 allele leads to increased functional activity of IIb/IIIa receptors and is associated with intense ADF-induced platelet aggregation *in vitro* (10).

The analysis of the relationship between the carrier-state of different genotypes and risk factors revealed a significant difference between genotypes A1A1, A1A2 and A2A2 carriers and the prevalence of smoking (53.90%, 48.68% and 69.84%, respectively, p <0.01) and mixed dyslipidemia (59.21%, 75.17% and 63.49%, respectively, p <0.05). Note the statistically significant difference between groups in terms of the share of old MI in the history of the study patients: A2A2 genotype was detected more frequently than A1A1 (20.63% *vs.* 9.21%, p <0.05).

Analysis of biochemical characteristics in relation with A1/A2 GPIIb/IIIa gene polymorphism showed that A2A2 genotype was associated with higher prothrombin levels as compared to A1A1 and A1A2 variants (106.96 ± 0.52% *vs.* 90.83 ± 0.59% *vs.* 80.00 ± 1.05%, p <0.05). Signs of grade II and III left ventricular failure (LVF) were present in 25.53% of A1A2genotype carriers, 15.87% of A2A2 genotype carriers, and 14.47% of genotype

A1A1carriers (p> 0.05).

It is noteworthy that one fourth of the risk A2A2 and A1A2 haplotype carriers presented Q wave acute MI compared with A1A1 carriers (28.36%, 22.22%, 19.73%, respectively, p <0.05).

It can be said that the presence of A_2 allele and A_2A_2 homozygous state are associated with the presence of dyslipidemia [rxy = 0.53, p(A2A2, A1A1) <0.05], smoking [rxy = 0.64, p(A2A2, A1A1) <0.01] - as risk factors, and a high frequency of previous IM.

CONCLUSIONS

Carrier state of DD genotype and D allele in ACE gene is a marker of increased risk for IHD and IM and is associated with an increased prevalence of hypertension, being positively correlated (rxy = 0.58, p <0.001) with the risk CC polymorphic variant of AT1R gene. A2A2 haplotype of GPIIb/IIIa receptor gene is associated with susceptibility to IHD and increasing prevalence of dyslipidemia, particularly in smokers. The impact of eNOS gene polymorphic markers for IHD was proved to be hypertension-mediated.

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LARYNGEAL MORPHOLOGICAL CHANGES DUE TO GASTROESOPHAGEAL REFLUX DISEASE

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LARYNGEAL MORPHOLOGICAL CHANGES DUE TO GASTROESOPHAGEAL REFLUX DISEASE (GERD) (Abstract): Chronic laryngitis may have life impact on professional voice users. Besides smoking and excessive alcohol intake, GERD is a determining factor in the etiology of dysphonia. **Aim**: To evaluate the laryngeal alteration due to GERD in professional voice users. **Material and methods**: The study included 96 vocal professionals (teachers, actors, singers and priests), 58 males and 38 females, with a mean age of 38.3 ± 7.5 years, presented for chronic laryngeal symptoms. The patients filled out a standardized questionnaire and were examined laryngoscopically. **Results**: Laryngeal changes were scaled 0 (absence) to 7 (maximum) – arytenoid edema (5.07±1.08), interarytenoid edema (6.18±1.12), vocal folds edema (5.67±1.04), ventricular bands edema (4.96±0.97), laryngeal edema 4.12±0.83). **Conclusions**: Laryngoscopic changes may suggest the concomitance of GERD in professional voice users with dysphonia. **Keywords:** GERD, DYSPHONIA, VOCAL PROFESSIONALS.

Even though most otolaryngologists are aware of the pathology induced by gastroesophageal reflux disease (GERD), this relationship was more intensely studied during the last two decades (13, 14). Otolaryngologists have become extremely cautious when performing the clinical ENT examination and look for reflux signs (rashes, edema in the arytenoid mucosa and interarytenoidian area) even when they prescribe treatment for chronic laryngitis. According to the most recent surveys, laryngopharyngeal reflux (LPR) displays a complex spectrum of pathological signs (1, 2, 3, 5).

Reflux laryngitis occurs as a result of a backward flow of acid and other stomach contents into the esophagus to the larynx, resulting in a chemical burn of the lining.

The diagnosis of LPR is based on a combination of symptoms and findings in the larynx during physical examination (4, 7, 8).

The opening of the esophagus sits behind the arytenoids in the posterior part of the larynx, and that is why these areas are the most affected by reflux. The larynx shows signs of irritation and inflammation, as evidenced by the presence of redness

(erythema) and swelling (edema) of the arytenoids, interarytenoid region (the area between the arytenoids, also referred to as the posterior glottis, or, incorrectly, as the posterior commissure), and the post-cricoid region (the area behind the arytenoids that separates the larynx from the esophageal opening).

In severe cases, the reflux material can contact other parts of the larynx causing erythema and edema of the folds.

The presence of edema of the true vocal cords is highly suggestive of LPR, even in the absence of laryngeal erythema (6, 9, 10, 11).

Any activity that increases intra-abdominal pressure may exacerbate the symptoms of LPR. Such activities may include weight lifting, sit-ups, abdominal crunches, sexual intercourse, running, dancing, aerobics, public speaking, acting, and singing.

Reflux-related symptoms and clinical presentation have been identified in 4 to 10% of all the patients seen by otolaryngologists (15, 16, 17), and most likely, as these estimates are not accurate, the percentage could be even much higher. Regarding the patients with laryngeal pathology, LPR is related with these conditions or represents an etiological factor in approximately 50% of the cases (11, 13).

The most common symptoms and clinical presentations of the reflux laryngitis are:

- morning hoarseness
- the voice warms in more than 20-30 minutes
- halitosis
- throat clearing
- oral dryness
- white tongue
- *globus faringeus*
- dysphagia
- regurgitations of the gastric content
- recurrent cough
- laryngospasm,
- complication of asthma
- acute upper airways infection in children.

In addition to longer vocal warm up time, the vocal professionals and actors may also suffer other interferences in their vocal practice – frequent throat clearing, abundant expectoration, especially during the first 10-20 minutes of vocal training (16).

Some authors noticed that LPR patients do not present esophagitis-related changes, so dyspepsia and heartburn are usually absent (8, 12, 17).

The prevalence of esophagitis is relatively low, as the esophageal mucosa of the distal esophagus has its own antacid protection mechanisms which include the following:

peristalsis (quickly pushing the acid off the surface of the esophageal mucosa)

structure of the mucosa (tolerating the intermittent contact with the gastric acid)

capacity of the saliva to neutralize the acid

However, the larynx and the pharynx do not have such protective mechanisms, thus exposure to acid and pepsin may cause substantial changes at this level, whereas the esophagus may not be affected at all. According to the preliminary data reported by Axford (14), the lining of the larynx has a cell defense mechanism which is different from the esophageal mucosa and there are specific differences in the expression of the MUC genes and carbonic anhydrase in the larynx and pharynx (1, 3, 8).

If the mucosa is affected, either directly or through secondary mechanisms, changes in the laryngeal mucosa may result. Thus, it

may be directly affected by the contact with the laryngeal lining with acid and pepsin, which results in mucosal lesions. Alternatively, the irritation of the distal esophagus by the gastric acid may activate a reflex mediated by the vagus nerve, which leads to chronic cough and throat clearing, affecting the larynx lining as well (16, 17).

The bile reflux may also affect the larynx. Additionally, according to recent surveys, there are a lot of question marks concerning the pathophysiology of reflux laryngitis. Some authors consider that the decrease in salivary epidermal growth factor may be associated with reflux laryngitis (14, 15, 17).

The aim of this research study was to evaluate the laryngeal alterations due to gastroesophageal reflux disease in professional voice users

MATERIAL AND METHODS

Selection of patients groups

The cases included in the research study were selected among the patients of the "Sf. Spiridon" Emergency Hospital Iasi, ENT Department. As study material, we used a group of 96 vocal professionals (teachers, actors, singers, priests) diagnosed with chronic laryngitis between 2008 and 2010. All patients filled out a standardized questionnaire and patient meeting the clinical criteria of gastroesophageal reflux disease were included in the study. A written informed consent was obtained from all patients. The control group included 54 age-, sex- and profession- matched ENT patients with no laryngeal complaints (16,17).

Selection criteria:

- vocal professionals (teachers, actors, singers, priests)
- dysphonia for at least 3 months (chronic dysphonia)
- absence of laryngeal benign or malignant lesions
- no drug treatment that could alter the esophageal motor function or acid secretion (anticholinergics, sedatives, prostaglandins, calcium channel blockers, potassium, antibiotics, NSAIDs)
- symptomatic GERD

Exclusion criteria:

- active smokers
- chronic alcohol intake
- upper airways infections during the last month (before inclusion in the research study)
- prior history of anti-reflux surgery
- asthma

Statistical analysis

Statistical analysis was performed using *SPSS 16.0*, on Windows XP Professional platform.

RESULTS

This research survey included 58 males and 38 females, aged between 21 and 60 years, mean age 38.3 years (±7.56) and median age 39 years.

The most affected professionals considering laryngeal changes - were teachers (37.5%), followed by singers (28.12%). There was no statistically significant difference between priests and actors, considering that priests are a male-only category.

The analysis of laryngeal changes revealed the prevalence of changes in the true vocal folds, as well as in the interarytenoid area ($p<0.001$), closely followed by changes at arytenoid level, false vocal folds, and laryngeal mucosa in general (table I).

Diagnostic sensitivity of laryngoscopy for laryngeal changes was of over 50% for 8 of the 10 laryngeal signs suspected to occur following the action of the reflux.

Interarytenoid and arytenoid edema followed by vocal folds edema and erythema were the changes with the highest sensitivity (83-97%).

TABLE I

Laryngeal changes (flexible endoscopic laryngoscopy)

Laryngeal change	Research group		Control group	
	$\dot{x}$	±SD	$\dot{x}$	±SD
Arytenoid edema/ erythema	5.07	1.08	1.15	0.92
Interarytenoid edema/ erythema	6.18*	1.12	0.93	0.89
Vocal folds edema/ erythema	5.67*	1.04	0.98	0.91
Ventricular bands edema/ erythema	4.96	0.97	0.87	0.73
Laryngeal edema/ erythema	4.12	0.83	0.77	0.69

$\dot{x}$ -arithmetic mean ±SD-standard deviation *p<0.001

Specificity was highest in the patients with true vocal folds and ventricular bands erythema, followed by ventricular bands edema and laryngeal erythema (90-94%).

DISCUSSION

According to our findings, laryngeal changes were more common at the level of the vocal and interarytenoid folds (p<0.001), followed by arytenoid area and ventricular bands both before, as well as after treatment. Interarytenoid and arytenoid edemas, followed by edema and erythema of the vocal folds were found to be the changes with the highest sensitivity (83 - 97%) (16, 17).

Despite numerous studies on reflux symptoms, those mentioned above as well as many others (16, 17), there is no objective data to support the significance of the various patient complaints and changes (as a result of the performed tests) in terms of diagnosis. This issue arose as there has not been any consensus as regards “normal” among the groups of patients included in the survey. Interdisciplinary debates (most particularly, otorhinolaryngology and gastroenterology), as well as multicentric surveys are necessary to accurately determine the sensitivity and specificity of the results frequently associated with laryngopharyngeal reflux, as well as the impact of LPR on the quality of life and general health status.

CONCLUSIONS

Patients presenting with laryngeal reflux symptoms must be treated in multidisciplinary way (otolaryngologist/gastroenterologist). The positive diagnostic requires modern and accurate testing methods: flexible endoscopic laryngoscopy, pH monitoring, esophageal impedance study, upper gastro intestinal endoscopy (UGIE).

Because there is no consensus on diagnosis and treatment, this condition is frequently either over diagnosed (resulting in various invasive, useless and expensive tests, as well as the application of inefficient treatment plans), or under diagnosed (resulting in the progression of symptoms because of the lack of adequate treatment).

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CORRELATION BETWEEN ASTHMA AND *TOXOCARA CANIS* INFECTION

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CORRELATION BETWEEN ASTHMA AND *TOXOCARA CANIS* INFECTION (Abstract): **The aim** of the current study was to examine the relationship between asthma and toxocariasis. **Material and methods**: We have studied 76 patients with asthma and 88 controls (without asthma) aged 5-16 years. An ELISA test based on the detection of anti-Toxocara canis (E/S antigen) serum immunoglobulin G (IgG) and E (IgE) was done in both groups. Ordinary allergens and E/S antigen of *T. canis* infections were used to evaluate cutaneous reactivity. **Results**: Seroprevalence in asthma patients was 68.42% and in the controls 13.63%. This difference was significant. The percentage of asthmatic patients with two anti-Toxocara antibodies was 26.31%, and of 4.54% in controls. All asthma patients with anti-Toxocara IgE had cutaneous reactivity to Ag E/S. **Conclusions:** Asthma patients with anti-Toxocara IgE and IgG may have toxocariasis. **Key words**: ASTHMA, HUMAN TOXOCARIASIS, CHILDREN, PARASITOSIS.

Human toxocariasis is a cosmopolite helminthic zoonosis caused by *Toxocara canis*, a dog nematode that accidentally infects man. Humans normally become infected with *Toxocara canis* S2 larvae by ingestion of eggs present in soil contamineted by dog faeces (1).

In the small intestine, at the level of duodenum or upper jejunum, eggs hatch to release S2 larvae that penetrate the small intestinal wall and begin to wander throughout the body entering in different tissues and organs: liver, lungs, brain, eyes, heart, kidney, muscle, etc., where they can survive for a long time, up to 11 years, remaining infectious and being free or encapsulated (2). S2 larvae which remain confined to the liver or other tissues and organs do not complete their life cycle in the human body, so they never reach the stage of mature adult worm (3, 4, 5).

A positive diagnosis of toxocariasis is difficult and the confirmation of this manifest or asymptomatic parasitic disease is possible by using ELISA serological test with *Toxocara canis* S2 larva secretory-excretory antigen for detecting in the blood IgG antibodies against this parasite. ELISA

immunoenzymatic reaction for the detection of circulating anti-Toxocara antibodies has high sensitivity and specificity (3).

The pathogenic action of *T.canis* in the lung parenchyma and asthma production can be explained by the effect of larval migration which results in necrosis, inflammatory reaction, and concomittantly, intratissue T. canis excretory/secretory (TES) deposits. The last reaction triggers an immunological cascade that induces inflammatory immune reactions even in the absence of larvae in the lungs. The clinical manifestations of toxocariasis, occurring after a variable period of time, are due to the fact that these larvae tend to remain dormant and continue migration, or to reinvade the tissues after a certain period of time (6, 7).

MATERIAL AND METHODS

In the study were enrolled 164 patients admitted to the clinics of Iasi „Sf. Maria" Children Hospital. The study database consisted in information obtained from patients medical records - clinical and laboratory findings and imaging data.

The patients were divided into two groups:

Group I: 76 patients diagnosed with asthma. The clinical signs were bronchoconstriction reversible spontaneously or by the administration of bronchodilator agents, wheezing, cough, dyspnea, and chest pain secondary to airway inflammation.

Group II: controls, including 88 patients infected with *T. canis* without asthma symptoms, but with symptoms of rhinitis (32 patients), dermatitis (26 patients) and rhinosinusitis (46 patients).

Asthma patients were divided into four stages: stage 1 (intermittent asthma) - 53.84%, stage 2 (mild persistent asthma) - 26.92%, stage 3 (moderate persistent asthma) - 7.69% and stage 4 (severe persistent asthma) - 11.53%.

In all patients IgG, IgE, and anti-Toxocara antibodies were determined and cutaneous hypersensitivity tests were done.

The obtained data were analyzed and expressed as percentages, and the associations between various factors were interpreted using correlation tests. *Statistica 8.0* software was used for statistical processing of the data.

RESULTS

Group I, asthma patients, had a mean age of 9.31 years, 57.9% of them were males and 42.1% females. Seroposivity rate for *T.canis* was 68.4% (52 cases), 24 female patients and 28 male patients.

Group II, controls, had a mean age of 8.12 years and a gender distribution of 27.3% females and 72.7% males. Seroposivity rate for *T.canis* was 13.6% - 12 patients, all males (tab. I).

TABLE I

Results of anti-Toxocara serology in the two groups

Serological markers for toxocariasis	Asthma	Other symptoms	p
IgE (+) IgG (-)	20 (26.31%)	4 (4.54%)	<0.01
IgE (+)	12 (15.78%)	4 (4.54%)	NS
IgG (+)	20 (26.31%)	4 (4.54%)	<0.01
IgE (-) IgG (-)	24 (31.57%)	72 (86.36%)	NS

Most asthma patients seropositive for *T.canis* had a positive IgE and IgG distribution of 26.31%.

In the control group, the distribution was uneven, two cases seropositive for *T.canis* associated with symptoms of rhi-

nosinusitis (persistent rhinitis determining a diagnosis of chronic rhinosinusitis) being identified. Of the patients with negative serology for *T.canis*, 31.6% (24 patients), were diagnosed with bronchial asthma, 75% (12 patients) had rhinitis, and 82.6% (38 patients) rhinosinusitis and all allergy tests were positive.

In asthma patients, allergen skin reactivity rate was higher than in controls: 82.6% (66 patients) and 75%, respectively. 22 patients had curataneous hypersensitivity to *T.canis* ES Ag, sex ratio 1:1.

Of the controls with positive skin reactions (tab. II), reactivity to ES Ag was found in 26 patients: 37.5% in those with a diagnosis of rhinitis, 30.4% with rinosinusitis, and 23.1% with dermatitis.

TABLE II

Results of cutaneous hypersensitivity tests in the 2 groups

	Asthma	Other symptoms	p
Toxocara canis ES *Ag*	44(57.89%)	26 (29.54%)	<0.01
Dermatophagia	42(55.26%)	40 (45.45%)	NS
Fungi	36(47.36%)	36 (40.9%)	NS
Other allergens	32(42.1%)	36 (40.9%)	NS
No reactivity	1013.15%)	22 (25%)	NS

All patients with asthma and seropositive for anti-Toxocara IgE (32 cases) showed skin reactivity to ES Ag. Similar results were seen in 4 cases belonging to the control group (tab. III). 70% (28 patients) asthma patients with positive IgG markers for T.canis showed positive skin reactivity to ES Ag.

Of the controls positive for anti-Toxocara IgG (8 patients), 6 had positive skin tests. Of the patients with asthma and negative *T. canis* serology (28 patients), 4 had positive skin reactivity to ES Ag.. Of the patients in the control group with negative serology for both immunoglobulins (76 cases), 16 reacted to *T.canis* ES Ag inoculation.

TABLE III

Results of cutaneous hypersensitivity test to ES Ag according to the positivity of serologic markers

Serologic markers for toxocariasis	Ag E/S (+)		Ag E/S (-)	
	Asthma	Other symptoms	Asthma	Other symptoms
IgE (+) IgG (-)	20	4	-	-
IgE (+)	12	4	-	-
IgG (+)	8	2	10	2
IgE (-) IgG (-)	4	26	20	60

DISCUSSION

In the group of asthma patients we noticed a positive correlation between anti-Toxocara IgE and/or IgG serological markers. In this study the determinations of anti-Toxocara IgE and/or IgG revealed a statistically significant association with asthma manifestations. This allows us to say that in asthma the clinical manifestations are more severe and aggravated on the atopic background determined by *T.canis* infection.

In the present study we found a correlation between the presence of anti-Toxocara IgE and skin reactivity both in asthma patients and controls. The results regarding IgG reactivity are not statistically significant to allow us to demonstrate a correlation between asthma, toxocariasis, and skin reactivity.

Antigenic specificity to cutaneous aller-

gens with negative serology for *T.canis* was below 20% in both study groups. The relationship between the presence of anti-Toxocara IgE, skin reactivity to ES Ag, and asthma is significant.

Migrating larvae can cause host sensitization to ES Ag with increasing IgE specificity. IgE couples to bronchial cell membrane and causes the release of an alveolar mediator that induces bronchial mucosa inflammation, and consequently bronchial asthma. A significant association between toxocariasis and high IgE levels was reported by other authors (8, 9, 10).

Asthma patients present skin reactivity to common allergens, but this reactivity is not significantly higher than in controls. On the other hand, reactivity of asthma patients to ES Ag is significantly different from that found in the control group. Although these results do not demonstrate a causal relationship, they express a significant association between cutaneous hypersensitivity to T. canis ES Ag and asthmatiform background.

CONCLUSIONS

The present study demonstrates an association between asthma and toxocariasis and suggests the need to include this parasitic disease in the differential diagnosis of asthmatiform syndrome. Determination of anti-Toxocara IgE and IgG becomes essential in the diagnosis of such patients.

Based on literature data should be consider toxocariasis when a patient, especially children older than 3 years, has asthma, hepatomegaly, hypereosinophilia, elevated IgE level, and positive epidemiology (11).

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TOXOCAROZA LA ADULT: STUDIU EPIDEMIOLOGIC PRIVIND INCIDENȚA ÎN ZONA MOLDOVEI

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ADULT TOXOCARIASIS (Abstract): **Aim**: Epidemiological study on a statistically significant population for determining the true degree of *Toxocara canis* infections in the adults from Moldova, Romania. **Methods**: 457 patients, 416 females and 41 males (91% and 9%, respectively) entered the study. Mean age of the investigated patients was 44.50 ± 12.706 years, with small differences between sexes. 49.2% were from rural and 50.8% from urban areas. Data on living conditions, animals in the household, and source of drinking water were obtained through direct questioning. At present there is no definitive diagnostic method for Toxocara infection, as the actual sensitivity and specificity of serological tests cannot be determined accurately. IgG antibody titer specific for *T. canis* was assessed by ELISA in all study patients. **Results**: Most infections were recorded in 2009 (51.4%), 15.3% in 2008, 17.1% in 2010 14.0% in 2011, and 2.2% (10 cases) in 2012. An IgG titer of 1/100 was found in 40.7% of the patients. Toxocariasis prevalence in our geographic area was 51.7%, without significant sex differences. However, while the percentages of negative determinations were similar for both sexes (42.5% in females and 46.3% in males), almost twice more men (9.8% *vs.* 4.6% women) had a 1/10 determination, while 21,4% of the women had a 1/50 determination as compared to 9,8% of the males. The source of water was not, at least in this study group, a major cause of disease in adults. The statistical results show a major and significant influence of human contact with the dog. Thus, in dog owners 1/50 and 1/100 determinations being found in equal percentages (26% of the total), while in those having both dogs and cats the 1/100 determination was the most common (40.7% of the total). **Conclusions**: Our data can be the basis for a nation-wide seroprevalence study and programs to eradicate this infection. **Key words**: *T.CANIS,* TOXOCARIASIS, SEROPREVALENCE, IMMUNOLOGICAL DIAGNOSTIC

Toxocaroza este o zooantroponoză cu evoluție de lungă durată, recidivantă, cauzată de parazitul *Toxocara canis*, având o simptomatologie nespecifică (1). În funcție de localizarea geografică, gradul eozinofiliei, semnele sau simptomele oculare sau pulmonare, toxocaroza poate fi întâlnită sub următoarele denumiri: *ocular larva migrans* (OLM), boala Weingarten, sindromul Frimodt - Moller sau pseudoleucemia eozinofilică (2, 3).

Toxocara canis, în stadiul adult, trăieşte în intestinul subțire al câinelui iar în stadiu larvar, accidental, la om, se poate localiza

în ochi sau viscere. Infecția se produce prin ingestia de ouă embrionate de pe sol, prin consumul de fructe sau legume contaminate cu ouă, consumul de apă contaminată sau introducerea în gură a mâinilor murdare. Omul este gazdă paratenică pentru *T.canis* (4, 5, 6).

Evaluând unele studii epidemiologice la nivel mondial, seroprevalența infecției cu *T.canis* variază între 1,6% în Marche, Italia şi 92,8% în Reunion, Oceanul Indian (7-8). Studii asupra toxocarozei au fost efectuate de Crețu et al. (.9) pe 85 de donatori de sânge, din mediul urban, evidențiind o seroprevalență de 30,6 %.

MATERIAL ŞI METODE

Studiul prospectiv, efectuat în intervalul 2008 - 2011, a cuprins un lot de 457 de pacienți adulți, urmărind evidențierea incidenței cazurilor de infestare cu *T.canis* şi relația câinelui ca sursă.

Informațiile despre geofagie, mediul în care trăiesc subiecții, condițiile de trai, contactul cu animalele, prezența sau absența manifestărilor clinice subiective, au fost obținute prin interogarea directă a subiecților. Contactul pozitiv cu *T. canis* a fost apreciat prin determinarea titrului anticorpilor IgG prin metoda ELISA.

Informațiile obținute din foile de observație ale pacienților selectați au fost introduse într-un fişier, folosind programul de calcul tabelar *Microsoft Excel*, ulterior acestea fiind prelucrate statistic cu ajutorul programului *SPSS 16.0*. Compararea valorii variabilelor numerice pe subgrupuri definite de variabilele calitative s-a realizat folosind testul ANOVA.

Pacienții incluşi în studiu au provenit din județele Iaşi (58,2%) şi Vaslui (40,7%), restul județelor fiind reprezentate cu câte un caz, Bacău şi Botoşani, şi 3 cazuri Neamț. Din totalul acestora, un număr de 416 (91%) au fost femei şi 41 (9%) bărbați. Vârsta medie a lotului investigat a fost de 44,50 ± 12,706 ani, la femei de 44,35 ± 12,527 şi respectiv 45,95 ± 14,490 la bărbați (fig. 1).

Majoritatea pacienților provin din anul 2009 (51,4%); în ceilalți ani incluşi în studiu cazuistica înregistrată a fost mai slab reprezentată (15,3%, 2008; 17,1%, 2010; 14,0%, 2011), cu un minim de 10 cazuri (2,2%) în 2012.

Lotul a avut o structură uniform repartizată şi în ceea ce privește mediul de proveniență: 49,2% rural şi 5 8% urban (fig. 2).

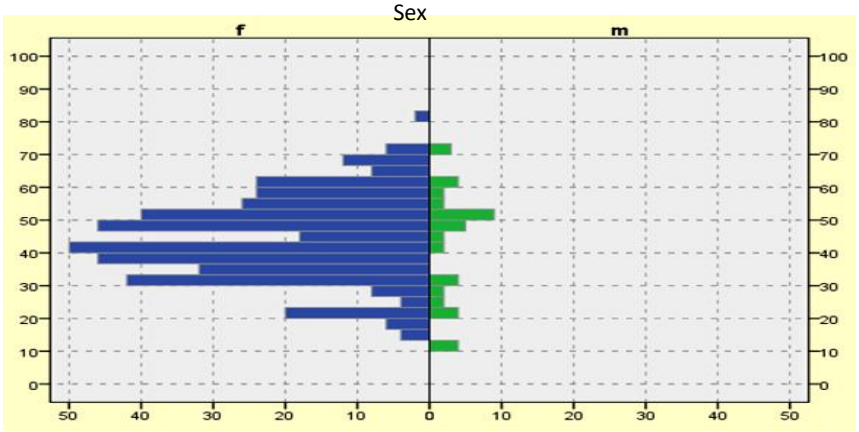

Fig. 1. Distribuția lotului pe sex şi grupuri de vârstă

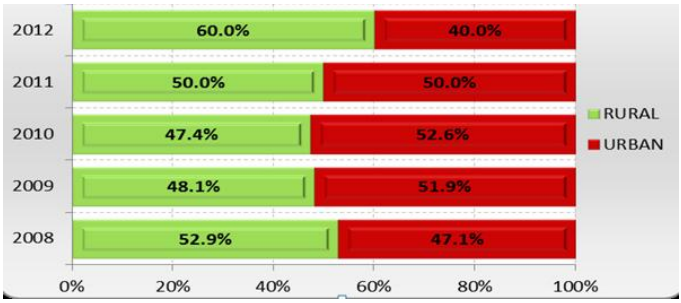

Fig. 2. Structura lotului inclus în studiu pe ani şi mediul de origine

În ceea ce priveşte sursa apei de băut, nu s-a decelat o caracteristică specifică a lotului; probabil pacienţii au consumat apă din mai multe surse, cu numai 2,2% apă de izvor. În rest, pacienţii au consumat apă de la sistemul centralizat (47,3%) şi din fântână (46,4%), iar un procentaj important au consumat apă îmbuteliată.

Pacienţii din mediul urban nu deţin animale în gospodărie în 45,1% din cazuri iar din mediul rural posedă cel puţin un câine (30.4%) sau atât câini cât şi pisici (23,6%), pentru ca să deţină numai pisici 4 (0,9%).

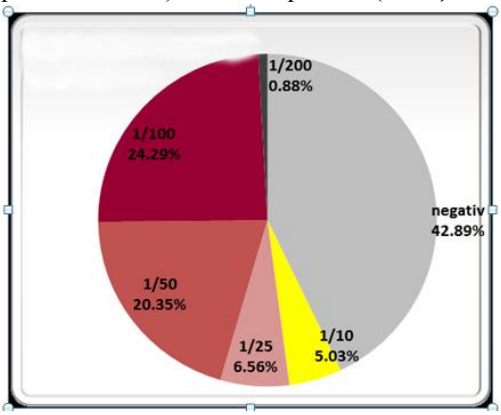

Fig. 3. Distribuţia pe titruri IgG /T.canis la lotul de paciente inclus în studiu

În ceea ce priveşte titrul anticorpilor IgG Toxocara, acesta a fost negativ în 42,9% din cazuri,1/100- 24,3% din cazuri, 1/50 - 20,4%, 1/25 -6,6%, 1/10 – 5,0% şi 1/200 -0,9% (fig. 3).

REZULTATE ŞI DISCUŢII

Studiul de faţă arată că, în lotul studiat 57,1% au avut o infecţie cu *T. canis*, 4 7 % prezentând un titru al IgG Toxocara de 1/100. Lotul a avut o distribuţie omogenă pe domicilii şi ani de studiu (fig.4).

Valorile titrului IgG Toxocara, comparative pe sex, nu a prezentat diferenţe semnificative statistic (χ^2= 6,664, p = 0,247). Se poate constata totuşi că procentajele de determinări negative sunt asemănătoare: 42,5% - femei, comparativ cu 46,3% - bărbaţi; un procentaj aproape dublu de bărbaţi (9,8%) faţă de femei (4,6%) au avut titrul de 1/1 în timp ce un procentaj dublu de femei (21,4%) a evidenţiat 1/50.

Pe medii au fost identificate,de asemenea, diferenţe semnificative statistic în ceea ce priveşte valorile titrului IgG Toxocara (χ^2 = 14,052, p = 0,015). În mediul urban prezenţa a fost 1/100 (31,6%), urmată de 1/50 (20,0%), în timp ce în rural mai frecventă este determinarea 1/50 (20,7%), urmată de 1/100 (17,2%).

Un rol major în transmiterea bolii l-a avut prezenţa câinelui în gospodărie. Se poate afirma că există o influenţă semnificativă a prezenţei animalelor în gospodărie asupra frecvenţei cazurilor cu titrul Toxocara pozitiv (χ^2 = 50,409, p = 0,000). Astfel, cel mai mare procentaj de determinări negative a fost înregistrat în rândul persoanelor care nu au animale în gospodă-

rie (55,3%), iar cel mai scăzut procentaj a fost identificat în rândul celor care dețin doar pisici (25,0%). De asemenea, tot la posesorii de pisici se observă un procentaj foarte mare de pacienți (50,0%) pentru care a fost identificată valoarea 1/5 la posesorii de câini determinările 1/50 şi 1/100 sunt în procentaje egale (26,6% din total), iar la cei care dețin atât câini cât şi pisici cea mai frecventă a fost determinarea 1/100 (40,7%).

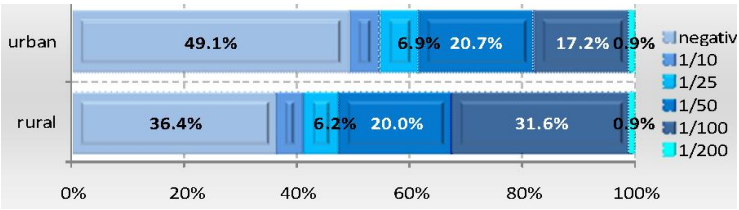

Fig. 4. Valorile comparative ale titrului IgG / *T.canis* pe mediul de origine

Datele epidemiologice, statistice şi de laborator au evidențiat ca sursă câinele. Sursa de apă potabilă nu reprezintă, cel puțin în lotul studiat, o cale de transmitere a infecției.

Seroprevalența infecției cu *T. canis* în cercetările noastre a înregistrat valori superioare celor semnalate de alți autori (7,8), dar în România nu s-au realizat cercetări pe pacienți atât din mediul urban cât şi rural şi care să nu aibă o imunodepresie secundară unei alte boli cronice, cum ar fi infecția cu HIV.

CONCLUZII

Rezultate studiului au evidențiat rolul major de sursă pentru *T. canis*, a câinelui şi transmiterea prin contact direct cu acesta.

Datele obținute pot sugera importanța unui studiu național privind seroprevalența, pentru elaborarea unor programe de eradicare a acestei zooantroponoze.

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CONTRIBUȚII LA CUNOAŞTEREA CLINICO-BIOLOGICĂ A LUPUSULUI ERITEMATOS SISTEMIC ŞI RELAȚIA CU INDICATORII DE ACTIVITATE ŞI DISTRUCȚIE TISULARĂ

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CLINICO-BIOLOGICAL ISSUES OF SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS (Abstract): **Aim:** to investigate clinical and biological aspects and to evaluate potential relation with activity and damage indexes in patients with Systemic Lupus Erythematosus (SLE). **Material and methods**: retrospective observational study in 30 consecutive SLE patients (ACR 1997 diagnostic criteria) assessed according to a standard protocol including demographic, clinical and biological (hematology, inflammatory, immunology) data, disease activity (SLEDAI) and damage (SLICC/ACR). **Results**: 90% SLE women with a mean age of 45.53±13.57 years and a mean age at onset of 31.10±9.20 years, presenting mainly with skin (43.33%) and small joint (56.66%) involvement, but also with renal (26.66%), cardio-vascular (26.66%) and neurological (6.66%); high anti-DNA double stranded antibodies (30% cases; mean 40.63±71.62IU/L) and low C3 levels (26.66%; mean 100.63±26.06 IU/L) have been reported, while more than 76% SLE patients displayed a low disease activity (mean SLEDAI 4±3.64) and limited damage (mean SLICC/ACR 1.4±0.85). Statistically significant correlation ($p<0.05$) have been identified between SLEDAI and SLICC/ACR ($r=0.477$), anti-DNA double stranded and SLEDAI ($r=0.515$) respectively SLICC/ACR ($r=0.404$), age at onset respectively current age and SLICC/ACR ($r_1=0.495$, $r_2=0.468$). **Conclusions:** The clinical and biological study based on data from consecutive SLE patients has offered a comprehensive approach of different disease subtypes in North-East Romania. The predominance of currently low disease activity and minimal damage disease profile could reflect either SLE particularities or the expected effects of early individualized therapy. **Key words**: SYSTEMIC LUPUS ERYTHEMATOSUS, CLINICAL MANIFESTATIONS, DISEASE ACTIVITY, DISEASE DAMAGE.

Lupusul eritematos sistemic (LES) este o condiție inflamatorie cronică, prototipul bolilor autoimune, numeroşi factori de ordin genetic, imunologic, hormonali şi de mediu fiind implicați în patogenia complexă a acestei afecțiuni. Cu o prevalență majoritară la sexul feminin în decadele treicinci de vârstă, boala prezintă un tablou clinic polimorf, cu afectare multisistemică ce se remarcă prin frecvența crescută a

manifestărilor cutaneo-mucoase, musculo-scheletale, renale, cardio-vasculare, pulmonare, neuro-psihice şi gastro-intestinale (2, 3). Bulversarea imună caracteristică bolii reține atenția printr-o paletă largă de auto-anticorpi, de la cei cu specificitate ridicată pentru lupus (anticorpi anti-ADN dublu catenar, anticorpi anti-Sm), la cei raportați şi în alte patologii reumatismale cu determinism imun (anticorpi antinucleari totali, anti-Ro/SS-A şi anti-La/SS-B, anticorpi anti-fosfolipidici), cu rol esențial în guvernarea proceselor patologice definitorii pentru LES (1). Mai mult, recentele progrese în înțelegerea imunopatogeniei afecțiunii au permis introducerea de noi instrumente de apreciere a activității şi distrucției tisulare, precum şi identificarea de noi ținte terapeutice.

Studiul de față şi-a propus analiza aspectelor clinice şi paraclinice precum şi analiza spectrului de relații dintre tabloul clinic, activitatea bolii şi indicele lezării organice la pacienți cu LES.

MATERIAL ŞI METODE

Am realizat un studiu observațional retrospectiv pe un lot de 30 pacienți consecutivi diagnosticați cu LES (criteriile *American College of Rheumatology* -ACR 1997) aparținând județelor din regiunea de Nord-est a țării, spitalizați în Clinica Reumatologie, Spitalul Clinic de Recuperare Iaşi în intervalul ianuarie-martie 2011.

Pacienții au fost evaluați conform unui protocol standard ce a inclus date demografice (sex, vârsta la debutul bolii şi la examinare), parametri clinici şi paraclinici (hematologici, inflamatori, imunologici), indicele de activitate al bolii (*Systemic Lupus Erythematosus Disease Activity Index,* SLEDAI), gradul de distrucție tisulară (*Systemic Lupus International Collaborating Clinics/American College of Rheumatology,* SLICC/ACR), precum şi particularitățile terapeutice. SLEDAI reprezintă un indice compozit de evaluare a activității bolii raportat la ultimele 10 zile, ce cuprinde 24 de variabile clinice şi de laborator, cu valori cuprinse între 0 şi 105, scorurile înalte definind activitatea înaltă a bolii. SLICC/ACR este un indice global de evaluare a gradului de distrucție tisulară la nivelul a 12 sisteme/organe, evidențiind modificări ireversibile cu impact pe termen lung asupra mortalității în lupus.

Studiul s-a desfăşurat conform normelor etice în vigoare. Studiul statistic (descriptiv, analitic) s-a realizat cu ajutorul programului *SPSS 19.0.*

REZULTATE ŞI DISCUȚII

*Date demografice***.** Lotul studiat a fost alcătuit majoritar din femei (90%) aparținând mediului urban (75%), cu o vârstă medie de 45,53±13,57 ani şi vârsta medie de debut a bolii de 31.10±9.20 ani, profil ce corespunde datelor raportate in literatură (1, 2, 3). Repartiția pe grupe de vârstă a demonstrat procente comparabile pentru decadele cinci şi şase (22% respectiv 25%), în timp ce unul din cinci pacienți cu lupus s-a încadrat în categoria de vârstă sub 40 ani (20%).

*Tablou clinic***.** Frecvența diferitor manifestări clinice specifice lupusului raportat la criteriile de diagnostic ACR 1997, incluzând patologia musculo-scheletală, cutanată, cardio-vasculară, renală şi neurologică, precum şi repartiția acestora pe grupuri (tab. I).

Manifestările cutanate (fotosensibilitate, *rash* malar "în fluture") şi cele musculo-scheletale (poliartrita non-erozivă afectând articulațiile mici ale mâinilor, bilateral şi simetric) au fost cel mai frecvent raportate

în lotul studiat, reprezentând 43.33% şi respectiv 56,66%, fiind prezente încă din etapa de debut a afecţiunii şi înregistrând severitate medie la momentul studiului; nici un pacient nu a prezentat afectare severă de tipul serozitei, manifestărilor psihiatrice sau renale active la evaluarea actuală. Mai mult, nu s-a raportat implicarea sistemelor gastro-intestinal, ocular şi nu au fost depistate adenopatii. Pentru definirea afectării cardio-vasculare au fost urmărite modificările ECG (aritmii, fenomene ischemice) şi trombozele venoase profunde, fenomene depistate în 26.66% cazuri. Aproximativ un sfert din pacienţii studiaţi au asociat comorbidităţi în sfera cardio-vasculară de tipul hipertensiunii arteriale (23%) şi în sfera metabolică, de tipul dislipidemiei (20%).

Tablou paraclinic. Principalele modificări paraclinice reflectă perturbări hematologice şi de natură inflamatorie şi imunologică (tab. II).

*Anomalii hematologice***.** Reţinem prezenţa izolată a citopeniei pe toate liniile hematologice, cu distribuţie variabilă pe serii celulare, procentul de pacienţi cu anemie (26,66%) fiind superior celui cu trombocitopenie (10%) şi respectiv leucopenie (10%), în timp ce bicitopenia s-a înregistrat la 16,66% pacienţi. Peste jumătate din cazuri nu au prezentat implicare hematologică la momentul iniţierii studiului.

TABELUL I

Frecvenţa manifestărilor clinice şi distribuţia pe grupuri de vârstă în lotul studiat

Manifestări evaluate	**N=30**	**%**
Cutanat (fotosensibilitate, rash malar)	13	43,3
Artrită (poliartritănon-erozivă simetrică)	17	56,66
Cardio-vascular	8	26,66
Afectare renală (nefropatie**)**	8	26,66
Afectare neurologică	2	6,66
Afectare gastro-intestinală	0	0
Serozite (pleurezie, pericardită)	0	0

	<40 ani (N=8)	**41-50 ani (N=9)**	**51-60 ani (N=10)**	**>60 ani (N=3)**
Cutanat	2	4	6	1
Articular	3	9	4	1
Cardio-vascular	0	1	5	2
Renal	3	1	4	0
Neurologic	0	1	1	0

*Sindromul imunologic***.** Deşi LES este prin excelenţă caracterizat de un profil complex de autoanticorpi, studiul actual a luat în atenţie doar bilanţul imunologic clasic ce presupune determinarea anticorpilor cu relevanţă pentru diagnostic, propuşi în cadrul criteriilor de diagnostic, şi anume: anticorpii anti-ADN dublu catenar (anti-ADNdc), anti-Sm, anticorpii anti-nucleari totali, precum şi anticorpii anti-fosfolipidici. Se remarcă pozitivitatea titrului anticorpilor anti-ADNdc în doar 30% din ca-

zuri (valoare medie 40,63±71,62UI/l, metoda imunoenzimatică cu detecție prin fluorescență) și a anticorpilor anti-fosfolipidici în 17% cazuri. Dat fiind importanța cuantificării nivelului complementului seric și mai ales a fracțiunilor C3 și C4 în relație cu activitatea LES, am luat în atenție în studiul efectuat și acești parametri, identificând valori scăzute ale C3 în 30% din cazuri, cu o valoare medie de 100,63±26,06 UI/l.

TABELUL II

Frecvența anomaliilor paraclinice în lotul studiat

Modificari hematologice	**Nr. cazuri**	**% ***
Anemie	8	26,66
Leucopenie	3	10
Trombocitopenie	3	10
Bicitopenie	5	16,66
Modificări inflamatorii		
CRP crescut	4	13,33
VSH crescut	10	33,33
Modificări imunologice		
C3 scăzut	9	30
Anticorpi anti-ADN dublu catenar crescut	9	30

***Pentru valorile mai mici de 10, procentele sunt orientative.

Formele active de boală se caracterizează prin valori crescute ale anticorpilor ADNdc și valori scăzute ale complementului, atestând consumul (complexe imune circulante, depozite tisulare de complexe imune fixatoare de complement), dinamica acestor parametri imunologici putând cuantifica răspunsul la tratament și anunța posibilitatea instalării unui nou episod de activitate (3,4). Analiza relației între titrul anticorpilor anti-ADNdc și concentrația serică a C3 a relevat o corelație indirectă, de intensitate medie, semnificativă statistic (Pearson, r=0,36 p= 0,047), confirmând datele din literatură (11): formele de boală cu un titru înalt al anticorpilor anti-ADNdc se caracterizează printr-un nivel scăzut al complementului seric.

Sindromul inflamator. Sindromul inflamator reține atenția doar într-o treime din cazurile studiate (33,33%), dominantă fiind creșterea VSH (valoare medie 24,73±19,84 mm/oră); menționăm un titru anormal al proteinei C reactive serice (valoare medie 1,05±0,9 mg/dl) detectat în doar 13,33% din cazuri. Lupusul este caracterizat de ascensionarea VSH și valori ale CRP în interval de normalitate (12), creșterea acesteia, și în mod particular a *high sensitivity* CRP (hsCRP) fiind importantă pentru evaluarea statusului infecțios intercurent la astfel de pacienți (13). Măsurarea CRP și a hsCRP în LES este utilă în diferențierea între infecție și etapele de recădere (12), nivelul hsCRP fiind mult mai mic în puseele de activitate (13); mai mult, hsCRP poate fi considerat factor de predicție al infecțiilor active în LES (13). Cu toate că literatura de specialitate citează o corelație de intensitate ușoară, în studiul de față s-a pus în evidență o corelație semnificativă statistic, medie, directă,între valorile celor doi markeri de inflamație (Pearson, r= 0,55

p=0,01), fapt explicat de posibile infecţii subclinice neevaluate în protocolul de studiu.

Indicele de activitate SLEDAI. Cuantificarea indicelui de activitate al lupusului conform SLEDAI a indicat prevalenţa majoritară a cazurilor de boală cu activitate uşoară (76.66%), valorile medii ale SLEDAI fiind 4±3,64. La polul opus se situează formele de boală cu activitate foarte înaltă (3,33%) şi cele inactive, aflate in remisiune (6,66%) (tab. III).

TABELUL III

Indicii de activitate (SLEDAI) şi distrucţie (SLICC/ACR) şi repartiţia pe grupuri de vârstă în lotul studiat

Grupuri vârstă (ani)	Subgrupe SLEDAI- număr de pacienţi				
	0 Fără activitate	**1-5 Activitate uşoară**	**6-10 Activitate moderată**	**11-19 Activitate înaltă**	**>20 Activitate foarte înaltă**
<40	1	7	0	0	0
41-50	1	6	2	0	0
51-60	0	8	1	0	1
>60	0	2	0	1	0

Grupuri vârstă (ani)	SLICC/ACR			
	0	**1**	**2**	**3**
<40	3	3	2	0
41-50	0	7	2	0
51-60	1	2	5	2
>60	0	1	1	1

Indicele de distrucţie organică tisulară SLICC/ACR. Distrucţia tisulară caracteristică LES are în atenţie trei componente esenţiale şi anume: procesul imunologic ce defineşte boala, potenţialul advers al medicaţiei folosite, precum şi intervenţia comorbidităţilor, datele fiind cel mai bine reflectate de indicele SLICC/ACR. Studiul efectuat a relevat valori medii ale SLICC/ACR de 1,4±0,85, variind în intervalul de 0 şi 3 puncte. Patru dintre cazurile evaluate nu au prezentat leziuni distructive permanente, în timp ce marea majoritate (76,66%) au cumulat punctaje de 1 şi 2.

Studiul potenţialelor relaţii între activitatea respectiv distrucţia tisulară din lupus, pe de o parte, şi aspectele demografice, clinice şi paraclinice, pe de altă parte, a permis caracterizarea extensivă a lotului de pacienţi. Dacă parametrii demografici respectiv vârsta şi vârsta la debutul afecţiunii nu sunt relevanţi pentru nivelul de activitate al LES (p>0,05), indicele de distrucţie tisulară se corelează direct cu ambii parametri (Pearson:r=0,495, p=0,005 respectiv r=0,468, p=0,004).

Principalul aspect clinic cu relevanţă pentru indicele SLEDAI a fost afectarea cutanată (Pearson, r=0,381, p=0,038), în timp ce componenta musculo-scheletală, statusul cardio-vascular şi manifestările renale nu au fost majoritar implicate în determinismul activităţii bolii (p>0,05). Mai mult, nu a fost demonstrată semnifica-

ție statistică nici pentru relația indice SLICC/ACR - elemente clinice (cutanate, musculo-scheletale, renale, cardio-vasculare) (p>0,05).

Anomaliile hematologice, izolate sau afectând mai multe linii celulare, nu s-au reflectat semnificativ în indicii de activitate şi de distrucție tisulară analizați (p>0.05). Deşi parametrii inflamatori (VSH, CRP) şi fracțiunea C3 nu au prezentat asociere cu SLEDAI respectiv SLICC/ACR (p>0,05), nivelul seric al anticorpilor anti-ADNdc a fost considerat apanajul formelor active de boala (Pearson, r=0,515, p=0,004), dar şi un element determinant pentru fenomenele distructive (Pearson, r=0,404, p=0,002), relația fiind directă, de intensitate medie.

Dacă pentru relația anticorpi anti-ADNdc – indice de activitate SLEDAI studiul realizat se înscrie în ceea ce raportează literatura de specialitate (14), pentru ceilalți parametri imunologici (în mod particular C3) şi inflamatori (VSH, CRP) datele obținute raportate la activitatea şi distrucția din LES au fost discordante față de literatură. Astfel, o serie de cercetări recente (14) demonstrează faptul că formele de boală cu activitate înaltă definite prin scoruri crescute ale indicilor de activitate ai lupusului, în particular pentru scorurile SLEDAI-2k (*Systemic Lupus Erythematsus Disease Activity Index 2000*) şi BILAG (*British Isles Lupus Assessment Group)* se asociază cu creşterea VSH, scăderea concentrației C3 şi C4, creşterea nivelului anticorpilor anti-ADNdc (p<0,01). În plus, toți aceşti parametri imunologici şi inflamatori sunt importanți pentru evaluarea şi monitorizarea răspunsului terapeutic la pacienți cu LES (14).

Relația semnificativă directă, de intensitate medie între activitatea şi potențialul distructiv tisular al LES (Pearson, r=0,477, p=0,008), validează faptul că unul din cele trei elemente generatoare de distrucție este procesul lupic *per se*, alături de efectele adverse ale medicației şi comorbiditățile sau complicațiile bolii.

*Terapie***.** Standardul terapeutic actual al LES este reprezentat de corticosteroizi, medicație remisivă (azatioprină, ciclofosfamidă, micofenolat mofetil, antimalarice de sinteză) şi mai nou introdusa în practica zilnică medicație biologică (belimumab), alegerea schemei terapeutice optime fiind guvernată de forma şi activitatea bolii. Majoritatea pacienților monitorizați în studiul de față a primit doze joase de corticoterapie cronică (5-20 mg/zi prednison sau echivalent), antimalaric de sinteză (200 sau 400 mg/zi) şi azatioprină (50-150 mg/zi, echivalentul a 2 mg/kg corp/zi), pacienții cu afectare renală având în protocolul terapeutic un al doilea imunosupresor, respectiv ciclofosfamida (800-1000 mg/administrare, *pulse*-terapie lunară).

Tripla asociere corticoterapie-azatioprină-hidroxicloroquin a fost raportată la 40% dintre pacienți, în timp ce monoterapia cu antimalaric de sinteză a fost indicată în 16,66% din cazuri. Nici unul dintre pacienții evaluați nu a primit micofenolat mofetil sau terapie biologică.

CONCLUZII

Studiul aspectelor clinico-biologice şi terapeutice la pacienții consecutivi cu LES internați într-o clinică de specialitate a permis o cunoaştere mai aprofundată a profilului cazurilor de lupus existente în populația din regiunea de nord-est a țării. Mai mult, cuantificarea activității şi distrucției tisulare prin indici validați, SLEDAI şi SLICC/ACR, precum şi analiza spectrului de relații între manifestările clinice, bulversarea biologică şi activitatea

sau distrucția caracteristice bolii a relevat impactul diferitelor opțiuni terapeutice disponibile asupra fenomenelor patogenice caracteristice afecțiunii.

Datele obținute confirmă aspectele demografice, clinice şi biologice consacrate şi anume: frecvența crescută la sexul feminin, cu debut în etapa de vârstă fertilă, polimorfismul clinic, rolul anomaliilor imune în evaluarea activității bolii; predominanța formelor de boală cu indici de activitate şi distrucție cu valori minim modificate este interpretată fie în contextul unor subtipuri de LES cu severitate joasă si evoluție benignă, fie în contextul unei terapii patogenice corect, precoce administrate şi strict monitorizate, cu impact asupra profilului de severitate şi evolutiv al bolii.

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QUALITY OF LIFE AND FECAL CALPROTECTIN IN INFLAMMATORY BOWEL DISEASES

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QUALITY OF LIFE AND FECAL CALPROTECTIN IN INFLAMMATORY BOWEL DISEASES (Abstract) Inflammatory diseases (IBD) are chronic disorders marked by periods of activity that require repeated in-hospital care, expensive surgical treatment with multiple side effects which affect the quality of life in these patients. Mucosal healing is associated with sustainable remission and a low risk of surgical intervention in these patients. **Aim**: To evaluate the correlations between fecal calprotectin (used as a marker of endoscopic inflammation) and quality of life (assessed by means of the IBDQ-32 questionnaire). **Material and methods:** A prospective study that includes 60 patients with IBD. Fecal calprotectin and Inflammatory bowel disease questionnaire-32 were performed in all patient. **Results:** The average value of fecal calprotectin was 247.38±345.77µg/g. The average total IBDQ was 137.42±38. There are negative statistical correlations between the activity of the disease assessed by means of the UCDAI and CDAI scores in the two studied disorders and the quality of life assessed by means of the IBDQ-32 score. We noticed significant correlations between the values of fecal calprotectin and the quality of life. **Conclusions:** The activity of the disease had a negative impact on the quality of life in the patients with IBD. Clinical manifestations are more likely to affect quality of life compared to the endoscopic activity of the disease. **Keywords:** INFLAMMATORY BOWEL DISEASE, QUALITY OF LIFE, FECAL CALPROTECTIN.

Inflammatory bowel diseases (IBD) are chronic disorders marked by periods of activity that require repeated in-hospital care, expensive surgical treatment with multiple side effects which affect the quality of life in these patients. It has been reported (1) the fact that mucosal healing is associated with sustainable remission and a low risk of surgical intervention in these patients. Until recently, colonoscopy assessment was essential in identifying the endoscopic activity in these patients.

Numerous studies (2, 3, 4) have demonstrated strong statistical correlations between the value of fecal calprotectin and the activity of the disease, this being also used as predictor of remission. Presently, fecal calprotectin is also accepted in the international diagnosis and treatment guides for IBD (5, 6).

Health is a very important component of quality of life, defined from several perspectives (7). Quality of life assessment tools have been increasingly used over the last period. These tools complement clinical and biological assessments of various disorders, in assessing medical services, the patient's perspective on the disorders they suffer from and the necessary treatments, the need - as perceived by the patient - regarding their healthcare, the patient's preference for a specific treatment, as well as the patient's expectations regarding the therapeutic results (8, 9). IBDQ-32 (Inflammatory Bowel Disease Questionnaire) is one of the most widely used questionnaires in assessing quality of life (QoL).

Starting from these prerequisites, we conducted a study assessing the correlations between fecal calprotectin (used as a marker of endoscopic inflammation) and quality of life (assessed by means of the IBDQ-32 questionnaire).

MATERIAL AND METHODS

We conducted a prospective study that included 60 patients with mean age 45±14.7 years, from whom 38.3% were women diagnosed with IBD. All subjects were performed a complete clinical-biological prophylaxis one day prior to the fecal sample collection for calprotectin measurement. The study included patients with a clinical, biological, endoscopic and histological diagnosis of IBD. The patients who had previously taken non-steroidal anti-inflammatory drugs, those with non-specific colitis, or with superinfections or colon neoplasm were excluded from the study.

In ulcerative colitis (UC), the activity was assessed based on the Ulcerative Colitis Disease Activity Score (UCDAI); this consists of three elements: frequency of the stools, rectal bleeding, the physician's global assessment and endoscopic activity. Remission was defied as a Mayo score ≤ 2. In Crohn's disease (CD), the disease activity was assessed based on the Crohn's Disease activity index (CDAI). Clinical remission was defined as a CDAI score <150. Fecal calprotectin was measured through the ELISA method. The samples were collected a day before the colonoscopy. Remission was considered for a fecal calprotectin value of < 30 µg/g.

Nineteen patients were diagnosed with CD (31.66%) and 41 with UC (68.33%). Thirty patients (50%) showed disease activity (58.53% UC and 31.57% CD) (fig. 1).

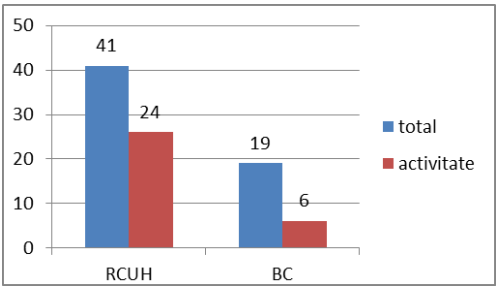

Fig. 1. Patients distribution based on disorder and activity

RESULTS

Based on lesion location, 68.21% of the patients with UC patients had pan-colitis and 29.26% had left colitis. About the patients with CD, 47.36% of patients had lesions located at colon level, 36.84% had ileo-colonic lesions and only 15.78% patients had lesions located at intestine level (fig. 2).

The average value of fecal calprotectin was 247.38±345.77 µg/g. The average total IBDQ was 137.42±38.

Analyzing the data obtained for the patients included in the study, we notice that there are negative statistical correlations between

the activity of the disease assessed by means of the UCDAI and CDAI scores in the two studied disorders and the quality of life assessed by means of the IBDQ-32 score (fig. 3).

We noticed significant correlations between the values of fecal calprotectin and the quality of life generally (fig. 4) and, in particularly in UC (fig. 5) as well as in CD (fig. 6).

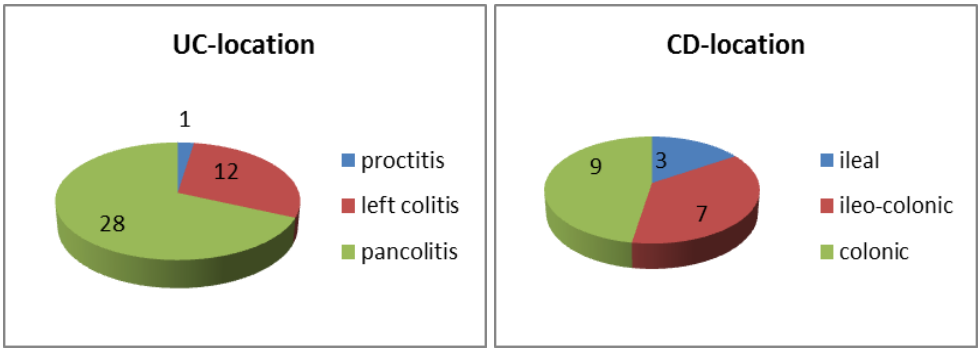

Fig. 2. Lesion location in patients with inflammatory bowel disease

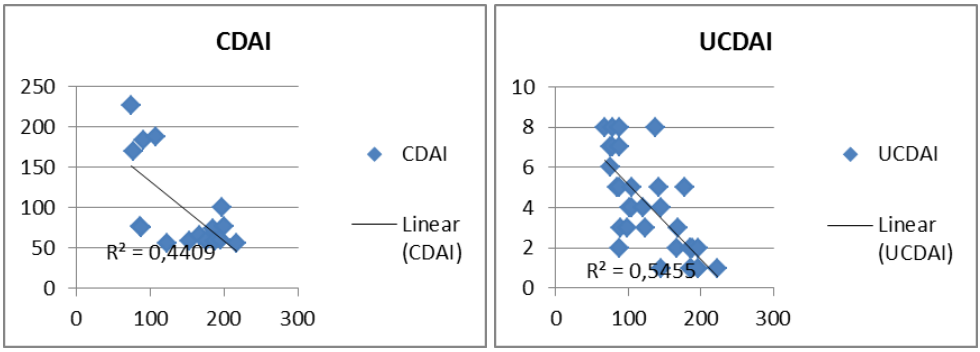

Fig. 3. Correlations between quality of life and disease activity in patients with inflammatory bowel disease

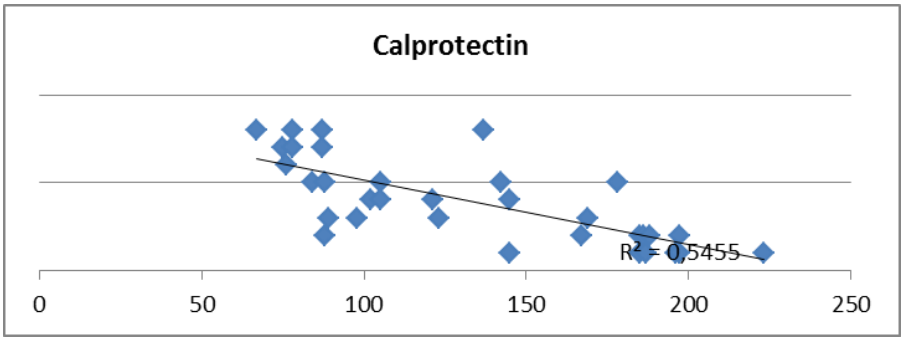

Fig. 4. Correlations between quality of life assessed by means of IBDQ-32 and the endoscopic activity of the disease assessed by means of fecal calprotectin

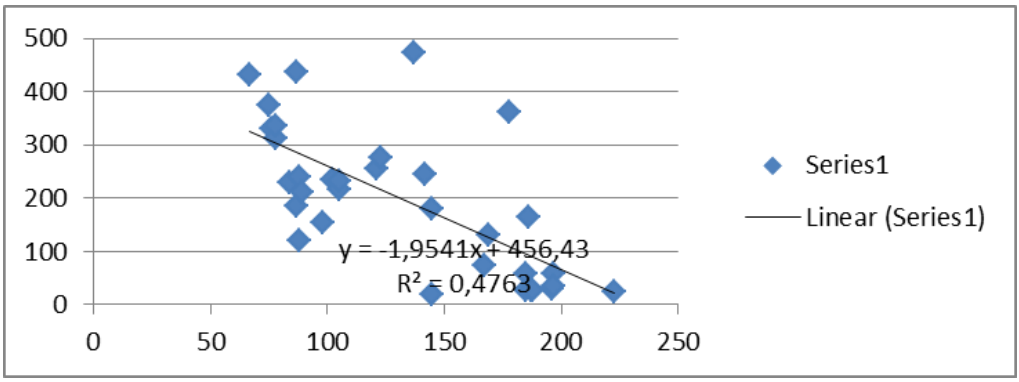

Fig. 5. Correlations between quality of life assessed by means of IBDQ-32 and the endoscopic activity of ulcerative colitis assessed by means of fecal calprotectin

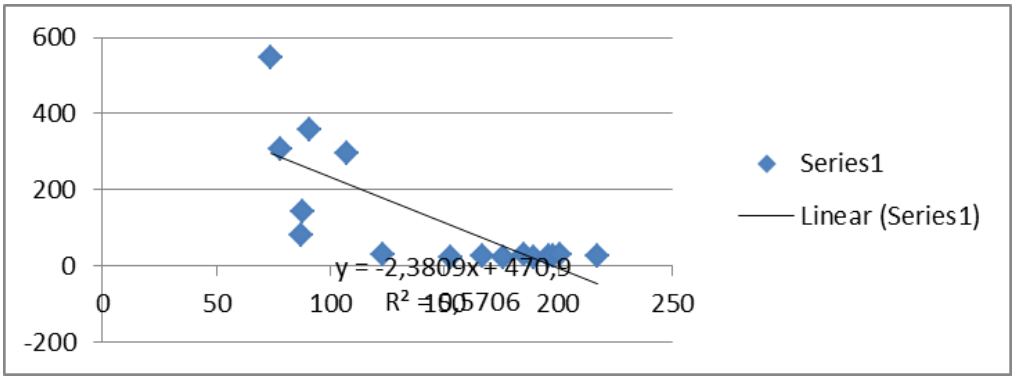

Fig. 6. Correlations between quality of life assessed by means of IBDQ-32 and the endoscopic activity of Crohn's disease assessed by means of fecal calprotectin

DISCUSSION

The concept of quality of life can only be discussed in correlation with health. If in the past, death causes and morbidities represented the main indicators of heath, presently, due to the increase in life expectancy, assessments are increasingly focused on the relationship between health and quality of life (10).

Despite its chronic evolution throughout life, IBD does not affect mortality, while it can influence he patient's morbidity and quality of life (11).

The early onset, the activity periods, the associated pathologies and their treatment are likely to impact the patients not only physically, but also by limiting their social and emotional life, their educational and professional activities. These diseases can lead in time to the impairment of their quality of life. In the studied samples, the total IBDQ-32 score varied between 67 and 207, with an average value of 137.42. To date, there is little data on establishing of a threshold of the total IBDQ-32 score. There is no defined interval within which the QoL of patients with IBD assessed by means of this instrument could be considered normal.

Compared to the study of Rubin *et al.* (12), in which the average value of the total IBDQ score was 94.1, the average value of the total IBDQ-32 score in the present study was significantly higher. Compared to other recent studies using the same tools for QoL assessment, and which reported average values of the total IBDQ-32 score of 186, respectively 159.5 (13, 14) the average value of the total IBDQ-32 score obtained in our study was lower.

Fecal calprotectin is an enterodimer present in the cytoplasm of the neutrophils. It is composed of two calcium-binding proteins. (S100A8 and S100A9) and it constitutes approximately 60% of the cytosoluble proteins of the neutrophils. Under these circumstances, calprotectin is a marker of neutrophil influx in the colon and thus, indirectly, a marker of intestinal inflammation (15).

Fecal calprotectin has been heavily studied over the last years, as a surrogate marker in inflammatory diseases about the differentiate diagnosis, the evolution of the disease, or treatment monitoring. The election method used in most of the studies was ELISA, and the cut-off values established by tests were considerably varied (16).

In our study, we used the cut-off value 30 μg/g, as the limit for predicting the endoscopic activity.

The data analyzed on the studied sample of patients confirms previous studies conducted in the clinic (17, 18). We notice a negative correlation between the quality of life and the disease activity, which translates in a more influenced quality of life in the patients with more severe forms of the disease.

About the use of calprotectin as a marker of endoscopic activity, the analyzed data demonstrated a negative correlation between it and the quality of life assessed by means of the IBDQ-32 questionnaire, lower than in the assessments performed by means of disease activity questionnaires, which shows that the endoscopic activity of the disease contributes less to the quality of life, while the clinical symptoms have a higher weight. Analyzing the two disorders separately, we notice a better correlation of quality of life with endoscopic manifestations in patients with CD compared to patients with UC. Nevertheless, the difference is not significant and, even more so, the sample of patients with CD was much smaller, and most patients were in remission.

Starting from this data, we can hope that improving quality of life and through specialized psychological intervention, we will succeed in reducing the number and severity of outbreaks. On the other hand, an efficient management of the disease can lead to significantly improved quality of life in these patients.

CONCLUSIONS

In our study, the activity of the disease had a negative impact on the quality of life in the patients with IBD. We found significant negative correlations between the activity of the disease evaluated by clinic-biological scores and the quality of life both in UC and in CD. Also, the endoscopic activity evaluated by fecal calprotectin correlates with the quality of life. Although, clinical manifestations are more likely to affect quality of life compared to the endoscopic activity of the disease.

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CORELAȚIA ÎNTRE DEFICITUL COGNITIV ŞI FACTORII DE RISC CARDIOVASCULAR LA PACIENTUL VÂRSTNIC DIALIZAT VS. NEDIALIZAT

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CORRELATION BETWEEN COGNITIVE IMPAIRMENT AND CARDIOVASCULAR RISK FACTORS IN DIALYSIS VS. NON-DIALYSIS ELDERLY PATIENTS (Abstract): The emergence or worsening of cognitive impairment is a consequence of the aging process. Geriatric depression occurs due to cognitive impairment associated with aging, and as it develops, it also affects the cognitive function. **Aim:** To analyze retrospectively over a period of 6 months the clinical parameters and biological differences of the depression and cognitive impairment in dialyzed and non-dialyzed elderly patients. **Material and methods**: There were 63 patients over 65 years included in the study (29 patients admitted to the Geriatric Department of the "C. I. Parhon" Hospital, Iaşi, and 34 patients that were in the renal dialysis program into the Transplant Centre Iaşi) that were evaluated in terms of cognitive status and level of depression through the following tests: MMSE (the cognitive impairment severity assessment), the Geriatric Depression Score, the modified Hachinski Ischemic Score (for vascular dementia). The resulting data were interpreted statistically by SPSS 12.0 software and the results were evaluated by t- Student test ($p < 0.05$). **Results**: The average age was 73.2 + / -6.1 for non-dialyzed patients group and 69.8 + / - 4.6 for dialyzed group. Body mass index (BMI), hemoglobin, glucose and lipids were similar for both categories of patients. In the dialyzed group, depression is correlated with an elevated blood triglycerides, and the vascular dementia is correlated with glucose levels ($p=0.04$). Cognitive impairment is more accentuated in the dialyzed group compared to the non dialyzed one. **Conclusions**: Elderly dialyzed people are likely to develop more frequently and more severely vascular dementia than non-dialyzed old people, probably in the context of the factors that are related with dialysis itself. **Key words**: CARDIOVASCULAR RISK FACTORS, COGNITIVE IMPAIRMENT, CHRONIC RENAL DISEASE, ELDERLY.

Înaintarea în vârstă se caracterizează prin apariția sau agravarea tulburărilor cognitive. Deficitul cognitiv uşor a devenit cel mai frecvent termen utilizat, fiind definit ca un declin silențios al funcțiilor cognitive cu conservarea activităților fizice zilnice. Acest tip de deficit cognitiv uşor se poate situa între starea cognitivă normală şi demență, majoritatea tipurilor de demență fiind precedate de o stare prodromală de deteriorare cognitivă uşoară (1).

Factorii de risc pentru tulburările cogni-

tive includ: sexul masculin şi prezenţa în antecedente a hipertensiunii arteriale, infarctul miocardic, ateroscleroza, diabetul zaharat, sindromul dislipidemic, accidentul vascular cerebral constituit sau tranzitor.

Depresia geriatrică apare pe fondul disfuncţiilor cognitive asociate înaintării în vârstă şi, pe măsură ce se dezvoltă, afectează ea însăşi funcţionalitatea cognitivă (2).

Studiul nostru îşi propune evaluarea relaţiei dintre gradul de afectare cognitivă la pacientul vârstnic cu boală cronică de rinichi aflat sau nu în program de supleere renală.

MATERIAL ŞI METODE

Studiul a fost realizat pe o perioadă retrospectivă de 6 luni, incluzând un lot de 63 de pacienţi cu vârsta peste 65 ani şi cu boală cronică de rinichi în diferite stadii, din care: 29 internaţi în Clinica de Geriatrie a Spitalului Clinic Dr."C.I.Parhon", Iaşi şi 34 în program de supleere renală în cadrul Centrului de Dializă şi Transplant Iaşi.

Au fost evaluaţi din punct de vedere al severităţii disfuncţiei cognitive prin testele: MMSE (Mini Mental Stage Evaluation), Scorul de Depresie în Geriatrie, Scorul Ischemic Hachinski modificat (pentru demenţa vasculară) (tab. I).

Evaluarea severităţii disfuncţiei cognitive se defineşte astfel: forma uşoară = MMSE > 21, moderată = MMSE 10 – 20 şi severă = MMSE < 10.

TABELUL I

Structura testului pentru lotul de pacienţi incluşi în studiu

I	*Orientare*	*Punctaj*
1	În ce an, anotimp, zi a săptămânii, zi din lună, lună, suntem	5
2	Unde ne aflăm: ţară, judet, oraş, spital, etaj	5
II	*Înregistrarea informaţiilor*	
1	Rostiti numele a 3 obiecte obişnuite (măr, masă, monedă). Între fiecare cuvânt faceţi câte o pauză de 1 secundă. Cereţi pacientului să le repete pe toate 3. Un punct pentru fiecare cuvânt corect. Repetaţi până le memorează	3
III	*Atenţie şi calcul*	
1	Numărare inversă: de la 100 scăzând 7, opriţi după 5 răspunsuri corecte Test alternativ: rostirea cuvântului AVION invers	5
IV	*Reproducerea informaţiilor*	
1	Întrebaţi cele 3 cuvinte memorate anterior	3
V	*Limbaj*	
1	Denumirea unui creion şi a unui ceas	2
2	Repetarea propoziţiei: capra neagră calcă piatra	1
3	Înţelegerea unei comenzi: luaţi o foaie de hârtie, împăturiţi-o şi aşezaţi-o pe podea	3
4	Citirea şi executarea comenzii: închideţi ochii	1
5	Scrierea unei propoziţii	1
6	Copierea unui desen	1
	Scor total	30

Factorii de risc cardiovascular analizaţi au fost: obezitatea (IMC), anemia (hemoglobina), diabetul zaharat (glicemie), sindromul dislipidemic (colesterol, trigliceride).

Datele obținute au fost interpretate statistic prin programul *SPSS 12.0* iar rezultatele au fost evaluate prin testul t-Student ($p<0,05$).

REZULTATE

Evaluări clinice şi de laborator. Vârsta medie la pacienții nedializați a fost de 73,2 ± 6,1 ani, respectiv 69,8 ± 4,6 la cei dializați (fig. 1).

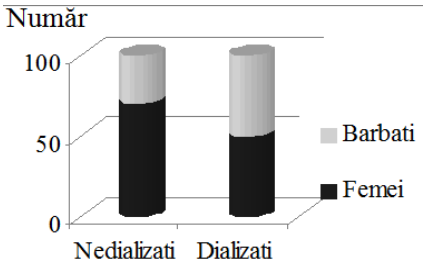

Fig. 1. Vârsta medie la cele două loturi

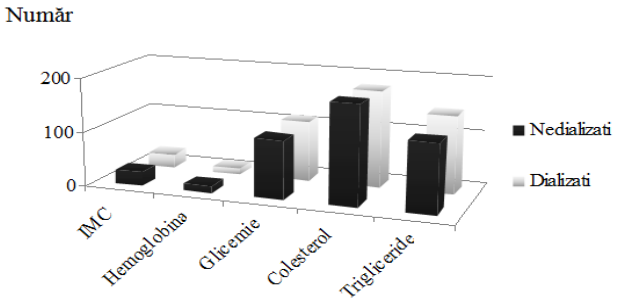

Fig. 2. Valorile parametrilor biologici comparativ la cele două loturi de pacienți incluşi în studiu

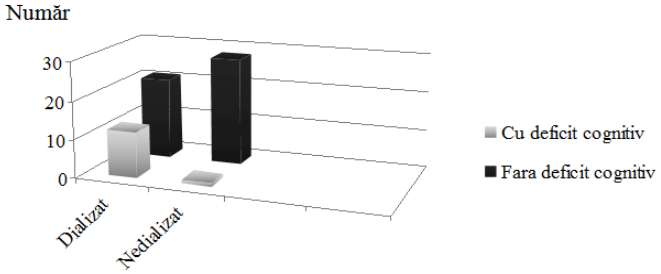

Fig. 3. Nivelurile comparative ale deficitului cognitiv la lotul dializat şi cel nedializat

La cele două loturi de pacienți, IMC-ul, valorile hemoglobinei, glicemiei şi ale lipidelor nu au prezentat diferențe semnificative (fig. 2).

În cadrul lotului dializat s-a constatat că afectarea cognitivă are niveluri de intensitate semnificativ mai ridicate, comparativ cu pacienții nedializați ($p=0,02$) (fig. 3).

Lotul dializat, conform testului Pearson, a evidențiat corelații semnificativ statistic între demența vasculară şi glicemie ($p=0,04$), respectiv depresie şi trigliceride ($p=0,04$) (tab. II).

Pe întregul lot de pacienți nivelul hemoglobinei s-a corelat cu deficitul cognitiv ($p=0,04$), aspect explicat posibil prin deficitul de vitamina B_{12} şi acid folic, ca şi cauză secundară a anemiei pacientului renal, alături de deficitul absolut sau relativ de fier, hemoliză, starea microinflamatorie, recoltări multiple, hemodializa *per se*.

TABELUL II

Corelația Pearson privind parametrii biochimici şi statusul cognitiv la lotul dializat

Statusul cognitiv	Corelația Pearson	Hemoglobina	Glicemia	Trigliceride
Disfuncție cognitivă	Total	0,151	-0,154	-0,064
	Sig. (2-tailed)	0,395	0,384	0,718
	N	34	34	34
Depresie	Total	-0,149	-0,068	**0,347***
	Sig. (2-tailed)	0,402	0,704	0,044
	N	34	34	34
Demență vasculară	Total	-0,076	**0,360***	0,218
	Sig. (2-tailed)	0,676	0,040	0,222
	N	33	33	33
*Corelația este semnificativă statistic sub nivelul 0,05 (2-tailed)				

TABELUL III

Corelația Pearson privind parametrii biochimici şi statusul cognitiv cumulativ la cele două loturi incluse în studiu

Statusul cognitiv	Corelația Pearson	Hemoglobina	Glicemia	Trigliceride
Disfuncție cognitivă	Total	**0,259***	-0,150	-0,037
	Sig. (2-tailed)	0,040	0,240	0,773
	N	63	63	63
Depresie	Total	-0,118	-0,024	**0,295***
	Sig. (2-tailed)	0,356	0,854	0,019
	N	63	63	63
Demența vasculară	Total	-0,183	0,220	0,127
	Sig. (2-tailed)	0,155	0,086	0,326
	N	62	62	62
*Corelația este semnificativă statistic sub nivelul 0,05 (2-tailed)				

De asemenea, nivelul trigliceridelor s-a corelat cu depresia (p=0,02), la fel ca şi la lotul dializat, fără a se evidenția o corelație semnificativă între statusul cognitiv şi glicemie (tab. III).

DISCUȚII

Modificările cognitive la vârstnicul cu afectare renală cronică se pot datora unor factori multipli: efectul direct al retenției azotate, nefropatia ischemică drept factor de avertizare asupra suferinței vasculare multiple, inclusiv cerebrale, tulburările asociate bolii cronice de rinichi (anemia, sindromul dislipidemic).

Mai multe studii (1, 5, 7, 9) au demonstrat afectarea diferitelor aspecte ale cogniției odată cu instalarea şi avansarea bolii cronice de rinichi, agravate odată cu inițierea metodelor de supleere renală. Date similare au fost evidențiate şi în studiu nostru, afectarea cognitivă fiind mai evidentă în lotul dializat comparativ cu cel nedializat (7,8). Urmează printr-un studiu ulterior să cercetăm şi evoluția tulburărilor cognitive după ce pacienții au fost scoşi din programul de dializă pentru a beneficia de transplantul renal. Datele existente sugerează o îmbunătățire a statusului cognitiv (3, 4, 9).

Diabetul este un factor de risc atât pentru bolile cardio-vasculare, renale cât şi

pentru cele cognitive aspect, în acord cu constatarea noastră privind unele corelații statistic semnificative între demența vasculară şi glicemie (10).

Toate aceste aspecte subliniază importanța evaluării statusului cognitiv şi al celui emoțional la pacienții vârstnici cu boală cronică de rinichi, indiferent de metoda de tratament, (supleerea renală prin dializă, inclusiv cea peritoneală sau hemodializă, tratamentul conservator sau transplant) (5, 6). De asemenea, este subliniat faptul că prezența bolii cronice de rinichi nu reprezintă o contraindicație pentru inițierea tratamentului tulburărilor cognitive, acestea având un prognostic mai bun cu cât acesta este inițiat mai devreme.

CONCLUZII

Cercetările au evidențiat că deficitul cognitiv este mai frecvent la pacientul vârstnic dializat datorită accelerării procesului de ateroscleroză, a comorbidităților (hipertensiune arterială, diabet zaharat) şi datorită asocierii în proporție ridicată a depresiei.

Nivelul hemoglobinei este corelat statistic semnificativ cu deficitul cognitiv, urmarea impactului anemiei renale cu posibilă cauză carențială secundară de vitamina B_{12} şi acid folic.

Este posibil ca pacientul nedializat să nu sesizeze deficitul cognitiv față de cel dializat, care este marcat de elementul depresiv şi care, de multe ori, ascunde deficitul cognitiv.

Este foarte importantă decelarea precoce a prezenței deficitului cognitiv la vârstnicii cu boală cronică de rinichi pentru a se iniția măsuri terapeutice adecvate ce încetinesc progresia spre demență. Prin aceste măsuri este posibil să crească perioada de independență a vârstnicului, să se amelioreze calitatea vieții şi să se reducă riscul de a dezvolta variate comorbidități.

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ASPECTE ETIOLOGICE ALE BOLII DIAREICE ACUTE- CONSIDERAŢII PE CAZURILE INTERNATE ÎN CLINICA DE BOLI INFECŢIOASE IAŞI ÎN PERIOADA 1.01. 2001-31.12.2010

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ETIOLOGICAL ASPECTS OF ACUTE GASTROENTERITIS – A TEN-YEAR REVIEW (1.01. 2001-31.12.2010) (Abstract): Acute gastroentritis is one of the most common diseases in humans, and continues to be a significant cause of morbidity worldwide. **Aims**: To determine the bacterial pathogens associated with gastroenteritis in patients admitted to the Iasi Infectious Diseases Hospital in the last ten years. **Material and methods**: A total of 40481 stool samples were examined using conventional methods. **Results**: Bacteria were found in 7.36% of cases, and parasites in 9.64%; 83% of the cases were viral, micotic, or disbiotic. The bacterial etiology was dominated by *Salmonella spp.*(58.34%), *Shigella* spp. (27.08%) , *Yersinia enterocolitica* O3 (8.53%), *Campylobacter spp*. (1.31%), other bacterial pathogens (EPEC, *Aeromonas hydrophilla*/ *caviae*/*sobria*, *Plesiomonas shigelloides*, *Bacillus cereus*, *Staphylococcus aureus*, etc) being detected in 3.74% of the cases. Of the *Salmonella species*, group B (51.99%) followed by group D (45.23%) were most common. *Shigella sonnei* and *Shigella flexneri* were found in almost the same proportion (49.45% and 49.70%, respectively*)*; *Shigella boydii* was isolated in only 0.85% of cases. The trend of gastroenteritis caused by bacterial pathogens is decreasing: from 355 cases in 2001 to 105 cases in 2010; three peaks have been recorded in 2002, 2005 (when *Salmonella typhi* was isolated in a patient), and 2008 (469, 409, and 252 cases, respectively). Bacterial gastroenteritis affected both sexes almost equally (122 males and 118 females). The most affected age groups were: 0 – 4 years, 15 – 24 years, 5 – 14 years and 25 – 39 years. **Conclusions**: Laboratory investigations are essential in determining the etiology of gastroenteritis. Its unpredictable incidence justifies the human and material efforts aimed at controlling the spread of potentially epidemic acute gastroenteritis. **Key words:** ACUTE GASTROENTERITIS, *SALMONELLA SPP*., ETIOLOGY

Bacteriile enteropatogene sunt responsabile de un număr mare de spitalizări şi decese în lume (în SUA, anual, 46 000 spitalizări cu 1.500 decese, cel mai frecvent implicate fiind *Campylobacter*, salmonelle non-tifoidice, *E. coli* producătoare de toxina shiga şi *Shigella*) (1, 2).

Scopul acestui studiu extins pe o perioadă de 10 ani, a fost acela de a releva etiologia bolii diareice acute în dinamică şi implicit, proporţia diferiţilor agenţi etiologici implicaţi, în zona Iaşi.

MATERIAL ŞI METODĂ

Analiza retrospectivă a înregistrărilor de laborator din perioada 01.01.2001 – 31.12.2010. Studiul a cuprins 40.481 de pacienţi internaţi în Spitalul Clinic de Boli Infecţioase Iaşi, în perioada sus menţionată. Materiile fecale au fost prelevate de la toţi pacienţii în mediu de transport Cary-Blair. Examinările microscopice (citologice, parazitologice, micologice) ca şi însămânţările bacteriene s-au executat în primele 2-3 ore de la prelevarea materiilor fecale. Prelevarea, transportul, examinarea şi însămânţările bacteriene s-au executat în concordanţă cu metodele standard de laborator. Prelevatele au fost însământate pe următoarele medii de cultură: Agar desoxycholat citrat lactoză, Bulion selenit acid de sodiu, Agar Wison-Blair, Agar Mac Conkey cu sorbitol, Agar TCBS şi Agar Karmalli cu supliment selectiv. Identificarea bacteriană s-a făcut utilizând medii convenţionale de identificare pentru Enterobacteriacee şi latex aglutinare pentru identificarea speciilor patogene de Campylobacter. Identitatea speciilor bacteriene patogene, a fost confirmată de Institutul Naţional de Microbiologie şi Dezvoltare „ I. Cantacuzino" Bucureşti.

REZULTATE

Etiologia bolii diareice acute a fost în proporţie de 7,36% (2.981 cazuri) bacteriană, 9,64% (3.901 cazuri) parazitară şi în proporţie de 83% de altă etiologie(virală, micotică sau disbiotică, fiind incluse în această categorie şi cazurile la care s-a instituit tratamentul antibiotic în ambulator, diagnosticul de laborator fiind în aceste situaţii, fals negativ) (fig. 1).

Etiologia bacteriană a fost reprezentată de *Salmonella* spp. în proporţie de 58,34% (1808 cazuri), *Shigella spp*. în proporţie de 27,08% (825 cazuri), *Yersinia enterocolitica* O3 în proporţie de 8,53% (114 cazuri), *Campylobacter* spp. patogene în proporţie de 1,31% (40 cazuri), iar restul de 3,74%, alte bacterii patogene (EPEC, *Aeromonas hydrophilla*/ *caviae*/*sobria*, *Plesiomonas shigelloides*, *Bacillus cereus*, *Staphylococcus aureus* etc.). (Fig. 2) Dintre salmonele , cele din grup B au fost dominante în pro-porţie de 51,99% (992 cazuri), cele din grup D în proporţie de 45,23% (863 cazuri), grupul A, C şi E fiind mai rar izolate. (Fig. 3)

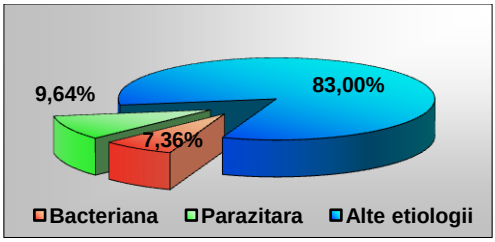

Fig. 1. Etiologia bolii diareice acute

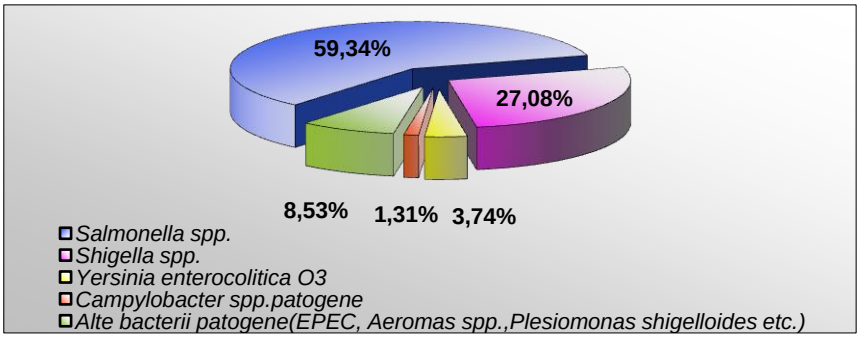

Fig. 2. Etiologia bacteriană

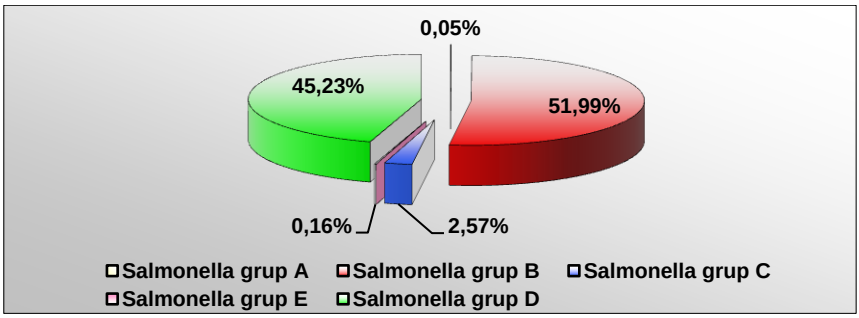

Fig. 3. Incidenţa etiologiei pe grup de salmonella

Tendinţa evolutivă a bolii diareice acute cauzată de bacterii patogene este descendentă, de la 355 cazuri în anul 2001, la 105 cazuri în anul 2010. Se remarcă totuşi trei vârfuri în anii 2002, 2005 (în acest an s-a izolat şi identificat inclusiv un caz cu *Salmonella typhi*) şi 2008 cu câte 469, 409 şi respectiv 252 de cazuri de boală diareică acută (fig. 5, 6).

Proporţia de *Shigella Sonne* şi *Shigella Flexner* a fost aproape egală, respectiv de 49,45% (408 cazuri) pentru *Shigella Sonne* şi 49,70% (410 cazuri) pentru *Shigella Flexner* iar *Shigella Boydi* a fost izolată doar în proporţie de 0,85% (7 cazuri) (fig. 4).

Incidenţa BDA de etiologie bacteriană a fost în general mai frecventă la sexul masculin, incidenţa medie în cei 10 ani studiaţi fiind de 122 de cazuri la sexul masculin şi 118 cazuri la sexul feminin (fig. 7).

Grupele de vârstă cele mai afectate au fost grupa 0 – 4 ani,15 – 24 ani, 5 – 14 ani şi 25 – 39 ani, cele mai puţine cazuri înregistrându-se la vârsta de peste 80 ani , la grupa 60 – 79 de ani şi la grupa 40 – 59 ani (fig. 8).

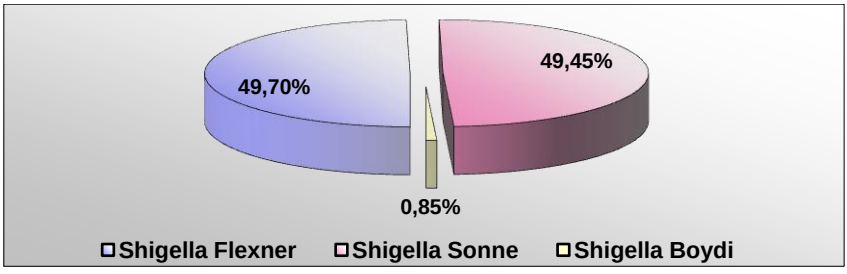

Fig. 4. Incidenţa speciilor de shigella

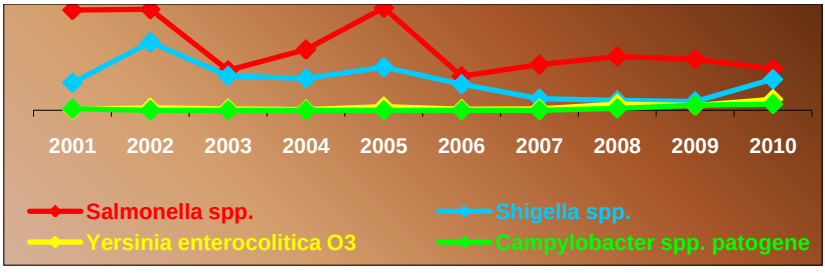

Fig. 5. Incidenţa anuală a bacteriilor patogene

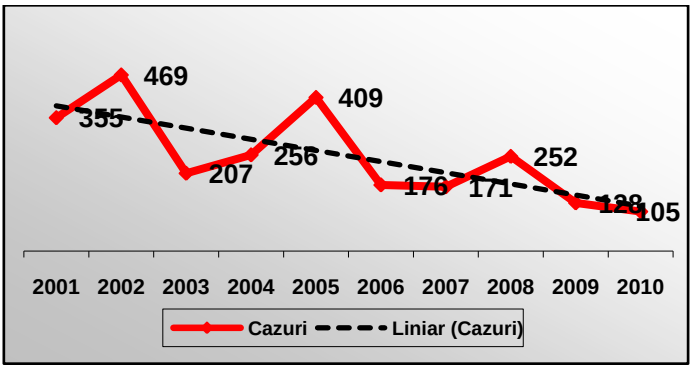

Fig. 6. Tendinţa bolii diareice de etiologie bacteriană

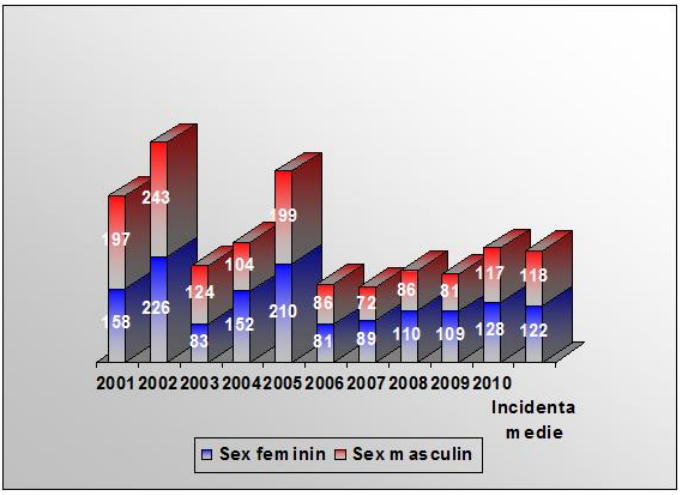

Fig. 7. Incidenţa BDA de etiologie bacteriană în funcţie de sexe

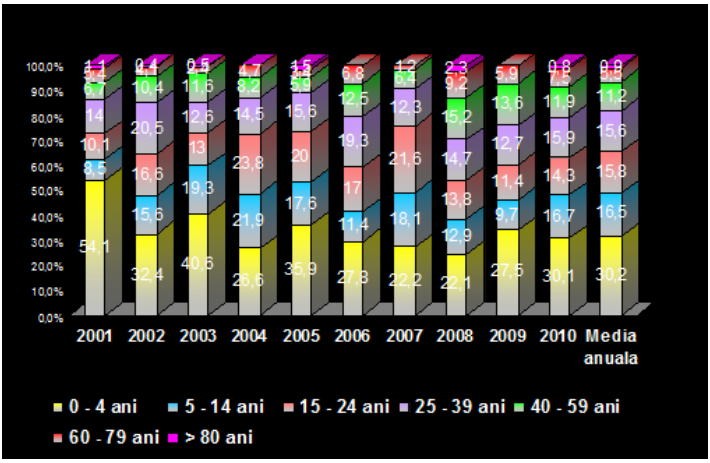

Fig. 8. Incidenţa BDA pe grupe de vârstă

DISCUŢII

Transmiterea bolii diareice se produce în proporţie de 80% cazuri prin intermediul alimentelor. Transmiterea interumană este posibilă pentru agenţii infecţioşi cu doză infectantă mică: *Shigella, E coli* producătoare de toxina shiga, protozoare şi virusuri. Clinicianul ar trebui să ia în consideraţie agenţii etiologici posibili în funcţie de tabloul clinic. Când se suspectează o bacterie enteropatogenă, examenul bacteriologic al materiilof fecale şi cercetarea toxinelor contribuie în mod crucial la stabilirea diagnosticului de certitudine. Majoritatea laboratoarelor sunt dotate pentru cercetarea de rutină a shigellelor, salmonellelor şi *Campylobacter* în coprocultură. Pentru diareea cu sânge, laboratorul ar trebui dirijat către determinarea *E coli* producătoare de toxina shiga. În diarrea asociată cu alimente marine sau de tip holeriform, ar trebui cercetaţi vibrionii holerici sau non-holerici. Indicaţiile coproculturii includ diareea severă (emisia de cel puţin 6 scaune pe zi), diareea persistentă peste 7 zile, febra, dizenteria sau o izbucnire epidemică. Coprocultura nu se recomandă în diareea apoasă sau diareea călătorului din cauza posibilităţilor etiologice restrânse. O singură coprocultură ar trebui să fie suficientă pentru un laborator competent (3).

Diareea bacteriană se poate asocia cu o serie de condiţii clinico-epidemiologice. Diareea acută apoasă este dată în principal dată de *Salmonella* şi *Campylobacter*, dar şi de *E coli* enterotoxigen, enteroagregant sau enteroinvaziv, vibrioni non-holerici sau norovirusuri (4). Tabloul clinic de dizenterie poate fi dat de *Shigella, Campylobacter*, salmonelle non-tifoidice şi *E coli* producătoare de toxina shiga, dar şi de *Aeromonas*, vibrioni non-holerici sau *Yersinia enterocolitica* (5). Toxiinfecţiile alimentare sunt date în principal de *S. aureus* şi de *C perfringens*. Diareea călătorului poate fi dată de *E coli, Shigella, Salmonella, Campylobacter, Aeromonas,* vibrioni non-holerici sau *Plesiomonas*. Diareea nosocomială este asociată cu *C difficile.*

Incidenţa mare a bolii diareice ridică o serie de probleme: transmiterea germenilor prin alimente într-o societate de consum şi o piaţă globală menţine posibilitatea unei morbidităţi şi mortalităţi ce pot deveni la un moment dat catastrofale (6). Susceptibilitatea la boala diareică (inclusiv factorii genetici şi microbiota intestinală), nu este pe deplin cunoscută (7). Factorii care influenţează riscul complicaţiilor pe termen mediu sau lung (deshidratarea, bacteriemia, sindromul hemolitic-uremic, sindromul Guillain-Barre, artrită sau irită reactivă, sindromul postinfecţios de intestin iritabil), nu au fost încă stabiliţi (1).

CONCLUZII

Investigaţiile de laborator (bacteriologice, virologice, micologice) sunt indispensabile stabilirii etiologiei bolii diareice acute şi instituirii terapiei adecvate. Incidenţa imprevizibilă justifică eforturile materiale şi umane angajate în limitarea extinderii bolii diareice acute cu potenţial epidemic şi pentru spitalele de boli infecţioase cea mai bună strategie este aceea de „expectativă armată”.

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NOUTĂȚI

NOI DIRECȚII TERAPEUTICE ÎN NEFROPATIA INDUSĂ DE CYCLOSPORINA A

Activarea sistemului renină angiotensină aldosteron (SRAA) joacă un rol important în nefropatia indusă de cyclosporina A. Scopul acestui studiu este de a demonstra că administrarea de extract de ceai verde poate preveni apariţia acestui tip de nefropatie. Pentru acest studiu au fost folositi şobolani care au fost împărţiţi în patru grupuri (6 în fiecare grup) şi trataţi 21 de zile: un grup de control (grupul 1) cărora li s-a administrat ser fiziologic, grupului 2 doar cyclosporină intraperitoneal 30 mg/kg/zi, grupului 3 cyclosporină şi extract de ceia verde 100 mg/kg/zi subcutanat iar grupului 4 doar extract de ceai verde. S-au observat niveluri plasmatice crescute de uree şi creatinină în grupul 2 comparativ cu grupul 3 şi cu grupul 1. Analizele biochimice au arătat că activitatea plasmatică a reninei şi concentraţia serică a aldosteronului au fost semnificativ crescute în grupul 2 faţă de grupul 1 şi semnificativ scăzute în grupul 3 faţă de grupul 2. Astfel, s-a demonstrat că administrarea cyclosporinei creşte nivelurile intrarenale ale reninei şi induce nefrotoxicitate. Efectele protective ale extractului de ceai verde în ceea ce priveşte nefropatia indusă de cyclosporină pot consta în blocarea SRAA. (Hyun HR, Hyun LK, Chung JH et al. Renoprotective effects of green tea extract on renin-angiotensin-aldosterone system in chronic cyclosporine-treated rats. *NephrolDial Transplant*. 2011; 26: 188-1193).

Ecaterina Enache

EFFICACY OF *HELICOBACTER PYLORI* ERADICATION THERAPY IN CIRRHOTIC PATIENTS WITH PEPTIC ULCER DISEASE

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EFFICACY OF *HELICOBACTER PYLORI* ERADICATION THERAPY IN CIRRHOTIC PATIENTS WITH PEPTIC ULCER DISEASE. (Abstract). Few studies have been focused on the role of *Helicobacter pylori* eradication in cirrhotic patients with peptic ulcer and the reported results are conflicting. **Aim:** To evaluate the efficacy of proton pump inhibitor (PPI)- based triple therapy on peptic ulcer course in patients with liver cirrhosis. **Material and methods:** This prospective study was carried out in a tertiary-care hospital. Of the 63 consecutive cirrhotic patients with peptic ulcer identified by endoscopy 39 (22 males, 14 females, aged 53 to 62 years) entered the study. The patients with peptic ulcer and *H. pylori* infection received eradication therapy, while *H. pylori negative* patients received PPI treatment. *H. pylori* eradication was confirmed by rapid urease test and histological examination. Follow-up endoscopies were performed at 6 and 12 months. The patients with peptic ulcer recurrence were treated with PPI. **Results:** Of the 39 selected patients 22 (56.4%) were *H. pylori* positive, and 17 (43.6%) were *H. pylori* negative. *H. pylori* was eradicated in 63.6% (14/22) of the patients. During the follow-up period 2 *H. pylori* negative patients died from variceal bleeding and hepatic encephalopathy, respectively, and one *H. pylori* positive patient did not return for further assessment). Ulcers recurring within 1 year were found in 14 of 36 (38.8%) patients. Peptic ulcer recurred in 4 of 21 (19.0%) *H. pylori* positive patients and in 10 of 15 (47.6%) *H. pylori* negative patients at study entry. Patients' age (p=0.018), Child-Pugh stage (p=0.019), peptic ulcer site (p=0.008), and *H. pylori* negative status (p=0.004) were significantly related to ulcer recurrence. **Conclusions:** Eradication of *H. pylori* infection in patients with liver cirrhosis and peptic ulcer disease could be helpful, but does not protect all cirrhotic patients from peptic ulcer recurrence. Most relapsed ulcers were gastric ulcers in *H. pylori* negative patients. **Key words:** PEPTIC ULCER, *HELICOBACTER PYLORI* ERADICATION, LIVER CIRRHOSIS

Peptic ulcer is a major problem in patients with liver cirrhosis and the risk of peptic ulcer is greater in these patients. (1). Factors related to peptic ulcer occurrence and the causes of the increased prevalence of the disease in cirrhotic patients are still poorly defined. Hypergastrinemia, a decrease in gastric prostaglandin E2 levels, portosystemic shunting allowing the ulcerogenic factors to escape hepatic clearance, an impairment of gastric mucosal defense secondary to portal hypertension and congestive gastropathy, alterations in gastric acid secretions have been postulated (2, 3).

In particular, the role of *H. pylori* in determining peptic ulcer disease in liver cirrhosis remains controversial; many studies have found no relationship between the two diseases, but others suggested that peptic ulcer disease is firmly linked to the *H. pylori* infection (4,5,6). It is well known that the eradication of *H. pylori* infection markedly decreases peptic ulcer recurrence in non-cirrhotic patients (7). Current strategies for the cure of *H. pylori* infection include triple therapy with a PPI, amoxicillin and clarithromycin or metronidazole (8). Moreover, eradication of *H. pylori* infection also decreases the risk of recurrence in patients with bleeding peptic ulcer (9). However, the role of *H. pylori* eradication in the course of peptic ulcer disease in patients with cirrhosis needs to be clarified. Therefore, an understanding of the determinants of the success or failure of attempts to cure *H. pylori* infection with triple therapy is clinically important in cirrhotic patients (10).

The aim of the present study is to assess the effects of *H. pylori* eradication in cirrhotic patients with peptic ulcer endoscopically detected during one-year follow-up period.

PATIENTS AND METHODS

From January 2006 to January 2011, 63 consecutive cirrhotic patients with peptic ulcer admitted at the Institute of Gastroenterology and Hepatology from Iaşi underwent to endoscopic examination in order to detect the presence of gastroesophageal varices or to investigate epigastric pain or unexplained anemia.

Patients with a history of malignancy, gastric surgery, *H. pylori* eradication, presence of significant comorbidities, active variceal bleeding, hepatic encephalopathy and those who had taken PPIs, antibiotics, bismuth compounds and/or non-steroidal anti-inflammatory drugs four weeks before endoscopy were excluded from the study.

Liver cirrhosis was diagnosed using a combination of clinical, biochemical and imagistic methods. The severity of cirrhosis was scored according to the Child-Pugh classification. The etiology of cirrhosis was defined as alcoholic, if viral markers (HBs Ag, anti-HCV) were negative and there was a history of ethanol intake of over 60 g/day for 5 years or more or viral in the presence of chronic viral infection markers.

The protocol of the study was approved by the Ethical Committee of our hospital and informed consent was obtained from all patients.

Endoscopy was performed using an Olympus GIF 240 videoendoscope. Endoscopic diagnosis of peptic ulcer disease was made when a distinct ulcer crater with fibrin-coated base larger than 5 mm was observed. The diagnosis of benign gastric ulcer was always confirmed by multiple biopsies of the ulcer.

Two biopsy specimens (one from the gastric antrum and one from the gastric corpus) were obtained to identify *H. pylori*, using the rapid urease test (CLO test) and histological examination (modified Giemsa). Results positive for *H.pylori* were defined as both a positive urease test and a typical microscopic appearance.

Enrolled patients were treated according to their *H. pylori* status: *H. pylori* positive patients received a triple therapy regimen that included omeprazole (standard dose, b.d.) combined with amoxicillin (1,0 g b.d.) and clarythromycin (500 mg b.d.) for 1 week, followed by a PPI 20 mg daily for 3 more weeks (if they were diagnosed with duodenal ulcer) or for 7 more weeks (if they were diagnosed with gastric ulcer); *H.*

pylori negative patients with duodenal or gastric ulcer received omeprazole 20 mg daily for 4, respectively 8 weeks. All patients had a second endoscopy at least 4 weeks after eradication treatment completion to assess ulcer healing and *H. pylori* status with rapid urease test and histology. *H. pylori* was considered eradicated if both rapid urease test and histologic examination results were negative. Patients with healed ulcer entered the follow-up period irrespectively of their *H. pylori* status.

Patients with unhealed ulcer were treated with a PPI 20 mg daily for a period of 4 weeks (duodenal ulcer) and 8 weeks (gastric ulcer). A new endoscopy was performed at the end of the therapy. Patients with complete ulcer healing were included in the follow-up period.

Clinical evaluations were performed monthly. Adverse effects of the therapy or symptoms indicating peptic ulcer recurrence were evaluated. The first follow-up endoscopy was performed at 6 months and the second at 12 months or earlier if they accused epigastric pain or other symptoms at any time during the follow-up. Recurrent ulcers were defined as the reappearance of ulcers during endoscopic examinations within 6 months after healing of index ulcers. Drug therapy for recurrent ulcers consisted of 20 mg PPI daily was given for 8 weeks.

Statistical analysis

Descriptive statistical values were expressed as number (%) for categorical data or median (range) for continuous data. Quantitative variables were compared according to the Student t test and qualitative variables were compared by using the chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

During the study period, 63 cirrhotic patients with peptic ulcer were screened. Twenty- four patients were excluded from the study because of systematic use of PPI within one month before endoscopy (N=7), use NSAID within one month before endoscopy (6), hepatic encephalopathy (N=5), active variceal bleeding (N=5), hepatocellular carcinoma (N=1).

Finally, 39 patients (25 male, 14 female, aged 47 to 63 years) with liver cirrhosis and peptic ulcer were enrolled in the study (Table 1). Their demographic characteristics and endoscopic findings are shown in Table I.

TABLE I

Demographic and clinical characteristics and endoscopic findings

	N = 39
Sex	
Male	25 (64.1 %)
Female	14 (35.9 %)
Age (median, range)	54 (47-63 years)
Child-Pugh class	
A	11 (28.2 %)
B	14 (35.9 %)
C	11 (35.9 %)
Etiology of liver cirrhosis	
Alcohol	17 (43.6 %)
HBV	6 (15,3 %)
HCV	16 (41.1 %)
Peptic ulcer site	
Duodenal	16 (41.1 %)
Gastric	23 (58.9 %)
H. pylori status	
Positive	22 (56.4 %)
Negative	17 (43.6 %)
Esophageal varices	
Yes	31 (79.5 %)
No	8 (20.5 %)
Portal gastropathy	
Yes	15 (38.4 %)
No	24 (61.6 %)

Among the 39 eligible patients, 22 patients (56.41%) were *H. pylori* positive and 17 patients (43.59%) were *H. pylori* negative on enrollment. Successful *H. pylori* eradication rate was 63.6% (14/22).

Eight patients (3 diagnosed with gastric ulcer and 5 with duodenal ulcer) with persistent *H. pylori* infection entered the follow-up period without receiving a second course of *H. pylori* eradication regimen.

Nine patients (40.9%) reported mild adverse effects included bitterness of taste and abdominal discomfort. However, the eradication therapy was considered safe since no serious side effects were identified during treatment.

Peptic ulcer was healed in 15/22 (68.2%) *H. pylori* positive patients. Patients with unhealed ulcer were treated with a PPI 20 mg twice daily; finally, they achieved ulcer healing after another 8 weeks of therapy.

Ulcer healing rate was higher in patients who achieved eradication than those who remained *H. pylori* positive, but these differences were not statistically significant (p=0.699) (Table II).

Among the 17 patients with *H. pylori* negative ulcers, peptic ulcer healing was found in 10 (58.8%) patients after 4 weeks of PPI 20 mg once per day treatment and the other 7 patients were cured after 8 weeks of PPI 20 mg once per day treatment.

TABLE II

Ulcer healing rate according to *H. pylori* eradication

Healed ulcer	N=15	*H. pylori* eradicated N=10 (66,7%)	*H. pylori* positive persistently N=5 (33.3 %)	p-value
Gastric ulcer	5	3 (60.0 %)	2 (40.0 %)	$\chi^2 = 0.15$
Duodenal ulcer	10	7 (70.0 %)	3 (30.0 %)	p = 0.699 (NS)

During the follow-up period, 3 patients were excluded from the study (2 patients *H. pylori* negative died from active variceal bleeding and hepatic encephalopathy, respectively and one patient *H. pylori* positive did not return for further evaluation).

Thirty-six patients (22 male, 14 female, aged 53 to 62 years) entered the follow-up period; peptic ulcer healing was noted in all patients, but 8 patients remained *H. pylori* positive despite of eradication therapy.

Recurrent peptic ulcers (12 gastric ulcers, 2 duodenal ulcers) within 1 year were found in 14 patients (38.88%).

Peptic ulcer recurrence was observed in 2 of 13 patients (15.4%) who had *H. pylori* eradicated and in 2 of 8 patients who remained *H. pylori* positive (25.0%). The ulcer recurrence did not differ in patients with liver cirrhosis whether *H. pylori* infection was present or not (p = 0.297) (Table III). Epigastric pain was noted in one patient, 3 of them were asymptomatics.

TABLE II

Ulcer recurrence rate according to *H. pylori* eradication

Reccurence	**Eradication rate**	
	Eradication	**Noneradication**
Yes N=4	2 (50.0%)	2 (50.0%)
No N=17	11 (64.7%)	6 (35.3%)
	$\chi^2 = 0,297$ p = 0,586 (NS)	

Among the 15 patients with *H. pylori* negative peptic ulcer, the recurrence was found in 10 (9 gastric ulcer, 1 duodenal ulcer) patients (66.6%).

Table IV compares baseline characteristics of patients with or without ulcer recurrence during the follow-up period.

The univariate analysis showed that patient's age (p = 0.018), severity of cirrhosis (p=0.019) (figure 1), peptic ulcer site (p = 0.008) (figure 2) and *H. pylori* negative status (p = 0.004) (figure 3) were significantly related to ulcer recurrence.

Moreover, the frequency of relapsers was increased in the presence of portal gastropathy (p = 0.016). Peptic ulcer recurrence was not related to gender or to etiology of liver cirrhosis. There were no complications during the follow-up period: this could be due to the regular timing of endoscopy which permitted early detection and treatment of the recurrence.

TABLE IV

Recurrence of peptic ulcer according to the characteristics of the patients who were included in follow-up period

	(N = 36)	**Ulcer recurrence (N = 14)**	**No ulcer recurrence (N = 22)**	**p-value**
Sex				
Male	22 (61.10 %)	10 (71.40 %)	12 (54.50 %)	0.311 (NS)
Female	14 (38.90 %)	4 (28.60 %)	10 (45.50 %)	
Age (median, range)	57 (53-62 years)	56 (52 – 61years)	54 (50 – 58 years)	0.018 (S)
Child-Pugh class				
A	11 (30.60 %)	2 (14.30 %)	9 (40.90 %)	0.019 (S)
B	14 (38.90 %)	4 (28.60 %)	10 (45.50 %)	
C	11 (30.60 %)	8 (57.10 %)	3 (13.60 %)	
Etiology of cirrhosis				
Alcoholic	16 (44.40 %)	8 (57.10 %)	8 (36.40 %)	0.411 (NS)
HBV	5 (13.90 %)	2 (14.30 %)	3 (13.60 %)	
HCV	15 (41.70 %)	4 (28.60 %)	11 (50.00 %)	
Peptic ulcer site				
Duodenal	15 (41.70 %)	2 (14.30 %)	13 (59.10 %)	0.008 (S)
Gastric	21 (58.30 %)	12 (85.70 %)	9 (40.90 %)	
H. pylori status				
Positive	21 (58.30 %)	4 (28.60 %)	17 (77.30 %)	0.004 (S)
Negative	15 (41.70 %)	10 (71.40 %)	5 (22.70 %)	
Esophageal varices				
Yes	28 (77.80 %)	12 (85.70 %)	16 (72.70 %)	0.361 (NS)
No	8 (22.20 %)	2 (14.30 %)	6 (27.30 %)	
Portal gastropathy				
Yes	12 (33.30 %)	8 (57.10 %)	4 (18.20 %)	0.016 (S)
No	24 (66.70 %)	6 (42.90 %)	18 (81.80 %)	

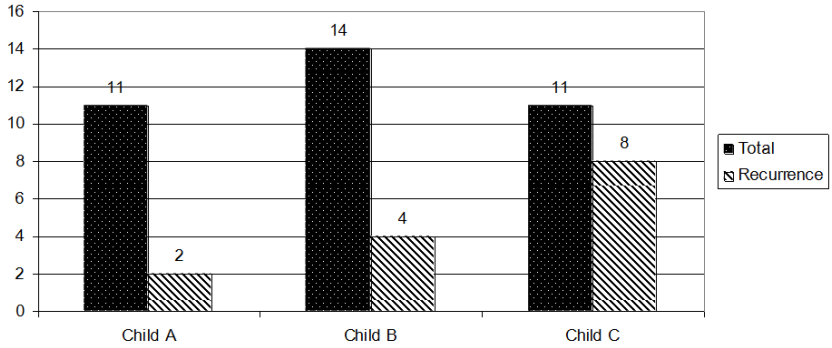

Fig. 1. Peptic ulcer recurrence according to Child-Pugh class (p<0.05)

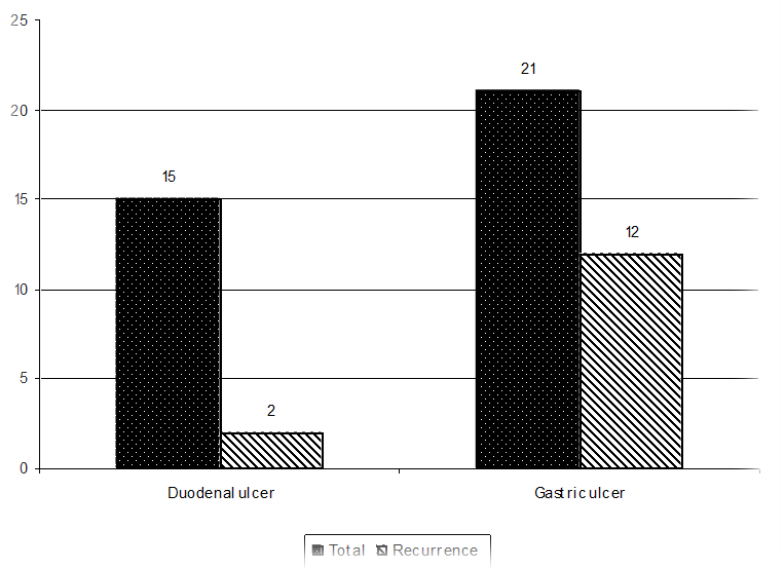

Fig. 2. Peptic ulcer recurrence according to ulcer site (p<0.05)

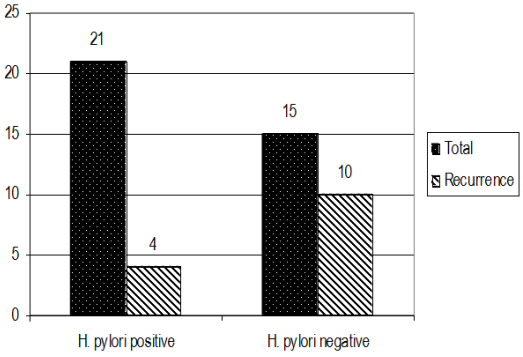

Fig. 3. Peptic ulcer recurrence according to *H. pylori* status (p<0.05)

DISCUSSION

Few data about *H. pylori* eradication and the clinical course of peptic ulcer in cirrhotic patients are avaible. Since most of patients with chronic liver disease required treatment with many drugs and the concentrations of the active metabolite of clarithromycin were found to be lower in patients with hepatic impairment, it could be difficult to choose a proper regimen for *H. pylori* eradication (11).

It is well known that eradication of *H. pylori* infection markedly changes the natural history of peptic ulcer in patients with duodenal or gastric ulcer and normal liver function. (12). In these patients, peptic ulcer recurrence can be greatly reduced from 60%-70% to 0%-10% when *H. pylori* is eradicated (7).

The impact of *H. pylori* on the recurrence of peptic ulcers in patients with cirrhosis is still uncertain. To our knowledge, the effects of *H. pylori* eradication on the recurrence of peptic ulcers among patients with cirrhosis have been investigated in only two studies (2, 9).

A recent publication from Greece reported peptic ulcer recurrence rate 41 % after successful eradication treatment and eradicated *H. pylori* infection was related with longer peptic ulcer relapse-free time. Recurrence rate was 90% (9 of 10 patients) in *H. pylori* negative patients and 44.4% (8 of 18 patients) in *H. pylori* positive patients at study entry. This peptic ulcer relapse rate is much higher as compared with that observed in the general population and could be explained by a long term of follow-up (2 years) and different course of peptic ulcer in cirrhotic patients. Another notable finding was that maintenance treatment with a PPI can effectively protect cirrhotic patients from ulcer recurrence and its complications (9).

Lo et al. detected duodenal ulcer recurrence rates 58% (21 of 36 patients) after successful *H. pylori* eradication therapy, 44% (8 of 18 patients) after failure of *H. pylori* eradication and 48% (24 of 50 patients) in *H. pylori* negative patients during 1 year of follow-up. So, the authors suggested that the policy of testing and eradication of *H pylori* universally is questionable in patients with cirrhosis and duodenal ulcers. It was also identified a statistically significant association of duodenal ulcer recurrence with the presence of portal gastropathy and the history of variceal bleeding. (2).

Our data showed that over the study period, ulcer recurrence in *H. pylori* positive cirrhotic patients was 19.0% (4/21); the ulcer recurrence did not differ in patients with liver cirrhosis whether *H. pylori* infection was present or not. We can conclude that eradication therapy does not protect all cirrhotic patients from peptic ulcer recurrence. According to our results, a subgroup of *H. pylori* positive patients may benefit from eradication therapy. Indeed, peptic ulcer relapsed in 15.4% of our patients after successful *H. pylori* eradication. Moreover, the ulcer recurrence in *H. pylori* positive patients was significantly lower than in *H. pylori* negative patients ($p = 0.04$). A strong correlation between ulcer recurrence and peptic ulcer site has been shown (gastric ulcer vs. duodenal ulcer = 85.7% vs. 13.4%, $p = 0.008$). Interestingly, we also found that the presence of portal gastropathy did seem to predispose to ulcer recurrence ($p = 0.016$). In addition, we identified a statistically significant association of ulcer recurrence with advanced stages of cirrhosis ($p = 0.019$). This agrees with a previous report demonstrating a shorter ulcer relapse-free time in patients

with Child B and C (9). Taken together, the pathogenetic mechanisms of peptic ulcer disease in cirrhotic patients appear multifactorial and are far from being elucidated.

In conclusion, eradication of *H. pylori* infection could be helpful, but does not protect all cirrhotic patients from the recurrence of peptic ulcers. Other factors than *H. pylori* could play a key role on peptic ulcer recurrence in cirrhosis such as advanced age, portal gastropathy and progression of liver disease.

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TESTING AND VALIDATION OF A NEW METHOD FOR THE INVESTIGATION OF FUNCTIONAL CHANGES IN HUMAN HEART AND SKELETAL MUSCLES

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TESTING AND VALIDATION OF A NEW METHOD FOR THE INVESTIGATION OF FUNCTIONAL CHANGES IN HUMAN HEART AND SKELETAL MUSCLES (Abstract): **Aim**: Analysis of the efficiency of the use of permeabilized fiber in clinical medicine. **Material and methods**: Mitochondrial respiration was performed in 680 patients with dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), inflammatory cardiomyopathy (ICM) and ischemic heart disease/Ischemic cardiomyopathy (IHD/Ischemic CM) with heart failure (HF) 0-IY NYHA, as well as in the deltoid muscle during fitness in athletes. Subendomycocardial and deltoid muscle biopsy, myocardial scintigraphy, quantitative immunoblotting and electrophoresis were performed. **Results**: Scintigraphy revealed left ventricular (LV) perfusion defects of 85.6% in Ischemic CM/IHD and 81% in DCM. The indices of myocardial hypertrophy showed a 42% increase in tubulin and 33% increase in desmin levels, and of skeletal myosin beta isoforms by 20%. SM22 was increased 1.5-fold, Kinase Related protein KRP by 52%, Myosin Light Chain Kinase (MLCK) by 70%. Nuclei were bizarre and oversized. CrKmit fraction - 12.8, MB - 27.24, in the intact myocardium, 21.08 and 19.7, respectively. Mitochondrial respiration, Vmax / Vo 2.27 ± 0.17 1.52 ± 0.08 <0.001. Tissue respiration of the deltoid muscle in athletes correlated with the amount and density of mitochondria and was related to the level of physical fitness. **Conclusions:** The study shows that the use of this method of approaching and interpreting the data obtained through the permeabilized cell technique, largely based on personal clinical results, makes it possible to identify the earliest mitochondrial functional changes and their consequences on the energy balance and mechanical function of the heart, thus validating the method as a reference in the investigation of various cardiac diseases and assessment of skeletal tissue capacity related to fitness level. **Keywords:** CARDIOMYOCYTE, MITOCHONDRIA, MITOCHONDRIAL RESPIRATION, ISCHEMIA, CARDIAC INSUFFICIENCY, CARDIOMYOPATHY.

The considerable advances in recent years in the fields of molecular biology and medical imaging in heart disease have led to the identification of new entities by a

better understanding of the mechanisms and causes of intrinsic and secondary myocardial diseases. The latest classification proposed by the American Heart Association (AHA) in 2006, reiterated and commented by the European Society of Cardiology (ESC) in 2008, is based on a more thorough understanding of molecular aspects and mutations in myocardial proteins. An imperative of this is the study of the mechanism of mitochondrial oxidative phosphorylation, ATP production and the modalities of intracellular transport of energy from mitochondria to its sites of use, in myofibrils, by phosphocreatine molecules, through the various creatine kinase isoferments. The method of recording the respiratory rate through the isolated mitochondria technique, which until recently has been used as a single procedure requires large amounts of muscle tissue (50-60 grams wet weight), making it inapplicable under clinical conditions, especially in cardiology. Recently, a new method for the study of mitochondrial function has been proposed (3) using saponin-permeabilized muscle fibers. On extremely small pieces of tissue, this technique offers the possibility of precisely controlling the extramitochondrial environment and the properties of mitochondria, which in isolation conditions may be lost. We are the first to use this method for more than two decades in the study the mitochondrial function, especially in clinical heart diseases and skeletal muscles.

MATERIAL AND METHODS

Prospective study on mitochondrial respiration, determined in 680 patients with circulatory insufficiency, stages 0-IY according to NYHA classification in various pathological conditions: dilated, hypertrophic, inflammatory, and ischemic cardiomyopathy; decompensated hypertrophy; coronary circulation and various forms of hemodynamic and intramyocardial disorders, in total – 655 patients; in acute myocardial ischemic injury during open heart surgery and in heart preservation problems – 25 patients with acquired heart defects, as well as the assessment of mitochondrial respiration in the deltoid muscle during exercise – 30 athletes. The control group consisted of 120 healthy individuals. Cardiac catheterization, coronaroventriculography, subendomycocardial and deltoid muscle biopsy, electron and confocal microscopy, myocardial scintigraphy, quantitative immunoblotting and electrophoresis were performed.

RESULTS

The coronarography study in DCM patients highlighted large, sinuous coronary arteries of typological significance. The passage of the contrast medium and its subsequent wash-out, both in the large arterial trunks and in third- and fourth-order arteries were slowed down. In patients with DCM and Ischemic CM the diameter of the coronary arteries in systole and diastole was significantly higher and the extensibility index was lower than in the control group. No clear index differences were noted between DCM and Ischemic CM patients, except for the diastolic diameter of the right descending coronary artery.

Positron emission computed tomography with TL-201 detected LV myocardial perfusion defects in DCM (81.1%), Ischemic CM (82.5%), and in 100% of ICM cases, the incidence of perfusion defects occupying over 20% of LV area; in DCM it was 35.2%, in Ischemic CM - 45.5%, and

in ICM - 69.5%. In 75% of the cases perfusion defects coincided with the localization of myocardial scarring changes, according to the data of instrumental investigation, ECG, ECOCG, CVG, necropsy, in Ischemic CM patients (tab. I).

TABLE I

Comparison of left ventricular myocardial infarction defect sizes in patients with Ischemic CM and DCM according to myocardial scintigraphy with TI-201 at rest

	Perfusion defects (% of LV area)			
	total per group	IC I-II (NYHA)	IC III-IV (NYHA)	P
DCM	n = 71 13,7 ± 1,3	n = 49 13,1 ± 1,6	n = 22 15,1 ± 2,3	NS
Ischemic CM /IHD	n = 63 14,2 ± 1,7	n = 28 9,5 ± 2,0	n = 35 17,7 ± 2,4	< 0,02
Accuracy of P differences	1-3 < 0,002 2-3 < 0,001 1-2 – NS	1-3 < 0,01 2-3 < 0,002 1-2 – NS	NS	

Expression of protein markers of myocardial hypertrophy in DCM showed an increase in tubulin level on average by 42%, in desmin by 33% and in skeletal myosin beta-isoform by 20%, in smooth muscle proteins, alpha-smooth muscle actin and SM22 (1,5-fold), KRP by 52%, MLCK by 70%, MHCβ by 21%. *The morphometric study* showed bizarre and oversized nuclei with highly elevated levels of membrane convolutions. *Electrophoretic analysis* of the total creatine kinase fraction revealed significant alterations in the expression of creatine kinase isoenzymes. The diseased myocardium had a lower mean creatine kinase content and a higher MB content compared to the healthy myocardium (fig. 1).

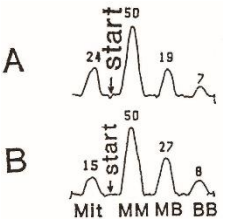

Fig. 1. Spectrum of creatine kinase isoenzyme in normal human myocardium (A) and cardiomyopathic myocardium (B).

In athletes there was a quantitative increase in mitochondrial density with a significant increase in mitochondrial respiratory capacity related to physical fitness level.

The increased cardiomyocyte mitochondrial respiration rate (Δ%) by the stimulation of *ADP* and creatine in patients with DCM HF-III-IV (NYHA) is certainly lower than that in those with HF-I-II (NYHA), demonstrating a more pronounced myocardial hypoxia in the patients with severe cardiac functional and morphological changes (tab. II).

Evaluation of mitochondrial status and efficacy of mitochondrial creatine kinase function in ischemia and after reperfusion during the surgical correction of acquired cardiac defects revealed have significant changes. More prominent was the three-fold increase of creatine-stimulated respiration, which demonstrates alteration of the mitochondrial creatine kinase system. These changes are reversible after reperfusion in the presence of phosphocreatine.

TABLE II

Indices of cardiomyocyte mitochondrial respiration in patients with DCM depending on heart failure stage

	HF – III (NYHA)	*HF* – IV (NYHA)	Accuracy of differences P
Basal mitochondrial respiration rate Vo ng atom O/min/mg dry weight	1,91 ± 0,29	3,86 ± 0,23	< 0,001
ADP-stimulated respiration rate 0.1 mM ADP VADP ng atom/ min/mg dry weight	3,77 0,47	5,13 ± 0,32	< 0,02
Δ %	119,1 ± 30,3	33,1 ± 2,8	< 0,01
Creatine-stimulated respiration rate 30 mM VCR ng atom 0/min/mg dry weight	7,38 ± 0,73	7,05 ± 0,42	*NS*
Δ %	95,8 ± 3,2	37,7 ± 1,1	< 0,001
Maximal ADP-stimulated respiration rate mM ADP Vmax ng atom 0/min/mg dry weight	10,72 ± 0,46	8,77 ± 0,50	< 0,01
Δ %	61,0 ± 23,9	24,9 ± 3,2	*NS*
Inhibition of ADP-stimulated respiration with CAT - Carboxyatractyloside ng 0/min mg dry weight	3,05 ± 0,65	2,96 ± 0,45	*NS*
Δ %	- 72,5 ± 5,8	- 66,5 ± 4,1	*NS*
Vmax / Vo	2,27 ± 0,17	1,52 ± 0,08	< 0,001

DISCUSSION

The success of models used to study differentiated complexity should be understood sequentially when applied to sufficiently large domains (1,2).

The topic that we have assumed as a sphere of interest and an object of a long-lasting study is mitochondrial respiration, a new method for the investigation of functional changes in human heart and skeletal muscles. By means of relating the subject to the whole issue, as well as to its structural connections with other adjacent problems, we are aimed at highlighting, with the help of combining the descriptive method with analytical and interpretative ones, the rich diversity and complexity of the topic which seems to us extremely important because of its considerable scientific and moral significance.

The permeabilized fiber method (3), used by us, proved to be very well suited to studying the functioning of mitochondria in patients with DCM, ICM and Ischemic CM; in decompensated cardiac hypertrophy; in acute myocardial ischemic injury and in heart preservation-related issues, as well as in the assessment of deltoid muscle mitochondrial respiration during physical fitness in athletes (4-8). In the latter, in addition to the stimulation of mitochondrial quantitative growth and density, training improves mitochondrial qualities as shown by physical fitness-related increase in mitochondrial respiration. Higher oxygen extraction in the working muscle reduces glycolysis and the

use of glycogen with the increase of aerobic resynthesize of ATP during exercise, decisive for delaying fatigue and ensuring performance. Mitochondrial function declines with age and may contribute to the loss of muscle strength and cardiorespiratory endurance. Lower mitochondrial capacity and efficacy are associated with a lower walking speed. At the same time, older adults who maintain a high level of physical activity have a greater mitochondrial capacity, like that of the very active young adults (9, 10). This suggests that by the assessment of the bioenergetic status mitochondria may be determinant in predicting performance in certain physical fitness situations, and an important therapeutic target for sedentary lifestyle- and aging-related conditions including sarcopenia, dynapenia and loss of physical function (11, 12). In most of the patients with primary myocardial diseases investigated by us there were coronary and/or intracardiac circulation disorders which confirmed the presence of chronic myocardial hypoxia and its role in primary and/or secondary cardiomyocyte injury. An evidence of this is also the cardiomyocyte mitochondrial respiration rate (Δ%) by stimulation of ADP and creatine in patients with HF-III-IV NYHA and in those with more marked cardiomyocyte hypertrophy and bizarre nuclei, which is certainly lower than in HF-I-II NYHA, Vmax / Vo 2.27 ± 0.17 1.52 ± 0.08 < 0.001. In the ischemic heart, the fastest cellular change is mitochondrial swelling which causes the disruption of the outer mitochondrial membrane or the dissociation of creatine kinase from the inner mitochondrial membrane. The lesion effect on the membrane is mainly exerted by lysophosphatidic which increase ischemic cardiomyocytes. Phosphocreatine completely inhibits the elevation of lysophosphatidylcholine and lysophosphatidylethanolamine in the sarcolemma membrane of the ischemic myocardium which may be one of the possible mechanisms that protect the adjacent membrane of contractile cardiomyocytes adjacent to phosphocreatine. For this reason, creatine-stimulated respiration is a very sensitive indicator of ischemic disease and, at the same time, of the efficacy of heart protection in heart conditions. The study of the aspects of myocardial morphological alterations in patients with cardiomyopathy and measured cellular creatine kinase function revealed low PCr/ ATP ratio only in DCM patients with severe heart failure due to insufficient ATP formation. To the same extent, the reduced mitochondrial creatine kinase function can be combined with low myofibrillar activity of ATPase in the diseased heart (5, 6). This assumption is confirmed by the decrease by up to 57% of creatine content in the DCM patients with severe heart failure and pronounced cardiomyocyte hypertrophy. The increase of end-diastolic pressure in our DCM patients can be largely related to the increase in myofibrillar ADP due to its reduced rephosphorylation resulting from the decreased efficiency of phosphocreatine pathway for energy transport. ADP in excess amounts is sufficiently tightly bound to myosin with the concomitant inhibition of ATPase activity and dissociation of cross-bridges. This may be the cause of the increase of end-diastolic pressure and decrease of ejection fraction in DCM patients with severe heart failure and pronounced hypertrophy, with larger cardiomyocyte nuclei of unusual shapes.

CONCLUSIONS

Our study demonstrates that the use of this method of approaching and interpreting the data obtained through the permeabilized cell technique, largely based on

personal clinical results, makes it possible to identify the earliest mitochondrial functional changes and their consequences on the energy balance and mechanical function of the heart, thus validating the method as a reference in the investigation of various cardiac diseases and assessment of skeletal tissue capacity related to fitness level.

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THE ROLE OF REAL-TIME ELASTOGRAPHY IN THE EVALUATION OF POST CHEMOTHERAPY HEPATHOTOXICITY IN CHILDREN WITH CANCER

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THE ROLE OF REAL - TIME ELASTOGRAPHY IN THE EVALUATION OF POST CHEMOTHERAPY HEPATHOTOXICITY IN CHILDREN WITH CANCER (Abstract) : The drugs hepatotoxicity represents a major problem of the iatrogenic pathology, with various manifestations, directly through the hepatotoxic effect or through idiosycrasy reactions. The hepatic affection induced by chemotherapy appears in children in cases of prolonged therapy, chronic diseases, or other associated conditions. Hepatotoxicity clinically develops through hepatic disorder, cholestatic or mixed hepato-cholestatic manifestations and systematic affection. There are no specific hystological or biochemical characteristics for diagnostic of hepatotoxicity. The international criteria for asessing the hepatotoxicity includes the bilirubin, the transaminasis, GGT, FA, albumin and the flow on the vein. It has been noticed that these parameters are not enough for the right assesssment of the chemotherapics' hepatotoxicity. Thus it is required the abdominal ultrasonography and computerised tomography for the identification of billiary tract, vascularisation, associated conditions and the degree of fibrosis ; also, the hepatic biopsy may be necessary. The ultrasound elastography is a method which can give information related to the elasticity/stiffness of the examined tissue and degree of fibrosis. Acustic radiation force imaging(ARFI) is an elastographic method which allows valid, accurate and flexible evaluation of liver stiffness, a quantification with a strong correlation with the fibrosis stage, not influenced by steatosis. In conclusion, the hepatic toxicity showed by alterated hepatic biochemical tests and by symptomes of hepatopathy needs a proper appreciation of the hepatic modifications, which can be obtained through hepatic biopsy or by assessing the hepatic elasticity through elastography. Thus, real-time elastography is an useful tool in assessing the chemotherapics hepatotoxicity in children with cancer. **Key words** : ELASTOGRAPHY, HEPATOTOXICITY, CANCER

The drugs hepatotoxicity represents a major problem of the iatrogenic pathology, because of a great increase of the number of drugs with toxic action on liver, and also the fact that the drug hepatopathology includes various clinic, biological and histological manifestations which can get the aspect of any kind of hepatobilliary disease, acute or chronic, and histologically necrosis, steatosis, fibrosis and vascular injury (1, 2, 3).

The hepatic toxins can lead in a great number of cases to hepatic fulminating, di-

rectly through the hepatotoxic effect or through idiosycrasy reactions (4).

Chimiotherapies against cancer, known as oncostatic agents, drugs against tumours, cytostatics are substances capable to improve the clinic condition, to decrease the tumour volume, to prolong the surviving period and sometimes to heal the patients with cancer affections, on the other hand the main effect of the oncostatic agents is cytotoxicity beside one or more aimed organs. (5)

The cytostatic treatment inevitably affects the liver, which is one of the tissues with the most intensive metabolic activity of the organism. In normal condition these effects are clinically unseen because of the liver's capacity of regeneration. Though the hepatic affection induced by chemotherapy appears more often in children in cases of prolonged therapy, debilitated chronic diseases, immunosupresion, viral hepatitis, malnutrition, infections, total parenteral nutrition. (3)

On the other hand we must take into consideration that the hepatic affection during the cytostatic treatment can be owed not only to it but also to other treatments that these patients benefit of: antibiotherapy, analgesics, antithermics, and so on (3, 6, 7).

Many of the chemotherapies can lead to hepatotoxicity, having an idiosincrasis action, due to the immunologic mechanisms or due to the variation of the host's metabolic response. These reactions do not typically depend on the dose. Less common, hepatotoxicity depends on the dose. Pre-existant hepatic disease leads to a more reduced toxins elimination and later it determines hepatic lesions up to cronic hepatitis and cirrhosis. (3, 8, 9)

The hepatotoxicity diagnosis is sometimes difficult, because the drug toxicity can mime any other hepatic disease. The estimation of the hepatic function and the hepatic injuries is indirectly made, the recognition of the hepatic injury sometimes being doubtful. There are no specific hystological or biochemical characteristics of a hepatic affection induced by drugs, and in many cases there are not simple and sure methods for the etiologic diagnosis. (3)

Hepatotoxicity **clinically** develops through *hepatic* disorder – signs and symptoms specific to hepatitis (fatigue, anorexia, nausea and increased transaminasis, sometimes asymptomatic, just the transaminasis being increased), *cholestatic manifestations* (jaundice, pruritus, the increase of alkaline phosphatase and a medium increase of the transaminasis), *mixed hepatic and cholestatic manifestations* and also shapes with *systematic affection* (fevre, Stevens-Johnson syndrom, renal dysfunction, myocardial disorder). (10)

The antineoplasis drugs used in the malignant treatments are divided in: alkilant agents (mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil), nitrozoureas (carmustine (BCNU), lomustine (CCNU) and streptozotocin), antimetabolites (currently in clinical use include cytosine arabinoside (ara-C), 5-fluorouracil (5-FU), 6-mercaptopurine, azathioprine, 6-thioguanine, methotrexate and gemcitabine), antitumours antibiotics (doxorubicin, daunorubicin, mitoxantrone, bleomycin, mitomycin, mithramycin (plicamycin) and dactinomycin), spindle inhibitors, topoisomerase inhibitors, platinums, interferon, and so on. In Chart no. I we mention the drugs more frequent associated with toxicity upon liver and the main consequences of hepatotoxicity. (3, 11-18)

The combined chemotherapy produces a higher hepatotoxicity by intensifying the effects thus determining changes from simply increasing the transaminasis, bilirubin, alkaline phosphatases to hepatic colestasis, hepatocellular necrosis, hepatic regenerative nodullary hyperplasia (combination of busulfan with 6-tiogunin), hepatic vein-oclusive disease, toxic hepatitis, hepatic cirrhosis and also hyperamoniemia. (3, 19, 20)

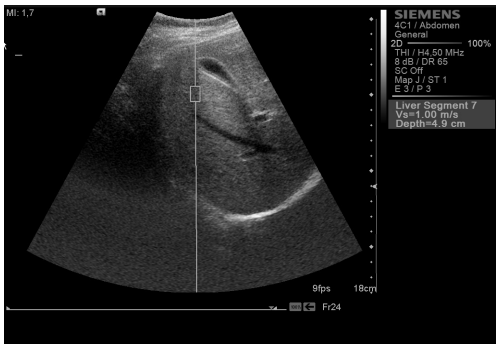

Fig. 1. Real time elastography in a normal liver to a five years old boy (personal collection)

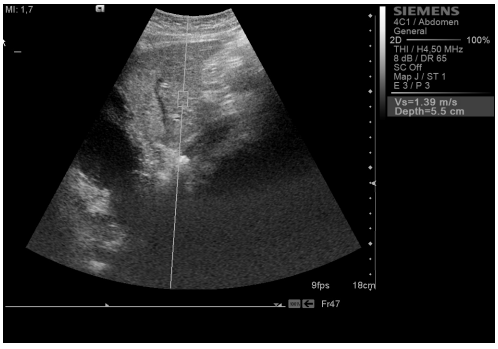

Fig. 2. Elastography of the left liver lobe to on obese girl (personal collection)

MATERIAL AND METHOD

The international criteria for asessing the hepatic toxicity according to the national Institute of Cancer includes the bilirubin, the transaminasis, GGT, FA, albumin, the flow on the vein (3).

Starting with the international studies (3, 6, 7) and those of our clinic on a number of 78 patients with different malignities treated with different treatment schedules including more cytostatics (1), it has been noticed that these biochemical parameters are not enough for the right assesssment of the chemotherapics' hepatotoxicity. Thus it is required the abdominal ultrasonography and computerised tomography, which are necessary for the identification of billiary tract, vascular system, tumoral associated conditions and the degree of fibrosis associated to the therapy. We mention that the hepatic biopsy may be necessary in order to appreciate the degree of hepatotoxicity. (3)

The ultrasonography examination which has a special place, is a noninvasive method for investigation, unpainful, repetitive and which allows a good exploration of the tissues and organs, in adults and children too.

The manual palpation was used a long time ago too in order to offer a qualitative evaluation of the tissues' stiffness. The need of a direct image led to the development of the imagistic methods ; "the palpation" through ultrasound (ultrasonography) is used for a longer period and the evolution of the technique concomitant to the increase of the necessity for the quantification of the pathological changes connected to the presence of an abnormal tissue, compared to the surrounding tissues (21) it led to the development of the ultrasonographic elastography.

The ultrasound **elastography** reffers to ultrasound methods which can produce images or measurement of tissue elasticity - the ability to resist to deformation, the application of tissue deformation on its strengths. The force applied to the tissue can be (22) the force applied by the ecografist's hand, the force applied by an external mechanical device, the force given by the physiological movements of the human body - breath, the cardiac activity, pulsed movements of large arteries or the force applied by a certain ultrasound issued by the ultrasound probe which makes the exploration itself**.** The response to the application of this force will vary according to the tissue elasticity.

This new method of diagnosis gives information related to the elasticity/stiffness of the examined tissue, degree of tissue fibrosis (eg. pre-stage liver cirrhosis), respectively the degree of stiffness of a tumor tissue compared to the free one, with promising results to differentiate between benign and malignant lesions or in assessing the

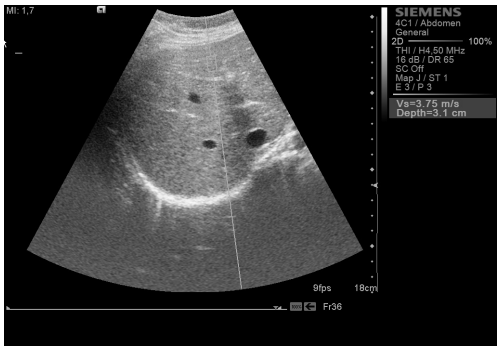

Fig. 3. Real time elastography to a child with three degree of fibrosis (personal collection)

tissue changes over time. (22)

There are different mechanisms of producing acoustic radiations and a lot of biomedical applications, especially in medicine, as diagnosis methods, in ultrasounds domain. (23)

The electromagnetic waves and the acoustic ones are capable to exert an equilibrum force called radiation force, this being a universal phenomina at any moving wave. A major interest in different biomedical applications of the acoustic force of radiation started in 1990 and it still works nowadays ; thus new applications have appeared, including the evaluation of viscous-elastic properties of the fluids and of the biological tissues, the imagery of the elasticity, molecular imagery and others. (24)

ARFI – "acustic radiation force imaging" (the diffusion impulse of the acoustic force) beside the elastography through compression "***eSie touch*** elasticity imaging" is a method which analyses the tissue area through ultrasound elastography. **ARFI** consist in generating an acoustic impulse in the tissue in order to assess/ interogate the mechanical properties of the tissue stiffness/ elasticity. The method has 2 possibilities of evaluation :

- ***Virtual touch tissue imaging*** – a method through which a diagram of the tissue stiffness is obtained, with a gray scale (reverse as the classical examination 2D) ;
- ***Virtual touch tissue quantification*** through which it is appreciated the speed of the transverse propagation of the waves triggered, allowing the quantification of the tissue elasticity.

The elastography methods previously available needed a manual compression of the tissue, an approach which could limit the depth of the image location and which could favour the appearance of artefacts. The ARFI method does not impose a certain degree of compression on the tissue, done by the examiner and which can vary (by hand fatigue), but it takes over the patient's respiratory movements, which means rhythmic and constant. The elastography using compression can assess just the superficial tissues, but with the ARFI method the tissue elasticity can be assessed starting with a depth of 1,5 cm up to 8 cm.

Unlike the conventional sonography B-mode which gives us anatomical details based on differences in the acustic impedance, the Virtual Touch image describes properties reffering to the tissue stiffness. In this respect, the Virtual Touch image is closer to a tissue evaluation through a physical palpation than through the conventional ultrasound.

ARFI technology is more and more studied in order to be used in different clinic application, including breast, liver, kidney, colon and rect tumours, the characterization of atheroscerosis plates, and also monitoring the ablation through radiofrequency. (25-31)

ARFI technology involves a mechanical compression of the tissue using acoustic impulses for a short time (pushing impulses), in a region of interest chosen by the examiner, which produces shear waves which spread from the interest area, perpendiculary on the acoustic impulse, generating localized displacements in the tissue, with a size of few microns. (32, 33) In the same time, detecting waves are generated of smaller intensity than the pushing impulse (1 : 100).

The moment of interraction between the shear waves and the detecting waves mark a short period between the shear waves and the crossing of the region of interest by this one. (34)

RESULTS

Registering the position of the shear waves in many locations and correlating these measurements with the short time, the speed of the shear wave can be quantified (shear wave velocity - SWV) (m/s), generally, the stiffer the region of the tissue is, the greater is the speed of the shear wave which passes through that region (fig. 1). Thus, the measured SWV is an intrinsic feature measured by an intrinsic and reproducible feature of the tissue. (35-37) The equipment measures SWV and the depth when the measurement was done too. Only one transductor (a diagnosis scanner) is used to generate the radiant force and to follow the displacement generated in the tissue.

Having in view the fact that technology is applied through software algoritms suplementary for control and imagary detection, the method can parallely offer a B-mode recording, color Doppler and ARFI images. (29)

Concerning the evaluation of difuse hepathopaty, there are a lot of studies, now being available preliminary results which were communicated at different conferences. (38 - 40)

The new ultrasonographic method of ARFI elastography allows valid, accurate and flexible evaluation of liver stiffness. Elastography of the left liver lobe is also possible which may be especially helpful in obese patients (fig. 2) (33, 41)

Thus the studies made by Sporea and the contributors who have quantified the speed of the contractual wave through ARFI method of the elastography showed, at healthy subjects, values of 1,24 $\pm$ 0,5 m/s, with values smaller than for the female gender (1,01 $\pm$ 0,15 m/s) and values higher than for the male gender (1,63 $\pm$ 0,59 m/s) and a little bit smaller in the younger subjects (0,93 $\pm$ 0,12 m/s in 18 – 29 years) compared to the oldest 1,55 $\pm$ 0,04 m/s for the group aged 50 – 59 years). (42)

ARFI allows SWV quantification of the shear wave velocity in strong correlation with the fibrosis stage. Steatosis does not influence SWV. (32, 33) The maximal performance of the method consists of the prediction in severe fibrosis and cirrhosis. The diagnostic accuracy is strongly comparable to transient elastography (Transient elastography, Fibroscan, EchoSens) only for the prediction of severe fibrosis and cirrhosis (for lower degrees of fibrosis, TE has better performances). (43)

Thus the studies made on adults' groups with chronic hepatitis found out the following values of the speed of the (42) shear waves for different stages of hepatic fibrosis: F 0: 1,31 $\pm$ 0,48 m/s; F 1: 1,52 $\pm$ 1,02 m/s; F 2: 1,61 $\pm$ 0,68 m/s; F 3: 1,76 $\pm$ 0,76 m/s; F 4: 2,81 $\pm$ 0,71 m/s (fig. 3).

ARFI method (the same with TE) has excelent predictive values for the presence of cirrhosis too. (20) The medium values of the speed of the shear waves in patients with compensated cirrhosis (Child A) are smaller (2,67 $\pm$ 0,73 m/s) beside the patients with decompensated cirrhosis. (Child B, C) 3,05 $\pm$ 0,8 m/s. (42)

Recent data show that the imagery through ARFI technology is a promising method based on ultrasounds for the evaluation of hepatic fibrosis in viral chronic hepatitis with a diagnosis accuracy similar to TE. (14, 44)

Elastography through ARFI method has a very good precision in the evaluation of the liver fibrosis and is superior to other noninvasive methods (APRI Index, FibroMax) in order to stage the hepatic fibrosis. (45, 46)

Elastography through ARFI method showed a good diagnosis performance than the visual evaluation by the radiologists

with experience in diagnosing the chronic hepatic diseases, and also the evaluation of their severity (it better correlates with the Child-Pugh scores and the tests of the hepatic functions, compared to an ultrasonography system of scoring based on the visual evaluation of the conventional ultrasonographic images in B- mode). (47)

CONCLUSION

The hepatic toxicity is discovered as routine by altering the hepatic biochemical tests, through the appearance of some symptomes characteristic for hepatitis, but the differentiation of hepatitis from hepatic cirrhossis and the right appreciation of the degree of the hepatic cirrhossis, useful for the therapy, can be obtained just through hepatic biopsy or by assessing the hepatic elasticity through so called "noninvasive biopsy" through elastography.

Thus, real-time elastography is useful and good in assessing the chemotherapics hepatotoxicity in children with cancer, which in future will get into a schedule of monitoring these children.

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MANAGEMENT OF EISENMENGER SYNDROME IN A LATE PRESENTATION OF TRANSPOSITION OF THE GREAT ARTERIES ASSOCIATED WITH INCIDENTAL ADRENAL TUMOR. CASE REPORT

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MANAGEMENT OF EISENMENGER SYNDROME IN A LATE PRESENTATION OF TRANSPOSITION OF THE GREAT ARTERIES ASSOCIATED WITH INCIDENTAL ADRENAL TUMOR. CASE REPORT (Abstract): We present the case of a 25-year-old patient with transposition of the great arteries (TGA), ventricular septal defect (VSD), without surgical correction, severe pulmonary hypertension and Eisenmenger syndrome, under vasodilatation therapy with Bosentan and Sildenafil, which presented for hemoptysis, shortness of breath at mild effort, myalgia, headaches and vertigo. Blood test showed important erythrocytosis and thrombocytopenia. CT examination revealed the presence of an adrenal incidentaloma (AI), which was proved to be nonfunctional after hormonal testing. Management of the patient included phlebotomy for the treatment of the hyper viscosity syndrome and recommending the addition of a prostacyclin analogue to the vasodilators scheme. The dimensions of the AI raised the question regarding the need of surgical excision, due to the risk of being an adrenal gland carcinoma. Due to the high risk of general anesthesia in Eisenmenger patients, a multidisciplinary team (endocrinologist, cardiologist, surgeon, and anesthesiologist) decided on a watch and wait strategy regarding the tumor, with regular monitoring. **Keywords:** TRANSPOSITION OF THE GREAT ARTERIES, EISENMENGER SYNDROME, PULMONARY HYPERTENSION, ADRENAL INCIDENTALOMA

Transposition of the great arteries (TGA) is the 2nd most frequent cyanotic congenital heart disease (CHD), after tetralogy of Fallot, with an incidence of 20-30/100,000 births and represents 5-7% of all CHD (1). Also, it is the most common CHD in the neonatal period, being responsible for 25% of the deaths caused by CHD in the first year of life. If it is associated with other congenital malformations which create a communication between the 2 circulatory systems, such as ventricular septal defect (VSD), the survival rate can be up to 50% in the first 3 years (1). However, these patients develop severe pulmonary arterial hypertension and Eisenmenger syndrome (ES) in the first year of life, therefore the survival till adulthood is uncommon without surgical interventions for correction or palliation.

CASE REPORT

We report the case of a 25-year-old female patient, diagnosed at birth with transposition of great arteries and peri membranous VSD, who didn't benefit from surgical correction for the congenital malformation. 10 years before admission the patient developed secondary hypertension and Eisenmenger syndrome, for which she was receiving chronic treatment with vasodilators Bosentan 125 mg b.i.d. and Sildenafil 20 mg t.i.d. At admission the patient presented for severe coughing with important hemoptysis, fatigability and shortness of breath at mild effort, myalgia, headaches and vertigo.

The physical examination showed a mildly altered general status, absence of fever, cyanosis, digital clubbing, congestive sclerae, heart rate 100 bpm, systolic murmur grade IV/VI, accentuation of the 2nd heart sound, BP 140/90 mmHg, SpO_2 68% in room air.

The electrocardiogram revealed: sinusal rhythm, 100 bpm, QRS axis-150 degrees, prominent P wave, 1st degree AV block, major RBBB (fig. 1).

We performed the 6-minute walking test with the following pretest parameters: SpO_2 65%, HR 100 bpm, BP 140/90 mmHg. During the test the patient walked 250 m, with the aggravation of the cyanosis, a 14/20 dyspnea on the Borg scale, a SpO_2 of 60%, HR 120/min, BP 130/90 mmHg.

The blood tests showed erythrocytosis (Hb 20g/dL, Ht 69%, RBC 9.3 million/mm^3), secondary thrombocytopenia (52,000/mm^3), iron deficiency (iron 16 µg/dL, ferritin 11 ng/dL).

The transthoracic echocardiography (TTE) revealed atrioventricular concordance and ventriculoarterial discordance, a large subaortic VSD (30 mm) with bidirectional shunt, preserved systolic function of the left and right ventricles, grade III tricuspid regurgitation (the septal cusp of the valve, floats in the left ventricle through the defect, during diastole), severe secondary pulmonary hypertension (sPAP 113 mmHg, mPAP 70 mmHg) (fig.2).

Right heart catheterization confirmed the pulmonary hypertension with increased pressions (mPAP 114 mmHg, sPAP 173 mmHg) and increased pulmonary vascular resistance (PVR 40 uW, 60 uWi and a PVR/SVR of 1.4).

One month before admission, the patient had undergone a thoracoabdominal CT examination, which revealed a tumoral mass of 50/35/40 mm, with delimitation of hypodense necrosis areas, on the topography of the suprarenal gland and splenic venous hemangiomas (fig. 3).

Considering the CT findings, we decided to investigate the secretor nature of the suprarenal tumor. The serum ACTH was slightly but inconclusively increased (55.2 pg/nL), serum cortisol morning/night ratio was normal (16.7µg/mL/6.78µg/mL), as well as free urinary cortisol (113.94 µg/24h) and metanephrine values (309 µg/24h). All these results pleaded for a nonfunctioning adrenal incidentaloma. After multidisciplinary evaluation (endocrinologist, surgeon and the anesthesiologist we decided to postpone the surgery and monitor the evolution of the tumor every 3 months, due to the high anesthetic risks.

Management of the patient consisted in phlebotomy, with the evacuation of 400 mL of blood, with normalization of red cell and platelets values. In addition to the vasodilatation therapy we added low doses of diuretics, which improved the dyspnea and also oral iron supplementation.

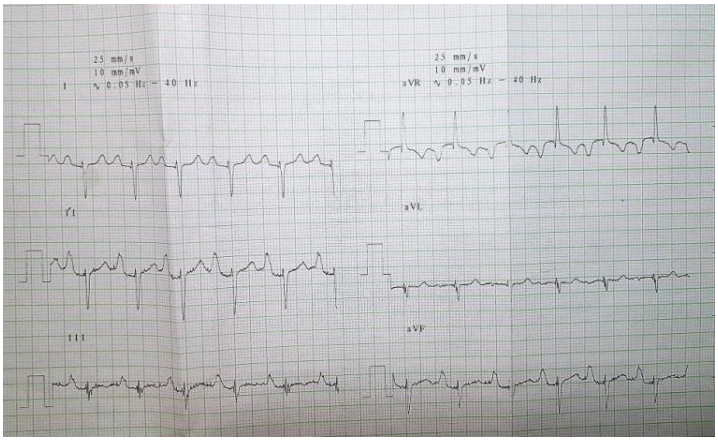
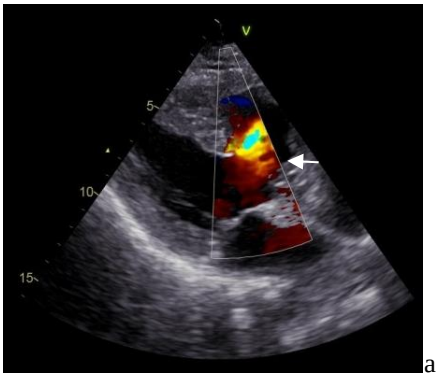


Fig. 1. Admission ECG



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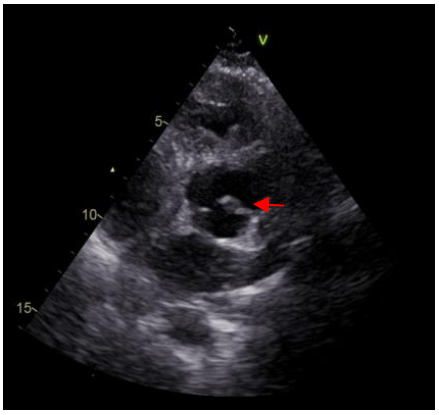

c

Fig. 2. TTE-a. Parasternal long axis view- important ventricular septal defect, with bidirectional shunt (white arrow). b. Apical 4 chamber view-the septal cusp of the tricuspid valve, floats in the left ventricle, during diastole (white arrow). c. Parasternal short axis view at the base of the great vessels-the aortic (white arrow) and pulmonary valves (red arrow) can be seen in the same echocardiographic plane (typical image for TGA).

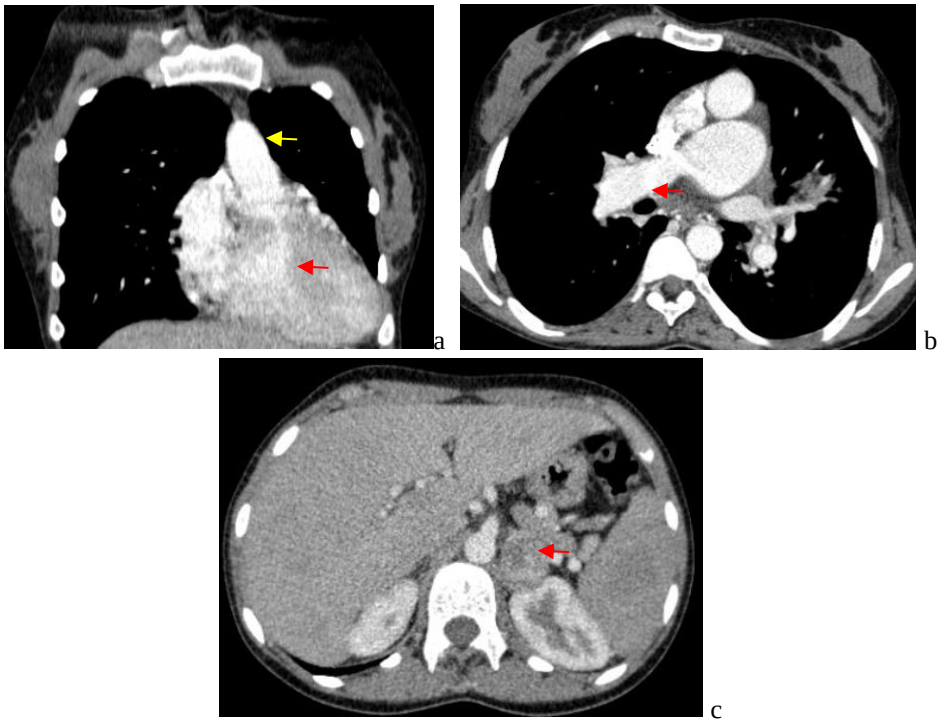

Fig. 3. Thoracic CT with intravenous contrast presents in arterial phase an important septal defect (red arrow), the aorta emerging the right ventricle (yellow arrow) (a) and the pulmonary artery emerging the left ventricle (red arrow) (b). Abdominal CT reveals a tumoral mass at the level of the left adrenal gland with inhomogeneous contrast enhancement (red arrow) (c).

DISCUSSION

This case represents a real exception from the typical natural history of patients with transposition with the great arteries. Most of the studies regarding this pathology date from the pre-surgical era, but they report a 90% mortality rate at 1 year after birth (2). This prognostic can be improved by the association of another defect that permits the communication of the blood between the two circulations, such as atrial septal defect (ASD), persistent ductus arteriosus and VSD. The best prognosis can be found in the patients with VSD and pulmonary sub valvular stenosis, when the debit in the 2 circulations is equilibrated with a life expectancy of 3 years. In the case of our patient, where only a ventricular septal defect is associated, the life expectancy is somewhat lower, but there are still reported cases of patients reaching adolescence or even the 3rd or 4th decade of life (1, 2).

The only curative treatment for patients with transposition of the great arteries remains the surgical correction in the neonatal period through arterial switch techniques and the correction of associated malformations (1). In our case, by presenting secondary pulmonary hypertension, a correction procedure is not indicated, and the pri-

mary option remains heart-lung transplantation. However, the 10-year life expectancy in patients who undergone transplant is only 30-40%, which is inferior to the life expectancy of patients receiving pulmonary vasodilatation therapy, thus the right timing for the surgery is debatable (3).

In the case of unoperated patients, with severe pulmonary hypertension, the therapeutic strategy consists in using vasoactive drugs that target, together with the prevention of the complications of hypoxemia (erythrocytosis, thrombocytopenia, coagulation disorders) (4, 5, 6). Our patient, presented with moderate hyper viscosity symptoms (headaches, myalgia) and important erythrocytosis, prompting us to perform a phlebotomy with the removal of 400 mL of blood together with isovolumic fluid replacement, with a good clinical and hematological outcome. Regular phlebotomies are not indicated, as they induce iron deficiency and microcytosis, which are associated with cerebrovascular events (4). The main classes of vasoactive therapy are endothelin receptor antagonists (Bosentan) (7), phosphodiesterase type 5 inhibitors (Sildenafil) (8), and prostacyclin derivates. In this case the patient already received chronic treatment with high doses of Bosentan and Sildenafil, with low efficiency on the symptomatology. Consequently, additional treatment with prostanoids should be tried, as current studies with intravenous epoprostenol showed improvement of the hemodynamics and of the exercise capacity, but with increased risk of paradoxical embolism and infections, due to the presence of a central venous catheter (9). Studies with other prostacyclin analogs such as subcutaneous treprostinil or oral selexipag, which are used for idiopathic pulmonary hypertension, are in the incipient phases, with favorable results (10, 11).

Another particularity of the case was the presence of the adrenal incidentaloma (AI), which posed problems regarding the necessity of a surgical intervention. Current literature suggests excision at a cut-off to 4 cm as most adrenocortical carcinomas (ACC) are > 4 cm in size. Also, this cut-off value provided a good sensitivity (93%) in predicting malignant processes; nevertheless, the specificity is a bit low (42%), as a result to the low prevalence of ACC (12).

Non-cardiac surgery for patients with Eisenmenger syndrome is a very controversial topic, due to the high perioperative death which is up to 19%. Hematocrit higher than 60%, arterial oxygen saturation lower than 80%, right ventricular hypertension, syncopal episode and a fixed pulmonary hypertension, not responsive to oxygen represent poor prognosis factors (13). The key to the anesthetic management of patients with ES is to maintain the balance between pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) during anesthetic induction, together with appropriate monitoring (13, 14). In order to reduce the elevated PVR patients should continue the chronic vasodilatation therapy in the perioperative period, with inhaled pulmonary vasodilators (nitric oxide, epoprostenol) used during the procedure. Another important problem is the hypotension which appears as a side effect of induction agents, which can increase the right-left shunt and the right ventricular dysfunction during the procedure, therefore vasopressor treatment should be associated during induction. Traditionally, general anesthesia has been recommended for these

patients because the relative sympathectomy that occurs with regional anesthesia would tend to decrease SVR (14). However, several cases were reported that documented the relative safety of epidural anesthesia for ES patients (15).

In the case of our patient we decided that a watch-and-wait approach is better for the management of the adrenal incidentaloma, deciding on careful imagistic monitoring every 3 months and hormonal evaluation annually, with the possibility of a surgical approach if the tumor changes in size or shows radiological characteristics of an adrenocortical carcinoma.

CONCLUSIONS

This case shows a rare presentation of an uncorrected cardiac congenital disease, with a natural evolution towards severe pulmonary hypertension and Eisenmenger syndrome. As a result, management of these patients is complex, requires regular and careful monitoring and it focuses both on the vasoactive, but also on the management of erythrocytosis, thrombocytopenia and the coagulation disorders.

The association of the adrenal incidentaloma poses additional problems regarding the management of the patient. Due to the high risk posed by general anesthesia, a multidisciplinary team (cardiologist, endocrinologist, radiologist, surgeon, and anesthesiologist) was required in order to decide on the proper approach and follow-up for the adrenal mass.

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NEWS

IS LIVER-LIMITED DISEASE IN METASTATIC COLORECTAL CANCER A PROGNOSTIC FACTOR?

Approximate 44-77% of colorectal cancer patients develop liver metastasis, one of the most frequently involved sites. Among these patients, only 10-30% have a potentially resectable disease that can be treated with curative intent. The resection of the liver metastasis leads to 5-year survival rates of up to 40%. Liver-limited disease (LLD) was investigated as prognostic and predictive factor in patients with initially unresectable disease treated with chemotherapy (FOLFIRI) and targeted therapy: cetuximab (anti-EGFR) or bevacizumab (anti-VEGF) in the FIRE-3 trial. Of 400 patients with RAS wild type tumors included in the analysis, 133 (33.3%) had LLD and 267 (66.8%) had non-LLD. Median overall survival (OS) was significantly longer in LLD compared to non-LLD patients (36.0 vs. 25.4 months) proving that LLD status is an independent prognostic factor in patients with metastatic colorectal cancer treated with targeted therapy and standard chemotherapy. About the predictive relevance of LLD status, the data from the analysis suggest that FOLFIRI and cetuximab improve overall survival and treatment response compared with bevacizumab regardless of the status of LLD (Julian Walter Holch, Ingrid Ricard, Sebastian Stintzing *et al*. Relevance of liver-limited disease in metastatic colorectal cancer: Subgroup findings of the FIRE-3/AIO KRK0306 trial. IJC DOI: 10.1002/ijc.31114).

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AFECŢIUNILE ATOPICE ŞI INFECŢIA INTESTINALĂ CU HELMINŢI

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ATOPIC DISEASES AND INTESTINAL HELMINTH INFECTIONS (Abstract): The purpose of the current study was to examine the relationship between past and current intestinal helminth infections, and asthma, allergic rhino-conjunctivitis, and atopic dermatitis. **Material and method**: The study was performed on 84 patients aged 2-18 years with a mean of 9.31 ± 3.75 years. Helminth infection was determined by stool examinations and questionnaire. Asthma was diagnosed by spirometry, atopic dermatitis and allergic rhino-conjunctivitis by skin prick testing. **Results**: Stool examinations revealed infection with *A. lumbricoides* in 5.95% of cases and *E. vermicularis* in 4.76% of cases. 8 patients had positive tests for at least a helminth. The most frequent respiratory symptoms were caught, wheezing and dispneea. Spirometry showed anormal results in 4.76% of patients and skin tests were positive in 20.24% of cases. 11 patients were diagnosed with allergic rhinoconjunctivitis and 5 with atopic dermatitis. A history of infection with *E. vermicularis* was associated with increased risk of atopic dermatitis (OR 1.86), asthma (OR 2.11), and allergic rhinoconjunctivitis (OR 1.34), and a positive stool examination for *A. lumbricoides* was negatively associated with asthma (OR 2.60). **Conclusions**: Current *A. lumbricoides* infection protects against asthma while past infection with *E. vermicularis* are risk factors for allergic rhinoconjunctivitis, asthma and atopic dermatitis. Interactions depend on the type of helminth and time of infestation. **Key words**: ASTHMA, ALLERGIC RHINOCONJUNCTIVITIS, ATOPIC DERMATITIS, HELMINTHS

Observaţia conform căreia în ţările dezvoltate se înregistrează o prevalenţă ridicată a afecţiunilor atopice şi o incidenţă scăzută a helmintiazei intestinale, spre deosebire de ţările în curs de dezvoltare în care situaţia este inversă, a condus la ipoteza existenţei unei asocieri între cele două fenomene. Deşi studiile de specialitate au demonstrat că expunerea la infecţii parazitare în prima copilărie reduce riscul de atopie, relaţia dintre afecţiunile alergice şi infecţia cu helminţi rămâne insuficient cunoscută şi deci, controversată (1,2).

Majoritatea studiilor din literatura de specialitate au încercat să elucideze corelaţia dintre helmintiaza intestinală şi astmul bronşic, existând puţine informaţii certe cu privire la asocierea cu alte afecţiuni atopice, cum ar fi dermatita sau rinoconjunctivita alergică. Mai mult decât atât, s-a analizat expunerea curentă la helminţi, ceea ce face dificilă investigarea secvenţialităţii temporale a celor două fenomene (3).

Studiul de faţă are drept obiectiv investigarea asocierii dintre infecţia curentă şi în antecedente cu helminţi şi prevalenţa ast-

mului, dermatitei atopice şi rinoconjunctivitei alergice la copii internaţi cu patologie respiratorie în clinicile de pediatrie din cadrul Spitalului Clinic de Urgenţă pentru copii „Sf. Maria" Iaşi în perioada 2007--2010. Concomitent au fost analizate contribuţiile altor factori de risc în determinarea afecţiunilor atopice descrise în literatura de specialitate.

MATERIAL ŞI METODĂ

Studiul curent s-a efectuat pe un lot format din 84 de pacienţi diagnosticaţi cu afecţiuni respiratorii în intervalul 2007-2010, spitalizaţi în clinicile de pediatrie din cadrul Spitalului Clinic de Urgenţă pentru copii „Sf. Maria" Iaşi. Etapele alcătuirii lotului şi analiza foilor de observaţie s-au desfăşurat în conformitate cu legislaţia medicală în vigoare.

Toţi copiii incluşi în lotul de studiu au fost investigaţi spirometric, înainte şi după efort, alergologic (*prick-test*) şi coproparazitologic. Adiţional, aparţinătorii au fost chestionaţi cu privire la factorii de risc ai sensibilizării alergice: administrarea de antibiotice în primul an de viaţă, contactul cu animalele de companie, prezenţa refluxului gastro-esofagian, expunerea la fum de ţigară, alimentaţia cu lapte de vacă.

Atopia a fost definită ca prezenţa unui test cutanat pozitiv la cel puţin unul din alergenii aplicaţi. Testul alergologic a implicat utilizarea a 7 alergeni: *Dermatophagoides pteronyssinus, Dermatophagoides farinale,* păr de pisică, polen mixt de copaci, polen mixt de iarnă, *Alternaria alternata* şi acarieni. Testul alergologic a fost realizat pe faţa ventrală a antebraţului stâng, iar răspunsul cutanat a fost cuantificat după 15 minute, luându-se în considerare cea mai mare papulă (diametru ≥ 3 mm), în absenţa reactivităţii semnificative. Pentru a demonstra hiperreactivitatea bronşică s-a realizat spirometria la 5 şi 10 minute după exerciţiul fizic, conform ghidului American Thoracic Society. Testul a fost considerat pozitiv dacă FEV1 (*forced expiratory volume*) a înregistrat valori mai mici de 70% din valoarea prezisă sau a scăzut cu mai mult de 15% la 5 sau 10 minute după exerciţiul fizic (alergare 6 minute). În acest caz, copilul a primit un bronhodilitator pentru a facilita recuperarea, iar spirometria a fost repetată la 20 minute de la administrarea bronhodilatatorului, pentru a ne asigura de atingerea valorilor bazale. Infecţia cu helminţi a fost confirmată prin certificarea prezenţei de ouă de paraziţi (*Ascaris lumbricoides* sau *Enterobius vermicularis*) la examenul coproparazitologic. În cazul fiecărui subiect s-au analizat antecedentele de infecţii cu helminţi prin chestionarea aparţinătorilor.

Datele obţinute au fost analizate şi exprimate procentual, diferenţele dintre valorile medii ale parametrilor investigaţi au fost analizate prin intermediul testelor *t--Student* şi *ANOVA*, iar asocierile dintre diferiţi factori au fost interpretate cu ajutorul testelor de corelaţie şi regresie. Prelucrarea statistică a fost realizată cu programul *Statistica 8.0.*

REZULTATE

Vârsta medie a pacienţilor incluşi în studiu a fost de 9,31 ani, 57,14% aparţinând sexului masculin, iar 42,86%, sexului feminin. Examenul copro-parazitologic a evidenţiat prezenţa *A. lumbricoides* la 5,95% dintre pacienţi şi a *E. vermicularis* la 4,76%. 8 copii au avut teste pozitive pentru cel puţin

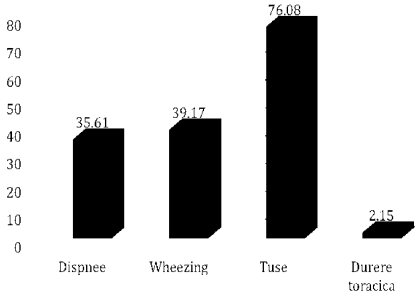

Fig. 1. Simptomele respiratorii întâlnite la pacienţii cuprinşi în lotul de studiu

TABELUL I
Rezultatele regresiei logistice multivariate privind asocierea afecţiuni atopice, infecţie cu helminţi

Variabilă/Factor de risc	Raportul cotelor	Interval de încredere 95%	*p*
Rinoconjunctivită alergică			
Istoric de infecţie cu *E. vermicularis*	1,34	1,00-1,79	0,048
Simptome de reflux gastro-esofagian	1,74	1,24-2,44	0,003
Atopie familială	1,67	1,07-2,59	0,025
Administrarea de antibiotice în primul an de viaţă	1,80	1,28-2,53	0,002
Dermatită atopică			
Istoric de infecţie cu *E. vermicularis*	1,86	1,34-2,58	0,001
Examen coproparazitologic pozitiv pentru *A. lumbricoides*	0,23	0,08-0,68	0,011
Atopie familială	1,56	1,09-2,24	0,017
Administrarea de antibiotice în primul an de viaţă	1,61	1,10-2,35	0,017
Contact cu animale de companie	0,68	0,48-0,98	0,039
Wheezing			
Examen coproparazitologic pozitiv pentru *A. lumbricoides*	2,60	2,00-3,38	0,001
Examen coproparazitologic pozitiv pentru *E. vermicularis*	2,11	1,55-2,86	0,001
Simptome de reflux gastro-esofagian	1,59	1,20-2,11	0,003
Teste spirometrice anormale			
Examen coproparazitologic pozitiv pentru *A. lumbricoides*	6,56	1,30-33,13	0,025
Examen coproparazitologic pozitiv pentru *E. vermicularis*	2,77	1,94-3,97	0,001

un helmint. Dintre simptomele respiratorii, cel mai frecvent s-a întâlnit tusea, urmată de wheezing şi dispnee (fig. 1).

Teste spirometrice anormale au fost întâlnite la 4,76% dintre pacienţi, iar reacţiile cutanate pozitive la 20,24%. 11 pacienţi (13,09%) au prezentat rinoconjunctivită alergică, iar 5 (5,95%) dermatită atopică.

După ajustarea testelor statistice pentru vârstă, sex, mediul de proveniență s-a evidenţiat asocierea istoricului de infecţie cu *E. vermicularis* cu risc crescut de dermatită atopică şi rinoconjunctivită alergică şi a istoricului de infecţie cu *A. lumbricoides,* cu risc de astm bronşic alergic. Prezenţa unui examen copro-parazitologic pozitiv pentru *A. lumbricoides* s-a asociat invers proporţional cu prezenţa dermatitei atopice. Prezenţa rezultatelor pozitive la testele efectuate nu s-a asociat semnificativ cu nici unul dintre helminţi în cazul regresiei multiple (tab. I).

DISCUŢII

Deşi există numeroase studii în literatura de specialitate care demonstrează asocierea inversă între prezenţa infecţiei cu helminţi şi afecţiunile atopice, relaţia dintre cele două afecţiuni este încă incomplet cunoscută şi demonstrată. Literatura de specialitate consultată oferă rezultate contradictorii conform căreia helmintiaza cauzează, inhibă sau chiar nu prezintă o asociere cu afecţiunile atopice (4). Studiul de faţă confirmă existenţa a diferite asocieri între infecţia cu helminţi şi afecţiunile atopice, natura acestora depinzând de tipul infecţiei helmintice şi al atopiei. Concomitent, momentul infecţiei cu helminţi deţine un rol important, aşa după cum o demonstrează analiza informaţiilor obţinute de la aparţinători în absenţa rezultatelor examenelor coproparazitologice anterioare. Această metodă este nespecifică şi are sensibilitate redusă având în vedere lipsa preciziei informaţiilor obţinute, fiind grevată de riscul cuantificării efectului infecţiilor masive. Cu toate acestea, considerăm că rezultatele obţinute constituie un indicator patent al importanţei momentului infectării cu helminţi în determi-

nismul afecţiunilor atopice.

Prezentul studiu demonstrează o asociere inversă între infecţia curentă cu *A. lumbricoides* şi dermatita atopică, sugerând că infecţia cu *A. lumbricoides* ar oferi protecţie împotriva dermatitei atopice. Cu toate acestea, reversul acestei relaţii nu poate fi exclus, în condiţiile în care istoricul de infecţie cu *A. lumbricoides* nu a exercitat nici un efect asupra incidenţei dermatitei atopice. O relaţie inversă, bidirecţională, între infecţia helmintică şi dermatita atopică a fost raportată de Schafer şi colab., care au utilizat rezultatele chestionarelor pentru a determina momentul instalării celor două afecţiuni. Includerea în analiză a secvenţialităţii temporale a condus la concluzia că prezenţa antecedentelelor de infecţie helmintică se corelează invers proporţional cu dermatita atopică, iar antecedentele de dermatită atopică, se asociază negativ cu infecţia helmintică.

Există puţine studii în literatura de specialitate referitoare la relaţia dintre parazitozele intestinale şi dermatita atopică. În cadrul prezentei cercetări am evidenţiat o asociere statistic semnificativă a istoricului de infecţie cu *E. vermicularis,* cu creşterea riscului de rinoconjunctivită alergică şi dermatitiă atopică, rezultate ce contrazic studiul efectuat de Schafer şi colab. care raportează o asociere negativă a dermatitei atopice cu antecedentele de infecţie cu *E. vermicularis* la copii de vârstă şcolară din Germania de Est (5). În mod similar, wheezingul a înregistrat o asociere puternică cu istoricul de helmintiază intestinală, aşa după cum o relevă rezultatele înscrise în tabelul I.

În cazul infecţiei curente cu *E. vermicularis* nu am reuşit să evidenţiem o astfel de relaţie, dar Huang şi colab. indică o asociere negativă în ceea ce priveşte asocierea cu rinita alergică şi astmul (4). Cu toate acestea, procentul de copii cu hiperreactivitate bronşică diagnosticată spirometric (7,14%) a fost semnificativ mai mic decât incidenţa declarativă a wheezingului (39,17%).

CONCLUZII

Reactivitatea cutanată la alergeni depinde de intensitatea şi statusul cronic al infecţiei helmintice, infecţia cronică, masivă protejând copilul împotriva atopiei (6,7,8).

Ingestia de antibiotice în primul an de viaţă se asociază semnificativ cu creşterea riscului de wheezing, dermatită atopică şi rinoconjunctivită alergică, substratul fiziopatologic fiind reprezentat de modificarea florei intestinale. Acest fenomen este întreţinut de diagnosticarea eronată a simptomatologiei alergice drept infecţie respiratorie şi administrarea nejustificată de antibiotice. Wheezingul apare frecvent la pacienţii cu reflux gastroesofagian datorită reactivităţii bronşice crescute şi microaspiraţiilor pe care acesta le antrenează (9,10). Contactul cu animalele de companie se asociază negativ cu dermatita atopică şi constituie un factor de protecţie împotriva afecţiunilor atopice. Alţi factori concuratori, alimentarea cu lapte de vacă, expunerea la fumul de ţigară nu au un impact semnificativ asupra afecţiunilor analizate.

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NOUTĂŢI

Noi opţiuni terapeutice în cazul infecţiilor produse de *Klebsiella pneumoniae* producătoare de carbapanemaze

Existenţa tulpinilor de *Klebsiella pneumoniae* producătoare de carbapanemaze a fost prima dată raportată în Carolina de Nord iar frecvenţa izolării acestora este în creştere. Polimixina B rămâne unul dintre antibioticile încă active asupra acestor tulpini dar, din păcate, nivelul concentraţiei minime inhibitorii este foarte ridicat lăsând clinicienilor puţine opţiuni terapeutice. În cadrul acestui studiu s-au investigat câteva antibitotice testându-se sinergismul lor antimicrobian atunci cand sunt asociate cu polimixina B împotriva a 12 tulpini de *Klebsiella pneumoniae* producătoare de carbapanemaze (identificarea genelor care codifică acest caracter făcându-se prin *Real Time* PCR). De asemenea valorile concentraţiei minime inhibitorii în acest caz au fost cuprinse în intervalul 16-128 μg/ml. Polimixina B a fost studiată în combinaţie cu cefazolin, ceftriaxona, cefepime, imipenem, gentamicina, tigecyclina, doxycyclina, şi rifampicina. Sinergismul antibacterian a fost observat în cazul combinaţiilor dintre polimixina B şi doxiciclină şi dintre polimixina B şi rifampicină (valoarea concentraţiei minime inhibitorii scăzând de patru ori). Pentru ambele combinaţii efectul sinergic antimicrobian a fost observat la concentraţii ale antibioticelor care pot fi atinse în mod fiziologic.Un efect sinergic mai scăzut a fost observat în cazul asociaţiei dintre tigecilina şi polimixina B, iar în restul cazurilor sinergismul antibacterian nu a putut fi evidenţiat. Astfel, în conformitate cu aceste rezultate, doxiciclina şi rifampicina pot fi asociate polimixinei B în vederea tratării infecţiilor produse de tulpini de Klebsiella pneumoniae producatoare de carbapanemaze, urmând ca viitoarele studii sa stabilească dacă rezultatele testării *in vitro* sunt asociate cu succesul terapeutic obţinut *in vivo* (Elemam A, Rahimian J, Doymaz M. *In vitro* evaluation of antibiotic synergy for polymyxin b-resistant carbapenemase-producing *Klebsiella pneumoniae*. *J Clin Microbiol* 2010 ; 48 : 3558-3562).

Ecaterina Enache

ONE-MINUTE SIT-TO-STAND TEST – A USEFUL TESTING TOOL FOR THE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ONE-MINUTE SIT-TO-STAND TEST–A USEFUL TESTING TOOL FOR THE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): Exercise tolerance of patients with chronic obstructive pulmonary disease (COPD) should be carefully assessed in order to gain an optimal quality of life through pulmonary rehabilitation programs. The 1-minute sit-to-stand test (1-min STST) is part of the larger family of sit-to-stand tests which were developed recently as feasible options to evaluate the physical capacity in COPD patients, based either on the time needed to perform a preset number of repetitions or on measuring the number of repetitions performed in a preset period of time. 1-min STST is easy to perform in clinical practice and the results are similar in estimating physical capacity as those generated by other tests, like the 6-minute walk test (6MWT). The 1-min STST performance is predicted by age, sex, smoking status, body mass index, and level of perspiration during physical activity. The results of the test are correlated with the quality of life scores and prognostic indices. The studies to date proved that 1-min STST is an alternative reliable tool to more traditional but also more exhausting exercise capacity tests in COPD patients. **Keywords**: SIT-TO-STAND TEST, CHRONIC OBSTRUCTIVE PULMONARY DISEASE, EXERCISE CAPACITY.

For the chronic obstructive pulmonary disease (COPD) patients the daily physical tasks can be a target sometimes too difficult to achieve. Thus, in order to gain an optimal quality of life for these patients, their exercise tolerance should be carefully assessed, and pulmonary rehabilitation programs should be included in optimal disease management along with smoking cessation (1-4).

In daily practice, exercise tests as the incremental shuttle walk test, endurance shuttle walk test, stair climbing test, 6-min walk test (6MWT), step test or sit-to-stand (chair rise) test (STST) should assess functional capacity, in addition to lung function tests. The gold standard of these usual tests for evaluating exercise tolerance in terms of dyspnea and oxygen desaturation as well as aerobic capacity in terms of walked distance or fatigue is the 6MWT (5).

In 1985, the sit-to-stand test (STST)

was described for the first time (6) and then was associated with increased popularity among researchers mainly due to the simplicity of the movement – standing up from a chair (7). There are several versions of STST available, the main difference being the number of repetitions performed and, as a consequence, the duration of the test. As a result, the mobilized physical resources of the patient and the exercise-induced symptoms and perceived difficulty are variables. The most accepted variant of the STST is the 1-minute sit-to-stand test (1-min STST) that outlasted the variant consisting of 5 repetitions.

MATERIAL AND METHODS

We performed a search of the medical literature, to find out the role and usefulness of 1-min STST in COPD patients. Data sources were represented by several renowned electronic medical databases-MEDLINE, Cochrane Library, Google Scholar and the search terms were SIT-TO-STAND TEST, CHRONIC OBSTRUCTIVE PULMONARY DISEASE, and EXERCISE CAPACITY. We have identified and critically selected relevant research (systematic reviews, meta-analysis, narrative reviews, clinical guidelines, randomized controlled trials and non-randomized but meeting scientific quality criteria) from the last ten years, written in English language, full text available, regarding STST (1-minute and other variants, like 30 seconds or 3-minute) (8, 9).

RESULTS AND DISCUSSION

1. A general view of STS. Different versions of the test

Due to the fact that for COPD patients the two commonly accepted and used exercise capacity tests (6MWT and incremental shuttle walk test) are difficult to use in outpatient settings and primary care due to special requirements needed (time, space and resources) (10), the development of a much simpler and easier to achieve evaluation tool was needed. A movement used in everyday life – the sit-to-stand movement, was the base for the development of a new family of tests by Csuka and McCarty (6). These tests are based either on the time needed to perform a preset number of repetitions (5 repetitions - STST) or on measuring the number of repetitions performed in a preset period of time (1-min STST). Over time, different versions of the STST were used in various populations, to asses lower extremity muscle strength and evaluate the functional capacity of the disabled patients, in order to provide useful information on their independence or disability in daily life (11).

2. Measurements and reference values for 1-min STST

1-min STST requires simple conditions: a chair and a stopwatch. Usually, to perform a 1-min STS test, a trained nurse has to ask the patient to sit down on a chair without armrests, height usually about 46-48 cm, the same chair used for all the patients. The height of the chair should be adapted to the morphology of the patient performing the test. The subject is invited to keep the feet parallel (the legs should not touch the chair) at least as wide apart as the hips and to have the arms hang down loosely, on the hips or crossed on chest (12). The assisted use of the arms is not allowed during the 1-min STST. The patient is asked to fully stand up with the legs straightened entirely (complete knee extension) and then to sit down the chair, which had to be touched by the buttocks.

The nurse asks the patient to complete as many as possible sit-to-stand cycles within 60 seconds at a self-determined speed. Usually, the person who supervises

the test informs the patient when 30 and respectively 15 seconds were left and is counting the number of fully completed STS cycles; not completed or incorrectly performed STS cycles are not counted. There should be a motivation of the patient to perform as many cycles as possible but without any pressure on participants with musculoskeletal or neurological conditions, who felt unable to complete the 1-min STST. The results of a Swiss population-based study (13), which included 6,926 participants which completed the test, have determined the sex- and age-stratified reference values for the 1-min STST for a European adult population, up to the age of 79 years old (tab. I).

TABLE I

Reference values of 1-min STST (adapted from Strassmann *et al.*) (13)

Age group (years)	Number of STS repetitions Men (p 97,5*)	Number of STS repetitions Women (p 97,5')
20-24	72	70
25-29	74	68
30-34	72	68
35-39	72	63
40-44	69	65
45-49	70	63
50-54	67	60
55-59	63	61
60-64	63	55
65-69	60	53
70-74	59	51
75-79	56	43

*97,5 percentile

The 1-min STST performance was predicted by age, sex, smoking status, body mass index, and level of perspiration during physical activity. Usually, there is a small learning effect, thus some researchers are suggesting that, if possible, the test should be performed twice, and the higher scores obtained to be used. However, other studies suggest that the learning effect is not important and reliable results can be obtained with a single performance of the 1-min STST (9).

3. 1-min STST *vs.* other functional tests in COPD patients

Only recently STS tests started to be used in COPD patients and their use increased markedly during the last decade, being also associated with the first studies analyzing the efficiency of STST use in COPD patients (12). These studies were designed to assess the relevance of STST in the evaluation of the functional status of COPD patients when compared with 6MWT. Many studies found significant correlations in terms of functional performance, dyspnea, and quadriceps strength between 6MWT and 1-min STST (6, 14-16). For other parameters, like heart rate, systolic blood pressure, and oxygen saturation, some studies found 1-min STST less stressful than 6MWT. These parameters were significantly modified during 6MWT, a situation not encountered in 1-min STST.

Data published by some researchers (9, 12) indicated that only the 3-min STST is equivalent with 6MWT from the point of hemodynamic stress of the patient during the test but similar results of 6MWT and 1-min STST were recorded when quadriceps strength, end-exercise oxygen consumption, carbon dioxide output, ventilation, breathing frequency, and heart rate were analyzed. These results demonstrated that 1-min STST can be considered a useful tool to assess functional status and lower limb activity in COPD patients, but the different versions of the STST are not equivalent, due to the fact that the length of the exercise is associated with different physiological processes (17).

4. STST in other pulmonary diseases

Radtke *et al.* (18) analyzed the use of 1-min STST in other respiratory diseases. In cystic fibrosis, the 1-min STST was

designed as a cheap, valid and promising alternative to asses muscle function of the patients. Due to the fact that these conclusions were obtained by a study realized on 14 patients with CF, the results must be confirmed by larger studies in different populations.

5. Value of STS in evaluation of the morbidity and prognosis in COPD

Van Gestel *et al.* (19) have confirmed that in patients with COPD, the ability to exert physical effort is correlated with the survival.

Except for the role in the evaluation of the functional status in COPD patients, the 1-min STST test has proved its utility as a tool for survival prognosis in these patients. A study (20) realized on 409 patients, showed that a lower number of rises on 1-min STST test correlates with a higher mortality rate at 2 years. The patients who weren't able to perform more than 12 rises in a 1-min STST have a lower metabolic power, which is associated with a higher risk of mortality. The 1-min STST performance is inversely correlated with the BODE index (21-23), that encompasses four variables: body mass index (BMI), dyspnea, obstruction, and exercise capacity and is proved to be associated with the mortality in COPD at 52 months (23).

Researchers (9, 15, 24) confirmed also that 1-min STST is an effective tool for measuring the efficiency of a pulmonary rehabilitation program; an improvement of 3 rises in 1-min STST is consistent with the benefits of a pulmonary rehabilitation program.

CONCLUSIONS

Our evaluation of the 1-min STST revealed that this test, based on a simple movement, can be realized in almost every clinical setting. Even under the conditions of COPD patients whose walking distance is reduced, this type of requested movement - sit-and-stand, can be performed several times during the day. The necessary equipment is not expensive, and the patients easily understand how to perform correctly the test. Recent studies indicate a good correlation between 1-min STST parameters and other functional tests or prognostic indices. Based on our research of the literature, we concluded that in COPD patients, the 1-min STST elicited a physiological response similar to that of the 6MWT, a more traditional but also more exhausting exercise capacity test in chronic respiratory patients.

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COLONOSCOPIC SCREENING OF ASYMPTOMATIC FIRST-DEGREE RELATIVES OF COLORECTAL CANCER PATIENTS

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COLONOSCOPIC SCREENING OF ASYMPTOMATIC FIRST-DEGREE RELATIVES OF COLORECTAL CANCER PATIENTS (Abstract): **Aim**: First-degree relatives of colorectal cancer (CRC) patients are at increased risk for developing colorectal neoplasm, and current guidelines recommend screening colonoscopy in such individuals. The aim of this study was to evaluate the use of colonoscopy as the screening test in asymptomatic first-degree relatives of CRC patients. **Material and method**: Colonoscopy was performed in 102 asymptomatic individuals who had at least one first-degree relatives with CRC. Subjects included in the screening program were aged between 36 and 72 years, and majority came from two counties (Suceava, Iaşi) located in north-eastern Romania. **Results**: Thirty colorectal lesions were found in 17 individuals: two (6.6%) had adenocarcinomas, and remaining 15 patients had 28 polypoid lesions: 14 (46.6%) adenomas, 5 (16.6%) tubulovillous adenomas, 3 (10%) adenomas with high grade dysplasia and 6 (20%) had hyperplasic polyps. **Conclusion**: Colonoscopy is a useful, feasible and safety initial screening tool for first-degree relatives of patients with CRC. **Key words**: COLORECTAL CANCER, COLONOSCOPY, FIRST-DEGREE RELATIVE, SCREENING

Colorectal cancer (CRC) is the second leading cause of death from malignancies in most western developed countries (1). In Romania, the incidence of CRC has significantly increased over the last two decades, and now it has the tendency to exceed the gastric cancer, which is still the most frequent malignancy encountered in our country (2).

The goal of cancer screening is to reduce mortality through a reduction in incidence of advanced disease. CRC screening can achieve this goal through the detection of early-stage of adenocarcinomas and the detection and removal of adenomatous polyps (3). Recent trends in CRC incidence and mortality reveal declining rates, which have been attributed to the effect of screening and early detection and prevention through polypectomy (4,5).

Colonoscopy performed by experienced endoscopists is regarded as "gold standard" method for CRC screening; it seems to outperform fecal occult blood test (FOBT) and flexible sigmoidoscopy in the detection of cancer and advanced neoplasia (5).

First-degree relatives of CRC patients are at increased risk for developing colorectal neoplasm, and current guidelines recommend screening colonoscopy in such individuals (6,7,8).

The aim of this study was to evaluate the use of colonoscopy as the screening test in

TABLE I
Characteristics of the screened group

Lesions	Number	%
Adenocarcinoma	2	6,6
Adenomas	14	46,6
Tubulovillous adenomas	5	16,6
Adenoma with high-grade dysplasia	3	10
Hyperplasic polyps	6	20

asymptomatic first-degree relatives of CRC patients.

MATERIAL AND METHODS

In January 2006 we set up a colonoscopy screening program of first-degree relatives of common ("sporadic") CRC patients (those cancers not associated with familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or inflammatory bowel disease). The potential screenees were invited to an interview and after detailed information about an increased risk for the development of CRC for first-degree relatives, a colonoscopy was suggested. All the subjects interviewed were asymptomatic, and those who agreed for screening received colonoscopic examination. All colonoscopies were performed by experienced endoscopists at Iaşi Institute of Gastroenterology and Hepatology and Suceava County Hospital. The majority of screened subjects came from north-eastern region of Romania (Iaşi and Suceava counties).

RESULTS

Out of 216 subjects interviewed, 114 (52.7%) refused colonoscopy or any other type of screening test, and 102 (47.3%) subjects agreed to have a colonoscopy examination. The characteristics of screenees are presented in table I.

The endoscopic findings are shown in Table II. Overall, there were 30 lesions in 17 patients: 2 (6,6%) adenocarcinoma (one patient with Dukes A stage, the other with Dukes B), and 28 polypoid lesions in 15 patients (14 adenomas, 5 tubulovillous adenomas, 3 adenomas with high grade dysplasia and 6 hyperplasic polyps).

DISCUSSION

Familial risk of CRC is well-known and current guidelines recommend screening colonoscopy in first-degree relatives of patients with CRC (6,7,8). Several studies investigated the effectiveness of colonoscopy screening in close relatives of patients with common ("sporadic") CRC (those cancers not associated with familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or inflammatory bowel disease) (9,10,11,12, 13,14,15,16,17,18).

Colonoscopy performed by experienced

TABLE II
Results of histological examinations of the colonoscopic findings

No. of participants	102
Sex (M/F)	57/45
Mean age (range)	52±9.6 yrs (36-72 yrs)
Origin of participants	
Iaşi county	51 (50%)
Suceava county	32 (31,3%)
Others	19 (18,6%)
Relative affected by CRC	
Parents	86 (84,3%)
Siblings	16 (15,6%)

endoscopists is regarded as "gold standard" method for CRC screening; although is invasive and operator dependent, colonoscopy has the highest sensitivity among current methods, examines the entire colon, is cost-effective, and offers diagnosis and therapeutic opportunity in a single-session and the longest interval protection. Therefore, we adopted colonoscopy as a screening procedure for CRC, and the compliance was acceptable (47.3%). We did not routinely use sedation, and the cecum was intubated in 92.15% of colonoscopies. Therefore were no complications during colonoscopic examinations.

We found 30 lesions in 17 patients, a figure which is certainly higher than found in the general population aged over 50 years in our region. Our study confirmed the results of the other studies (9,10,12,13,15, 16,17). Thus, Baker et al. (15) reported 166 lesions (27% neoplastic) in 85 (42%) out of 201 asymptomatic patients who underwent colonoscopy based solely on a family history of CRC. Guillem et al. (12) prospectively colonoscoped 181 asymptomatic first-degree relatives of CRC patients and 83 asymptomatic controls, and found an increased prevalence of adenomas in first group. Pezzoli et al. (10) reported the results of a 5-years CRC screening program in asymptomatic subjects at increased risk and found colonoscopy-based screening feasible, effective and well accepted. Ruthotto et al. (9) investigated the compliance for colonoscopy in first-degree relatives of patients with CRC and found that less than 40% of them participate in colonoscopy screening for CRC; the authors suggested that better information of CRC patients about the potential risk for their relatives will increase participation in screening colonoscopy in their first-degree relatives. Wu et al. (17) found a high acceptability and safety of colonoscopy in screening persons with family history of CRC, and suggested the use of colonoscopy as an initial screening procedure. Similar results were reported by Syrigos et al, and other studies (16,18).

CONCLUSION

Our study shows that screening colonoscopy in first-degree relatives of patients with CRC is feasible, safe, and generally well accepted. First-degree relatives of CRC patients have a higher percentage of neoplasms than average-risk population from our region. The compliance rate in screening colonoscopy was acceptable and may increased by direct physician-patient contact.

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NOUTĂŢI

NIVELURILE SERICE ALE VITAMINELOR B SUNT INVERS PROPORŢIONALE CU RISCUL DE CANCER BRONHO-PULMONAR

Studiul european prospectiv de tip caz-martor EPIC (European Prospective Investigation into Cancer and Nutrition), desfăşurat între 1992 şi 2000, a cuprins 519978 subiecţi din 10 ţări europene, din care 385747 erau donatori de sânge. La aceştia au fost puse în evidenţă 899 cazuri de cancer bronho-pulmonar (CBP), ce au fost comparate cu 1770 de cazuri martor, luând în considerare data naşterii, sexul, ţara de origine şi momentul prelevării de sânge. Au fost dozate vitaminele B2, B6, B9 (folaţi) şi B12 ; metionina, homocisteina şi cotinina. După considerarea tabagismului cronic drept un factor potenţial confuzional, compararea între cele două grupuri a arătat că riscul de CBP a fost foarte scăzut în următoarele cazuri : nivel crescut al vitaminei B6, cu odds ratio (OR) – 0,44, $p < 0,000001$; nivel crescut de metionină (OR – 0,52, $p < 0,000001$), ambele tendinţe fiind independente de existenţa tabagismului sau de durata de supraveghere. Riscul de CBP a fost scăzut şi în cazul creşterii folaţilor (OR 0,68, $p = 0,001$) doar la fumători. Acest studiu caz- martor pledează în favoarea unei asocieri inverse între nivelul seric crescut al Vitaminei B6 şi al metioninei şi riscul de cancer bronho-pulmonar. O ipoteză seducătoare pentru eventuale aplicaţii terapeutice (Johansson M et al. Serum B vitamin levels and risk of lung cancer. *JAMA* 2010, 303 : 2377-2385).

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PSYCHIATRIC SYMPTOMS IN A PATIENT WITH VON RECKLINGHAUSEN DISEASE. CASE REPORT

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PSYCHIATRIC SYMPTOMS IN A PATIENT WITH VON RECKLINGHAUSEN DISEASE. CASE REPORT (Abstract) Neurofibromatosis represents a group of three diseases characterized by tumors of the nervous system. Of these three diseases type I neurofibromatosis (von Recklinghausen disease) is probably the best-known form and it is one of the most common genetic diseases. The clinical manifestations of the disease differ significantly from patient to patient, from the monosymptomatic to the plurisystemic form, and so does diseas e severity. We report the case of a 44-year-old male patient diagnosed with type I neurofibromatosis, his brother and father being also diagnosed with this disease, who during the di s-ease course had many hospitalizations for a depressive syndrome and suicidal ideation. Although the disease course is favorable in most patients, there are numerous complications and a negative impact on the quality of life. **Keywords**: NEUROFIBROMATOSIS TYPE I, DEPRESSION, QUALITY OF LIFE

Von Recklinghausen neurofibromatosis (type I neurofibromatosis) is a chronic progressive inherited disease with an autosomal dominant pattern of inheritance. The disease affects about 1 in 3000 individuals and is mainly characterized by *"café au lait"* spots, neurofibromas and optic glioma (1, 2). The phenotypic expression of type I neurofibromatosis (NF1) is quite variable, with some patients meeting only the minimum criteria necessary for diagnosis, whereas other patients are severely affected by cutaneous symptoms or other manifestation of this disease. NF1 gene has the potential for multiple system involvement and there is often an increase in the number of neurofibromas during puberty, at the time when young people are mostly concerned about their body image (3). A person`s physical appearance, along with his/her sexual identity represent the personal characteristic that are most obvious and accessible to others in social interactions. Generally, empirical research unequivocally documents that greater physical attractiveness is favorable for a person and lesser physical attractiveness is unfavorable. Society is often prejudicing against those with an atypical facial appearance or a disabil- ity. Thus, a person with a facial disfigure- ment may experience severe social rejec- tion (4, 5). Many patients described re-

striction in social contacts and lifestyle changes as they tried to hide peripheral neurofibromas. They are concealing the lumps in order to avoid explaining the condition to other people (6).

There are numerous reports about physical aspects of neurofibromatosis, but information about psychological features of these patients are rare and the reported findings are often based on highly selected groups of patients, sometimes not assessed in a systematic manner. Thus, psychiatric and developmental problems arise in patients with neurofibromatosis, when they are compared with other chronic neurological patients (7). Concomitant occurrence of neurofibromatosis and autism spectrum disorder has also been described in several reports (8, 9). Other studies assessing the psychiatric and social aspects of neurofibromatosis in a carefully defined population showed that the most commonly associated psychiatric conditions were depressive syndrome, anxiety state with vegetative dysfunctions and organic brain syndrome (10, 11, 12). In addition, the data also indicated an excess of occupational disability, unemployment or employment in a position characterized as not requiring special skills.

CASE REPORT

The patient I.F., a 44-year-old male is in the database of the "Socola" Institute of Psychiatry Iasi since 2005. But he was known with depressive symptoms since 2002 and he was treated like an outpatient. The last presentation at the hospital, in January 2018, was for an acute exacerbation of depressive symptoms with moderate depressive mood, psychomotor inhibition, anxiety, mixed insomnia, psycho-emotional lability with frequent crying, bradylalia, bradyphemia, depressive ideation, feelings of worthlessness, reduced self-esteem, hypobulia with social isolation and difficulties to integrate and function in social and family environment. During the current hospital stay (January 15-January 23, 2018) the psychological evaluation revealed astheno-dysthymic state with symptoms of anxiety in a psychosomatic context and emotional lability. The Hamilton Scale for Depression score was 18 points (13, 14). The global assessment functioning scale (GAF) score was 30 points (13). Woodworth-Mathews questionnaire: emotivity = 280, psychasthenia = 240, depression = 280, irritability = 212 (15). The questionnaire for the determination of accentuated personalities: emotivity = 87.5%, anxiety = 87.5% (16).

Also, the patient was diagnosed with several somatic conditions which influenced the current psychiatric symptoms: Recklinghausen disease (neurofibromatosis type I), elephantiasis of the left leg (Virchow neurofibromatosis), hepatic hemangioma, hypercholesterolemia and a minor traumatic head injury, without any immediate or tardive consequences. Physical examination revealed multiple growths all over his body, superficial and deep, of soft consistency, and multiple *"café au lait"* spots on the whole body, but especially at the posterior part of the thorax (fig.1). The patient also presented with left leg and ankle increased in volume suggestive of elephantiasis and venous dilatation in the left leg (fig. 2). We noticed discrete distal trophic changes in both lower limbs and pain at the Arnold points at distal compression (17). Neurological examination revealed active movements in normal ranges, deep tendon reflexes were symmetric, bilateral, normal coordination, and no patho-

logical findings at cranial nerve examination.

Fig. 1. Multiple growths on the skin with a soft consistency and multiple *"café au lait"* spots

Fig.2. Left leg increased in volume, appearance of elephantiasis

Electroencephalogram revealed a series of polymorphic theta waves (6 Hz, 20-30 microV) in the frontotemporal derivations that persisted when the eyes were opened. Basic tracing had a dominant alpha rhythm (11 Hz, 70-90 microV) especially at tempo-ro-parieto-occipital level bilaterally. Attenuation of pupillary reaction to light was normal. Hyperpnoea did not influence the EEG tracing.

The native computed tomography (CT) of the brain revealed: mild supratentorial cerebral atrophy, consistent with patient age. Ventricular system is slightly dilated and symmetrical. A small hypodense area corticalized at left frontopolar level (fig. 3). Numerous small inclusions of adipocyte density, thinning of the cerebral fissure as well as of the superior sagittal sinus and right sinus.

There is an area with the density of the cerebrospinal fluid, without walls, of 21/15/16 mm, probably an arachnoid cyst.

Also, frontal left sinus agenesis and right frontal sinus hypoplasia are observed; numerous oval tumors located bilaterally at occipital level and posterior cervical level, the largest of them of 10/9/16 mm, being located right posterolateral (fig. 4 a, b). All these images observed on the brain scan can have clinical consequences, regarding the future complications or the risk to develop a brain tumor, but the physician did not considered that a contrast-enhanced brain scan is necessary, at this moment of the evolution of the disease.

Fig. 3. Small sequel hypodense area corticalized at left frontopolar level

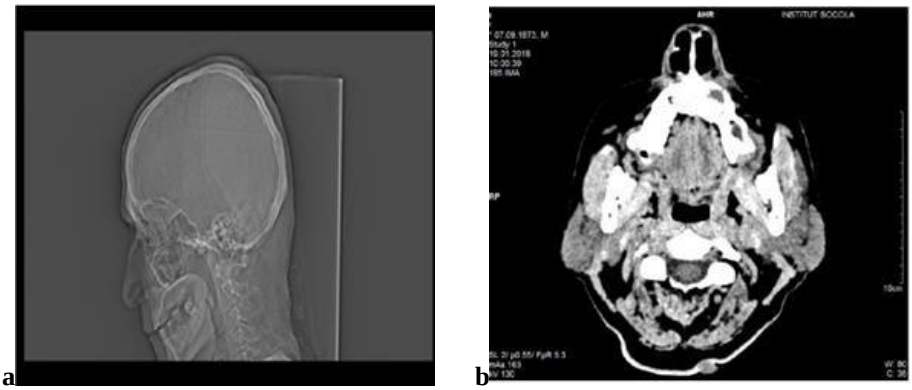

Fig. 4. a. Side image-subsegmental oval tumors located posterior cervical and bilateral occipital; b. Cross section-subcutaneous occipital formation located occipital subcutaneous.

Family history - both father and one brother were diagnosed with the same disease; father died in 2002 from a stroke, his brother was diagnosed with a brain tumor, already surgically removed.

History of the disorder

The onset of the psychiatric disorder was in 2002 after the death of his father, but in 2005 he was admitted to hospital for the first time due to suicidal thoughts. At that time, the symptoms were characterized by suicidal thoughts, marked psychomotor inhibition, mixed insomnia, feelings of guilt. Psychological assessment revealed severe depressive configuration in a person with avoidant personality with self-aggressive manifestations and a major suicidal risk. The treatment initiated at that moment consisted in antidepressant Paroxetinum 40 mg/day, benzodiazepine Diazepam 10 mg/day, sleep inducer Zolpidem 10 mg/day, cerebral trophic and vitamins group B. After his discharge from the hospital, the antidepressant treatment was continued in the outpatient unit; the symptoms remitted, but there was a dysthymic deficit explained by the fact that the patient only partially complied with the treatment.

During disease progression some complications were recorded, including a surgical intervention for a perianal abscess and a neurological symptom (vertigo) accounting for the exacerbation of the depressive symptoms which required a new hospitalization at the psychiatric department of the Iasi Military Hospital. Thus, at that hospitalization, the diagnosis of recurrent depressive disorder was established because the patient had more than two episodes of severe depression, without any manic or hypomanic episodes. The psychiatrist restarted the treatment with antidepressant medication Paroxetinum. His last hospital admission was in 2018 to the "Socola" Institute of Psychiatry Iasi caused to an exacerbation of the depressive symptoms and also because he didn't took any antidepressive medication for almost two weeks. Thus, we confirmed the diagnostic of recurrent depressive disorder.

In the case of a typical depressive episode, irrespective of its mild, moderate or severe intensity, depressive mood, lack of interest or pleasure, decreased energy, which causes increased fatigue and decreased activity are usually found. Marked tiredness after only slight physical effort is

also common. Other common symptoms are: (a) poor concentration and reduced attention span; (b) low self-esteem and self-confidence; (c) ideas of guilt and unworthiness (even in a mild intensity episode); (d) bleak and pessimistic views of the future; (e) thoughts or acts of self-harm or suicidal ideation; (f) disturbed sleep; (g) diminished appetite.

The positive diagnosis of "recurrent depressive disorder" was according to the criteria of International Statistical Classification of Diseases and Related Health Problems (ICD) 10th revised version and these are (18): the criteria for recurrent depressive disorder should be met; the current episode should meet the criteria for depressive episode, moderate severity and at least two episodes that should have lasted a minimum of two weeks and should have been separated by several months without significant mood disturbance. The disorder is characterized by repeated episodes of depression (mild, moderate or severe), without any history of independent episodes of mood elevation and overactivity that meet the criteria for mania.

The patient was unmarried, had no children, graduated ten classes, worked for 15 years, but had been medically retired for the past three years and lived with his family. He retained the ability to organize and plan spending and income, self-care and home care, but had a small group of friends. Currently, he is under antidepressant treatment with Tianeptine 12.5 mgx3/day and Lorazepam 1.0 mg/day for anxiety symptoms, he is compliant to treatment and come regularly to follow-up visits.

An important role in the therapeutic management of the case has been the use of some psychotherapeutic measures besides the pharmacological ones. Thus, psychoeducation aimed at informing the patient and his family about neurofibromatosis, a chronic, disabling illness. Information received by the patient from people around or from the internet was sometimes inappropriate, overwhelming, given the fact that the risk of many complications is exhaustively presented to him (19). Also, the frequent use of the term tumor, even if the majority of the diagnosed tumors are benign, has a negative connotation regarding the perception of the disease by the patient. That is why it has been specifically discussed how the patient assesses the risk of tumor malignancy but explains it in the context of general risk in the population.

By presenting the patient, but also his family to the therapist, the symptomatology of the disease and its possible later complications can be controlled, and the patient can create ways of coping for the situations in which anxiety occurs and they anticipate the unpredictability of the evolution of the disease.

Another important step in patient therapy was the use of cognitive psychotherapy techniques, considering the differences in the responses of patients to the diagnosis of chronic disease. Thus, a focused, structured, short-term psychotherapy, aimed at facilitating problem solving and restructuring dysfunctional thoughts and behaviors, was envisaged. This therapy also targeted depressive symptomatology, adopting and learning active measures to coping symptoms, and establishing an appropriate collaborative relationship with the therapeutic team.

The patient's thoughts related to his diagnosis are "nothing can be good any- more"; if someone has a chronic illness that supports depressive symptoms with suicid- al ruminations there is an important risk of suicidal behavior; also, depression can be

emphasized by avoiding various daily activities. In addition to cognitive techniques, behavioral strategies have been aimed at reducing factors that maintain and exacerbate both depressive-anxious and physical symptoms. Each meeting with the patient, which was initially weekly, and then two weeks, but later for a monthly therapy session to date, had a well-established working agenda. Of course, this agenda has been modified according to the patient's new needs, specific to each meeting, but also to our need to cover certain points of the meeting. Thus, the patient made journals in the form of homework that were discussed, by focusing on dispositional, behavioral changes, as well as thoughts with negative, intrusive or ruminative connotations correlated with the diagnosis of chronic or suicide-related disorders.

Thus, the patient noted the thoughts and emotions determined by the fact that he was diagnosed with a chronic, genetic transmission disorder requiring multiple treatments and periodic clinical assessments, with many risks and complications for the future. It also quantified how different somatic complications had an impact on daily functionality and various activities as a result of pain or other physical symptoms and how often it needed reassurances regarding its subsequent family evolution or different medical professionals. The fact that the patient has a genetic disease with uncertain evolution for the future makes him depressed, anxious, so our role has been to improve our patient's own coping mechanisms, the long-term self-management of this disease. Bearing in mind the lower compliance of the patient to drug therapy, there have been special sessions dedicated to this aspect of the therapeutic plan. In this regard, he has been taught to keep a journal about the times when he is taking his medication, as well as those in whom he has depressive and anxious symptoms, and to see if there is a correlation or not between these periods. Thus, it was intended to increase therapeutic long-term compliance as we found that discontinuation of medication was a trigger in worsening of psychiatric symptomatology.

DISCUSSION

Neurofibromatosis, a chronic progressive genetic disease, has a dominant autosomal transmission and its penetrance is almost complete by five years of age. Our patient was diagnosed with the disease from birth. The disease is characterized by benign tumors in any organ, especially on skin, but they can also be present in the nervous system, eyes or bone system. The number and size of these tumors increase in time and spread out over the whole body. The presence of brown, *"cafe au lait"* spots and nervous malformations can also be encountered. The treatment is being symptomatic, and it is represented by surgical removal of the tumors.

In the patients with this disease, psychiatric disorders can be more frequent than in general population and include dysthymia, depressive mood, anxiety, and personality disorders. In the here-reported case, the prognosis of the psychiatric disorder is influenced by the future course of the neurofibromatosis complications and especially by the cerebral complications.

The psychiatric symptoms and their impact to functioning were evaluated using some psychometric scales. The Hamilton Scale for Depression score was 18 points, means a moderate depression. This scale is used to determine the severity of depres- sion and can be also used in psychophar-

macological research to evaluate compliance and response to treatment. It was published in 1960, revised in 1967 by Max Hamilton and is one of the first instruments to evaluate the severity of depressive symptoms. It is a 17-item scale which measures the intensity of symptoms specif- ic to a depressive syndrome according to DSM IV (Diagnostic and Statistical Manual of Mental Disorders 4th edition) criteria for diagnosis. It is quite sensitive to changes in symptoms during an antidepressant treat- ment (13, 14).

The global assessment functioning scale (GAF) score was 30 points, means his inability to function in almost all areas of life, like occupational, familial or social functioning caused to the severity of the psychiatric symptoms. The global assessment functioning (GAF) scale evaluates the correlation between social functioning, adaptive incapacity and working capacity.

The dysfunctions can be primary, like in the case of a disease, lesions or brain trauma, or secondary, like in a systemic disease which affects the brain and other organs (13).

Woodworth-Mathews questionnaire: emotivity = 280, psychasthenia = 240, depression = 280, irritability = 212. The *Woodworth Mathews Questionnaire* contains 76 questions that highlight the psychopathological tendencies of personality. The Romanian standard of multifactorial profile for psychopathological tendencies reveals a marked risk of inadequacy for scores between 180-260 and a pathological risk for scores between 260 and 360 (15).

The questionnaire for the determination of accentuated personalities: emotivity = 87.5%, anxiety = 87.5%. This questionnaire was developed by H. Schmiescheck for the identification of accentuated personality traits according to the theory of the neuropsychiatrist Karl Leonhard (16).

The short-term prognosis of the depressive syndrome is favorable given that adequate antidepressant therapy has been instituted, the patient is compliant with treatment, the intensity of depression is moderate, and the patient has good social and family insertion. Reduced quality of life is determined by the motor deficit - elephantiasis and disfigurement is determining a tendency to social isolation.

Medium and long-term prognosis is worsened by the probability to develop an expansive intracranial process, suggested by the changes observed on CT brain scan. Also, a family history of cerebral tumors additionally increases this risk. If an intracranial expansive process is diagnosed, secondary cognitive dysfunction can be developed even if the tumor can be resolved surgically or not. Additionally, there is a risk of intracranial hypertension and we recommended the patient to be evaluated by a neurosurgeon in order to create a multidisciplinary team together with the general practitioner, neurologist and psychologist to prevent any other severe complications. Moreover, the symptoms of anxiety or depression developed in an organic context may have negative consequences in terms of the therapeutic response to psychotropic medication comparing with a case without any somatic issues. Considering the patient's history of suicidal behavior, he will have suicidal ideation in the future, suicidal risk being one of the most severe complications.

Risk of progredient development of new tumors will increase the risk of affecting major peripheral nerves with secondary functional impotence, limiting activities with negative consequences on psychiatric

symptoms and especially on the depressive syndrome. Antidepressant treatment will be necessary for a long period of time with regular follow-up and regular psychiatric visits to increase the quality of life of the patient.

CONCLUSIONS

In this case, a multidisciplinary team will be essential for improving the prognosis of this patient. Society is often prejudicing against those with lesser physical attractiveness or atypical facial appearance or handicap. It is important for the patient to be encouraged to have an insertion in his socio-familial environment, to keep the capacity of organizing and planning of income and expenses, the capacity of self-care and the upkeep of the house. Any psychological difficulties regarding self-esteem or feelings of rejection from others must be discussed and the patient can learn how to accept and deal with the problems related to the physical aspects of the dis- ease as well as the somatic and psychiatric ones (20).

The particularity of this case is due to the relatively early onset of depressive symptomatology, which occurred in a reactive-situational context and which was aggravated by the pre-existing chronic disease, neurofibromatosis. Thus, psychological counseling and periodic psychiatric evaluation of these patients, who are diagnosed with chronic hereditary diseases, such as neurofibromatosis, should diagnose and treat, as soon as possible, the psychiatric symptomatology that can be associated.

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NEWS

MAGNETIC RESONANCE SPECTROSCOPY – CLINICAL APPLICATIONS

The basic principle of magnetic resonance spectroscopy is that the distribution of electrons around an atom cause nuclei in different molecules to experience a slightly different magne t-ic field. This results in slightly different resonant frequencies, which in turn return a slightly different signal. Magnetic resonance spectra can be acquired from any "active" nucleus: protons, carbon-13 and phosphorus-31 and. In clinical practice only proton spectra (which enable the resolution of metabolite profiles in vivo) are encountered. Magnetic resonance spe c-troscopy (MRS) is performed with many pulse sequences, for example spin echo sequences, can measure the peaks of N-acetyl aspartate, creatine, choline, lactate in different tissues and has various clinical applications. Can help increase our ability to predict glioma's grade: as the grade increases, N-acetyl aspartate (NAA) and creatine decrease and choline, lipids and lactate increase. We can differentiate radiation change and tumor recurrence: in recurrent tumor choline will be elevated, whereas in radiation change, NAA, choline and creatine will all be low. When brain infarction takes place lactate will increase, then lipids are released, and peaks appear. Within bacterial abscess cavities, lactate is elevated.

THE INCIDENCE OF ADVERSE EFFECTS ON ANTIEPILEPTIC MEDICATION IN CHILDREN DIAGNOSED WITH EPILEPSY

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THE INCIDENCE OF ADVERSE EFFECTS ON ANTIEPILEPTIC MEDICATION IN CHILDREN DIAGNOSED WITH EPILEPSY (Abstract): The treatment of epilepsy is complex and its purpose being to achieve the best possible control of seizures with minimal side effects. The presence of side effects of antiepileptic drugs is one of the major problems of antiepileptic therapy. The aim of the study was to evaluate the incidence of side effects of antiepileptic drugs in children diagnosed with different types of epilepsy. **Material and methods:** The authors followed 247 patients aged between 0 and 17 years old, diagnosed with epilepsy between 01.01.2010 - 01.12.2018. The study analyzed retrospectively the evolution of the seizures under treatment and the presence of the side effects of antiepileptic drugs. The Composite Index of Impairments was used in order to properly evaluate the problems caused by the medicine used. **Results:** From the study group 47.77% of the patients reported side effects, more commonly in children receiving polytherapy (88.46%). In 96.61% of children, the severity of the side effects was low/medium and 4 patients (3.39%), adverse reactions were severe, the score at Composite Index of Impairments being ≥50. The most frequent adverse reactions were represented by behavioral changes, sleepiness, gastrointestinal disorders. **Conclusions:** Patients who received antiepileptic therapy in polytherapy had more frequent side effects than those who undergone monotherapy. The presence of adverse events has an undeniable negative effect on the quality of life of children diagnosed with epilepsy. **Keywords:** ANTIEPILEPTIC DRUGS, ADVERSE EFFECTS, CHILDREN.

The treatment of epilepsy is complex and the improvement of the diagnostic methods as well as the discovery of new antiepileptic drugs have led to the development of therapeutic possibilities of epileptic seizures in the child. Antiepileptic therapy is symptomatic, its purpose being to achieve the best possible control of seizures with minimal side effects. The presence of adverse reactions is one of the major problems of antiepileptic therapy. They alter the clinical picture, adding its own symptoms which negatively affect the quality of life of the patient. Complete seizure control is important for most patients suffering from epilepsy; they are generally

accepting the adverse effects of medication and continue antiepileptic treatment.

The aim of the study was to evaluate the incidence of side effects of antiepileptic medication in children diagnosed with different types of epilepsy given the fact that most studies in pediatric patients with epilepsy are focused on the clinical effectiveness of antiepileptic medication.

MATERIAL AND METHODS

In our study we followed children aged between 0 and 17 years old, diagnosed with epilepsy in the Pediatric Neurology Department of the "Sf. Maria" Emergency Clinic Hospital for Children, Iasi, Romania between 01.01.2010-01.12.2018. The study analyzed retrospectively the evolution of the seizures under treatment and the presence of the side effects of antiepileptic drugs. Patients were included in study had two or more epileptic seizures diagnosed by a doctor (family doctor or neuropediatrician). Repeated seizures (two or more) within 24 hours were considered to be a single seizure. Children who had only simple febrile seizures, without presenting any other seizures without fever, were not included in the study. Those with febrile epileptic status were considered to have epilepsy only if they also had seizures without fever. The diagnosis of epilepsy was based on electro-clinical data. As for the children who displayed several types of seizures, it was decided which type was the most significant from the clinical perspective. However, information about the other forms of seizures was recorded as well.

Epileptic seizures and syndromes have been classified according to the International League Against Epilepsy Classification of 2010 (1). The study group included 247 children, out of which 106 girls and 142 boys. 102 (41.29%) were diagnosed with presumed genetic epilepsy, 13 (5.27%) with unknown cause of epilepsy and 132 (53.44%) with structural-metabolic epilepsy. Of the children studied, 89 (36.03%) presented partial seizures, 152 (61.53%) generalized seizures, and 6 (2.44%) other types of seizures.

169 (68.42%) of the patients received monotherapy and 78 (31.58%) of the patients received polytherapy. All antiepileptic drugs were prescribed in therapeutic doses. Sodium valproate (VPA), Lamotrigine (LTG), Topiramate (TPM), Oxcarbazepine (OXC), Levetiracetam (LEV) and Carbamazepine (CBZ) were used in both monotherapy and polytherapy. Benzodiazepines (BZD), Phenobarbital (PB), Ethosuximide (ETX), Stiripentol were used only in association.

The Composite Index of Impairments was used in order to properly evaluate the problems caused by the medicine used - table I, which contains 3 subscales, Seizure Activity Index which analyzes the type and frequency of the crises, The Neurotoxicity Rating Scale (neurotoxicity index) which assesses the severity of the secondary neurological effects and the Systemic Toxicity Rating, which assesses the severity of systemic side effects of antiepileptic drugs (2).

The score for each symptom ranges from 0 to 50. The 0 score indicates complete seizure control and the absence of side effects, while a score of 50 or >50 stands for low seizure control and the presence of side effects which required the modification of the therapeutic regimen (tab. II).

TABLE I

The range of the toxicity score for each symptom used in CII

Effect	Score	Effect	Score
Neurotoxicity		*Systemic toxicity*	
◆ diplopia	15-30	◆ oral and gingival impairments	3-20
◆ nystagmus	5-10	◆ gastrointestinal impairments	3-50
◆ dysarthria	5-30	◆ hematopoiesis disorders	50
◆ ataxia	5-50	◆ dermatological impairments	20-50
◆ tremor	10-50	◆ renal impairments	50
◆ emotional instability and mood disorders	5-50	◆ liver impairments	25-50
◆ cognitive and behavior disorders	5-50	◆ weight gain or weight loss	3-20
◆ dizziness	3-50	◆ hair loss	5-50
◆ headache	3-50		
◆ sedation	5-50		

TABLE II

Side effects of antiepileptic drugs correlated with the values of the CII score

CII	Neurological and/ or systemic side effects	Epileptic seizures control
0	0	good
1-10	easy	acceptable
11-49	moderate	acceptable
≥ 50	severe	absent

RESULTS

In our study, 129 (52.23%) of patients did not report any adverse effects of antiepileptic drugs, the score measured by the Composite Index of Impairments being 0. 118 (47.77%) of patients reported adverse effects, out of which 67 (56.77%) of patients only one adverse effect and 51 (43.23%) of patients at least two adverse reactions.

Side effects were more commonly reported in children receiving antiepileptic treatment in polytherapy (88.46%) compared to those receiving monotherapy (28.99%).

In 114 (96.61%) of children, the severity of the side effects was low/medium, in these cases the score at Composite Index of Impairments ranging between 1-49. In 4 patients (3.39%), adverse reactions were severe (2 cases of Steven Johnson syndrome in combination with Lamotrigine, one case of acute renal failure at the initiation of Topiramate treatment, one case of toxic hepatitis in combination with Stiripentol), this time the score at Composite Index of Impairments being ≥50.

Antiepileptic medication was discontinued and considered responsible for adverse reactions in children with skin rash following the administration of antiepileptic drugs (Sodium Valproate, Phenobarbital, Levetiracetam) and in all those with severe side effects. In other 20/118 cases, the antiepileptic drugs dose was reduced in case of side effects.

The most frequent adverse reactions were represented by behavioral changes, sleepiness, gastrointestinal disorders (tab. III).

TABLE III

The distribution of adverse effects associated with antiepileptic medication

Adverse effects	VPA	CBZ	LTG	TPM	LEV	OXC	PB	ETX	Stiripentol	BZD
Neurotoxicity										
diplopia		3								
nystagmus						1				
dysarthria										
ataxia					1	1				
tremor	3		3							
emotional instability and mood disorders		2					3			
cognitive and behavior disorders	6	8	1	4	3		5			
dizziness		2				2	3			6
headache			5			3	2			3
sedation	3	5		3	1	4	4			5
Systemic toxicity										
oral and gingival impairments	2	3								
gastrointestinal impairments	8	10	4	4	3	6		2		
hematopoiesis disorders										
dermatological impairments	5	2	2		1		5			
renal impairments				1						
liver impairments	4	5							1	
weight gain or weight loss	8	7		4						
hair loss	6		1							

DISCUSSION

According to The Food and Drug Administration's definition, the adverse effects are regarded as "any adverse event associated with the use of a drug in humans, even if it is not considered to be associated with the drug" (3). From the classifications of adverse effects, the most useful one is the idiosyncratic or dose-related classification because it manages to identify the drug as the cause of the adverse effect; if there is a connection with its dose, it results that a dose reduction will cause the reduction/disappearance of the adverse reaction (4). At the same time, classification in connection with the organ that may be affected is useful in the clinical and paraclinical monitoring of the specific organ which must be carefully monitored during treatment (4, 5).

The presence of adverse effects in antiepileptic drugs is an important problem of children diagnosed with epilepsy who require chronic treatment.

In the study we conducted, 47.77% of evaluated patients had adverse reactions: 56.77% reported a single adverse event, while 43.23% at least two side effects.

The occurrence of side effects varied with the type of antiepileptic drugs used. For example, in patients who received VPA, gastric discomfort and weight gain were the most usual problems caused by medication; in the case of LTG treatment, headache and gastrointestinal disorders; in patients treated with TPM, anorexia and weight loss, emotional lability, difficulty concentrating. In the case of antiepileptic medications, the presence of the side effects depended on the way the medication was introduced, thus the rapid increase of the dose of LTG favored their occurrence.

Depending on the therapeutic scheme used, there were differences regarding the occurrence of adverse effects, more frequent in patients who received polytherapy (88.46% of patients), but there was no distinction regarding the intensity of issues caused by medication. Sedation and cognitive disorders were two of the most usual neurological side effects in children with polytherapy.

This data are similar to that which was reported in the literature. In a prospective study which included 180 children with epilepsy, 135 of whom received monotherapy, and 45 with polytherapy, Anderson and co-workers (2015) reported the presence of adverse effects in approximately 1/3 of the patients. In 61% of the cases, the adverse reactions were average, in 3 cases being severe (2 cases with Steven Johnson syndrome and one case with respiratory arrest). The most common adverse events reported were behavioral disorders (12%), sleepiness and sleep problems (10%) (6).

Also, Peruca *et al*. (2009) examined the existence of at least one side effect in 88% of the patients followed and at least two adverse reactions in 82% (7). The most prevailing adverse events observed were fatigue (63.3%), memory disorders (58.3%), difficulty concentrating (44.4%), nervousness or agitation (43.2%), headache (42.7%), drowsiness (41.1%).

Jayalekshmi *et al*. (2016) reported the presence of side effects in 56 cases, of which 42.85% were represented by manifestations due to central nervous system damage. These observations were made in a study in which 180 patients with epilepsy (8).

In our own study, adverse effects occurred most frequently at the initiation of treatment. In no patient was it observed the increase of the risk of occurrence of new secondary events, simultaneously with the increase of the duration of treatment. There were no specific side effects in connection with a certain age.

CONCLUSIONS

Antiepileptic medication is generally well-tolerated, with adverse effects being usually minimal or medium, thus not requiring treatment interruption. The presence of severe side effects imposed a break in the administration of the antiepileptic drug responsible for their occurrence and the modification of the therapeutic regimen. Patients who received antiepileptic therapy in polytherapy had more frequent adverse effects than those who undergone monotherapy. The presence of adverse events has an undeniable negative effect on the quality of life of children diagnosed with epilepsy.

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NEWS

A PERSPECTIVE ON NANOPARTICLE UNIVERSAL INFLUENZA VACCINES

Annually recurring seasonal influenza causes massive economic loss and poses severe threats to public health worldwide. The current seasonal influenza vaccines are the most effective means of preventing influenza infections but possess major weaknesses. Seasonal influenza vaccines require annual updating of the vaccine strains. However, it is an unreachable task to accurately predict the future circulating strains. Vaccines with mismatched strains dramatically compromise the vaccine efficacy. In addition, the seasonal influenza vaccines are ineffective against an unpredictable pandemic. A universal influenza vaccine would overcome these weaknesses of the seasonal vaccines and abolish the threat of influenza pandemics. One approach under investigation is to design influenza vaccine immunogens based on conserved, type-specific amino acid sequences and conformational epitopes, rather than strain-specific. Such vaccines can elicit broadly reactive humoral and cellular immunity. Universal influenza vaccine development has intensively employed nanotechnology because the structural and morphological properties of nanoparticles dramatically improve vaccine immunogenicity and the induced immunity duration. Layered protein nanoparticles can decrease off-target immune responses, fine-tune antigen recognition and processing, and facilitate comprehensive immune response induction. Herein, we review the designs of effective nanoparticle universal influenza vaccines, the recent discoveries of specific nanoparticle features that contribute to immunogenicity enhancement, and recent progress in clinical trials (Lei Deng and Bao-Zhong Wang. A Perspective on Nanoparticle Universal Influenza Vaccines. *ACS Infect Dis* 2018; 4(12): 1656-1665).

OVARIAN ENDOMETRIOSIS AND VITAMIN D SERUM LEVELS

The aim of this study was to assess the vitamin D serum level in women with ovarian endometriosis; specifically, a possible correlation between the dimensions of ovarian endometriomas and vitamin D serum levels was evaluated. This was an observational study of childbearing-age women diagnosed with singleton ovarian endometrioma. Women diagnosed with multiple ovarian endometriomas or extraovarian endometriosis were excluded. Results: Forty-nine women constituted the initial study cohort. In these women, the mean 25-OH-D3 serum level was 22 ng/mL, and 42 of them (85,7%) were diagnosed with “hypovitaminosis D women” the mean diameter of ovarian endometriomas was 40.2±22.6 mm while in the “normal vitamin D serum level women” it was 26.7.12,1 mm. However, a significant linear correlation between 25-OH-D3 serum level and the diameter of ovarian endometriomas was found. (Ciavattini A, *et al. Gynecol Endocrinol* 2017; 33(2):164-167).

BLEEDING EVENTS IN PATIENTS WITH HCV- RELATED LIVER CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRALS - FACT OR NATURAL COURSE ?

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BLEEDING EVENTS IN PATIENTS WITH HCV- RELATED LIVER CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRALS-FACT OR NATURAL COURSE ? (Abstract) **Aim**: We aimed to assess the incidence and risk factors of bleeding events in cirrhotic patients with sustained virological response (SVR) treated with direct-acting antivirals (DAAs) therapy. **Material and methods:** We retrospectively analyzed a cohort of patients with HCV-related liver cirrhosis treated with paritaprevir/ritonavir, ombitasvir and dasabuvir(PrOD) ± ribavirin and ledipasvir/sofosbuvir (LED/SOF) ± ribavirin for 12/24 weeks, in a tertiary gastroenterology referral center from North-Eastern Romania, between January 1st2016 and January 1st, 2019. All patients with presumption of digestive bleeding (hematemesis or melena) were evaluated and confirmed by upper digestive endoscopy. Patients known with thrombophilia were not included in the study group. **Results:** The study included 874HCV-infected cirrhotic patients treated with PrOD or LED/SOF ± RBV, with documented SVR, mean age 58,7±6,2years, predominantly female (58%). Of the total number, 443 (50.68%) received PrOD and 431 (49.31%) patients were treated with LED/SOF ± RBV. From all, 572 (65.34%) patients had Child-Pugh class A, 226 (25.96%) class B and 76 (8.7%) class C cirrhosis. Mean period from SVR and the occurrence of bleeding events was 230±121 days. Bleeding complications after SVR were reported in 16(1.83%) patients: 9 (56.25%) with variceal hemorrhage and7 (43.75%) with non-variceal hemorrhage. There was no significant change in prothrombin serum levels (baseline values in patients treated with PrOD was 11.67 ± 0.91 versus 11.70 ± 0.83 at SVR, p=0.993, respectively 11.5 ± 0.84 sec at baseline versus 11.4 ± 0.68 at SVR, p=0.715 in patients treated with LED/SOF+RBV) and platelet count (126 000 (101 500-162 000)*vs*. 131 000 (101 000-165 000), p=0.818 in patients treated with PrOD, respectively 94857.14±32 *vs*. 92428.57 ± 35, p= 0.853, in patients treated with LED/SOF+RBV).**Conclusions**: Bleeding events in patients with HCV-related liver cirrhosis treated with DAAs are not influenced by the variations of coagulation parameters, rather correspond to the hemodynamic changes induced by the status of advanced liver disease. It should be underlined that the bleeding events were mostly variceal bleeding, due to persistent portal hypertension despite viral eradication. **Keywords**: DIRECT ACTING ANTIVIRAL THERAPY, SUSTAINED VIROLOGIC RESPONSE, BLEEDING COMPLICATIONS, LIVER CIRRHOSIS.

The complications of liver cirrhosis frequently occur in the evolution of the disease, with a negative impact on the prognosis of patients.

In daily practice, we frequently meet cirrhotic patients who associate bleeding complications, especially variceal bleeding sustained biologically by prolonged bleeding and coagulation times, which is why it was assumed that these patients are characterized by a state of hypocoagulability induced by the deficiency of coagulation factors (1, 2).

Although thrombotic events may occur in the development of liver cirrhosis, bleeding is much more frequent and is based on a complex mechanism that involves increased pressure in the portal system, changes in vascular endothelium and coagulation disorders (3).

The vascular endothelium plays a central role in the pathophysiology of portal hypertension and in the control of coagulation. Physiologically the hepatic sinusoidal endothelial cells are different from the rest of the endothelial cells in the body because they have fenestrations, do not have platelet adhesion molecules (PECAM1 or CD31), E-selectin, and have no basement membrane. Capillary hepatic sinusoids determine their transformation into vascular endothelial cells with loss of fenestration, basal membrane formation, and expression of new surface adhesion molecules. All these changes contribute to an increased resistance to flow and the development of portal hypertension in cirrhotic patients (4).

The bleeding complications encountered in cirrhotic patients may be due to portal hypertension (bleeding from the esophageal, gastric, ectopic varices, portal gastropathy) or, in the advanced stages of the disease, by the status of hypocoagulability that could be associated with the appearance of cutaneous, mucosal or intracranial bleeding(5).

Portal pressure, assessed by hepatic venous pressure gradient (HVPG) measurement, determines the development of liver-related complications and mortality in patients advanced chronic liver disease. Variceal bleeding is one of the most severe complications associated with liver cirrhosis, being the most significant complication of portal hypertension, which is associated with a high early mortality (20% at 6 week)(6).

Since the onset of esophageal varices, the risk of bleeding is mainly related to local hemodynamic factors and mechanical parameters such as the pressure gradient between the hepatic vein and the portal vein, the degree of varicose veins, the presence of *cherry spots* and the severity of liver cirrhosis (7).

Direct-acting antivirals (DAAs) have radically changed the management of chronic HCV infection over the past years through high effectiveness and safeness, with a major impact on the prognosis and burden of the disease. The DAAs mechanism of action directly targets viral protease, polymerase and nonstructural proteins, thus allowing a high rate of viral clearance without significant side effects (8).

Sustained virologic response (SVR) is now achieved in >90% of the patients and is associated with improvements in liver function, fibrosis and overall survival. Portal hypertension is also expected to improve with virological response, along with the improvements in liver inflammation and liver fibrosis (9). The knowledge of the effects of hepatitis C virus (HCV) elimination on clinically significant outcomes like portal hypertension and its

complications is thus of unremarkable importance since it can influence management after SVR (10).

Recent studies have shown the positive impact on liver function, as measured by the CHILD-PUGH and MELD score, as well as the decompensation rate after obtaining SVR with the new oral direct-acting antivirals, but there are no conclusive data regarding the effect on clotting disorders due to liver cirrhosis (11). In our study, we aimed to assess the incidence and risk factors of bleeding events in cirrhotic patients with SVR treated with DAAs therapy.

MATERIALS AND METHODS

This is a retrospective study in which we included patients with HCV-related liver cirrhosis which fulfilled the criteria for antiviral therapy, conducted in a tertiary gastroenterology referral center from North-Eastern Romania, between January 1st, 2016 and January 1st 2019. The inclusion and exclusion criteria was according to our National Protocol. The regimens used were: Paritaprevir/Ritonavir, Ombitasvir and Dasabuvir (PrOD) ± Ribavirin in patients with HCV-related compensated liver cirrhosis (LC) and Ledipasvir/Sofosbuvir (LED/SOF)+Ribavirin (RBV) for 12/24weeks, in patients with compensated and decompensated LC. Diagnosis of cirrhosis was established based on clinical features, blood tests and imaging methods.

The study protocol was approved by the Ethical Research Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi and by the Ethical Committee of the "Sf. Spiridon" County Clinical Emergency Hospital. All patients were required to sign an informed consent.

The diagnosis of liver cirrhosis was established by abdominal ultrasound (Philips HD 11xe ultrasound system) and non-invasive techniques for assessing liver fibrosis (Fibroscan and Fibromax). Blood tests were performed at baseline,at12/24 weeks after end of treatment (sustained virological response- SVR12) and during the presentation for digestive bleeding. All patients with presumption of digestive bleeding (hematemesis or melena) were evaluated and confirmed by upper digestive endoscopy performed in emergency and blood samples.

Statistical analysis. Data collected was statistically analyzed using *SPSS 20.0* (Chicago, IL, SUA). tests were two-tailed with p-value <0.05 was considered statistically significant. The quantitative variables were compared using the t-Student test, and the qualitative parameters were evaluated by the chi-square test.

RESULTS

The study included 874 HCV-infected cirrhotic patients treated with PrOD or LED/SOF± RBV, between January 1st, 2016 and January 1st, 2019, with documented SVR, mean age58,7 ± 6,2 years, predominantly female (58%). Of the total number, 443 (50.68%) received PrOD and 431 (49.31%) patients were treated with LED/SOF ± RBV. From all, 721 (82.49%) had compensated LC and 153 (17.50%) had decompensated LC. Child-Pugh score at baseline was 8.64 ± 1 in the LED/SOF ± RBV group versus 5.06±0.24 points (tab. I).

All patients had SVR documented by undetectable HCV-RNA. In patients treated with PrOD, GGT, AST and ALT had significant decreased values at SVR12 compared to baseline (p<0.001). Also, in the LED/SOF±RBV group, transaminases levels had an improving trend (p<0.001) (tab II, III).

TABLE I

Demographic characteristics at baseline

Demographic characteristics at baseline	All (n=874)
Females, n (%)	507 (58%)
Age (mean ± SD)	58,7 ± 6,2
Treatment Regimen	
PrOD, n (%)	443 (50.68%)
LED/SOF+RBV, n (%)	431 (49.31%)
CHILD-PUGH Score	
PrOD (mean ± SD)	5.06 ± 0.24
LED/SOF±RBV (mean ± SD)	8.64 ± 1
Stage of liver disease	
Compensated LC	721 (82.49%)
Decompensated LC	153 (17.50%)
Demographic characteristics at baseline	**All (n=874)**
Females, n (%)	507 (58%)
Age (mean ± SD)	58,7 ± 6,2
Treatment Regimen	
PrOD, n (%)	443 (50.68%)
LED/SOF+RBV, n (%)	431 (49.31%)
CHILD-PUGH Score	
PrOD (mean ± SD)	5.06 ± 0.24
LED/SOF±RBV (mean ± SD)	8.64 ± 1
Stage of liver disease	
Compensated LC	721 (82.49%)
Decompensated LC	153 (17.50%)

TABLE II

Dynamic of biochemical parameters in patients treated with PrOD at baseline *vs.* SVR12

Parameters	Baseline	SVR12	p
PLT (/mm^3), median (IQR)	126 000 (101 500-162 000)	131 000(101 000-165 000)	0.818
INR, median (IQR)	1.1 (1.06-1.2)	1.19 (1.12-1.21)	0.002
TP (sec)	11.67 ± 0.91	11.70 ± 0.83	0.993
AST (U/L), median (IQR)	80 (55.5-120)	24 (21-32)	<0.001
ALT (U/L), median (IQR)	85 (62-119.5)	23 (20-33)	<0.001
GGT (U/L), median (IQR)	66 (40-108)	35 (23-55)	<0.001
Bilirubin (mg/dL), median (IQR)	0.93(0.77-1.38)	0.89 (0.61-1.1)	0.038
Creatinine (mg/dL) (mean ± SD)	0.76 ± 0.1	0.83 ± 1.67	0.004
Hb (g/dL)	14.27 ± 1.54	13.48 ± 1.59	0.002
Albumin (g/dL)	4.06 ± 0.43	4.01 ± 0.29	0.557
MELD	7.8 ± 1.79	7.39 ± 1.82	0.192

TABLE III

Dynamic of biochemical parameters in patients treated with LED/SOF at baseline *vs.* SVR12

Parameters	Baseline	SVR12	P
PLT (mm^3), (mean ± SD)	94857.14 ± 32	92428.57 ± 35	0.853
INR, (mean ± SD)	1.28 ± 0.12	1.20 ± 0.08	0.05
TP (mean ± SD)	11.5 ± 0.84	11.4 ± 0.68	0.715
ALT (U/L), (mean ± SD)	86 ± 49.72	24.07 ± 13.33	< 0.001
AST (U/L), (mean ± SD)	76 ± 32.45	25 ± 18.29	< 0.001
GGT (U/L), (mean ± SD)	43.64 ± 19.66	33.71 ± 15.34	0.148
BT (MG/dL), (mean ± SD)	2.05 ± 0.66	1.50 ± 0.71	0.044
Creatinine (mg/dL) (mean ± SD)	0.7 ± 0.22	0.73 ± 0.15	0.626
Hb (g/dL), (mean ± SD)	12.82 ± 1.51	13.17 ± 1.50	0.544
Albumin (g/L), (mean ± SD)	3.30 ± 0.52	3.71 ± 0.39	0.026
MELD (mean ± SD)	12.46 ± 3.07	9.5 ± 3.32	0.024

Mean period from SVR and the occurrence of bleeding events was 230±121 days.

Bleeding complications after SVR were reported in 16 (1.83%) patients: 9 (56.25%) with variceal hemorrhage and 7 (43.75%) with non-variceal hemorrhage (3 (18.75%)- portal hypertensive gastropathy, 2 (12.5%)- gastric ulcer,1(6.25%)- duodenal ulcer, 1 (6.25%)- Mallory-Weiss syndrome). Four patients had previous episodes of variceal hemorrhage before initiating direct antiviral therapy (fig.1).

From all patients, 572 (65.34%) patients had LC Child-Pugh class A, 226 (25.96%) class B and 76 (8.7%) class C cirrhotic patients (fig. 2).

There was no significant change in prothrombin serum levels in both groups: baseline values in patients treated with PrOD was 11.67±0.91 versus 11.70±0.83at SVR, p=0.993, respectively 11.5±0.84 sec at baseline versus 11.4±0.68 at SVR, p=0.715 in patients treated with LED/SOF + RBV (fig.3).

Platelet count had no significant changes at baseline versus SVR in both groups (126 000 (101 500-162 000) *vs*. 131 000 (101 000-165 000), p= 0.818 in patients treated with PrOD, respectively 94857.14±32 *vs*. 92428.57±35, p= 0.853, in patients treated with LED/SOF+RBV) (fig. 4).

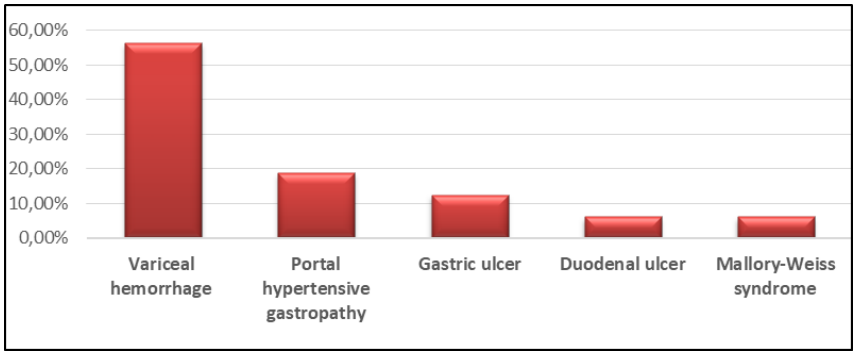

Fig. 1. Bleeding events after SVR12

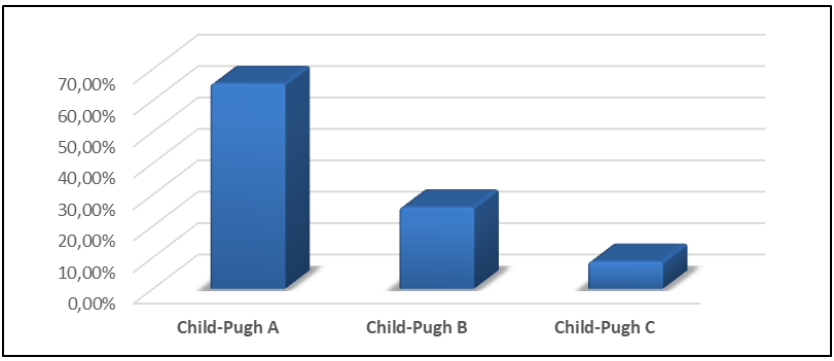

Fig. 2. Distribution of patients according to severity of liver disease evaluated with CPS

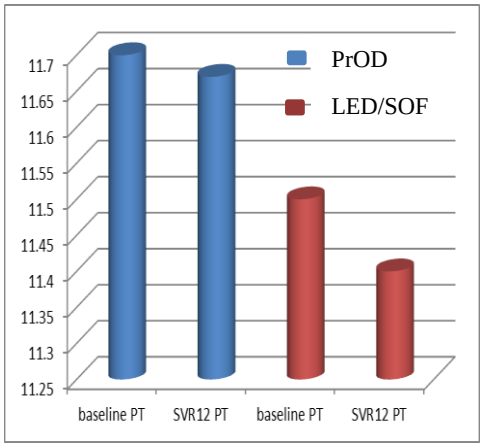

Fig. 3. Prothrombin serum levels at baseline and SVR12

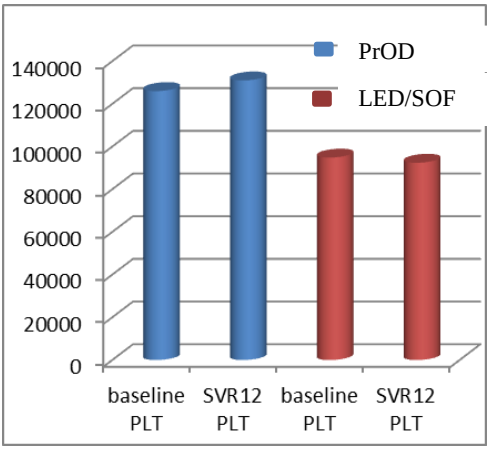

Fig. 4. Platelets count at baseline and SVR12

DISCUSSION

The last few years brought an important change in the therapeutic management of chronic HCV infection. Direct antiviral agents (DAAs) are highly effective and safe and are changing the prognosis and burden of the disease. Sustained virologic response (SVR) is now achieved in more than 90% of cases and is associated with improvements in liver function, fibrosis and overall survival (12).

In order to accomplish the goal of HCV elimination as an important health public threat by 2030, HCV treatment is nowadays recommended in almost all infected patients, even those without significant fibrosis. This, together with the high SVR rates and the safety profile of DAAs, can change the history of HCV infection and improve clinical outcomes namely by avoiding the development of portal hypertension and improving it in patients with patients with established portal hypertension (13).

Although thrombotic events may occur in the natural course of liver cirrhosis, bleeding is much more frequent in clinical practice. The available data from the last few years showed that there is an imbal-

ance concerning the hemostasis status in cirrhotic patients which are considered to be predisposed to bleeding events; many studies have demonstrated a disturbance of the balance between pro- and anticoagulants factors in cirrhotic patients. There are very few studies regarding the coagulation status of cirrhotic patients with SVR treated with DAAs (11). In our study we aimed to assess the incidence and risk factors of bleeding events in patients with SVR treated with DAAs.

Our study showed an incidence of 1.83% of bleeding events in patients with HCV related liver cirrhosis treated with DAAs.

There was a slight improvement of platelet count after SVR in patients treated with PrOD, but not significantly statistic. We didn't find any improvement in platelet count at SVR compared to baseline in patients treated with LED/SOF+RBV, but on the contrary we identified a slight decrease in platelets that can be explained by splenic sequestration and exaggerated destruction, representing a characteristic status in patients with advanced liver disease. Studies that used radiolabeled platelets have shown a low survival to which is added the low level of thrombopoietin, determined by a decreased synthesis in liver cirrhosis. Tripodi *et al* (14), did not found an appreciable increase in platelet count in their study lot, incriminating the short period of follow-up and the fact that viral eradication and improvement of liver function does not change the splenic pool. None of the currently used coagulation parameters in cirrhotic patients (PT, INR) had a notifiable statistic change. This finding confirms the idea that the anticoagulant status in cirrhotic patients cannot be reflected only by these laboratory parameters.

The majority of biochemical parameters showed an improvement on SVR12 compared to baseline. Transaminases and GGT had significantly decreased values in both groups of patients, revealing the disappearance of continuous liver injury due to the viral clearance. Also, we observed a decreasing trend for MELD score, showing an improvement in global liver function.

The evaluation of the severity of the liver disease according to the Child-Pugh score (CPS) demonstrated that the majority of the patients who had variceal hemorrhage were in the moderate and severe stages (CPS class B/C).

The assessment of the risk of development or of the prognosis of bleeding of esophageal varices, depending on the ratio between the spleen/platelet diameter, showed that the majority of patients who had variceal hemorrhage had a value > 3.

The bleeding events were mostly variceal bleeding, which is in fact and rightly the cause of portal hypertension; despite SVR liver fibrosis remains, portal hypertension remains and thus the risk of bleeding.

The clinical use of hepatic venous pressure gradient (HVPG) measurement is limited by its invasiveness. Moreover, its availability is mostly restricted to academic centers. Thus, the non-invasive monitoring of the regression of liver fibrosis and portal hypertension after HCV eradication will be a major challenge in the post-HCV era.

A well-designed retrospective study conducted by Libânio and Marinho (15), evaluated the changes in HVPG and liver stiffness in 60 cirrhotic patients (84% Child A) treated with various combinations of DAAs. SVR led to a reduction in HVPG in 80% of the patients. This beneficial effect was found in all strata of HVPG, although

portal hypertension was less likely to improve in Child B patients; therefore, a point of no return seems to exist from which viral elimination is no longer capable of preventing portal hypertension progression and liver decompensation.

Although non-invasive methods cannot substitute HVPG measurement, non-invasive evaluation of liver fibrosis by transient elastography (Fibroscan) and serological tests (Fibromax and APRI test) showed that patients with variceal hemorrhage had decreased hepatic stiffness after SVR, but with a preservation value above 12.5 kPa, which shows that liver fibrosis remains after SVR, portal hypertension remains and thus the risk of bleeding (16).

According to Calvaruso V *et al*. (17), although portal hypertension improves in the majority of people with hepatitis C and cirrhosis after hepatitis C is cured, not all patients experience improvement, and esophageal varices may continue to progress despite successful hepatitis C treatment.

What is less clear is how frequently portal hypertension and varices improve after hepatitis C is cured. Two studies presented at The International Liver Congress (Paris, 2018) reported on the evolution of these conditions and attempted to identify predictors of regression or progression (18).

Puente Á *et al.*(19), found that clinically significant hypertension persisted in 68% of people with cirrhosis cured of hepatitis C, despite a significant improvement in liver stiffness. Persistence of portal hypertension was associated with a higher hepatic venous pressure gradient and a smaller reduction in liver stiffness, which involve an increased risk of variceal bleeding.

Another study conducted by Sabela *et al*. (20), showed that once patients develop cirrhosis with severe portal hypertension (HVPG $\geq$ 10 mmHg), regardless of SVR in hepatitis C therapy, they remain at risk for hepatic decompensation within the first 5 years after treatment.

Due to the novelty of DAAs therapies (and thus short follow up times) there are only few studies assessing the effects of HCV novel treatments on portal hypertension and clinical decompensation. In patients successfully treated with DAAs, MELD and CHILD-PUGH scores were shown to improve, fact also demonstrated by the literature data (21).

Future studies should focus on predictors of portal hypertension resolution, as it may influence management and monitoring in the subset of patients with a high probability of having clinically significant portal hypertension after treatment, objectified by the appearance of bleeding causes variceal as well as by the portal hypertensive gastropathy.

CONCLUSIONS

Bleeding events in patients with HCV-related liver cirrhosis treated with DAAs are not influenced by the variations of coagulation parameters, rather correspond to the hemodynamic changes induced by the status of advanced liver disease. It should be underlined that the bleeding events were mostly variceal bleeding, which is in fact and rightly a consequence of portal hypertension; despite SVR liver fibrosis remains, portal hypertension remains and thus the risk of bleeding.

It remains to be further demonstrated if the mechanism of DAAs can influence the development of bleeding events in these patients or are part of the natural course of advanced liver disease.

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ASPECTE DE INTERFERENŢĂ ÎNTRE PATOLOGIA VIRALĂ ŞI MANIFESTĂRILE ORALE

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VIRAL PATOLOGY AND ORAL MANIFESTATION (Abstract): Although the medical and biological importance of ringworm infections was acknowledged a long time ago, the putting-up-to-date of some clinical notions is justified for at least three reasons: the improvement and accessibility of molecular biology techniques, the consideration of the major pathogenic role of these viruses in circumstances of immune depression, the possibility to control these infections by means of specific chemotherapy. **Material and method**: A prospective study was carried out in the interval 1.01.2006 – 31.12. 2008, of all cases of ringworm infections with oral eclat, hospitalised in Infectious Diseases Hospital Iaşi. The diagnosis was established according to the clinical manifestations, the hematological modifications and, if the case, serological examination. **Results**: The study included 291 cases.The infection with EVB (infectious mononucleosis) was diagnosed within the age interval 8 months–60 years (median–10 years). The oral manifestations were mainly located at the level of the palatine and velopalatine vault. Chickenpox was diagnosed within the age interval 7 days–52 years (median–8 years). The chickenpox enantem was observed in 90.42% of the cases, being represented by varix wounds, or "hemorrhagic spots", mostly located at the level of the fibrous mucose membrane of the palatine vault – 40.2%. **Conclusions**: The knowledge of the oral manifestations in ringworm infections with highly contagious potential and a high risk of severe complications represents a fundamental method in early diagnosis as well as an important step in establishing the prophylaxy measures meant to prevent these pathogens from being transmitted to the dentist office. **Key words**: EPSTEIN-BARR VIRUS, VARICELO-ZOSTERIAN VIRUS, HERPES SIMPLEX VIRUS

IS THERE A ROLE FOR A NEW SET OF PROGNOSTIC MARKERS IN ACUTE PANCREATITIS?

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IS THERE A ROLE FOR A NEW SET OF PROGNOSTIC MARKERS IN ACUTE PANCREATITIS? (Abstract): The lymphocyte-monocyte ratio, platelet-lymphocyte ratio and neutrophil-lymphocyte ratio are parameters that are simple and may easily benefit to assess the inflammatory condition of a patient that is suspected of having acute pancreatitis. **Material and methods***:* In a group of 337 patients, laboratory and imaging investigations were performed to diagnose and determine the severity score of acute pancreatitis and lymphocyte-monocyte ratio, platelet-lymphocyte ratio and neutrophil-lymphocyte ratio. **Results:** The mean for neutrophil-lymphocytes ratio and for platelet-lymphocyte ratio in patients without complications were lower compared to patients with complications. **Conclusions:** It is important for patients to be aware of the evolutionary stage and to follow the treatment and the appointment and make an abdominal or pancreatic CT scan with contrast to control and prevent complications with late onset. Determining neutrophil-lymphocytes ratio, platelet-lymphocyte ratio and lymphocyte-monocyte ratio is a simple investigation and can help control and prevent complications with late onset. **Keywords:** ACUTE PANCREATITIS; LYMPHOCYTE; NEUTROPHIL; PROGNOSIS; NEUTROPHIL-LYMPHOCYTE RATIO.

Acute pancreatitis (AP) is histologically defined as an acute inflammatory process of the pancreas, varying from interstitial edema to necrosis of the parenchyma and peripancreatic tissues. AP can range from mild disease requiring mainly supportive therapy, occurring in the majority of cases, to severe condition with major, potentially fatal complications.

According with the revised Atlanta classification, acute pancreatitis is classified as mild acute pancreatitis (MAP), moderate severe acute pancreatitis (MSAP) and severe acute pancreatitis (SAP) (1).

In the treatment of AP, an early determination of severity is an important factor. The assessment of the severity of pancreatitis is made standardized by severity scores, biological markers and imaging explorations. The neutrophil-lymphocyte ratio (NLR) is an inflammatory status parameter that has been used as a prognostic factor for various cardiovascular (2, 3) or neoplastic diseases, such as esophageal,

lung, colorectal, and pancreatic cancer (4-6), as well as a strong predictor for postoperative complications (7-9).

The platelet-lymphocyte ratio (PLR) is also a simple parameter that can be used to assess the inflammatory status and the prognosis of patients with acute pancreatitis, especially in case of acute gallstone pancreatitis (10). PLR has also been used as an efficient predictor marker of mortality and morbidity after heart transplantation, obstetrical conditions, and stroke (11-13).

The lymphocyte-monocyte ratio (LMR) is a less studied parameter that can be used to assess the inflammatory condition of a patient suspected of having acute pancreatitis (14, 15). It has been used to predict the evolution of tumors, in oncohematology, and myocardial infarction (16-18).

The role of NLR, PLR and LMR as predictors in acute pancreatitis has not been thoroughly studied, and they may offer a simple and efficient tool for assessing the severity of the disease.

In this study we investigated the role of NLR, PLR, and LMR as predictors in complicated and uncomplicated forms of acute pancreatitis.

MATERIAL AND METHODS

A retrospective analysis of all patients admitted for acute pancreatitis between 1st of January 2013 and 31st of December 2017 in the Ist Clinic of Surgery of Craiova Emergency University Hospital was conducted in order to identify those patients which developed specific complications during their hospital stay. The cases were structured in two subgroups: uncomplicated cases (group 1) and complicated cases (group 2).

The information was collected from the electronic database of the hospital, as well as from the medical registries and medical files of the patients.

The data collected included: Balthazar score, lymphocytes, neutrophils, platelets, monocyte, complications, co-morbidities.

Inclusion criteria were acute abdominal pain, elevated serum enzymes and urinary pancreatic enzymes and / or radiological evidence of pancreatitis

Exclusion criteria were patients with sepsis, infections, pneumonia, chronic inflammatory diseases, chronic myocardial infarction or patients with incomplete clinical and paraclinical data.

Complications that have been considered were local pancreatic complications have been defined as acute development of peri-pancreatic fluid collection, pancreas pseudocyst, acute necrotic collections and wall-off necrosis, visualized by computed tomography (1, 19, 20). Systemic complications have been defined as exacerbation of pre-existing co-morbidity, such as coronary artery disease or chronic pulmonary disease.

All relevant data were collected into Microsoft Excel database. Statistical analysis of the results was performed using *SPSS version 20*. We compared the values of NLR, PLR and LMR for patients in the two subgroups by using independent samples t-test, the cut-off value using ROC curve and Chi-square for odd ratio, in order to determine a maximum normal limit with the goal of improving the specificity and sensibility of these parameters as predictors for early complications. We considered p-values of < 0.05 to be statistically significant.

The statistical analysis has considered several parameters, such as the degree of

severity, the value of the neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and lymphocyte-monocyte ratio, as well as the overall complication rate.

RESULTS

Only 58 (17.21%) from the 337 patients had complications, with a mortality rate of 8.90% (29 patients).

Cardiovascular disease represented 35% of the co-morbidities, the most common cardiovascular disease being hypertension, followed by atrial fibrillation and chronic heart failure. Other co-morbidities were pancreatic fistula, oral or gastrointestinal cancer, diabetes.

The mean value of neutrophil-lymphocytes ratio in uncomplicated patients was 6.38, compared to the complication group of 18.33 with $p < 0.001$ at independent samples t-test (tab. I, fig. 1). We determined that for a cut-off value of 9.14 for NLR the sensitivity resulted 81% and the specificity was 76.7%, with a Chi-square odd ratio for developing complications by 8.58. The NLR is an inflammatory marker that can be used to predict the occurrence of acute pancreatitis with a surface over the Receiver Operating Characteristic (ROC) of 0.830 (0.766-0.894) ($p < 0.001$); (fig. 2).

TABLE I

NLR without complications *vs.* with complications

	NLR without complications	**NLR with complications**
Sample size	279	58
Arithmetic mean	6.3887	18.3302
Standard deviation	5.6098	13.5644
Cut-off value (ROC Curve)	**9.1483**	
independent samples t test	P < 0.001	

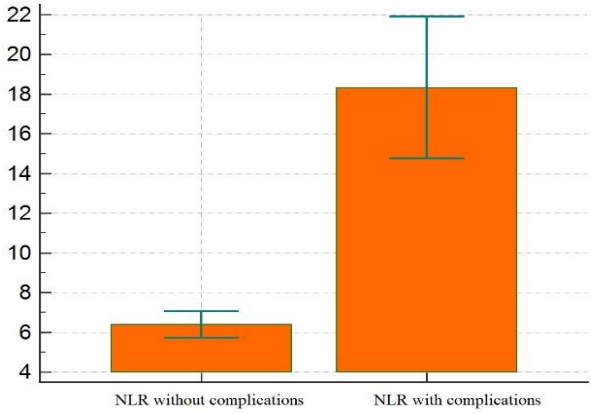

Fig. 1. NLR without complications *vs.* with complications

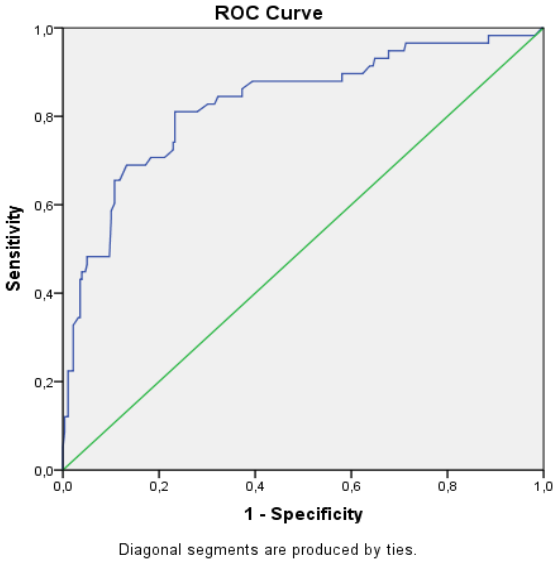

Fig. 2. ROC for NLR without complications *vs.* with complications

The mean platelet-lymphocyte ratio in patients without complications was 141.56 compared to 175.97 for the patients with complications (p=0.021) (tab. II, fig. 3). We determined that for a cut-off value of 123.82 the sensitivity resulted 72.4%, but with a low specificity of 47%, with a Chi-square odd ratio for developing complications by 1.88. The PLR is a marker that show inflammation and can be used to predict acute pancreatitis occurrence with a surface over the Receiver Operating Characteristic (ROC) of 0.587 (0.501-0.673) (p=0.037); (fig. 4).

The mean lymphocyte-monocyte ratio of 3.8 resulted for patients without complications compared with value 2 for patients with complications (Table 3, Graph 5). We determined that for a LMR cut-off value of 1.88 the result for sensitivity was 66% and specificity was 72.9%, with a Chi-square odd ratio for developing complications by 3.56. The LMR can be used as an inflammatory marker that can predicted the occurrence of acute pancreatitis with a surface over the Receiver Operating Characteristic (ROC) of 0.303 (0.229-0.377) (p <0.001); (Graph 6).

TABLE II

PLR without complications *vs.* with complications

	PLR without complications	**PLR with complications**
Sample size	279	58
Arithmetic mean	141.5692	175.9790
Standard deviation	70.9545	106.2958
Cut-off value (ROC Curve)	**123.8267**	
independent samples t-test	p = 0.021	

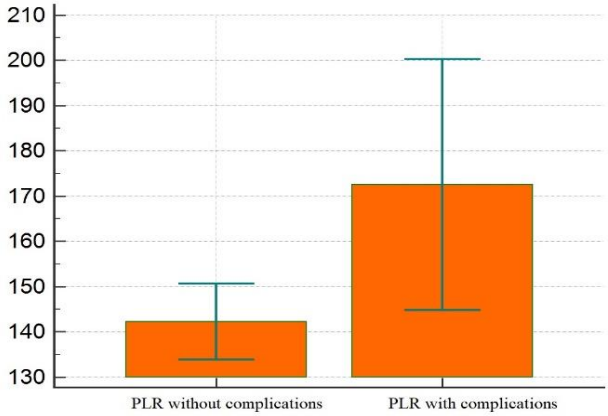

Fig. 3. PLR without complications *vs.* complications

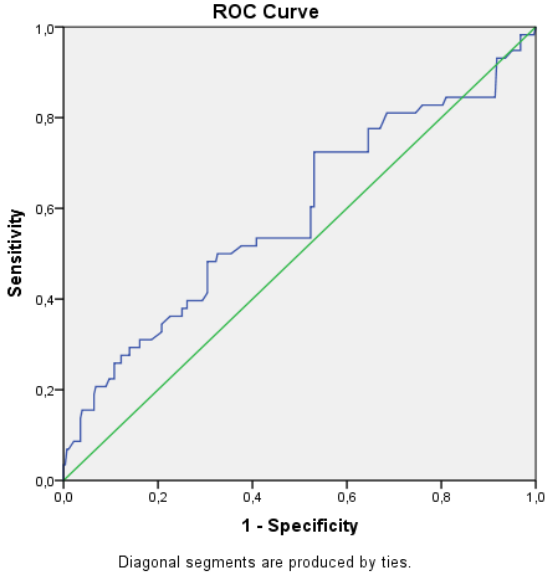

Fig. 4. ROC for PLR without complications *vs.* complications

TABLE III

LMR without complications *vs.* with complications

	LMR without complications	**LMR with complications**
Sample size	279	58
Arithmetic mean	3.8742	2.0004
Standard deviation	5.0025	1.3116
Cut-off value	**1.8803**	
F-test for equal variances	$p < 0.001$	

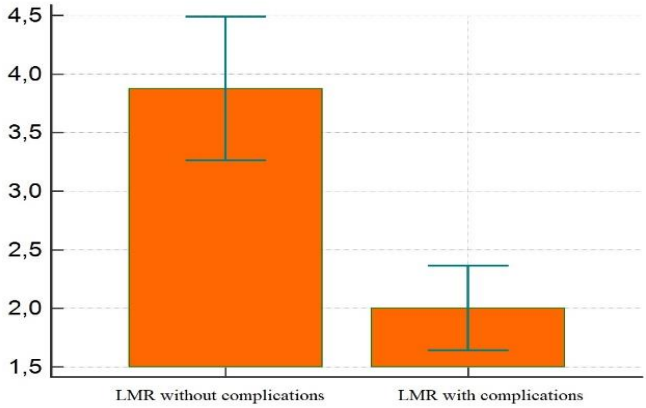

Fig. 5. LMR without complications *vs.* complications

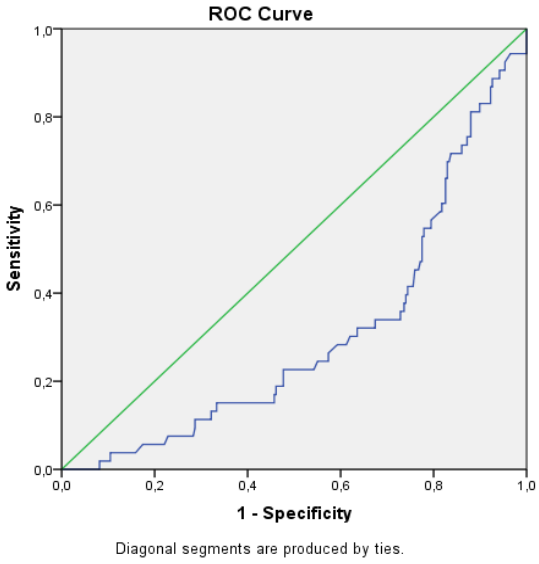

Fig. 6. ROC LMR without complications *vs.* complications

DISCUSSION

Patients with acute pancreatitis usually recover without having further complications, but patients that have severe form of acute pancreatitis, in evolution, may develop serious complications (such as pancreatic infection, bile obstruction, pseudocysts, pancreatic duct obstruction, pancreatic necrosis, pulmonary edema, splenic vein thrombosis, adult respiratory distress syndrome). It is important for patients to be aware of the evolutionary stage and to follow the treatment and to accept the appointment and realization of an abdominal or pancreatic CT scan with contrast to control and prevent complications with late onset. Determining neutrophil-lymphocytes ratio, platelet-lymphocyte ratio and lym-

phocyte-monocyte ratio is a simple investigation and can help control and prevent complications with late onset.

Thirty-five percent of patients were diagnosed with severe acute pancreatitis. The overall mortality rate was 8.90% of all cases in the study group.

Only twenty-five percent of patients didn't suffer from co-morbidities, which could explain the mortality rate. Cardiovascular disease represented 35% of the co-morbidities, the most common cardiovascular disease being hypertension, followed by atrial fibrillation and chronic heart failure.

Eighteen percent patients developed complications: patient under treatment with anticoagulant, patients under platelet anti-aggregant treatment, patients requesting discharge on request, patients that developed pancreatic fistula, patient leaving the hospital without the agreement of the medical staff, death.

Patients should be made aware of their evolutionary status and follow a treatment, accepting an imaging investigation such as abdominal CT or pancreatic CT scan to control and prevent the onset of complications with late onset.

Different genetic mutations (p.N34S in SPINK1) may be predisposing for acute pancreatitis, mostly alcohol induced acute pancreatitis, and could develop a more severe form of acute pancreatitis (21). One study suggests a risk for developing acute pancreatitis for patients having colorectal cancer that are undergoing chemotherapy with FOLFOX6 (22).

Since blood counts are routinely performed on admission, determining the neutrophil ratio of lymphocytes is a simple, quick and inexpensive investigation that can help to control and prevent complications with late onset.

The role of NLR, PLR and LMR as predictors in acute pancreatitis has not been thoroughly studied and may offer a simple and efficient tool for assessing the severity of the disease.

The NLR is a simple parameter that has been used as an inflammatory or infectious predictor and marker in various pathologies and postoperative complications (8, 9).

The PLR is also a simple parameter that can be used to assess easily the inflammatory condition of a patient suspected of having acute pancreatitis, specially to predict acute gallstone pancreatitis (10).

The LMR is an easy and simple parameter that can be used to assess the inflammatory condition of a patient suspected of having acute pancreatitis.

The mean value for NLR in patients without complications was found to be lower compared with that of the patients with complications.

The PLR mean for patients with complications was found to be higher than in patients without complications.

Patients without complications had a higher mean LMR than patients with complications.

Forget *et al*. determined that normal NLR values in a non-geriatric adult (21 to 66 years of age) in the healthy population are between 0.78 and 3.53 (23).

Most patients had multiple other health problems or came in an advanced stage of acute pancreatitis, and so they had a high NLR value. Like Azab B *et al.* (24) suggested that patients with NLR above 7.6 have more than one presentation in the ICU and suggest using an NLR limit value above 4.7 as a simple indicator of severity in patients presenting with acute pancreatitis.

The cut-off value for NLR mean was 9.14 with a sensitivity of 81% and specific-

ity of 76,7% with an odd ratio for developing complications by 8.58. The cut-off value for PLR mean was 123.82 with a sensitivity of 72.4% and specificity of 47% with an odd ratio for developing complications by 1.88. The cut-off value for LMR mean was 1.88 with a sensitivity of 66% and specificity of 72.9% with an *odd ratio* for developing complications by 3.56.

CONCLUSIONS

Determining the neutrophil to lymphocytes ratio, platelet-lymphocyte ratio and lymphocyte-monocyte ratio are a quick, simple and inexpensive investigation that can help to control and prevent complications with late onset.

Patients with complicated acute pancreatitis had a higher mean value for neutrophil-lymphocytes ratio and platelet-lymphocyte ratio. Patients without complications had a higher mean LMR than patients with complications.

The following cut-off values for over which it is more likely to have complications have been found: NLR mean > 9.14, PLR mean > 123.82, LMR mean < 1.88.

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INFECTION AND PREGNANCY - THE EXPERIENCE OF THE HOSPITAL OF INFECTIOUS DISEASES IASI IN 2018

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INFECTION AND PREGNANCY - THE EXPERIENCE OF THE HOSPITAL OF INFECTIOUS DISEASES IASI IN 2018 (Abstract). **Aim**: To evaluate the incidence of different infections diagnosed in the pregnant women admitted at the "Sf. Parascheva" Clinical Hospital of Infectious Diseases, from Iasi. **Material and methods**: We conducted a retrospective study using the hospital-based medical records of the pregnant patients admitted in our hospital. We analyzed clinical and paraclinical data regarding symptomatology, treatment and evolution. **Results:** In 2018, 11 707 patients were admitted to the clinic, of which 6 102 were female. 102 of them were pregnant women (1.67%) diagnosed with a variety of conditions. Specifically, 52.94% suffered from respiratory tract infections (including bacterial pneumonia, interstitial pneumonia, confirmed influenza, tracheobronchitis), 15.68% had urinary tract infections, and 15.68% presented digestive pathologies. Symptomatic and antibiotic treatment was initiated, with a favorable evolution in 99% of cases. **Conclusions**: Pregnancy-related immunosuppression seems to predispose pregnant women to certain infections. **Keywords:** INFECTION, PREGNANCY, ANTIBIOTIC.

Pregnant women have been known for centuries to manifest a unique susceptibility to infectious diseases, which may lead to different outcomes compared to the general population and especially non-pregnant woman at childbearing age. It is thought that a combination of adaptive alterations in immunity combined with changes in anatomy and physiology accompanying pregnancy underpin this vulnerability (1). During pregnancy, several mechanical and pathophysiological changes occur (e.g. a decrease in respiratory volumes and urinary stasis due to an enlarging uterus) and immune adaptations are required to accommodate the fetus (2). Immunologic alterations during pregnancy may help explain the modified severity of and susceptibility to infectious diseases. As pregnancy progresses, hormone levels change dramatically and are considerably higher than at any other time. The interplay between sex hormones and the immune system is complex and multifactorial, and it affects many organ systems (3).

Several issues are pertinent with regard to the threat of infectious disease during pregnancy. First, as previously mentioned, pregnant women are more susceptible to infectious diseases due to the changes in

immunity and physiology brought about by the pregnancy itself. Second, the effects of infectious diseases on the fetus may be unknown and difficult to predict, and the diagnosis of infection in the fetus or infant can be challenging. Third, the standard approaches to prophylaxis and treatment appropriate for the general population may not be appropriate for pregnant women (4).

This retrospective analysis represents a first step towards a longer-term data collection regarding the most frequent infections during pregnancy, considering that we found no specific data about infections affecting pregnant women in North-East Romania.

MATERIAL AND METHODS

We conducted an observational retrospective study of the patients admitted to the "St Parascheva" Hospital of Infectious Diseases Iași over a 1-year period (January 1st, 2018 - December 31st, 2018). Only pregnant women admitted to this hospital were included in the study. Data regarding the symptomatology at the moment of presentation, paraclinical data, treatment administered, and subsequent evolution were collected from the hospital-based medical records of the patients.

Patients that presented to the triage room of the hospital but were not admitted to our clinic as well as HIV-positive women were excluded from the study. The follow-up of the discharged patients was performed by the obstetrician monitoring the pregnancy, and we are not aware of any further developments or consequences. Mention should also be made that not all infections of pregnant women are treated in our clinic, and the primary physician that the pregnant female approaches is usually her obstetrician. This can influence how accurately hospital data is able to reflect the real incidence of infections during pregnancy.

RESULTS

In 2018, 11 707 patients were admitted to the "Sf. Parascheva" Hospital of Infectious Diseases in Iași, Romania, of which 6 102 (52.12%) were female. The 102 pregnant women represent 1.67% of the total number of admitted female patients, the majority coming from the urban area (65.5%). We also noticed that 60.78% (62 cases) presented to our hospital in the cold months of the year (January and February, and from September to December) (fig. 1).

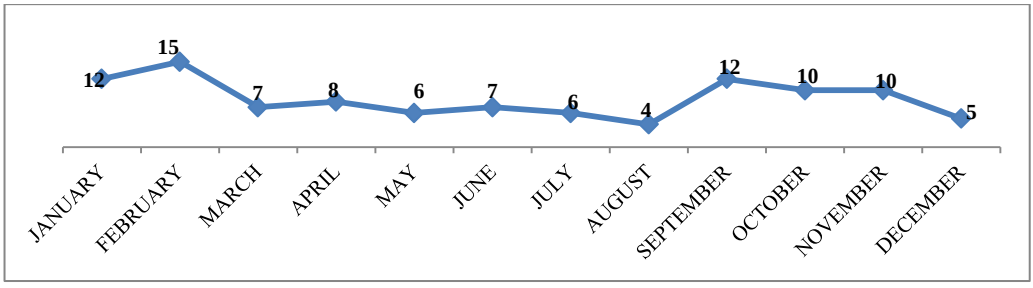

Fig. 1. Number of cases admitted by months

The pregnant patients' age ranged from 15 to 43 years old, with a mean age of 29.8 and the most affected age group being 26-35 . (fig. 2)

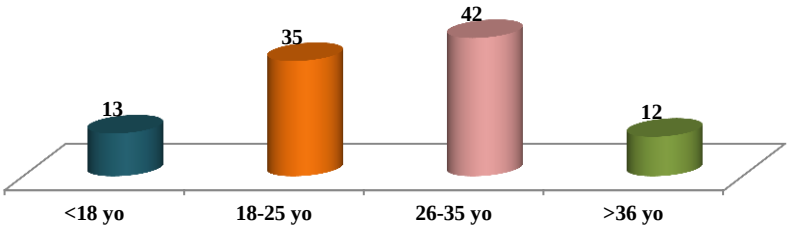

Fig. 2. Age categories

Regarding the pregnancy stage of the presenting women, there were no significant differences: 35 women were in the first trimester (34.31%), 40 were in the second (39,21%) and 27 in the third (26.47%).

The predominant symptomatology included shivers in 76 cases (74.5%), followed closely by fever in 65 cases (63.72%). The patients also complained of nausea (32 cases, 31.37%)), dysuria (18 cases, 17.6%), pollakiuria (16 cases, 15.68%), arthralgia and myalgia (7 cases, 6.8%).

The primary pathology that pregnant women were suspected upon admission to our hospital consisted of pneumonia in 31 cases (30.39%). In 10 of these cases (9.80%), the etiological agent was identified as *Streptococcus pneumoniae*, and in 6 cases (5.88%) it was *Hemophilus influenzae*. The other 15 cases (14.70%) were diagnosed as interstitial pneumonia.

Other respiratory pathology admitted in our hospital was tracheobronchitis (15 cases - 14.70%), seasonal influenza (5 cases - 3.92%) and bronchopneumonia (3 cases- 2.94%)

Apart from these, the respiratory pathology included tracheobronchitis (15 cases, 14.70%), seasonal influenza (5 cases, 3.92%) and bronchopneumonia (3 cases, 2.94%). Another pathology requiring admittance was urinary tract infection, present in 17 cases (16.66%). In most of them (12 cases, 11.76%), *Escherichia coli* was the causing agent, followed by *Klebsiella pneumoniae* (4 cases, 3.92%) and one case with *Staphylococcus saprophyticus.* Digestive symptoms were the main cause of presentation in another 16 cases (15.68%), of which 15 (14.70%) were due to severe gastroenteritis and of the other turned out to be food poisoning. The diagnosis of sepsis upon admittance was encountered in 11 cases (9.27%). Last but not least, 4 patients presented with different types of rashes: two cases of maculopapular rash were diagnosed as measles, one case of small bumps, blisters, and pustules was identified as chicken pox, and the other was a case of allergodermia.

Antibiotic or antiviral therapy was administered according to the clinical presentation and data from the antibiogram, in accordance with the European guidelines: Ampicillin, Amoxicillin, Clarithromycin, Ceftriaxone, Oseltamivir. In the majority of cases, the patients responded well to the treatment and their evolution was favorable. Only one patient with several prior comorbidities passed away after developing due to multiorgan dysfunction syndrome.

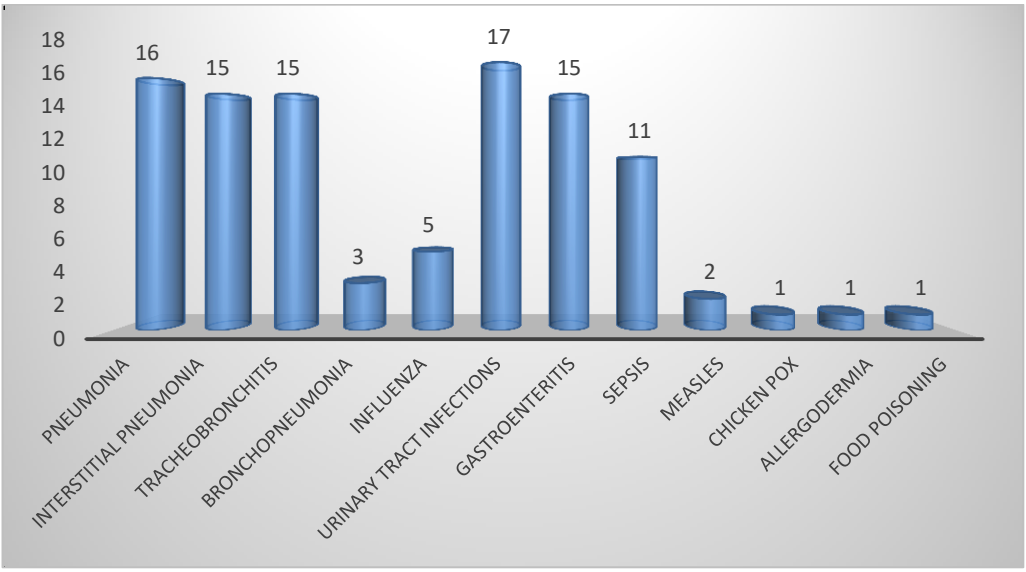

Fig. 3. Diagnosis at admittance

DISCUSSION

The most common upper respiratory disease, coryza, pharyngitis, and others, acts similarly in both pregnant and non-pregnant women, without affecting the fetus. Diseases of the respiratory system caused by acute infections are among the most common maternal diseases during pregnancy (5). In our study, the majority of cases which required admission were upper and lower respiratory infections, 54 (52.94 %)in all. The medium forms of respiratory infection hospitalized were represented by tracheobronchitis and interstitial pneumonia.

In the literature, bacterial pneumonia is most commonly attributed to the *Streptococcus pneumoniae, Hemophilus influenzae, Mycoplasma pneumoniae* (5). The signs (fever, crackles, abnormal breath sounds) and symptoms (cough, sputum production, dyspnea, pleuritic chest pain) of bacterial pneumonia in pregnancy are the same as in non-pregnant individuals.

In our study, the data showed that the primary etiological agent of bacterial pneumonia was *Streptococcus pneumoniae* (10 cases), followed closely by *Hemophilus infuenzae* (6 cases). The treatment was initiated empirically and then adjusted with the appropriate antibiotics according to the results of the antibiogram.

Pregnant women tend to suffer from more severe forms of illness and higher mortality from influenza than the general population. (6) This is theorized to be due to the physiologic changes brought about by the pregnancy, including decreased lung capacity, increased oxygen consumption, and decreased cell-mediated immunity (7). Studies on pregnant women in New York City, for instance, showed that the severity of illness and the timing of antiviral treatment are correlated, which would justify the early use of antivirals in cases of suspected or proven influenza infection (8, 9). The clinical presentation of influenza does

not seem to be altered by pregnancy; symptoms include cough, fever, malaise, rhinitis, myalgias and arthralgias, headache, chills (10).

In our study, five women diagnosed with influenza were admitted to hospital upon presenting with severe symptoms and low oxygen saturation. Their condition required specialized treatment and continuous observation, and the antiviral of choice was Oseltamivir together with specific support treatment.

Urinary tract infections (UTIs) are common in pregnant women and pose a great therapeutic challenge, since the risk of serious complications in both the mother and her child is high. At around week 6 of pregnancy, physiological changes occur, and the ureters begin to dilate (11). Although the incidence of bacteriuria in the pregnant population is only slightly higher than in non-pregnant women, its consequences for both the mother and the unborn child are more severe (12,13). Organisms causing UTI in pregnancy are the same uropathogens which commonly cause UTI in non-pregnant patients. Most infections are caused by *Enterobacteriaceae*, with *Escherichia coli* being responsible for 63-85% of cases, and the other culprits are *Klebsiella pneumoniae* (in approximative 8% of cases), coagulase-negative *Staphylococcus* (around 15%), *S. aureus* (up to 8%), and group B streptococci (up to 7%) (14-16). Our results are in accordance with the literature, *E.coli* being the primary etiological agent of UTIs found in three quarters of the samples (12 cases).

Studies have shown that gastroenteritis affects a large proportion of pregnant women. While gastrointestinal disease such as Crohn's disease (17) and untreated celiac disease (18) could affect neonatal outcomes, acute gastroenteritis during pregnancy has not been associated with any adverse outcomes for the newborn (except for a slight decrease in the duration of pregnancy and an increased risk of preterm birth in mothers with several episodes of gastroenteritis) (19). The signs and symptoms of viral gastroenteritis in pregnant women are no different from those reported by non-pregnant women, and they include diarrhea (> 3 watery stools/ day), nausea, vomiting. In our study, the hospitalized women showed dehydration signs and were admitted. In only one case a preterm birth was initiated, which required urgent transfer to the obstetrics and gynecology hospital.

According to numerous studies, pregnant women are at increased risk of morbidity and mortality associated with measles and are also more likely to develop pneumonia and to be hospitalized (20-24). The signs and symptoms of measles in pregnant women are usually similar with those in the general population, namely a prodrome of fever, cough, conjunctivitis malaise, coryza and the appearance of Koplik's spots towards the end of prodrome, followed by the appearance of a maculopapular rash which then spreads from the head to the trunk and ultimately down to the lower extremities. Fever and elevated liver enzymes were reported as more common among pregnant women with measles than among non-pregnant women suffering from the same illness (25).

Similarly, the clinical presentation of chicken pox is the same in pregnancy as generally. The infected patient will develop high fever accompanied by small bumps, blisters, and pustules (26). However, chickenpox during pregnancy may cause

fetal infections (27), and abnormalities diagnosed as congenital varicella syndrome are expected in about 12% of infected fetuses. Prospective studies have estimated the incidence of fetal and congenital varicella syndrome as 2.2% (28).

Last but not least, psychosocial stress and disruptive life events have been shown to affect birthweight and fetal development (29) enough to require the intervention of a hospital psychologist (30, 31).

CONCLUSIONS

Most of the pregnant women admitted to the hospital of infectious diseases from the city of Iași in NE Romania were diagnosed with mild forms of various pathologies. Adequate and timely treatment, together with careful monitoring, resulted in favorable outcomes in 99% of the cases.

Normally, pregnancy involves a certain degree of physiological adjustment and immunosuppression. Any febrile sign or symptom that points to an infectious disease requires an urgent response and a holistic approach involving the collaboration between obstetricians and specialists in infectious diseases. Early diagnosis followed by immediate and appropriate therapy is essential during pregnancy in order to avoid compromising maternal and neonatal health.

Because the effects of emerging infections in pregnant women may differ from those in the general population, pregnancy must be considered a potential risk factor for infectious disease and, if left untreated, even death.

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POSTPROCESSED COMPUTED TOMOGRAPHY ANGIOGRAPHY IMAGES - AN UNDERUSED MODALITY OF BETTER UNDERSTANDING ANEURYSMS

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POSTPROCESSED COMPUTED TOMOGRAPHY ANGIOGRAPHY IMAGES - AN UNDERUSED MODALITY OF BETTER UNDERSTANDING ANEURYSMS (Abstract): **Aim**: Intracranial aneurysms are the leading cause of non-traumatic subarachnoid hemorrhage which can have devastating consequences. Imaging techniques improved greatly and now play a very important role in detection and management of aneurysms. The aim of this study was to share our experience with applications of three-dimensional reconstructions from computed tomography angiography images for diagnosis and surgical planning of brain aneurysms. **Material and methods**: We retrospectively analyzed a series of 244 aneurysms in 210 patients treated from January 2019 to December 2019 in a specialized neurosurgical center. We used Toshiba Aquilion 32 slice CT scanner for image acquisition and Vitrea software for three dimensional reconstructions. **Results:** we found 244 aneurysms and the anterior communicating artery was the most frequent aneurysm location, followed by middle cerebral artery, the posterior communicating artery and internal carotid artery. Maximum Intensity Projection and volume rendering techniques provided for the neurosurgeons and neurosurgery residents a very good detection and assessment of aneurysm morphology with its proximal and distal vessels. **Conclusions:** Postprocessed images and three-dimensional reconstruction should be used more often in neurosurgery in order to better understand the morphologic details and for a better treatment planning of intracranial aneurysms. **Keywords**: POSTPROCESSED IMAGES, 3D RECONSTRUCTIONS, COMPUTED TOMOGRAPHY ANGIOGRAPHY, MAXIMUM INTENSITY PROJECTION, VOLUME RENDERING TECHNIQUE.

Intracranial aneurysms represent a serious medical condition defined by localized dilatations within the wall of cerebral arteries and are prone to rupture. The resulted subarachnoid hemorrhage (SAH) is associated with a high mortality and morbidity rate (1).

The ongoing neurosurgical progress with surgical and endovascular treatment techniques had a major impact in patient outcome and found an important place in case management (2). Important factors in treatment planning are the imaging techniques and three dimensional (3D) reconstructions used as tools for a better understanding of the morphology of the aneurysms.

Although sectional imaging was a revo-

lutionary discovery especially for brain and cerebral arteries evaluation, the lack of a third dimension (sagittal and coronal) can be a limitation in appreciating anatomy and pathology for many physicians, including neurosurgeons and mostly for inexperienced ones. Since the development of three-dimensional computer rendering algorithms in the 1970s this limitation could be overcome and used to make exceptional 3D reformats for a better treatment planning (3).

Computed tomography angiography (CTA) and magnetic resonance angiography (MRA) are used for an initial evaluation of cerebral vessels in case of an aneurysm suspicion even though the digital subtraction angiography (DSA) is the "gold standard" because they are less invasive techniques. Maximum intensity projection (MIP) and 3D volume rendering (3D VR) are very useful tools that can provide an overview of the aneurysm and this way help the neurosurgeon think to the best surgical approach.

In this article, through case studies and illustrations, we describe the use of tools for 3D reconstructions with MIP and VR techniques and also highlight their advantages, disadvantages and some of the pitfalls that can lead to misinterpretation.

MATERIAL AND METHODS

We analyzed retrospectively 244 aneurysms in 210 patients from January 2019 to December 2019 that were treated in a dedicated neurosurgery hospital that serves as a care center in Moldova region of Romania. All aneurysm were confirmed with DSA.

All imaging studies (CTA) were acquired in our hospital using a Canon (Toshiba) Aquilion 32 slice CT scanner (continuous slice with slice thickness of 0.5 mm with zero gap).

We selected a series of cases and did 3D reconstructions with MIP and VR using Vitrea software. These cases were later discussed in multidisciplinary teams that included radiologist, neurologist, neurosurgeon and interventional staff.

RESULTS

Of 210 patients, 118 (56%) were females and 92 (44%) were males, with an age range from 21 to 86-year-old. From a total of 244 aneurysms, the anterior communicating artery was the most frequent aneurysm location (33%), followed by middle cerebral artery (23%), the posterior communicating artery with 16% and internal carotid artery with 14%. Other localizations were found in less than 10% of cases (fig. 1). We also found 15 patients with multiple aneurysms.

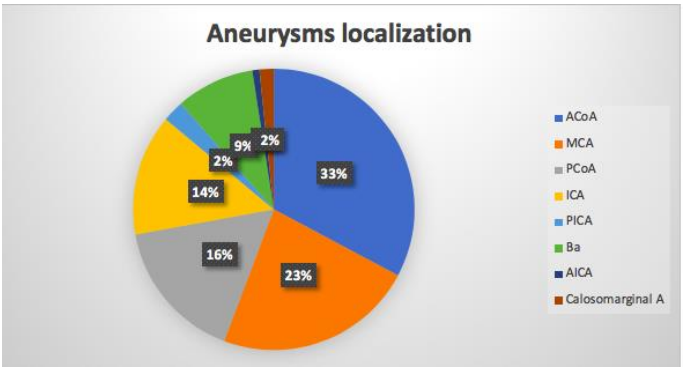


Fig 1. Aneurysms localization

The postprocessing techniques that are discussed in this article are MIP and VR which are most helpful in the diagnosis and assessment of intracranial aneurysms.

DISCUSSION

Although DSA remains the gold standard for diagnosis of intracranial aneurysms (4) even in recent studies (5), Willinsky *et al*. identified a rate of 1.3% neurologic complications in a number of 2,899 procedures with the mentioning that the patients had age-related vascular diseases (6). In another study with a more selected series of patients, Dawkins found a lower rate of complications, 0.34% in 2924 diagnostic procedures (7).

Wintermark *et al*. discovered a sensitivity of 94.8% of CTA in detecting intracranial aneurysms with scanners with 8 and 16 detectors but with the ongoing development of multi-slice imaging (8), Wang *et al*. found an overall sensitivity, specificity and accuracy of 3D CTA of 96.3, 100 and 94.6% of intracranial aneurysms using a 320-detector row scanner (9).

It is very important to go through all axial images before using the reconstructions, but the use of MIP or 3D VR afterward can improve the report by adding information about aneurysm morphology, size, orientation, neck and other aspects that neurosurgeons need for treatment planning.

In our study most aneurysms were found on anterior communicating artery, like in the Hwang *et al*. study (10).

In most studies was used 64-row spiral CT unit (11, 12, 13). In our study was used 32 slice CT unit.

Maximum intensity projection is a technique in which the brightest voxel from a volume of CT data is collected and therefor used to reconstruct an image (14). Therefore, it is not a 3D method, but it creates 2D images and depth information is lost (15). This technique can facilitated the view of arterial tree in an "angiographic" image and vascular particularities can be better displayed. It offers an easy visualization of small caliber intraparenchymal arteries in an enhancing organ, such as brain but also kidney, liver (16, 17) and it is very useful for the evaluation of arteries in potential living transplantation donors (18).But MIP reconstructions can be edited using only windowing parameters and not opacity, color or shading.

Regarding intracranial aneurysms, MIP reveals smaller vessel branches, and this makes it well suited for rapid evaluation of collateral vessels (17).

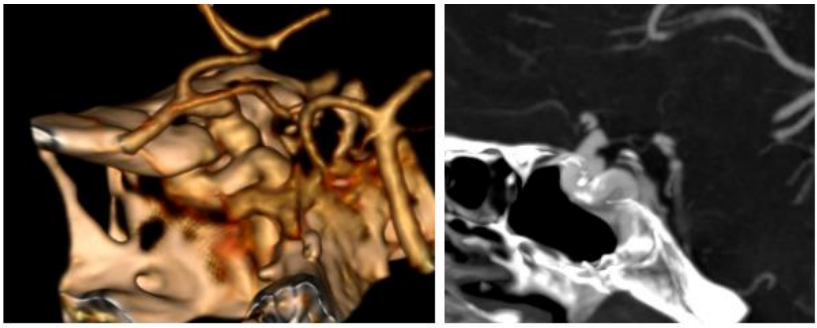
A limitation of the technique is represented by wall calcifications because the lumen of the vessel can be masked, and the stenosis overestimated (19) (fig. 2). Also, skull base has a higher attenuation than arteries and the disadvantage of this technique is that bone removal from the data set is necessary and it is a time-consuming task (20).

MIP is easy to use but it must be remembered that it uses only 10% of the information from the given volume.

MIP imaging of CTA has proven its usefulness in diagnosing cerebral vasospasm following subarachnoid hemorrhage in a study conducted by Kim *et al*. that compared the accuracy of MIP images of CTA with DSA. Therefore, the number of unnecessarily invasive DSA can be reduced (21).

Volume rendering is a technique used to visualize three-dimensional scalar fields so unlike MIP, VR maintains depth and spatial relationship (22). Ever since 1987, Fishman reported that VR is more useful than axial and 2D multi-planar reformatted images in assessing subtle acetabular fractures (23)

and later on, Levin *et al*. reported better evaluation of brain cortical lesions (24). VR is also useful for liver, knee, aorta imaging (25, 26).

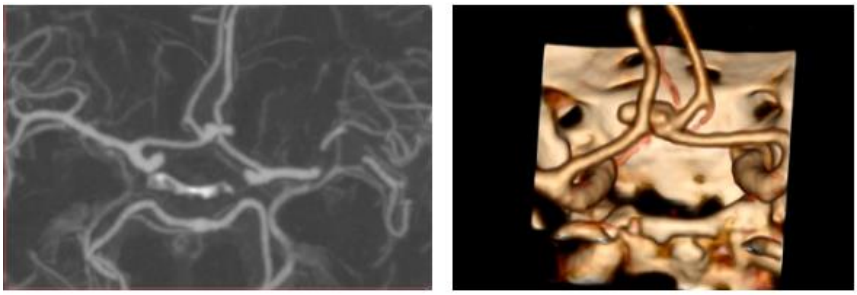


a b

Fig. 2. Another case is that of a 65-year-old woman with an internal carotid artery aneurysm. MIP reconstruction creates the vascular map, but the vessel lumen and wall calcifications cannot be individually defined (a). The volume rendering improves the visualization by enabling a color display with differentiation between lumen and calcification (b).

There are many software that can provide 3D reconstructions from CT data sets and almost all have similar interface and tools to edit the volumes. In this study we used Vitrea software 4.1.52 to obtain useful 3D reconstruction for neurosurgeons (fig. 3).

VRT shows vascular anatomy but also provides information about soft tissue, bone, muscle and other structures that can play an important role in a more comprehensive understanding of the pathology or surgical approach (fig. 4). Some parameters such as window width and level, opacity, brightness can be modified to depict more accurately the vasculature (19). This may also represent a challenge because adjusting these parameters is an interactive process that has an interobserver variability and is dependent of user's experience (27).



a b

Fig. 3. A 56-year-old patient with an anterior communicating artery saccular aneurysm, injected from right and oriented to the left. The aneurysm is better seen on MIP reconstruction in axial plane (a) but VR provides a better understanding of the morphology of the aneurysm (b).

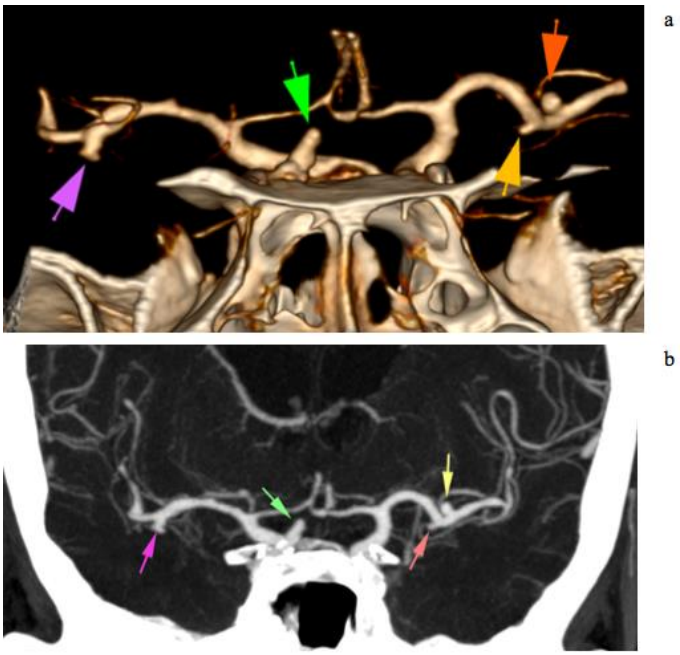


Fig. 4. A 48-year-old female patient with multiple aneurysms (two aneurysms on left MCA, one aneurysms on right MCA and one aneurysm on right PCoA). Volume rendering has the advantage of revealing all the aneurysms and rotate the 3D reconstruction in different planes (a) while MIP can display in full detail only one or two aneurysms, depending on the location of the aneurysmal dilatations (b).

Most software provide simple interface with user-friendly command icons in the left side of the screen and some also include a few preset functions for 3D VR. For example, after a region of interest is selected in the multiplanar reconstruction, when using Vitrea, we can select the Circle of Willis protocol and a 3D reconstruction will be made with only vessels present. A "bone" preset can be selected and the bone structure that is in the region of interest is added to the 3D model. Another benefit of VR is that the colors of the arteries and bones can be chosen to be distinctive and a better appreciation of the vessel direction within an osseous canal can be made. The software also has a knife tool that gives options to cut inside, cut outside and clear area in order to better edit the image. The user can also use this tool to remove the bone structure and make a simulation for a surgical approach.

Consequently, preoperative understanding of the anatomic individualities of each patient can be of major advantage in achieving a more precise and a less invasive approach. Furthermore, this may help with anticipating potential complications and intraoperative difficulties (28).

In another study, Jha *et al*. supports the use of VRT for training residents and in surgical planning of arterio-venous malformations, aneurysms, skull-base lesions, lesions of the brain and spine lesions (29).

Overall, many studies report that using VR is a very useful method to analyze vascular structure in detail, understand the

morphology, size, orientation of the neck of the aneurysm, the parent vessel, the emerging vessels and their direction and for choosing the treatment plan, either surgical or endovascular (30, 31).

In our experience, both reconstruction methods are useful, and we routinely use both in our practice but, because of its limitation, we would not use only MIP images without a correlation to volume-rendered images.

CONCLUSIONS

Postprocessing tools with 3D reconstructions should be used more often in neurosurgery because it enables a more comprehensive understanding of aneurysms, the morphologic details, complex anatomy of vascular structures and 3D relationships.

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MYOCARDIAL ISCHEMIA AND COMPONENTS OF OXIDATIVE STRESS IN DIABETES – AN ACCIDENTAL ASSOCIATION? CASE REPORT AND LITERATURE REVIEW

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MYOCARDIAL ISCHEMIA AND COMPONENTS OF OXIDATIVE STRESS IN DIABETES - AN ACCIDENTAL ASSOCIATION? CASE REPORT AND LITERATURE REVIEW (Abstract): The global epidemic of diabetes can be alarming and extremely severe. Diabetes, especially in type 2 cells, is officially recognized as a major and independent cardiovascular risk factor. We are presenting the case of a 55-year-old female diabetic patient, with postoperative status, admitted to the internal medicine clinic for polymorphic symptomatology and elevated blood pressure values at home. From an evolutionary point of view, the patient presents electrocardiographic and enzymatic dynamics, and the therapeutic approach was non-interventional. This case was particular due to the postoperative status, cardiovascular risk factors and important prognostic implications through pain perception disorders. The assessment of the overall cardiovascular risk is important to determine the optimal time to initiate the therapy, its intensity, and therapeutic targets. The role of the multidisciplinary team is essential in the complex management of the patient. The aim of this paper is to make a brief review of the role that endothelial dysfunction and oxidative stress have in diabetes and cardiovascular disease. **Keywords:** ENDOTHELIAL DYSFUNCTION, OXIDATIVE STRESS, DIABETES, CARDIOVASCULAR DISEASE, THERAPEUTIC TARGET.

World Health Organization predicts that diabetes and coronary heart disease will remain the leading causes of death over the next two decades. Correct and early risk stratification is essential and decides the therapeutic management (1). Long-term monitoring of the coronary and diabetic patient should include lifestyle changes, correction of risk factors, and secondary prevention. Diabetes mellitus, a complex and multifactorial metabolic disorder, is characterized by hyperglycemia and the development of micro and macrovascular complications. One of the factors that contribute to the development of insulin resistance is oxidative stress. People with diabetes have a higher risk of silent myocardial ischemia and to develop myocardial

infarction compared to the non-diabetic population. Most often, myocardial ischemia is asymptomatic in diabetic patients (2).

There is a close relationship between oxidative stress, diabetes, and coronary heart disease. Diabetes increases the risk of cardiovascular disease. An early indicator of heart disease in diabetic patients is the endothelial dysfunction. The pathophysiological factors incriminated in the endothelial dysfunction in diabetes are dyslipidemia, inflammation, and oxidative stress.

Endothelial dysfunction, commonly associated with diabetes, is an early indicator of diabetic vascular disease, and the pathophysiological mechanism is complex (3). Current studies point out that diabetes, insulin resistance and atherosclerosis are common pathogenic mechanisms, and endothelial dysfunction could explain each of these pathological conditions. Metabolic syndrome, insulin resistance and hyperglycemia lead to damage to the vascular wall and the occurrence of cardiovascular events caused by oxidative stress, inflammation, platelet hyperactivity and endothelial dysfunction (4).

Decreased NO bioavailability can be caused by several factors such as: decreased production of endothelial nitric oxide synthase (eNOS), blocking the cofactors necessary for eNOS synthesis, subsequently causing degradation or inactivation of excess NO by reactive oxygen species. The main cause of decreased bioavailability of NO in cardiovascular disease is the increased production of reactive oxygen species, peroxide ion, superoxide, or peroxide free radicals (5). Cardiovascular risk factors (hyperglycemia, hypertension, dyslipidemia) contribute to oxidative stress and the development of endothelial dysfunction (fig. 1). Therefore, a number of profibrotic and cell growth factors are released, stimulating the proliferation of smooth muscle cells and the production of excess collagen, with the initiation of atheroma plaque formation. Consequently, there is arterial remodeling, increased intima-media thickness and decreased arterial distensibility, also called early arterial stiffening (6, 7).

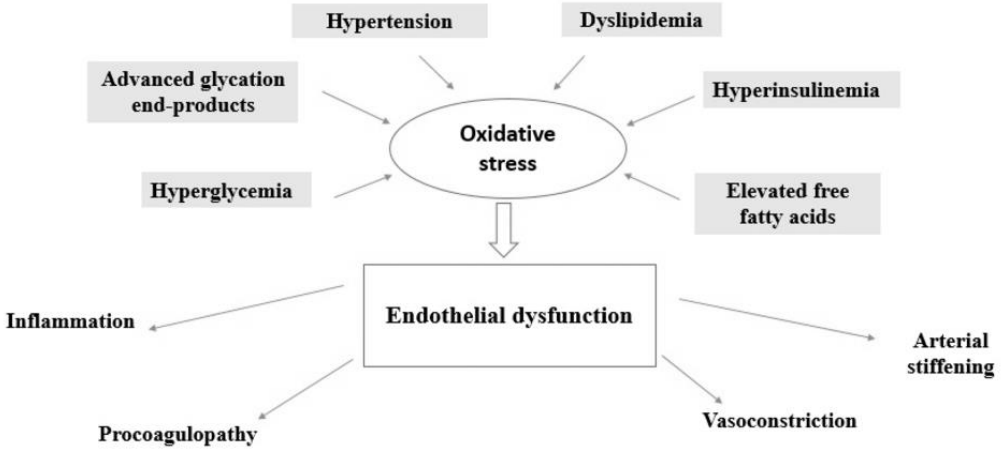

Fig. 1. Endothelial dysfunction in type 2 diabetes - consequences and pathogenesis. (adapted from Sandra J. Hamilton and Gerald F. Watts).

One of the pathophysiological mechanisms that lead to insulin resistance and diabetes is the oxidative stress. Under physiological conditions, pancreatic B-cells secrete and release insulin in response to increased postprandial blood glucose. Blood glucose is normalized by the penetration of glucose into the cell due to the increase in peripheral tissue expression of glucose transporters, especially GLUT 4 (8). But if the hyperglycemia is chronic, there is a chronic increase in insulin secretion that will cause the expression of GLUT 4 transporters to decrease, through a strong positive feedback mechanism. This is how insulin resistance appears. Reactive oxygen species (ROS) are implicated in the initiation and maintenance of insulin resistance (9).

CASE REPORT

The patient aged 55, from the urban area, is admitted in elective regime for: vertigo, physical asthenia, occipital headache, elevated blood pressure at home (self-monitoring systolic BP max = 180 mmHg, symptomatology with onset of about 3-4 days before presentation).

From the personal pathological history, we retain grade 3 essential hypertension (sBP max = 180 mmHg), type 2 diabetes mellitus treated with oral antidiabetic drugs, chronic venous insufficiency class C3 CEAP, endometrial adenocarcinoma for which total hysterectomy was performed, bilateral adnexectomy and lymphadenectomy approximately 30 days prior to the current presentation, grade I obesity. Perioperatively, the patient showed increased blood pressure (sBP max= 200 mmHg) and symptomatic episodes of sinus tachycardia (HR=120-130 beats/min). The patient is non-smoker, does not consume alcohol and the home treatment consists of a fixed combination of Olmesartan 40 mg / Amlodipine 10 mg, 1 cp/day, Metformin 1,000 mg 1cp/day.

At the objective physical examination, we note: the obese patient with BMI = 31.9 kg/m^2, malarial telangiectasis, postoperative sub-umbilical scar on the median line without inflammatory signs at this level, hyperemic conjunctiva, varicose inferior limbs bilaterally, rhythmic cardiac noise, BP = 250/80 mmHg, HR = 90 beats/min, bilateral pulsatile peripheral arteries, abdominal scar, increased abdominal volume on account of adipose tissue, without signs of peritoneal irritation.

From a biological point of view, on admission, the patient has hyperglycemia (fasting blood glucose = 135 mg/dL), liver dysfunction (SGPT = 74 U/L, SGOT = 39 U/L, GGT = 93 U/L), D-Dimer 0,968 µg/ml, TnT = 26,9 ng/L, CK = 143 U/L, CK-MB= 22 U/L, LDH = 244 U/L, Hb A1c = 6,8%, hypercholesterolemia (229 mg/dL), complete blood count, coagulation parameters and urine summary within normal limits. In order to exclude the diagnosis of secondary HBP, during hospitalization, additional investigations were performed: urinary catecholamine (adrenaline), vanillyl mandelic acid, cortisol, ACTH with results within the normal limits and quantitative proteinuria also with normal results.

Electrocardiogram on admission (fig. 2): sinus rhythm, intermediate QRS axis, HR = 83 beats/min, symmetrical wide negative T V_3-V_6.

We mention that the patient presented at admission an electrocardiogram performed postoperatively that showed a normal morphology in all derivations (fig. 3).

Chest X-ray showed normal lung transparency and heart within normal limits.

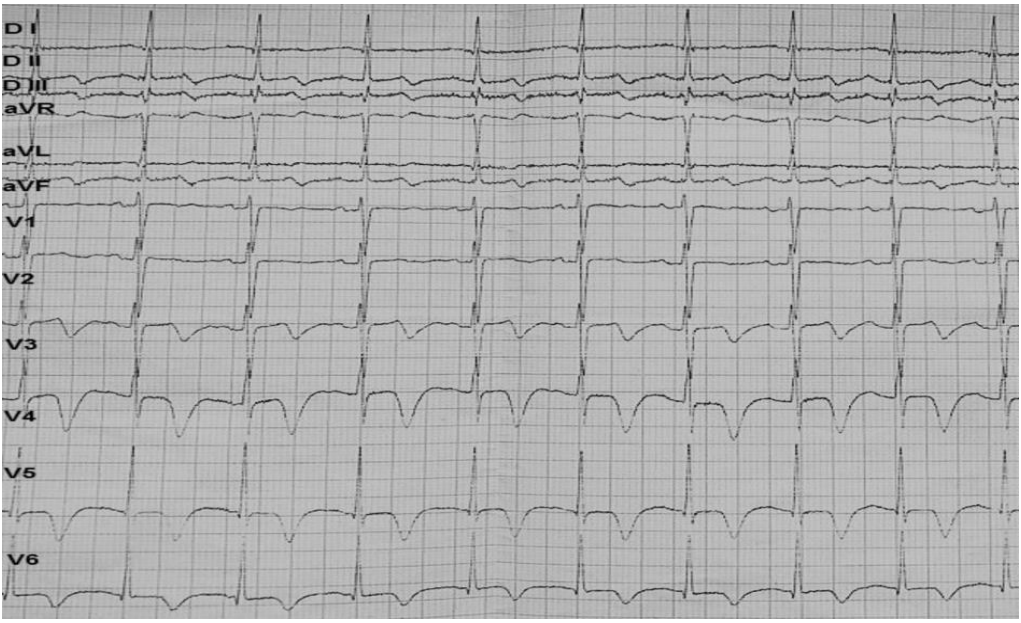

Fig. 2. Electrocardiogram on admission

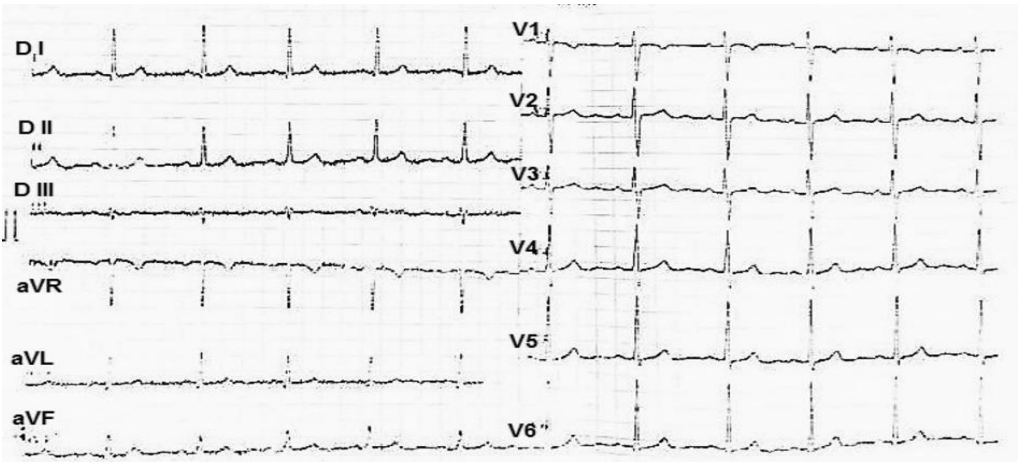

Fig. 3. Postoperative electrocardiogram

Abdominal-pelvic ultrasound reveals some infra-centimetric calculi at the cholecystic level and the post-surgical absence of the uterus and the annexes, without any further changes.

We can observe on the transthoracic echocardiography the left ventricular hypertrophy with preserved systolic function, apical hypokinesis at the 1/3 apical inferior wall and type I diastolic dysfunction delayed relaxation, without significant hemodynamic valvopathy, ejection fraction = 51%.

Vasodilator treatment with glycerol

trinitrate infusion solution, beta-blocker, angiotensin receptor blocker, dihydropyridine calcium blocker with progressive control of BP values was initiated. On the third day of hospitalization the patient discreetly manifested a myocardial cytolysis syndrome with remission on the following day; the electrocardiographic aspect is constant throughout the hospitalization. The particularity of the case: patient with postoperative status (radical gynecologic-oncologic intervention 30 days ago), known with diabetes mellitus controlled by oral antidiabetics, hypertensive, who is admitted for increased blood pressure manifested by headache and vertigo, an opportunity with which electrocardiographic (?) and via myocardial ischemia enzymatic turn in the absence of suggestive symptomatology and in the absence of coronary lesions.

DISCUSSION

Diabetes is a well-known risk factor for cardiovascular disease, doubling the risk of acute vascular events (coronary heart disease, ischemic stroke, and vascular death) independent of other risk factors. The relative and absolute risk is increased especially in women and young people, being higher in patients with a long evolution of diabetes, in those with poor control of glycemic values and in those with microvascular impairment (1). As diabetes progresses, it causes endothelial dysfunction and changes in energy metabolism leading to early atherosclerosis in the middle and large arteries affecting the coronary arteries.

The vascular endothelium is a complex organ, with an important metabolic, secretory, and immunological role, with auto- and paracrine function that thus provides a "first line" of defense against atherosclerosis (10, 11). It intervenes in the regulation of vascular tone, fibrin-coagulant balance, cell proliferation and inflammatory processes, being considered the main regulator of homeostasis of the vascular wall (12, 13). Damage to the vascular endothelium thus causes atherosclerosis and increases the risk of cardiovascular events (14, 15).

Endothelial dysfunction, defined as a reduction in the bioavailability of vasodilators, especially nitric oxide (NO) and an increase in vasoconstrictors, causes, along with structural and functional consequences, stiffness, and remodeling of the arterial wall. This is the link between the onset of atherosclerosis and cardiovascular risk factors (16). Endothelial dysfunction is initiated by oxidized LDL which influences the adhesion of monocytes and their migration into the subendothelial space, their transformation into macrophages and subsequently the formation of foam cells.

Insulin resistance and hyperinsulinemia play a particularly important role in endothelial dysfunction. Increased insulin levels resulting from insulin resistance activate the renin-angiotensin system as well as the sympathoadrenergic system which causes important metabolic, hemodynamic, and vascular changes, including endothelial dysfunction. Although, physiologically, insulin stimulates the production of nitric oxide by endothelial cells (by increasing the activity of nitric oxide synthetase by activating phosphatidylinositol-3 kinase) this action is reversed or decreased in patients with insulin resistance (17).

Another mechanism by which insulin resistance causes changes in the vascular endothelium is to increase the production of oxygen free radicals and the release of excess fatty acids from adipose tissue that eventually produce oxygen free radicals

that inhibit the production of NO (18).

Current studies highlight the role of insulin resistance and the initiation of inflammation, mainly the serum level of C-reactive protein. In the presence of C-reactive protein, LDL-cholesterol particles are taken up by macrophages and transformed into foam cells in the subendothelial space. Oxidation of LDL-cholesterol particles may increase protein levels that stimulate the endothelium to preferentially enhance ADMA production instead of NO (19, 20, 21).

Although the precise mechanism by which insulin resistance causes endothelial dysfunction remains a controversial topic and remains to be elucidated, in addition to the decreased response and nitric oxide synthesis, insulin resistance has been associated with increased endothelin 1 production, which may cause vasoconstriction, with a particularly important role in the development of hypertension. Endothelial dysfunction may also involve vasoconstrictor substances (endothelin 1 and angiotensin II) (22) and vasodilators such as the endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (23, 24, 25, 26).

Although so strongly involved in various pathological conditions, endothelial dysfunction is not yet fully investigated and used in medical practice, because for a long-time endothelium-dependent relaxation could not be documented in humans, and due to the complexity and adaptability of this phenomenon.

The symptoms of a patient with diabetes and acute myocardial ischemia are more often atypical. This may present as silent ischemia or with a variety of nonspecific symptoms. Silent myocardial ischemia is more common in the diabetic patient than in the general population and seems to be the main form of atypical presentation of both acute and chronic myocardial ischemia (2). The exact prevalence of myocardial ischemia remains unknown, but an analysis of data from the Framingham study shows in the group of asymptomatic patients with risk factors, 3% cardiovascular mortality (27).

The atypical symptomatology of acute myocardial infarction in diabetic patients is a subject under investigation in major clinical trials and the conclusions are diverse which makes definitive statements difficult (28). Autonomic neuropathy, disorders of the metaiodo-benzylguanidine metabolism, pain perception disorders through sympathetic denervation are some of the possible explanations for the atypical symptoms of myocardial ischemia in diabetic patients (29, 30, 31). In patients with diabetes, a resting electrocardiogram can identify silent myocardial infarction in 4% of cases, which is associated with an increased cardiovascular risk and increased all-cause mortality in men but not in women (32).

Another important factor that contributed to the atypical presentation of the patient was the post-surgical status. Despite optimal surgical and medical care, approximately one million patients worldwide die annually shortly after undergoing surgery. A major contributor to this postoperative mortality is “silent myocardial ischemic events“ which are often unrecognized. Routine assessment of cardiac biomarkers is an important step in identifying high-risk patients and improving perioperative care (33).

Major gynecological interventions (34) have an estimated intermediate risk (1-5%) of cardiovascular death and myocardial infarction at 30 days postoperatively. This risk only considers the surgery undergone without considering the patient's comorbid-

ities (35).

Another feature of this case is the absence of significant coronary changes. The absence of obstructive coronary artery disease in patients with signs of myocardial ischemia is a common clinical scenario that may be due to several mechanisms (epicardial coronary vasospasm, structural or functional changes of microcirculation). Due to the prognostic and therapeutic implications, the pathophysiological mechanism and a complete diagnosis must be followed. The intracoronary acetylcholine test or the adenosine test can evaluate the involvement of the epicardial coronary arteries as well as the endothelial dysfunction of the coronary microcirculation (36).

CONCLUSIONS

Diabetes is a huge public health problem that puts a lot of financial pressure on public health systems. It is the most common metabolic disorder that has a multifactorial etiology. Hyperglycemia and inadequate peripheral tissue response to insulin contribute to micro and macrovascular complications, which decrease quality of life and life expectancy. There is a close relationship between diabetes, oxidative stress, and endothelial dysfunction. Only understanding the pathophysiological mechanisms underlying the development of diabetes and knowing the risk factors can prevent the appearance of this condition and the development of complications.

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ERCP-RELATED PERFORATIONS: COVERT OR OVERT ADVERSE EVENTS? A PROSPECTIVE CASE SERIES

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ERCP-RELATED PERFORATIONS: COVERT OR OVERT ADVERSE EVENTS? A PROSPECTIVE CASE SERIES (Abstract): **Background** Perforations related to endoscopic retrograde cholangiopancreatography are a rare but feared adverse events, with significant morbidity and mortality. In order to describe the potential patient profiles related to post-ERCP perforations we present a case series of three patients, documenting several particular aspects related to their ERCP related complications. **Material and methods:** Between 01.01.2018 and 31.08.2020 a total of 403 patients underwent therapeutic ERCP in the tertiary medical center represented by "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania. Three duodenal and pancreaticobiliary perforations were identified (0.74%). **Results:** Three types of perforations (according to Stapfer *et al.*, 2000 classification) were described: type 2, type 3 and type 4. All patients presented patient-related risk factors and developed post-ERCP comorbidities that proved to be of significant impact to their prognosis resulting in two cases of fatal outcome. Given each case particularities, positive diagnosis was established using cross-sectional imaging. The therapeutical approach was complex and included surgical treatment, as advocated in current literature. **Conclusions:** Post-procedural diagnosis of perforations can prove to be challenging but an early diagnosis, an appropriate therapeutic approach and a well conducted post-procedural patient surveillance can significantly improve patient outcome. **Keywords**: ERCP, ADVERSE EVENTS, SURGERY, INFECTIONS.

Perforations represent a significant part of the possible complications related to upper gastrointestinal procedures, with an increased incidence among interventional endoscopic techniques (1). Both endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy have an overall complications-associated rate between 0.09% and 1.67% (2, 3) with duodenal perforations representing 0.08 % to 0.6% (4, 5, 6) or roughly one third of the total ERCP related perforations. What delineates them from other ERCP-associated complications are the high mortality rates ranging from 10% to 23% (6, 7, 8) making post-ERCP perforations the complication with the highest lethal outcome. However, the overall low incidence as well as the diversity in the risk factors lead to multiple epidemiological biases in the *evidence-*

based facts related to post-ERCP perforations.

Although perforations are known complications of ERCP for which endoscopic extended sphincterо-papillotomy and post papillotomy sphincteroplasty are well known risk factors, recent publications, including some guidelines, have shown that sphincteroplasty is characterized by a lower perforation risk than sphincter-papillotomy (6, 9, 10, 11). In order to describe the potential patient profiles related to post-ERCP perforations and due to the relatively lesser frequency of such complications, we aimed to present a case series of three patients, documenting several particular aspects related to their ERCP related complications.

MATERIAL AND METHODS

Patients

A prospective study was conducted that included 403 patients who underwent therapeutic ERCP from 01.01.2018 to 31.08.2018 in the tertiary medical center represented by *Saint Spiridon* Emergency Hospital, Iasi, Romania. Three post-ERCP perforations were identified and classified defined according to Stapfer *et al.*, 2000 classification (tab. I). Only duodenal and pancreaticobiliary perforations were detected within the study cohort.

TABLE I

Classification of post-ERCP perforation after Stapfer *et al.*, 2000

Perforation	Type 1: Lateral or medial duodenal wall perforation, endoscope related
	Type 2: Periampullary perforations, sphincterotomy related
	Type 3: Ductal or pancreatic perforations due to endoscopic instruments
	Type 4: Due to excess compression of air in the duodenum with presence of retroperitoneal air

From the total patients reviewed (tab. II) 19.1% presented post-ERCP complications, most of which were of infectious nature (8.93%). Acute pancreatitis occurred in 8.68% of the cases, post-ERCP hemorrhage in 2.98% and perforation in 0.74% of the cases with significant low incidence compared to other ERCP related complications.

TABLE II

Post-ERCP complications in the conducted study

Procedure ERCP (n=403)	Complications	Pancreatitis	Hemorrhage	Infection	Perforation
N	77	35	12	36	3
%	19.11%	8.68%	2.98%	8.93%	0.74%

In the conducted study three cases of post-ERCP perforation were identified, representing 0.74% of the total patients. Given the impossible task of carrying out an univariate study assessing the problems associated with this type of complication, we will present the case-studies of the three patients analyzing the evolution of their clinical and paraclinical status.

CASE PRESENTATIONS

I. First case C. E.

The first case involves a 27 years-old female patient, referred from the surgical department for biliary stones extraction. She experienced recurrent 48-72 hours

history of right upper quadrant colic pain, and altered blood works suggestive for biliary obstruction, without jaundice. The abdominal ultrasound and CT scan prior to the procedure revealed multiple gallstones less than 1 cm in diameter in the gallbladder and in the common bile duct (CBD) with CBD dilated up to 8 mm and biliary radicles dilated up to 3 mm. The patient consented to the procedure and elective ERCP was performed.

During the procedure, a modified papillary anatomy with a small infundibulum (type 2) papilla was identified, the cannulation proved to be difficult and required free-hand *needle knife precut* sphincterotomy.

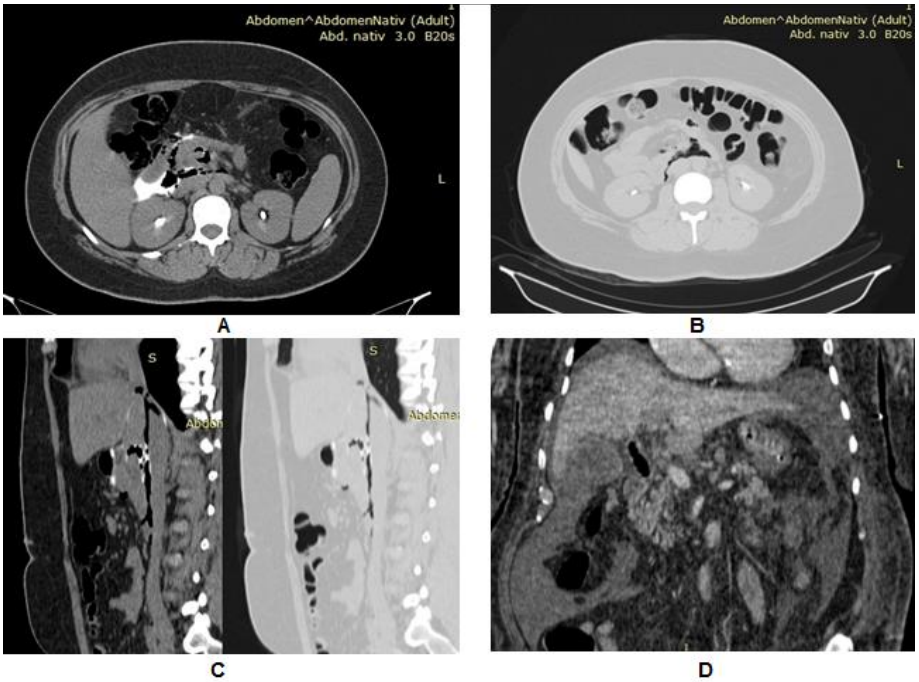

Fig. 1. CT scan images recovered from the C. E. case

Within the 24 hours evaluation following the procedure, the patient presented altered clinical status with tachycardia, tachypnea, tenderness in the right upper quadrant, dorsal thoracic pain, nausea and absence of bowel movements with a qSOFA score of 1. Blood panel showed systemic inflammatory response syndrome (SIRS) elements, with persistent elevation of C-reactive protein (CRP) from 0.89 mg/dL to 3.56 mg/dL. Given the clinical progression of the patient, a second emergent thoraco-abdominal computed tomography (CT) scan was performed, showing ERCP-injected contrast outside the biliary tract and gas bubbles in the retroperitoneum (fig. 1. Section A: axial section CT scan without contrast of the inferior part of the duodenum). There were also air bubbles surrounding the aorta and inferior vena cava, (fig. 1. Section B: axial section CT scan without contrast of the of the renal hilum using the lung window), with air dissecting through the retroperitoneal compartments tracking the inferior vena cava (fig. 1. Sec-

tion C: sagittal section abdominal CT scan without contrast). Fluid collections containing a mixture of hydro soluble contrast substance and air bubbles were present in the hepatic hilum, tracking the hepatoduodenal and pericaval ligaments.

A diagnosis of type 2 post-ERCP perforation was established. The patient underwent classic laparotomy with retroperitoneal approach, drainage and duodenal juxtapapillary suture. Also, cholecystectomy was performed with biliary-digestive and choledochal-duodenal anastomosis. After the surgery, the patient presented gradually favorable clinical course and was discharged after 22 days of hospitalization. She maintained a favorable clinical and paraclinical outcome at the 60 days follow-up.

II. Second case C. V.

The second case involved an 82 years-old male, admitted to the gastroenterology department for common bile duct stones associated with jaundice, acute cholangitis and acute biliary pancreatitis, and as a result an emergency ERCP was arranged in the endoscopy unit. The diagnosis was established based on clinical, biological and imagistic features including abdominal ultrasound and emergency abdominal CT scan. Prior to the procedure the patient presented poor clinical status, with an ASA score of 4.

The procedure revealed modified papillary anatomy with a hooded (type 3) papilla and the presence of a juxtapapillary duodenal diverticula. A total of 8 standard guidewire cannulation attempts were made lasting 6 minutes, followed by *needle knife* fistulotomy and successful CBD cannulation. The diagnosis cholangiogram confirmed multiple gallstones, the largest one measuring 15 mm and CBD diameter discreetly exceeding it. Sphincteroplasty was performed with a 13-15-18 mm controlled radial expansion balloon (CRE) and using an extraction balloon 4 stones were retrieved measuring 10 to 15 mm.

On the 24-hour evaluation post-ERCP the patient presented stable hemodynamic status, was conscious but somnolent, physical exam disclosed tachycardia and tachypnea with a qSOFA score of 3. Clinical examination of the abdomen remained inconclusive. Laboratory studies revealed improvement in the cholestatic syndrome, decreasing levels of the total bilirubin and cholestatic enzymes but with the presence of SIRS parameters, rising levels of CRP from 11.27 mg/dL to 32.18 mg/dL and acute renal failure with elevated serum creatinine from 1.07 mg/dL to 3.2 mg/dL. Also, high levels of sepsis markers were identified, with a presepsin value of 12,038 pg/mL. Blood cultures were collected with prior broad spectrum antibiotherapy from the admission with 3rd generation cephalosporin, isolating *Pseudomonas aeruginosa* with extended antibiotic resistance pattern, susceptible only to Amikacin and Colistin. An emergent abdominal CT scan was performed showing a moderate-density radiopaque image located in the Vater ampulla and an upstream dilated CBD suggestive for residual lithiasis (fig. 2. Section A: axial and coronal section abdominal CT scan without contrast). There was also bilateral portal branch thrombosis (red arrow) with rim contrast enhancement of the vessel wall suggestive for pylephlebitis (blue arrow), small gas bubbles surrounding the choledochal (yellow arrows) and peripheral hypoattenuating areas indicating perfusion abnormalities (fig. 2. Section B: post-contrast injection, central venous phase of the liver). Diagnoses of type 4 post-ERCP perforation and severe acute post-ERCP cholangitis were established.

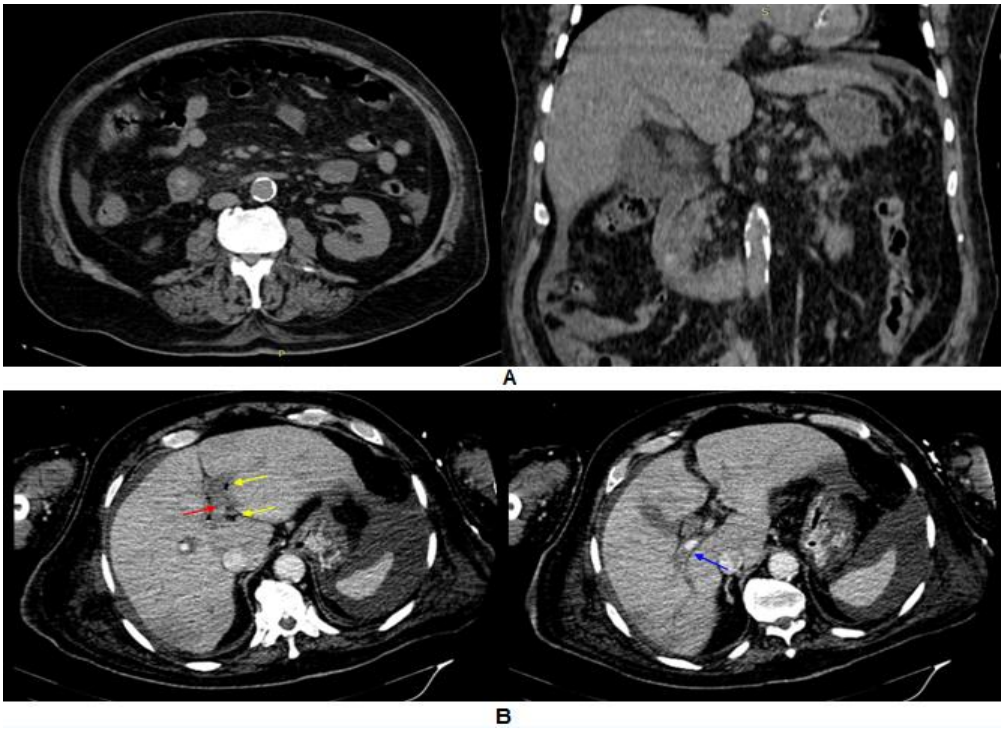

Fig. 2. Emergency CT scan images recovered from the C. V. case

The patient was admitted into the Intensive Care Unit (ICU). Exploratory laparotomy was performed with external abdominal transmural biliary drainage, gastrojejunostomy, a jejunal feeding tube was placed and the peritoneal and retroperitoneal cavities were drained. Postoperatively, the patient's functional status progressively declined, and he subsequently passed away due to multiple organ failure and septic shock.

III. Third case P. E.

The third case is of a 54 years-old female patient, with no significant personal medical history except some infrequent dyspeptic symptoms. She was referred from the surgical department for biliary obstruction and jaundice, given that prior to her admittance an elective abdominal ultrasound showed dilated intra and extrahepatic bile ducts, a 12 mm CBD with no apparent obstruction and no cholecystolithiasis. Tumoral markers assessment revealed a CA19-9 of 12,000 U/L and subsequently an abdominal CT scan was performed given the high suspicion for a cephalic pancreatic tumor.

During the procedure there was modified papillary anatomy with no infundibulum (type 1) papilla resulting in a difficult cannulation and after bile duct cannulation the cholangiogram revealed distal Bismuth type 1 biliary stenosis measuring 2-3 cm, with macroscopic irregular surface suggestive of malignancy. On the attending physician's recommendation only bile duct brushing for cytology was performed and the patient was scheduled for pancreaticoduodenectomy. Shortly after the proce-

dure the patient maintained a favorable clinical state, however, 24 hours after ERCP she developed subcutaneous emphysema located on the chest, in the supra and sub clavicular fossae and the CT scan revealed dilatation of the biliary tree, with absence of filling in the distal part of the CBD, important asymmetrical mural thickening and contrast enhancement (red arrow) corresponding to a malignant lesion. (fig. 3. Section A: abdominal CT scan of the liver in portal venous phase). CT findings included procedure injected contrast extravasation around the gallbladder with free gas bubbles in the retro pancreatic, right peri-renal and para-renal areas extending to the mediastinum, with emphysema of the soft tissue in the right hemiabdomen and bilateral pleural effusion (fig. 3. Section B: post-procedural abdominal CT scan without contrast using lung and abdomen windows).

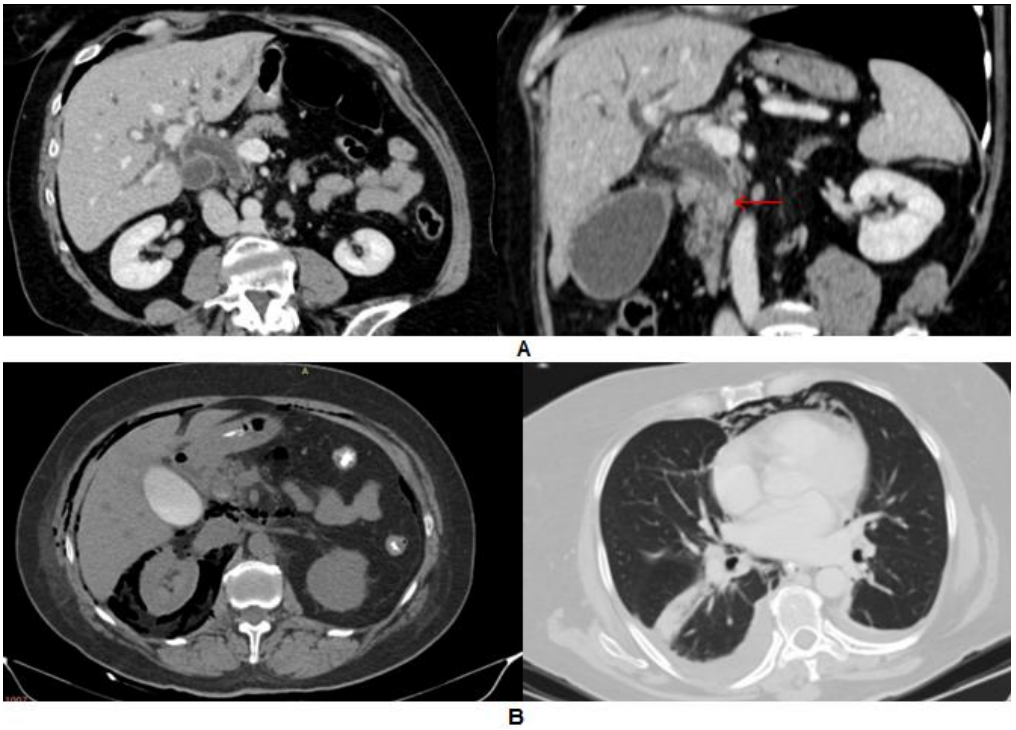

Fig. 3. Emergency CT scan images recovered from the P. E. case

Laboratory findings at the 24-hour evaluation included SIRS elements with elevated white blood cells 23,000/mmc, a CRP value of 13 mg/dL, aggravating cholestatic syndrome, a total bilirubin of 6.78 mg/dl (conjugated bilirubin 5.2 mg/dL) and elevated sepsis markers, with a presepsin value of 6,870 pg/dL. A diagnosis of type 3 perforation was established, with pneumoperitoneum, pneumomediastinum, subcutaneous emphysema, and severe acute post-ERCP cholangitis given the underlying malignant biliary stenosis. Blood cultures were collected. The patient received antibiotherapy with Piperacillin - Tazobactam and Amikacin, followed at 32 hours

post ERCP by acute renal failure onset, with serum creatinine of 5.8 mg/dL forcing discontinuation of Amikacin administration.

The patient underwent emergency laparotomy with cholecystectomy, external trans cystic biliary drainage, drainage of the retroperitoneal and peritoneal cavities and gastrojejunostomy. Bile samples were collected for culture. After the procedure, the patient showed signs of septic shock, with impaired hemodynamic function requiring two vasopressors. The blood and bile cultures isolated multidrug-resistant *Pseudomonas aeruginosa* susceptible only to Colistin and Amikacin. Histological examination of the endoscopic tissue sample was suggestive of malignancy showing high atypical mitoses. Despite the maximum multi-organic support, the patient passed away 3 days after the surgical intervention.

DISCUSSION

All three cases presented patient-related risk factors such as female gender (cases I and III), older age (case II), as well as procedure-related risk factors such as difficult cannulation and needle knife precut access papillotomy (cases I and II); balloon sphincteroplasty and bile duct brushing (cases II and III). The contribution of each procedure-related factor is relatively difficult to be quantified given the low incidence rate of 1% reported in the literature (6, 12, 13) for such adverse events. Such complication rates are similar to those described in our cohort. One common aspect of the three cases is represented by the difficulty in establishing a positive intraprocedural diagnosis, understandable given the small perforation size (for cases I and II) and the choledochal localization due to the underlying tumor (case III). For all the three cases demonstration of perforation was established using cross-sectional imaging that showed free extra-digestive air (pneumorethroperitoneum, pneumomediastinum, subcutaneous emphysema), and for cases II and III descriptive elements of severe acute cholangitis and hepatic abscesses. For the complications that occurred in cases I and III, acute post-ERCP cholangitis proved to be a comorbidity with significant impact. Another common feature was that all patients underwent surgical treatment, as advocated in current literature. Such decision was based on the experience of our tertiary center and on the patient's overall course following the perforation (6, 13, 14).

Post-procedural diagnosis of perforations can prove to be challenging as there are no specific symptoms. Therefore, very frequently such complications are considered to be covert. Epigastric pain radiating to the back is the most common finding, without being reliable given it's attribution to acute secondary pancreatitis, but often progressing to an acute abdomen accompanied by severe systemic inflammatory response (3, 13). Presence of subcutaneous emphysema gives a high index of suspicion for perforation whereas pain and systemic inflammatory response are a common find in the first 12 hours after the procedure (6). Signs and symptoms of peritonitis develop in first 4-6 hours post ERCP leading to shock if not promptly managed (6, 15). Moreover, in peri-ampullary perforations the diagnosis may be delayed if there are elevated enzymes levels wrongly attributed to acute pancreatitis (16) which is a frequent comorbidity. Contrast-enhanced computed tomography is the diagnostic imaging method of choice for detecting the presence of free air of contrast material in

the peritoneal or retroperitoneal cavities, regardless of the injury (16).

To what extent anatomy of the papilla is relevant to the occurrence of perforative adverse events is questionable. Nevertheless, as stated by our study protocol we implemented a four types of classification system of the macroscopic appearance of the papilla that seems to influence the overall complication rates after ERCP (17).

CONCLUSIONS

Among the ERCP-related complications, perforations represent adverse events that do not solely involve the level of technical endoscopic skills, but also require the ability to achieve an early diagnosis, to establish an appropriate therapeutic approach and a well conducted post-procedural patient surveillance. Given that this type of adverse events usually precedes or is linked to infections or post-procedural hemorrhages their impact on overall post-ERCP mortality is high, with important subsequent potential implications on liability and malpractice.

ETHICAL APPROVAL

The research project, the motivation, objectives, and research methodology have been reviewed and approved by the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi Board of Ethics. The subjects in this case report have given their informed consent to publish their cases along with the ERCP consent. In some cases, the consent for the interventional procedure was acquired from the closest legal representative, when emergent medical intervention was required, and the patient's status temporarily prevented them from giving their consent.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

DEMENTIA - ROLE OF MAGNETIC RESONANCE IMAGING

The role of neuroimaging in dementia extends beyond its traditional role of excluding neurosurgical lesions. Neuroimaging contributes to the early diagnosis (which allows early treatment) of neurodegenerative diseases (Alzheimer's disease), recognizes pre-dementia conditions (mild cognitive impairment), assesses disease progression and is adopted in current trials investigating these diseases. The coronal images show the hippocampus, the main structure involved in many forms of dementia. Magnetic resonance protocol includes: coronal-oblique T1-weighted images (used for the assessment of medial temporal lobe and hippocampal atrophy), obtained in a plane orthogonal to the long axis of the hippocampus, parallel to the brainstem; sagittal reconstructions will enable the assessment of midline structures, parietal atrophy, which may be involved in certain neurodegenerative disorders; FLAIR images assess global cortical atrophy, vascular white matter hyperintensities and infarctions; T2-weighted images evaluate lacunar infarctions in the thalamus and basal ganglia, which can be missed on FLAIR images; T2*-weighted images detect microbleeds in amyloid angiopathy, depict calcifications and iron deposition; diffusion weighted image (DWI) is a supplemental sequence used for young patients or in rapidly progressive neurodegenerative disorders (for differential diagnosis with vasculitis) (Barkhof F, Hazewinkel M, Binnewijzend M, Smithuis R. Dementia - Role of MRI. https://radiologyassistant.nl/neuro radiology/dementia/role-of-mri).

MULTIDISCIPLINARY MANAGEMENT OF OVARIAN TUMORS IN CHILDREN AND TEENS - A RETROSPECTIVE STUDY

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MULTIDISCIPLINARY MANAGEMENT OF OVARIAN TUMORS IN CHILDREN AND TEENS - A RETROSPECTIVE STUDY (Abstract): Ovarian tumors are the main pathology in the genital area among children and teens, their proper management remains a challenge due to the need for a multidisciplinary approach. **Aim:** The aim of the study was to evaluate the multidisciplinary management of ovarian masses in two reference centers. **Material and methods:** Files of patients, aged between 0-18 years, diagnosed with an ovarian tumor that required hospitalization between 2008 and 2019, were analyzed retrospectively. The following data were evaluated: demographic aspects, clinical symptoms, used imaging methods, tumor markers, type of treatment, presence of complications and their management, as well as the histological outcomes. **Results**: The study was performed on 803 patients with a mean age of 13.45 ± 4.06 years. 339 patients (42.21%) required surgery for tumoral pathology, this being most frequently located in the right side in 521 patients (65%). This surgical treatment was performed by laparoscopy in 55 of the operative cases (16.36%). The main symptom is abdominal pain, 85.42% of diagnosed patients being postmenarch. The most important complication is torsion, found in 22.12% of all ovarian tumors. The primary surgical intervention was cystectomy, which was performed in 53.09% of all surgical cases, in benign pathology, adnexectomy or oophorectomy being preferred in the case of complications or malignant tumors, the latter representing 17.69%. Regarding the histological results, 58.99% were serous cysts, and the main histological type among malignant tumors was represented by dysgerminoma 9.14%. **Conclusions**: Ovarian tumors in the analyzed age category are mostly benign, being more common in post menarche. The symptoms are nonspecific, dominated by pain in the lower abdominal floor, and the main complication is adnexal torsion. Preservation of fertility is the main goal in the management of ovarian tumors, conservative surgery with ovarian preservation being the main therapeutic concern. **Keywords**: CHILD, OVARIAN TUMORS, TORSION, TUMOR MARKERS, ADNEXAL, MASS, CYSTECTOMY, FERTILITY.

Ovarian tumors are the main pathology of the genital area among the infant population, their characteristics being different from those present in the adult population.

The annual incidence of ovarian tumors is 2.2-2.4 / 100,000 (1). Surgery among this population is also rare with a global incidence of 2.6 / 100,000 (2). Ovarian tumors range from simple serous cysts to malignant tumors, the latter accounting for 10-20 % of cases (3, 4). The incidence of malignant tumors is 0.102 / 100,000 in children under the age of 9 years and 1,072/ 100,000 respectively for the age group 10-19 years, observing an increase in the incidence with age (5), malignant pathology representing 60-70% among gynecological cancers present in this age category (6). The vast majority of ovarian tumors are benign and self-limited, surgical treatment is necessary only in complicated cases (7). Torsion is the main complication of these ovarian mases, especially due to the anatomical particularity represented by the presence of a longer ovarian pedicle compared to the adult population (8). Ovarian tumors in children are a challenge both from a diagnostic and therapeutic point of view, at this age group it is essential to preserve fertility and so a detailed analysis of the preoperative characteristics of tumors is necessary. In the case of benign tumors, it is recommended to excise them with the preservation as much as possible of functional ovarian tissue, in the case of neoplasia with an increased risk of malignancy ovariectomy is most often preferred (9). Minimally invasive surgery has become the gold standard in the treatment of benign ovarian tumors in the last decade due to the short recovery time, minimal pain (especially difficult to manage in children) and last but not least, the aesthetic aspect (10, 11). Signs and symptoms are most often nonspecific, mimicking multiple abdominal-pelvic medical or surgical pathologies (12). The aim of the study was to evaluate the multidisciplinary approach of ovarian masses and to compare the obtained surgical results with those in the literature.

MATERIAL AND METHODS

The study was carried out in two reference centers involved in the management of ovarian tumors, the "Sf. Maria" Clinical Emergency Children's Hospital, Iasi and the "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology Iasi and included 803 patients (children and teens), under 18 years, diagnosed with ovarian tumors during the 2008-2019 period, from which complete data could be obtained. From this group, 339 patients required surgery, the remaining 464 patients benefited from conservative management. Being two regional centers, the study group also included patients addressed from the neighboring areas for adequate treatment. The patient files/electronic databases, the operating protocols and the histological results were analyzed retrospectively, being collected by the investigators involved the following data: demographic aspects (age, environment of origin, weight, height), clinical symptomatology, laboratory analyzes / tumor markers, imaging used in diagnosing tumoral pathology, type of treatment (conservative or surgical), tumor characteristics (location, uni/bilateral, size), case evolution, the presence of complications and their management and histopathological aspects. Patients with simple cysts, with a diameter < 5 cm were treated conservatively, recommending imaging reassessment at 6 months, while in the case of those with tumors with a diameter ≥ 5 cm, complex structure or clinical signs of acute abdomen, surgical treatment was performed. Patients with signs of acute abdomen benefited from emergency surgery, and in the

case of stable hemodynamics, elective interventions were performed. Regarding preoperative evaluation, patients requiring emergency surgery were clinically and paraclinically evaluated according to specific protocols, including complete blood count, coagulogram, inflammatory tests, beta HCG and ultrasound examination. In the case of patients who received elective surgical treatment, determinations of tumor markers (β HCG, AFP, CEA, Ca-125) were performed preoperatively as well as additional imaging evaluations by computed tomography / MRI, especially in situations where malignant tumors have been suspected. Although specific assessment of ovarian masses imaging by CT or MRI is higher compared to ultrasound (to distinguish signs of malignancy), these investigations were performed only in 26 cases (5.4% of surgical cases) due to reduced accessibility during the on-call shifts. The exclusion criteria were represented by the cases with incomplete documentation and those in which the intraoperative exploration revealed the presence of a pathology other than the initially considered ovarian tumor. The study was developed and conducted in accordance with the guidelines of the Helsinki Declaration, and approved by the ethics committees of the 2 centers. The rights of all patients included in the study were respected. Statistical analysis was performed using *SPSS version 17.0*, and data was analyzed for frequency by descriptive analysis and expressed as mean ± SD. Categorical variables were expressed as number and percentage of patients.

RESULTS

Analysis of demographic basic characteristic features of the patients included in the study revealed a mean age of 13.45 ± 4.06 years, an asymmetry was observed of the right and a right-tilted distribution, which means a higher frequency of this condition in teens over 13 years of age and in newborns (fig. 1). The examined patients had weights between 1.7 kg and 82 kg with an average of 42.87 kg. There is a preponderance of patients from rural areas, respectively 540 cases (67.24%), compared to those living in urban areas, 263 cases (32.75 %).

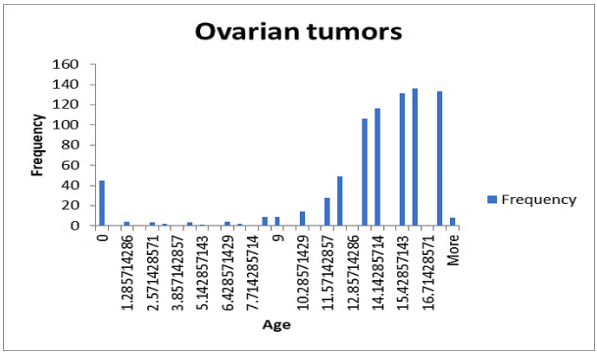

Fig. 1. Age distributes of the tumor pathology in the study group.

Regarding the site of tumor, they were highlighted especially on the right side (65%), in 11% of cases being bilateral. 85.42% of patients were diagnosed in postmenarche. Of the total number of patients enrolled in the study, only 42.21% required

surgery for the pathology, the rest benefiting from conservative management. Surgical treatment was performed by classical approach in most cases (83.77%), and in terms of complications of ovarian tumors, the most important one was torsion, present in 75 cases that required surgery (22.12%) (tab. I).

TABLE I

Integrated analysis of ovarian tumors in terms of location, diagnosis in relation to menarche, type of treatment performed and complications

Characteristics		Number	Percent
Location	Right	521	65%
	Left	192	24%
	Bilateral	90	11%
Menarche	Pre-menarche	117	14.57%
	Post-menarche	686	85.42%
Treatment	Surgical	339	42.21%
	Laparotomy	284	83.77%
	Laparoscopy	55	16.36%
	Conservative	464	57.78%
Complications	Torsion	75 (339)	22.12%

In patients who required surgical treatment, different interventions were performed depending on the symptoms, suspected malignancy or the presence of complications. The most frequent intervention was represented by cystectomy (53.09 %), followed by adnexectomy and oophorectomy (30.97%), adnexal detorsion saving the ovary was possible in a relatively small number of cases (11.19 %) (tab. II).

TABLE II

The types of surgery used in patients with ovarian tumors in the study group

Surgical procedure	Number	Percentage (%)
Detorsion - cystectomy	30	8.84%
Detorsion - fixation of the ovary	8	2.35%
Cystectomy	180	53.09%
Exhaust puncture	16	4.71%
Adnexectomy	56	16.51%
Ovariectomy	49	14.45%

In the case of ovarian torsion (75 cases), pelvic ultrasound was performed preoperatively in 48 cases (64 %) that showed loss or reduction of ovarian vascularization and which was later confirmed intraoperatively. In the remaining 24 cases (36%) it was not possible to perform imaging investigations due to hemodynamic instability, requiring emergency surgery, the diagnosis of torsion being confirmed intraoperatively. In case of torsioned tumors, the average age of the patients was 8.43 years old for benign tumors, respectively 7.25 years old for malignant tumors. Adnexal detorsioning to its

preservation was performed in 33 cases (44%), adnexectomy carried out in 16 cases (21.33%), while in the remaining 26 cases (34.66%) the removal of the ovary was necessary, without revascularization following detorsion. This complication was found in 8 cases with malignant pathology (10.66% of all twisted tumors), the remaining 89.33% being benign (tab. III).

Regarding the histological types, 57 surgically treated patients had malignant pathology (16.81%), representing dysgerminomas in 31 cases (9.14%), immature teratomas in 14 cases (4.12%), adenocarcinomas in 10 cases (2.94%) and 2 case of stromal tumors (0.58%). Benign tumors are represented by simple serous cyst in 200 cases (58.99%), necrotic ovarian tissue in 50 cases (14.74%) and mature teratoma in 29 cases (8.55%) (tab. IV).

TABLE III

Integrated analysis of the 75 cases with ovarian torsion in terms of location, diagnosis in relation to menarche, the type of treatment performed and the histological type

Characteristics		Number n / 75	Percent
Topography	Right	51	68%
	Left	24	32%
Menarche	Pre-menarche	39	52%
	Post-menarche	36	48%
Procedure	Adnexectomy / oophorectomy	37	49.33%
	Detorsion - fixation	8	10.66%
	Detorsion - Cystectomy	30	40%
Histology	Simple serous cyst	33	44%
	Necrotic tissue	30	40%
	Mature Teratoma	6	8%
	Dysgerminoma	5	6.66%
	Carcinoma	1	1.33%
	Borderline	1	1.33%

TABLE IV

Histological profile of ovarian tumors that required surgery (339 cases)

Histology		Number	Percent
Benign	Simple serous cyst	200	58.99%
	Necrotic ovarian tissue	50	14.74%
	Mature teratoma	29	8.55%
Malignant	Dysgerminoma	31	9.14%
	Immature teratoma	14	4.12%
	Adenocarcinoma	10	2.94%
	Stromal tumors	2	0.58%
Borderline tumors		3	0.88%

Conservative surgery represented by cystectomy was performed in 185 patients with benign pathologies, in malignant tumors preferring excision of the adnexa. Among the tested tumor markers, AFP had a specificity of 78%, while the specificity of β-HCG was 89%. Malignant tumors represented 17.69%, borderline tumors representing < 1%. The mean age for malignant tumors is 13.77 years ± 4.6 years, while for benign ones the mean age decreases to 12.59 ± 4.9 years. Conservative management of ovarian tumors was used in 464 cases (57.78%), with a mean length of hospital stay of 6.3 days. Clinical-paraclinical monitoring was accompanied in such cases by symptomatic treatment with analgesics, non-steroidal anti-inflammatory drugs, antipyretics and antibiotics. 48 cases that were initially managed conservatively, needed to be rehospitalized in an interval between 3-9 months and subsequently underwent surgery due to the size of the tumor and the symptoms, being included in the group of patients who needed surgical treatment. The most common symptoms of ovarian tumors were diffuse or localized abdominal pain (82.6%), nausea / vomiting (29.3%), palpable tumoral mass (19.3%), low-grade fever/fever (12.3%), intestinal transit disorders (11.6%), amenorrhea (3.4%) and vaginal bleeding (3.1%).

DISCUSSION

Ovarian tumors among children and teens are most commonly a benign pathology, which regresses after drug treatment, surgery being necessary in less than half of the cases, a situation highlighted in our study. The main symptom for presentation is pain in the lower abdomen, fact supported by other studies such as the study performed on 266 cases by How *et al.* or I. Banli-Cesur *et al.* on 146 cases (13). Numerous studies claim that transit disorders and the perception of a palpable abdominal mass are associated with an increased risk of malignancy, so the presence of one of these findings justifies an additional assessment (4, 11, 14, 15). Virilization and early puberty may be present in malignant tumors, and sex cord stromal ovarian tumors may secrete estrogen, androgens, or corticosteroids with specific clinical and paraclinical changes (14, 16, 17). Thus, in the presence of a palpable ovarian mass accompanied by signs of hyperandrogenism or hyperestrogenism, the suspicion of a malignant tumor of stromal origin arises. A predominance of tumor masses on the right side is observed in our study, which predisposes to an increased rate of torsion-type complications on this side. The torsion occurs when the ovary twists around its own ligaments, stopping the blood flow that served the ovary. Preoperative diagnosis is a challenge among the pediatric population, with acute pain requiring a complex differential diagnosis (18). Thus, pain located in the right iliac fossa can be associated with acute appendicitis, delaying diagnosis and surgical treatment, resulting in devascularization of ovarian tissue, with its necrosis, making it impossible to preserve it, reflected in the relatively high incidence of adnexectomies highlighted by us.

Also taken into account the role of the sigmoid located on the left side, could play a "protective" role against torsion. The rate of this complication was 42% in the study led by Cass *et al*, 10% in the study of Liu *et al.* and 23.5% in that of I. Banli-Cesur *et al.* (13, 18). In our case the torsion was present

in 22.12% of the cases. The use of ultrasonography, computed tomography or magnetic resonance imaging are implemented in clinical practice as diagnostic imaging methods, complementing each other provided they are accessible, which was not possible in all cases in our study (19).

Ultrasonography is, however, the main imaging method used in the diagnosis of ovarian tumors, especially in an emergency, as well as possible complications. Due to the accessibility and non-invasiveness of the method, it is the main tool in assessing the topography and vascularization of the affected ovary (20). Melcer *et al.* showed that ovarian torsion is associated with enlargement of the ovary on the basis of edema and free fluid (21). However, in the case of ovarian torsion, the gold standard is diagnosis laparoscopy. Practiced when the first signs appeared with the advantage of allowing detorsion and preservation of the adnexa and in order to preserve fertility in this age category, laparoscopy was used in our study only in 16.36% of operative cases, in correlation with surgeon experience regarding the use of this technique (22).

The torsion is a rare complication of malignant tumors, ranging in frequency from 1-6% in children and teens (23), the most common in germ cells tumors. In our study, the frequency of torsion in patients with malignant tumors is 7.99%, 6.66% of which are dysgerminomas. At the same time, dysgerminomas are the most common malignancies encountered in this age group, an aspect supported by the literature (24).

The use of a tumor marker both as a screening and as a diagnostic test for ovarian cancer is based on the identification in serum of an abnormal level of a specific marker, which reflects the systemic effect of the disease (25). There have been studied many tumoral markers: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carcinoid antigen 19.9 (CA 19.9), carcinoid antigen 125 (CA-125), β human chorionic gonadotropin (β-HCG) in order to detect and monitor malignant tumors, which are especially elevated in germ cell and epithelial tumors (26). Thus, they are essential tools in pediatric practice to exclude germinal tumors. In teenagers AFP and β-HCG are essential for assessing ovarian pathology. AFP is produced, in addition to germinal cells tumors by other tumors such as the pancreatic and liver neoplasia. However, the study led by Papic *et al.* demonstrated the specificity of AFP and β -HCG in ovarian malignancies (27). In the literature the sensitivity of this marker is 90%, with a specificity of 60%. In our study, we turned our attention to AFP, observing a preponderance of teens in the study group, thus obtaining a specificity of 70%. This marker is also used in staging and determining the doses and duration of administration of chemotherapeutics (28). In the case of tumors that do not produce AFP or β-HCG, studies have shown the possibility of using markers such as LDH, inhibin, TAG 72 glycoprotein or human epididymis 4 (HE 4) (29). In the management of malignant ovarian tumors in children, the desire essential is represented by conserving ovarian tissue without compromising cancer surgery principles being difficult to perform. In our study, the rate of ovariectomy is 30.95%, demonstrating the attempt to preserve ovarian tissue in all cases where this was possible. Interventions involving preservation of the adnexa were indicated in benign tumors, excision was recommended in case of malignancy.

In this study, the rate of interventions that preserved ovarian tissue was 68.99%, lower compared to the study led by I. Banli-Cesur, with a rate of 96.7% (30-33). The omission of a possible malignant tumor is the main concern of surgeons, the signs and symptoms being nonspecific. Tumor markers and imaging methods are a real support, but in the case of an emergency they cannot always be performed.

Benign tumors are most common in children and teens. Although there are no prospective and randomized studies on the management of ovarian tumors in this age group, the low incidence of malignant tumors and the preservation of fertility, advocate the approach of a surgical treatment after which the ovarian tissue is preserved. On the other hand, conservative non-surgical management can be prioritary, as highlighted in our study, considering the age, the possible implications on fertility and why not, the possible legal implications. Some of the ovarian initially evaluated neoplasia and which required symptomatic treatment may increase in volume later and may develop complications that this time will require surgery, as happened in 48 cases in our study. Surgery can be performed by laparoscopy, mini-laparotomy or laparotomy, ranging from aspiration of the cysts to salpingo-oophorectomy. Radical interventions such as adnexectomy in pediatric patients should be performed only if necessary, following the histological result. Laparoscopy is the method of choice for treating benign ovarian tumors without septa or vegetation, measuring less than 8 cm in maximum diameter (34). Preservation of fertility is essential, as surgery involving preservation of ovarian tissue is preferable whenever possible, as after an ovariectomy, castration may occur before the age of reproduction if torsion or neoplasm of the contralateral adnexa occurs (35). In the cases of torsion, the current approach is aimed at detorsioning and preserving the adnexa, despite its ischemic aspect. In this case, in one study conducted by Santos *et al.*, 97% of patients with a history of torsion in which detorsion and preservation of the adnexa were performed, did not show side effects, the ovary resuming its function later (36). Regarding the fixation of the ovary, the technique is controversial, some authors claiming that the fixation affects both the ovary and the connection between the follicles and the salpinx. This procedure is, on the other hand, recommended in cases where there is a recurrence, long ovarian ligament or single ovary (37). However, studies show an ovariectomy rate of 54-62.5% in case of torsion, in our study there is a decrease of this intervention, the rate being 49.33% (11, 38).

In our study we can also see an increased incidence of ovarian tumoral pathology in postmenarch patients, this can be explained by hormonal changes that occur at this age, a finding that is supported by other studies (36, 39). The pathology most frequently encountered in the literature is benign, as can be certified in the present study, the simple serous cyst being the histological type with the highest incidence of 58.99%. Functional ovarian cysts caused by anovulation or hormonal disorders are considered normal in adolescence, however in the case of persistent mass the possibility of a malignancy should not be ignored (27).

CONCLUSIONS

Ovarian tumors in children and teens are mostly benign, sometimes with nonspe-

cific symptoms, the most common being abdominal pain. In the management of these cases, the first step consists in the anamnesis and the clinical examination, later the ultrasonography representing an important investigation in the evaluation of the mass. Dosing of tumor markers is a second essential step in the evaluation, especially in the case of tumors that show signs of malignancy. Last but not least, the evaluation of suspicious tumors by computed tomography or magnetic resonance imaging is an important element that can dictate the conduct in such cases. Following these steps in the evaluation of ovarian tumors, allows their evaluation and a reduction in surgical interventions for benign tumors. Surgical management of ovarian masses should focus on ovarian preservation whenever possible. In the case of ovarian torsion, detorsion of the adnexa and preservation of the ovary is the recommended therapeutic approach.

CONFLICT OF INTEREST AND FUNDING

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NEWS

POST-MORTEM GENETIC INVESTIGATION OF CARDIAC DISEASE-ASSOCIATED GENES IN SUDDEN INFANT DEATH SYNDROME (SIDS) CASES

The sudden infant death syndrome (SIDS) is still one of the leading causes of post neonatal infants death. SIDS is defined as sudden death of an infant under 1 year of age, and the cause of death still remained unexplained after autopsy. In most cases, death occurs during sleep. A "triple risk model" for SIDS has been proposed and implies that the risk of SIDS increases if three factors concur: (1) a vulnerable infant, (2) a critical period in the development during the first months after birth, and (3) exogenous stress factors such as sleeping in prone position, smoking of the parents, and premature birth. Despite some successful campaigns to reduce these risk factors, SIDS is still the leading cause of death in post neonatal infants. Genetic variants affecting autonomic functions, neurotransmission, energy metabolism, and cardiac repolarization have been suggested to contribute to SIDS. These studies also showed that there exists a complex relationship between SIDS and inherited cardiac diseases. Long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and hypertrophic cardiomyopathy (HCM) have been described as a monogenic cause in a certain number of SIDS cases. The development of next-generation sequencing (NGS) offers new opportunities to investigate potential pathogenic sequence variations as an underlying cause of death in SIDS cohorts. Several studies suggest that in up to 30% of SIDS cohorts pathogenic mutations in cardiac channelopathy-associated genes contributed as a monogenic cause of death. In 3.5% of SIDS cases, a pathogenic variation in a cardiomyopathy-associated gene was detected. The detection of variants and its evaluation is important when counseling the family members in a SIDS case, but special precautions have to be made when a variant is considered to be pathogenic. In a recent study of variant analysis, 4.3% of pathogenic variations and 13% of "informative variations" have been found, which is a much lower percentage than that reported in the previous studies. In the present study, an analysis of genes associated with genetic heart diseases in a cohort of 31 SIDS cases was performed to investigate the presence of potentially causative variants, which may represent a predisposing risk factor for sudden death in infancy (Köffer J, Scheiper-Welling S, Verhoff MA, *et al*. Post-mortem genetic investigation of cardiac disease-associated genes in sudden infant death syndrome (SIDS) cases. *Int J Legal Med* 2021; 135: 207-212).

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HOW NEGATIVE IS THE PRESSURE USED IN THE SURGICAL WOUND TREATMENT WITH NEGATIVE PRESSURE THERAPY ?

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HOW NEGATIVE IS THE PRESSURE USED IN THE SURGICAL WOUND TREATMENT WITH NEGATIVE PRESSURE THERAPY (Abstract): The study aims to assess the significance of negative pressure therapy in the treatment of surgical wounds and using the ANSYS simulation software for fluid dynamics calculation will give us the possibility to determine the fluid flow dynamics inside the vacuum kit during its use. **Material and methods**: To predict the speed, pressure and temperature of the fluid inside a vacuum kit, we used numerical modeling, using the finite element method, of the gas fluid flow phenomenon. **Results:** The pressure of the liquid fluid is higher in the area of contact with the wound, compared to the pressure of the liquid fluid in the outlet area of the capillary tube, accentuating with increasing pressure applied to the end of the tube. 32% of the fluid pressure at the interface between the sponge and the wound. **Conclusions:** The rate of pressure transmission inside the sponge is variable favoring the appearance of ischemia areas on contact with the wound. **Keywords:** NEGATIVE PRESSURE, NPWT, WOUND TREATMENT.

Over time, the products used have become extremely complex and to find solutions to optimize them, such as solving problems of fluid dynamics, systems with moving parts (valves, pistons), where a transient analysis of phase systems is required due to heat exchange (cold / hot) and systems with complex physical phenomena, such as interference between liquids and surfaces requires the use of such computer programs because high quality products can no longer require engineers to rely on the study of phenomena the system is subject, addressing each one separately, but they must be thought of as a unitary whole taking into account in particular the dynamic interaction between them (eg the program is used in the conversion of nuclear waste into stable residues, having the ability to accurately predict mixtures required for stabilization).

The ANSYS simulation software for fluid dynamics calculation (CFD) will give us the possibility to determine the fluid flow dynamics inside the vacuum kit during its use. The capabilities of the software product in fluid flow analysis can be used to both design and optimize new devices

and to troubleshoot existing negative pressure systems, regardless of the phenomena studied.

A study, published by Maier *et al.*, Provides information showing that positive pressure also develops on the surface of the wound that meets the sponge of the kit, this being in contradiction with the theoretical considerations of device operation. In the study, pressure-measuring sensors placed on the wound were used, which recorded changes of up to + 26 mmHg in the contact areas between the sponge and the wound (1).

Starting from this study, we aim to analyze the conditions for the development of pressure both inside the sponge and at the contact surfaces between it and the wound, to evaluate the homogeneity and distribution of suction at different set suction values, the flow rate of secretions from the wound to the collecting container, using the polyurethane (PU) sponge.

MATERIAL AND METHODS

The analysis of the flow of liquid fluids through isolated systems demonstrates that the velocity, pressure and temperature of the fluid change continuously. To predict the speed, pressure and temperature of the fluid inside a vacuum kit, we used numerical modeling, using the finite element method, of the gas fluid flow phenomenon.

To solve the problem, we chose the ANSYS-FLUENT program which consists of the solver specialized in fluid flow phenomena.

The three-dimensional geometric model was made using the INVENTOR design program, developed by Autodesk USA. The geometric pattern was assimilated with a porous polyurethane medium, which has an open surface - in contact with the wound, and on the other surface is provided a port that comes into contact with a thin tube with variable geometry.

Discretization of the domain was performed using the unstructured tetrahedral networks from the existing software package in the Fluent solver, according to the rules of good practice in the field. The first figure presents the network of "finite elements", created for the considered model, which contains a number of 159,474 nodes, respectively a number of 205,093 tetrahedral elements.

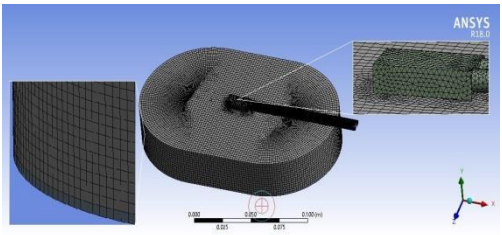

Fig. 1. The discretization networks applied to the model

The establishment of the limit conditions and the selection of the turbulence model was made according to the lines of good practice, valid in the field.

RESULTS

Figure 2a-d shows the variation of the fluid pressure at a negative pressure of 100 mmHg in the xOy –fig planes. 2a, and xOz - fig. 2b, as well as at two different points in the xOy plane figures 2c and d, under conditions imposed by the simulation. In figures 2e and 2f are represented the variation of the fluid pressure at a negative pressure of 150 mmHg in two different points in the xOy plane - figures 2e and 2f, under conditions imposed by the simulation.

Inside the model, the fluid has variable pressure, high values being registered towards the lower area of the polyurethane sponge, below the isosurface with the pressure of 13520 Pa (fig. 3).

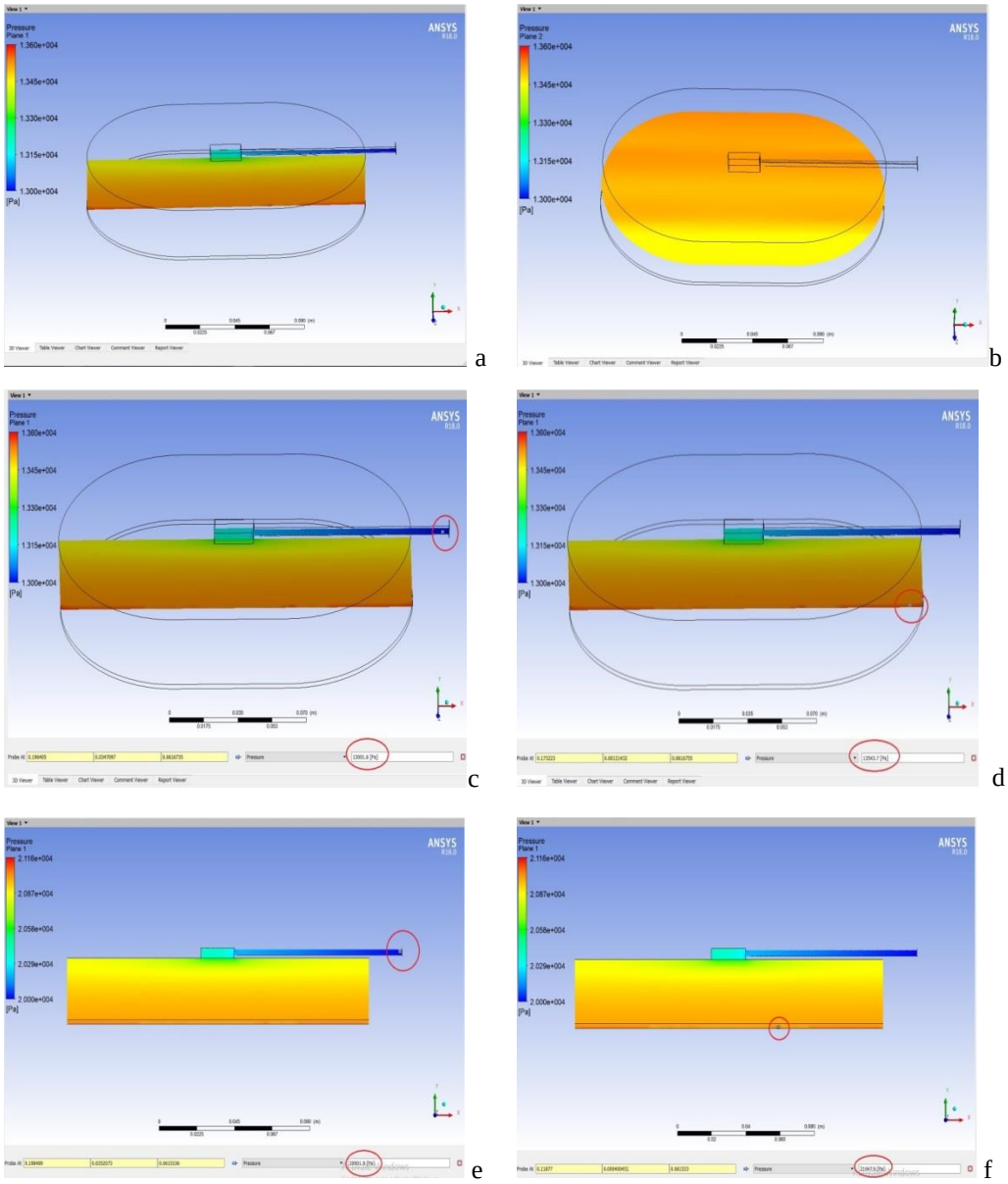

Fig. 2 a-f. Fluid pressure variation when setting the negative pressure system at 100 mmHg in: a xOy plane (bending = 13332 Pa); b) xOz plane (skew = 13332 Pa); c) at a point in the xOy plane located at the outlet of the suction tube (bending = 13332 Pa); d) at a point in the xOy plane located at the contact with the wound (skew = 13332 Pa); e) at a point in the xOy plane located at the outlet of the suction tube (bending = 19999 Pa); f) at a point in the xOy plane located at the contact with the wound (bending = 19999 Pa).

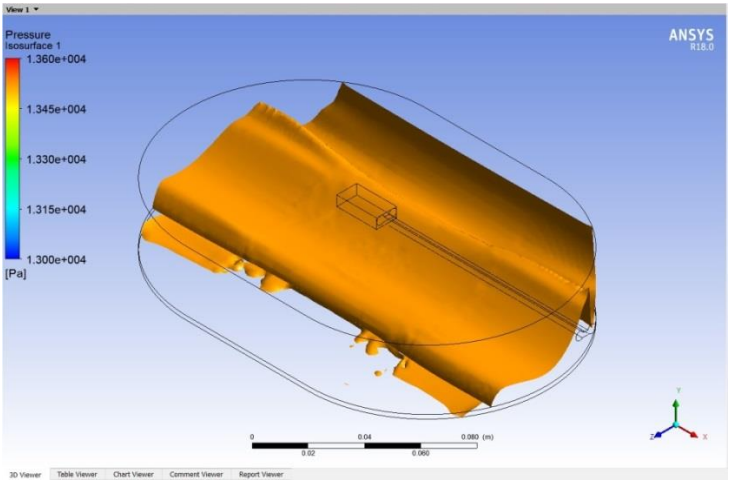

Fig. 3. Isosurface with average fluid pressure p = 13520Pa - system set at 13333Pa (100 mmHg).

DISCUSSION

The theoretical results obtained by finite element analysis show that, at the level of wounds, the suction pressure of the fluid is unevenly distributed, which determines, according to Willy *et al.*, the appearance of ischemic areas.

The size of the ischemic areas increases with increasing suction pressure. Fluid velocity is minimal at the sponge-wound interface, which causes slow fluid flow, which favors the appearance of edema and ischemic areas (2). The low flow rate of the fluid, both at the wound-sponge interface and inside it, determines the inability of the liquid flow to detach and mobilize the debris.

The theoretical results obtained, combined with existing clinical studies in the literature, converge to the fact that the application of negative pressure therapy in the treatment of wounds requires the use of an optimal fluid flow regime to prevent the formation of edema and ischemic areas of wounds.

Theoretical considerations and contributions made in the study refer mainly to the physical phenomena and interactions that take place inside a vacuum kit used in the negative pressure therapy of acute and chronic surgical wounds, because we consider that the postoperative results are closely related to the configuration his.

In the xOy plane, the pressure of the liquid fluid is higher in the area of contact with the wound, compared to the pressure of the liquid fluid in the outlet area of the capillary tube (fig. 2a). This increase, of approximately 4% (fig. 2c *vs.* fig. 2d), is accentuated with the increase of the pressure applied at the end of the tube and is due to the porous elastic environment and the complicated capillary system. Thus, the increase of the pressure applied at the end of the tube (from 13333 Pa = 100 mmHg) - to 19999 Pa = 150 mmHg) determines the increase by over 32% of the pressure of the liquid fluid at the interface between the sponge and the wound (fig. 2e and fig. 2f).

Our own studies, performed with the

help of finite elements, on the phenomenon of flow of liquid fluids through porous media, show that the rate of pressure transmission inside the sponge is variable (fig. 3), an aspect that contradicts the results obtained by Willy *et al.*, in the study on the suction effect of the polyurethane (PU) sponge. Measurements were performed on a sponge, at different distances (0-52 cm), in which he inserted 8 pressure sensors, arranged at distances equal to 7.5 cm, and applied different pressures, ranging from 50-200 mmHg , the measurements being performed at intervals of 25 mmHg. Each measurement involved stopping the experiment by setting the parameters to 0 and depressurizing the vacuum system, including the sponge, at which the information collection probes are located. The authors concluded that, when applying pressures of up to 150 mmHg, the transfer of pressures inside the sponge was performed at a rate of 1:1 over the entire length studied. Changes in the results obtained are highlighted at pressures above 150 mmHg, where, at a distance greater than 7 cm from the connector port, there are small deviations of the recorded pressure from the set one, but, as the study authors note, they are insignificant.

Wackenfors *et al.* and Saxena *et al.* showed, through animal experiments, the existence of areas of hypo- and hyperperfusion, in contact with the sponge with the wound. They observed hypoperfused areas and areas of ischemic tissue in the immediate vicinity of the wound, areas that had depths of about 2 cm in depth, which were directly related to the negative pressure set in the treatment of wounds. The increase in pressure causes the depth of the ischemic affected area to increase in depth (3, 4, 5). At that time, it was not possible to issue a uniform hypothesis from these studies, regarding the conditions of pressure and suction that lead to these changes, one of the reasons that prevent the issuance of such hypotheses being the limitations of the studies.

In the literature, there are postulated several theories on the action of vacuum on wounds in such systems applied in medical practice, based on observations made in the treatment of wounds, some of them being contradictory. The ischemia caused in these cases may be due to high negative pressures used in therapy.

Overall, there are uneven variations in the pressure of the liquid fluid inside the model, due to the complicated capillary system existing in the porous environment. The uneven differences in the velocity and pressure of the liquid fluid, the unevenness and anisotropy of the medium, as well as the deformability, elasticity, porosity of the sponge and the small wall thickness between two pores, make it work differently in the whole volume.

Based on the studies of Isago *et al.* and Maier *et al*, it can be suggested that the variation of the fluid pressure at the wound-sponge interface favors the appearance of ischemia areas, an aspect encountered in the group of patients introduced in the study (1, 6).

CONCLUSIONS

The elasto-plastic behavior of the porous sponge material during the operation of the kit allows the appearance of a pressure gradient inside the sponge accentuated appearance by increasing the pressure applied to the end of the tube. Thus, the increase of the pressure applied at the end of

the tube (from 13333 Pa = 100 mmHg to 19999 Pa = 150 mmHg) determines the increase by over 32% of the pressure of the liquid fluid at the interface between the sponge and the wound.

The existence of the pressure gradient inside the porous sponge determines the change of the sponge absorption rate and, implicitly, the favoring of the appearance of ischemia areas.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

EARLY CRITICAL RECURRENCE OF CARDIAC MYXOMA DEGENERATED INTO RHABDOMYOSARCOMA

Is presented the 50-year-old lady case, who underwent surgical resection of an intracardiac tumor occupying the left atrial cavity, which was histopathologicaly diagnosed as cardiac myxoma with moderate atypia. Monthly echocardiographic examinations demonstrated that there was no evidence of recurrent tumors during the first 5 months after operation, and the patient remained asymptomatic for further 3 months. However, she was sent to other emergency medical institution for the treatment of her sudden cardiogenic shock. It was informed that a redo operation for a recurrent intracardiac tumor was performed in an emergency basis and the second histopathological diagnosis was rhabdomyosarcoma. The present case implies that a follow-up schedule should be determined according to an individual basis, especially in a case suspicious of malignant pathology. In addition, other diagnostic methods independent of echocardiography would be expected for easier screening of possible recurrence (Hayashi Y, Yokoyama Y. Early critical recurrence of cardiac myxoma degenerated into rhabdomyosarcoma. *Chirurgia* 2021; 34(1): 55-57).

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THE IMPACT OF COVID-19 LOCKDOWN ON THE LEVEL AND INTENSITY OF PHYSICAL ACTIVITY: A STATISTICAL ANALYSIS ON ROMANIAN YOUNG ADULTS

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THE IMPACT OF COVID-19 LOCKDOWN ON THE LEVEL AND INTENSITY OF PHYSICAL ACTIVITY: A STATISTICAL ANALYSIS ON ROMANIAN YOUNG ADULTS (Abstract): COVID-19 pandemic is affecting the lifestyle of billions of citizens and within this context, the measures of social distancing are prone to bring worrying shifts in physical activity habits. The present research aims to evaluate the changes in sport and physical activity among Romanian young adults. **Material and methods**: The sample includes 417 respondents (most of the interviewees being around 20 years of age) which filled the questionnaire between 5 to 11 March 2021 through an internet application. Our analysis evaluated the frequency and duration of physical activity and how it changed during the lockdown due to COVID-19 pandemic. **Results**: The results show that most of the respondents no longer perform any other physical activity except for walking, within the three studied time frames, with a slight increase during the state of emergency. There are no significant differences between sex and area of residency. The regression model revealed that a higher variation of the average frequency of physical activity after the state of emergency is explained by the model. **Conclusions**: Our study revealed that the average frequencies of physical activities before, during, and after the state of emergency are moderately correlated. **Keywords:** COVID-19, LOCKDOWN, PHYSICAL ACTIVITY, ROMANIA, YOUNG ADULTS.

Sudden introduction of physical distancing measures together with severe restrictions imposed to recreational areas lead to a radical lifestyle change of many individuals (1). The effects of such measures included increased anxiety, stress, and depression (2).

Exercising helps people maintain a positive emotional status as well as a bio clinical equilibrium of different comorbidities - diabetes, hypertension, cardiovascular diseases, and respiratory diseases (3, 4). Moreover, regular exercising increases emotional resilience to stress through the decrease of the heart rate and free cortisol (5). On the other hand, sedentary lifestyle and physical inactivity increases chronic disease risks and mortality risk associated with SARS-CoV 2 infection (6, 7).

The Centre for Disease Control points out various benefits of 30 minutes of moderately intense daily physical activity for all adults including a reduced risk of coronary heart disease, heart attack, diabetes mellitus,

colon cancer, hip fracture and high blood pressure (8, 9). Despite the benefits of physical activity, "only 1 in 5 adults and 1 in 5 high school students fully meet physical activity guidelines for aerobic and muscle strengthening activities" (10).

During the lockdown imposed due to Covid-19 rapid spread, the WHO recommended 150-300 minutes of moderate physical activity or 75 minutes of vigorous-intensity physical activity weekly (11). In a meta-analysis comprising 31 studies Bentlage *et al.* (2020) showed that physical inactivity due to lockdowns is a serious public health issue (12). Moreover, physical inactivity is a major factor in reducing life quality and life expectancy as it worsens pre-existing diseases of leads to new pathologies (13). Yan and Spaulding (2020) pointed out that regular exercising reduces the risk of severe acute respiratory distress, one of the main causes of death in SARS-COV 2 patients (14).

Countries all over the world implemented restrictions to address the COVID-19 pandemic. These restrictions resulted in a significant reduction in the intensity and amount of physical activity (15,16).

According to data from Eurostat (2014), in Romania, 71.8% of the adults from 18 to 29 years old registered 0 minutes of aerobic physical activity, the highest rate among the EU countries. Moreover, 67.1% of the adults aged 20 to 24 years old, 78.6% of the adults aged 25 to 29 years old and 81.5% of the adults aged 25 to 34 years old didn't perform any physical activity outside work. In 2018, according to the Special Eurobarometer on Sport and Physical Activity, 81% of the Romanian adults never or seldom exercise (17).

Schmidt *et al.* (2020) point out that sport activity of children and adolescents declined while recreational screen time increased, during the first (and strictest) measures of the COVID-19 lockdown in Germany. The study used self-reported data from children and adolescents between 4-17 years of age participating at two measurements points (before and during the first COVID-19 lockdown). The authors conclude that physical activity is highly context-driven (18). Research conducted by Wunsch *et al.* (2021) points out that there is a negative association between pre COVID-19 screen time and within COVID-19 physical activities. The authors advocate that a general reduction of screen time is recommended combined with an increase of the physical activity) in children and adolescents (19).

González-Valero *et al.* (2020) argue that physical activity is positively correlated to self-concept. Their research investigating physical, mental and emotional status in adolescents in relation with gender and type or amount of sport and physical activities points out a reduction in the overall self-concept for boys and girls during the COVID-19 pandemic. Additionally, academic self-concept for girls was higher during the lockdown (20). Bowden Davies *et al.* (2019), report that physical inactivity in healthy young adults is associated with cognitive decline and other deficiencies (21). Woods *et al.* (2020) state that circumstances pose significant challenges on the lifestyle during the COVID-19 pandemic (22). Encouraging people to engage in physical activities in a modern society generally affected by sedentarism, is urgent in the context of social distancing (23).

Given this special context, as well as the situation in Romania, the aim of this paper is to perform an analysis on the frequency and duration of physical activity

and how it changed during the lockdown due to COVID-19 pandemic in this country. More specifically, several research questions were formulated:

Q1: To what extent the frequency of physical activities after the state of emergency is correlated with the frequency of physical activities during and before the state of emergency?

Q2: What factors influence the frequency of physical activities after the state of emergency?

MATERIAL AND METHODS

Questionnaire design

In order to address the research questions, a questionnaire was distributed via social media between 5 March 2021 and 11 March 2021. It includes 16 questions grouped as following: 3 questions aimed at measuring the frequency of each selected physical activity before the state of emergency, during the state of emergency and after the state of emergency; 3 questions aimed at measuring the duration of physical activity before the state of emergency, during the state of emergency and after the state of emergency; 1 question aimed at measuring to what extent adults aged 18 to 35 enjoy physical activity; 1 question aimed at determining the most important factor that needs to be addressed in order to make physical activity part of the daily routine; 2 questions aimed at measuring the use of technology in tracking calories or physical activity; 5 demographic questions and one question aimed to determine whether the respondent worked from home or not. As this is not a standard questionnaire on physical activity level, one can find it in Annex 1.

Regarding the types of physical activities, we chose to collect data on walking, running, cycling, roller-skating and fitness/bodybuilding. The first four activities were chosen because scientific literature proposes various solutions cantered around them to increase physical activity levels for adults. For example, Collins and Fulton (2015) point out that walking is one of the simplest activities that can be easily added in adult's daily routine (24). Public bicycle sharing initiatives contributes to increase use of bicycles instead of cars and increases mobility in urban areas (25,26). Kardiyono *et al.* (2018) point out that roller-skating can be promoted as a recreational sport and socializing activity by the municipalities (27), as it has been acknowledged as an efficient cardiovascular activity (28). Fitness/bodybuilding was chosen because muscle training twice a week is essential for adults' mental and physical health (29), especially in women's case (30).

The extent adults enjoy physical activity might be an important motivational factor. Phoenix and Orr (2014) and Chelladurai (2011) argue that good mood during physical activities contribute significantly to initiating and maintaining a regular sports routine and might be the only motivator for some people to engage in physical activities (31, 32). Besides enjoying physical activity, scientific literature points out several other factors that might influence the extent adult exercise: appropriate health education (33), initiating national campaigns (34), study-work-free time balance (35). Therefore, our questionnaire accounted for these factors as possible predictors for incorporating physical activity into daily routine.

Regarding the use of technology, one should note that using technology to measure the level of physical activity and encourage people to do more exercise is an area which has been widely researched. For

example, Pal and Ho (2011) concluded that using pedometers along with establishing measurable objectives in terms of steps increases the level of physical activity (36). Moreover, several apps use gamification to encourage youths to exercise: BunnyBolt (37), Go Run Go (38), FitPaly Games (39), etc (37-39).

Sample size

According to the National Institute of Statistics there are 4821927 persons with age between 18-35 years in Romania in 2020. Based on this population, the sample size was computed for a 95% confidence level and a 5% confidence interval using the Sample size calculator proposed by Creative Research Systems (40), resulting in a required sample size of 384 persons. However, our sample size is higher, as 417 respondents completed the questionnaire.

Statistical methodology

In order to answer the first research question, questions 2, 3 and 4 of the survey were recoded as follows: points from 0 to 7 were assigned to each frequency (never=0, Less than once a week = 0.5, Once a week = 1, 2-3 times a week = 2.5, 4-6 times a week = 5, everyday = 7).

Next, three variables were computed as follows:

$$Average\ frequency\ of\ physical\ activities\ before\ the\ state\ of\ emergency = \frac{\sum_{i=1}^{11} Points_i}{11}$$

$Points_i = number\ of\ points\ per\ activity\ i$

i=1 for Walking outdoor, i=2 for Running outdoor, i=3 for Roller-skating outdoor,

i=4 for Cycling outdoor, i=5 for Other sports outdoor, i=6 for fitness or bodybuilding at a gym, i=7 for Cardio activities at a gym, i=8 for Other sports at a gym,

i=9 for Fitness/bodybuilding at home, i=10 for Cardio activities at home,

i=11 for Other sports at home

$$Average\ frequency\ of\ physical\ activities\ during\ the\ state\ of\ emergency = \frac{\sum_{i=1}^{8} Points_i}{8}$$

$$Average\ frequency\ of\ physical\ activities\ after\ the\ state\ of\ emergency = \frac{\sum_{i=1}^{8} Points_i}{8}$$

$Points_i = number\ of\ points\ per\ activity\ i$

i=1 for Walking outdoor, i=2 for Running outdoor, i=3 for Roller-skating outdoor,

i=4 for Cycling outdoor, i=5 for other sports outdoor, i=6 for Fitness/bodybuilding at home, i=7 for Cardio activities at home, i=8 for Other sports at home.

Next, the Pearson correlation coefficient and the significance test were computed between these three variables, for the overall database, by area of residency and by sex. The significance level was set to 1%.

In order to answer the second question several regression models were computed using questionnaire data with Average frequency of physical activities after the state of emergency as dependent variable. Independent variables were excluded if the coefficients were not significant.

RESULTS

Analyzing the responses of the questions at the end of the questionnaire, one can observe several characteristics of the respondents. Approximately 30% of the interviewees use an app for tracking calories while 44% use an app for tracking physical activity. Despite the benefits in terms of increasing the amount of physical activity of such apps, Lopez-Bueno *et al.* (2020) observed a slight reluctance in using such devices by the adult population (41). About 84% of the respondents worked or studied from home during the state of emergency and 60% of them still work or study from home full time at the moment of data collection. Additionally, 20% of the of the interviewees work or study from home part time at the moment of data collection.

With regard to the respondents' demographic characteristics, the answers show that approximately 32% of those participating in the study are males and 68% are females. 84% of the respondents live in urban areas and 16% live in rural areas. The distribution by age is skewed to the left, most of the interviewees being 20 years old. Regarding the employment status, 60% of the respondents are students, 26% of them are employees and 11% are both students and employees. According to the National Institute for Sports Research (2020) most of the Romanian adults that used to practice physical activity before the pandemic did not continue their routine during the pandemic (42).

Figure 1 displays the distribution of all the respondents by the frequency of performing specific physical activity before, during and after the state of emergency. As one can observe most of the respondents never perform any other physical activity except for walking. The situation is similar within the three studied time frames, with a slight increase during the state of emergency, when most of the respondents worked from home. Working/studying from home might be an important risk factor for inactivity and increased screen time (43). However, our results show that 54% of the respondents enjoy physical activities for recreational purposes. One possible explanation for this paradox might be the lack of time.

As one can observe from figure 2, 96% of all the respondents stated that maintaining work/study-free time balance is important or very important in order to integrate physical activity into the daily routine.

Before the pandemic, 92.7% of the permanent resident population aged 5 years old and over practiced walking at least 10 minutes/day for several purposes not only physical activity (44). According to the same source, 40.5% of the females aged 10 to 29 years old practiced regular walking, 5.8 pp higher than males. Our study showed that below 10% of the respondents practiced regular walking in 30 minutes daily sessions.

In 2019, 54.7% of the persons aged 18-35 years old practiced 180 minutes of regular walking (44). Our study showed that during the emergency state, 10% of the respondents walked 90 minutes per week, while after the emergency state, this value increased to 25%. Thus, one can observe that there a significant decrease in the share of persons that practiced walking after the emergency state as well as in the duration of such activities compared to the pre-pandemic year.

Considering cycling, roller-skating and other outdoor activities, data from the National Institute of Statistics (2021) showed

that 17.6 of the adults under 35 years old and teenagers practiced such activities on a weekly basis (44). Our study showed that 5.3% of the respondents went cycling, roller-skating or practiced other outdoor activities during the emergency state while 14% of them declared practicing after the state of emergency.

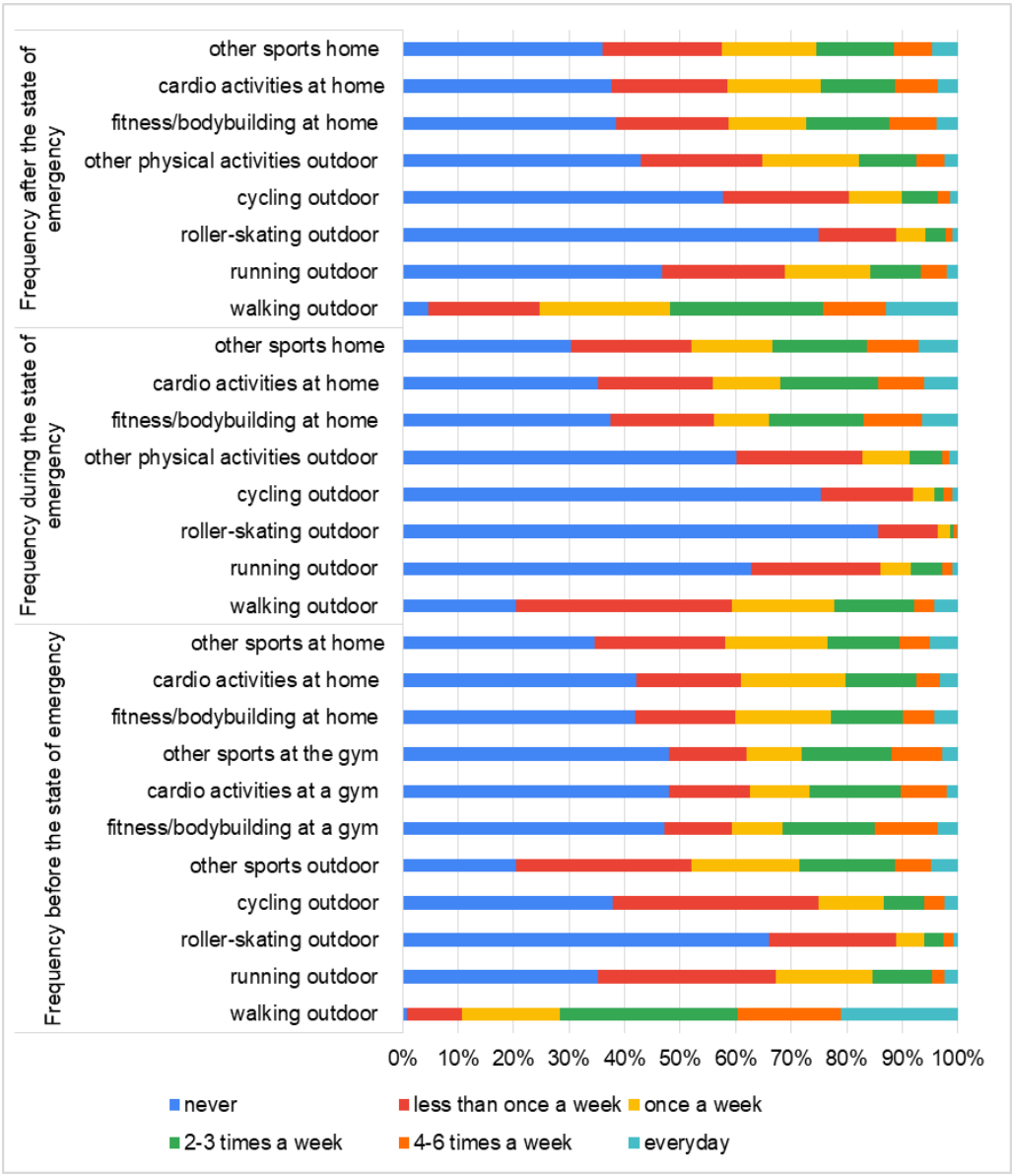

Fig. 1. The distribution of respondents by the frequency of performing specific physical activity before, during and after the state of emergency
(Source: designed by the authors based on questionnaire data)

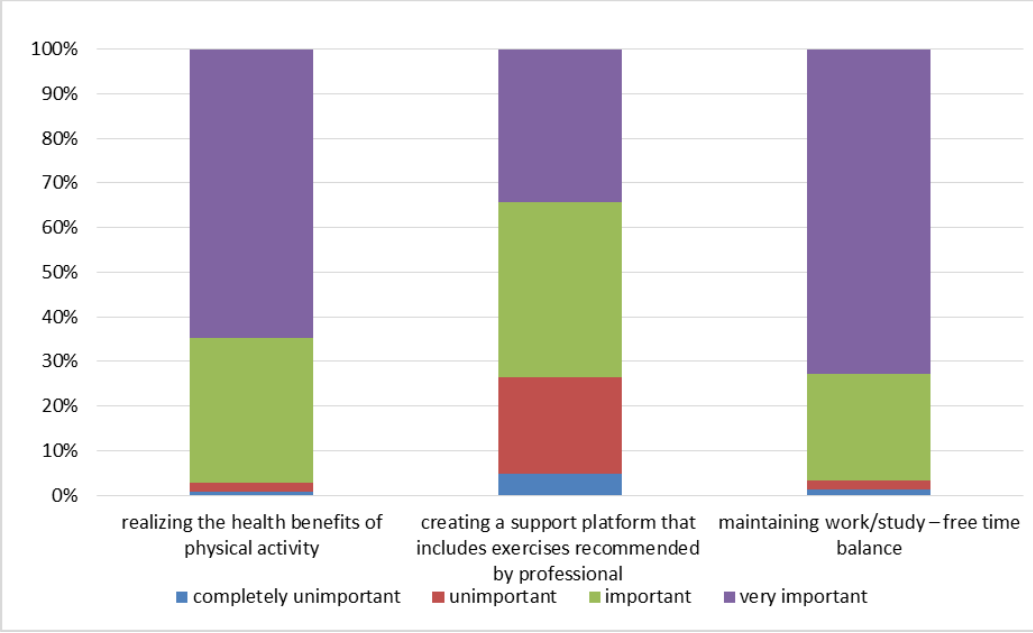

Fig. 2. The distribution of respondents by their attitude towards the factors that influence the process of integrating physical activity into the daily routine (Source: designed by the authors based on questionnaire data)

Also, realizing the health benefits of physical activity is important or very important for most of the respondents. As a consequence, authorities should actively promote the double advantage of staying active during lockdown, even if physical activities are mostly performed indoor: increased immune system and lower risk of non-communicable disease (34).

Table I presents the distribution of respondents by the duration of physical activities before, during and after the state of emergency. These activities include any kind of recreational physical activity. As one can observe, most respondents stated that the total amount of physical activity is at most 30 minutes daily. Our results are consistent with the results obtained by the National Institute for research in Sports (2020), that concluded most of the respondents exercised 60-90 minutes weekly (42).

Table II presents the correlation coefficients between the average frequencies of physical activities before, during and after the state of emergency. As one can observe there is a significant positive moderate correlation between the three variables. The results are similar when broken down by sex and area of residency.

The frequency of physical activity after the state of emergency is significantly influenced by the respondent's attitude towards such activities, the frequency of physical activity during the state of emergency and the frequency of physical activity before the state of emergency (tab. III). As the correlation coefficient between the last two variables is below 0.8, there is no risk for multicollinearity (45). More specif-

ically, 58% of the variation of the average frequency of physical activity after the state of emergency is explained by the model. All factors have a positive influence over the dependent variable, however the frequency of physical activity during the state of emergency has highest impact. The t-statistics is computed for each coefficient as the estimated coefficient divided by its standard deviation. The values for the t-statistics show that the coefficients for all variables are statistically significant.

TABLE I

The distribution of respondents by the duration of physical activities before, during and after the state of emergency

(Source: designed by the authors based on questionnaire data)

	Less than 10 minutes/day	10-30 minutes/day	30-60 minutes/day	More than 60 minutes/day
Before the state of emergency	14.39%	24.46%	36.45%	24.70%
During the state of emergency	28.78%	35.73%	26.62%	8.87%
After the state of emergency	18.23%	33.57%	32.61%	15.59%

TABLE II

The correlation coefficients between the average frequencies of physical activities before, during and after the state of emergency for the entire dataset, males, females, urban and rural residents respectively

(Source: designed by the authors based on questionnaire data)

	Average frequency of physical activities before the state of emergency	Average frequency of physical activities during the state of emergency	Average frequency of physical activities after the state of emergency
Average frequency of physical activities before the state of emergency	1	0.522** (total) 0.546** (females) 0.465** (males) 0.447**(rural) 0.534** (urban)	0.624** (total) 0.653 ** (females) 0.570 ** (males) 0.562** (rural) 0.647 (urban)
Average frequency of physical activities during the state of emergency	-	1	0.679** (total) 0.684** (females) 0.685** (males) 0.681**(rural) 0.694** (urban)
Average frequency of physical activities after the state of emergency	-	-	1

TABLE III

Coefficients and t-Statistics using the Average frequency of physical activities after the state of emergency as dependent variable

(Source: designed by the authors based on questionnaire data)

	Coefficient	t-statistics
Constant	-0.176	-1.481
Attitude towards physical activity	0.09*	2.557
Average frequency of physical activities before the state of emergency	0.374**	8.385
Average frequency of physical activities during the state of emergency	0.545**	12.448
*Significant at 10% level; ** Significant at 5% level		

DISCUSSION

Our analysis shows a significant change in physical activity among young adults in Romania. These findings are consistent with similar studies that examined related topics in surrounding countries. In general, the majority of the studies confirm that there is a concern on the changes in physical activity in healthy young adults as a result of social distancing imposed by the COVID-19 pandemic. Our findings are consistent with those of Barkley *et al.* (2020) who concluded that a well-established physical activity before the pandemic decreases the likelihood of a borderline sedentary life during the lockdown (46).

Recently, Sekulic *et al.* point out that the curve of physical activities among Croatian adolescents is driven by characteristics of the living communities, with a larger decrease in urban adolescents (47). Such aspect is in line with the research findings of Zenic *et al.* which point out that this decrease is mainly driven by the pandemic rules of social distancing as physical activity levels of Croatian teenagers rely on formal sport and organized recreational activities such as the ones in fitness centers or gyms (48).

In the light of such aspects, Guan *et al.* point out the importance of analyzing the situation of children from low income and middle-income countries during the COVID-19 pandemic, as per the known fact that a greater proportion of their population live in living-spaces that are exposed to overcrowding, making physical distancing challenging or virtually impossible (49).

One can observe a myriad of policies implemented by Governments to maintain some level of physical activity for the population. Governments have not always adopted measures in line with WHO recommendations for physical activity during this period. For example, in Belgium, people were actively encouraged to exercise at home and outdoor, in spite gym closures (50). Meanwhile, in Romania and Canada no such policies were issued (51).

Engaging in a daily exercise routine, socializing, offering support to people in difficult situations as well as using health apps are crucial in maintaining a good physical and mental health, therefore, physical activity at home is essential (52). A key element in ensuring an optimal level of physical activity is an efficient communication campaign through simple messages

(53). As our study showed that 90% of the respondents consider important or very important knowing the benefits of physical activities and 60% of them appreciate health digital platforms, promoting healthy physical activities should be focused on short and simple messages though dedicated pages.

Sport has proven to be an important element for development. The United Nations recognise the growing contribution of sport to the realization of the 2030 Sustainable Development Goals (SDGs) in resolution 70/1, entitled "Transforming our world: the 2030 Agenda for Sustainable Development". Physical activity and sport contribute to the general population well-being regardless of gender, age or ethnicity (54). The "Global action plan on physical activity 2018-2030" (55) pledge to introduced measures to promote and increase the global physical activity for a more active society. Regular physical activity should be integrated on a daily basis and can play a directly contribution to achieving many of the SDGs and promote United Nations values (55).

Considering that our study as most of the studies reported a decrease in physical activity and an increase in sedentarism, a public policy conducted by the World Health Organization and implemented in affected countries would be highly beneficial. Such policy should include online courses, walking, cycling and other outdoor activities. Moreover, considering the benefits of physical activity upon the positive evolution of patients infected with SARS-COV 2, targeted physical activity programs by comorbidities and supported by therapeutic options for the treatment of SARS-CoV2 infection (56) should be designed and implemented.

While this study examined a specific sample of the population, young adults (persons ages 18-35 years old), future research studies should be carried to evaluate the changes in sport and physical activities for other populations and groups of people. Literature suggests that social media can be further used to obtain statistical data (57, 58, 59). As countries develop economically, there is a rising concern of the sedentariness (55). With special attention focus on examining the changes in sport and physical activities beyond the COVID-19 pandemic and on sociodemographic factors driving these changes.

CONCLUSIONS

Our study analyzed to what extent the frequency of physical activities after the state of emergency is correlated with the frequency of physical activities during and before the state of emergency. Moreover, the factors that influence the frequency of physical activities after the state of emergency are identified. Our study revealed that the average frequencies of physical activities before, during and after the state of emergency are moderately correlated. Moreover, the average frequency of physical activities after the state of emergency is positively influenced by the attitude towards physical activity, the average frequency of physical activities before the state of emergency and the average frequency of physical activities during the state of emergency.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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60.

PREOPERATIVE PROGNOSIS OF COLON CANCER SURGERY: CAN THE RESECTABLE ARTERIAL LENGTH BE A VALID PREDICTOR OF LYMPH NODE COUNT?

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PREOPERATIVE PROGNOSIS OF COLON CANCER SURGERY: CAN THE RESECTABLE ARTERIAL LENGTH BE A VALID PREDICTOR OF LYMPH NODE COUNT? (Abstract) The number of harvested lymph nodes (LN) is an independent factor for colon cancer prognosis surgical quality. The vascular length from tumor margin to the central vascular ligature as measured pathologically is claimed to be prognostic factor and an indicator of surgical quality. We aimed to evaluate the anatomical variability of the colic artery from origin to the tumor margin (resectable arterial length - RAL) and to investigate its prognostic value by correlation with LN parameters. **Materials and methods**: We retrospectively included patients from the 2nd Clinic of Oncological Surgery, at the Regional Institute of Oncology, Iași, operated through complete mesocolic excision (CME), for colon cancer stages I-III, between 2017-2018, with available preoperative CT-scan. Ileocolic and inferior mesenteric artery RALs were measured on CT reconstructions. LN-parameters were extracted from electronic registries. Separately, on 50 normal CT-scans we measured RAL variation as generated by different tumor position on 6 standardized colon regions. **Results**: 36 colectomies were included, 25 right and 11 left hemicolectomies. Right colon RAL was mean (±SD) 98.9mm (±30.5) with a mean 39.7 (±12.1) LN count. Left colon RAL was 126.5mm (±29.0), with a mean 36.1 (±14.8) LN count. The *Pearson*-test showed no significant RAL-LN correlation (p=0.861). Large interindividual and intraindividual variation resulted from the 300 standardized RAL measurements, (mean±SD) 112.8mm ±42.1. **Conclusions**: Given the lack of correlation with a proven prognostic factor (LN), we cannot consider the RAL a reliable prognostic tool. Furthermore, its large anatomical variability makes it a difficult to use, unfeasible, surgical quality instrument. **Keywords:** COMPUTED TOMOGRAPHY COMPLETE MESOCOLON EXCISION, ARTERIAL LENGTH VARIABILITY, ILEOCOLIC ARTERY, INFERIOR MESENTERIC ARTERY, RESECTABLE ARTERIAL LENGTH, COLIC ARTERY TUMOR MARGIN LENGTH, SURGICAL QUALITY EVALUATION.

Complete mesocolic excision (CME) gradually becomes a new standard in the treatment of colon cancer and recently several indicators have been proposed to

evaluate the surgical quality (1, 2). Some studies propose the arterial length from the vascular tie to the tumor margin as a viable indicator for surgical quality of CME (2, 3, 4). Lymph node (LN) count and derived parameters are considered an independent prognostic factor in colon cancer (5, 6, 7, 8). Complete mesocolic excision (CME) with central vascular ligation (CVL) provides more LNs and a longer tumor to vascular tie length as measured on the pathological specimen, compared with conventional colectomies (2, 9, 10). However, CME is susceptible to surgical technique variability regarding the vascular ligature site that implies variable lymphadenectomy heights: D2 level (resection of the ileocolic artery up to the right side of the superior mesenteric vein or D3 level (at the origin of the ileocolic artery, on the lateral side of the superior mesenteric artery) as reported by various groups and national colorectal cancer societies (11, 12, 13).

Given that the mesocolic lymph nodes locate around the main vascular pedicle, some authors suggested that the pathological tumor to vascular tie length could also be a prognostic factor and derived from this that the length from vascular tie to tumor margin implemented its use in standardized pathological examination protocols evaluating the surgical quality of CME specimens (2, 14). However, this cannot accurately reflect the length of the arterial stump remaining in situ or its associated LNs, because the length of the main pedicle is variable and thus the arterial stump may be longer than the pathologist suggests (14, 15). Nevertheless, the vascular length could be a prognostic factor of the vascular length generated by patient anatomy and not by the quality of the surgical gesture.

We defined the resectable arterial length (RAL) as the distance from the D2 level of ligation to the nearest tumoral margin. We aimed to demonstrate the large intra-individual and inter-individual anatomical variability of the colic artery from origin to the tumor margin. Secondly, we investigated its prognostic value by correlation with LN parameters. Furthermore, we compare the CT measurement of RAL against the measurements on the photographed fresh specimen to validate the radiological measurement technique.

MATERIAL AND METHODS

We retrospectively investigated 2 groups of patients from the Regional Institute of Oncology (ROI), Iasi. For the 1st group we included patients from the 2nd Oncological Surgery Clinic, ROI, Iasi, operated by CME for stage I-III colon cancer, between 2017-2018, with a preoperative CT scan available. Images were extracted from the *"Patient Archiving and Communication System"* (PACS) of the hospital intranet. On the 2nd group we measured the inter-individual and intra-individual variability of the RAL on normal abdominal-pelvic CT scans.

CT measurement technique

Using Radiant DICOM Viewer (v.2020.2.3, Poznan, Poland) we identified and measured the length from the artery to the tumor margin as follows (fig. 1):

- the ileocolic artery (ICA) for those undergoing right hemicolectomy;
- the Inferior mesenteric artery (IMA) for those undergoing left hemicolectomy;
- full resectable arterial lengths (RAL) and theoretical D2 central vascular ligation corresponding lengths to tumor margin were measured.

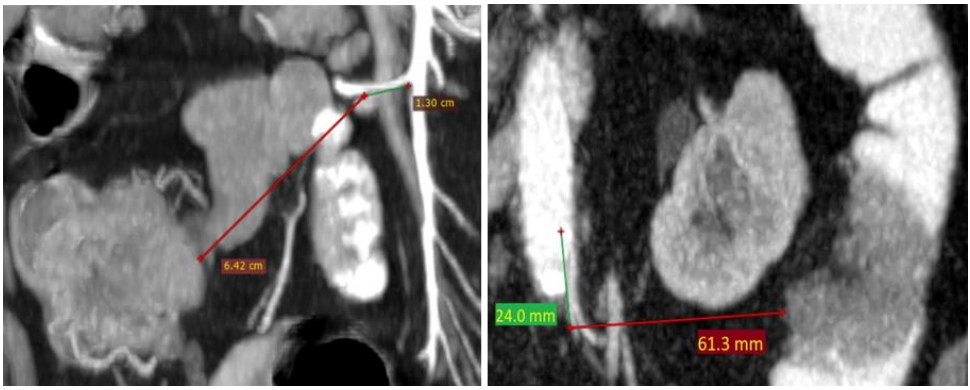

Fig. 1.A. Coronal CT view of resectable arterial length (RAL) measurement from the D2-theoretical central vascular ligation (D2-CVL) landmark to the tumor margin for colon cancer of the caecum. **B.** RAL from the D2 - CVL for descending colon.

We extracted pathological data regarding lymph node number and status from the integrated electronic system of our institution.

In order to validate the CT measurements, we performed a radio-pathological correlation of RAL on a subgroup of patients, by comparing the CT generated RAL with paired pathological measurements of resected arterial length up to the tumor margin, using fresh specimen photographies (figs. 2 and 3).

To investigate the anatomical variability of these arteries, we employed a retrospective study design on a 2nd group of patients which was investigated in order to account for the impact of the variation of the tumor's position on the colic frame upon the measurement of the resectable arterial length.

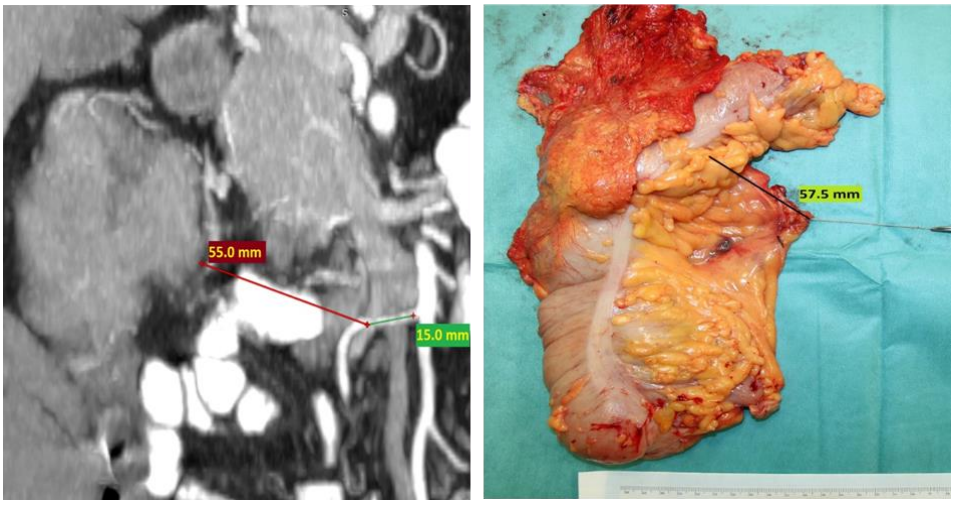

Fig. 2. Resectable arterial length correlation for colon cancer of the right flexure – image A - CT coronal reconstruction, compared with the measurement on the fresh specimen - image B.

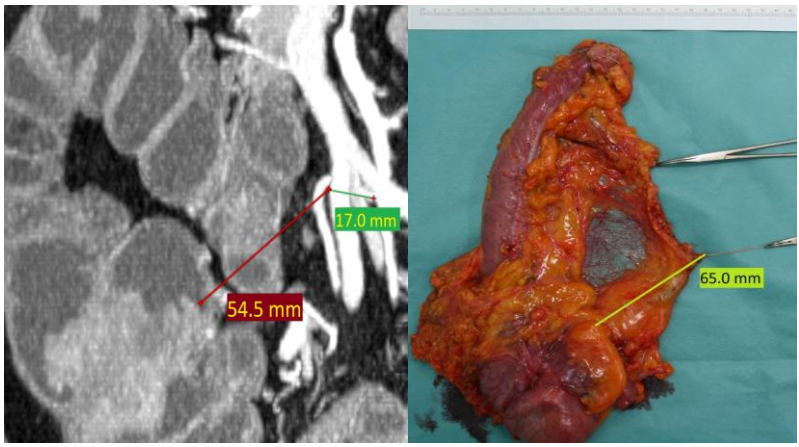

Fig. 3. The resectable arterial length correlation for colon cancer of the caecum; **A** - CT coronal reconstruction; **B** - fresh specimen

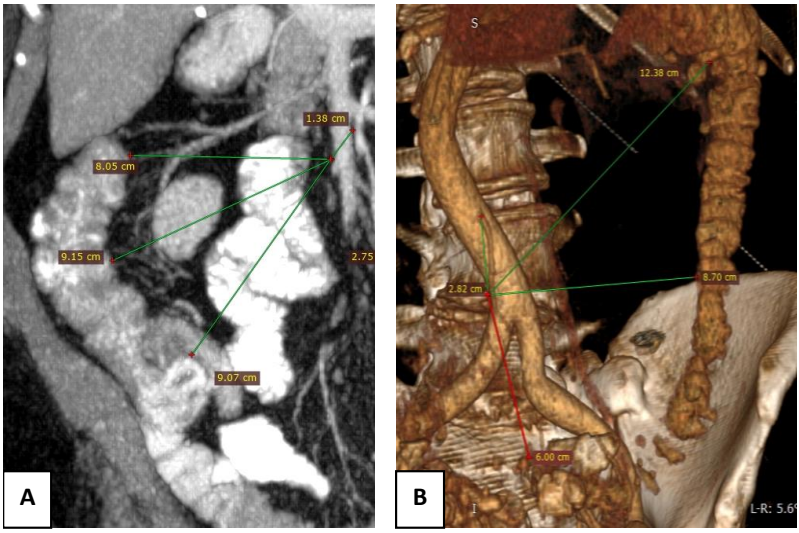

Fig. 4.A. Coronal CT reconstruction shows measurement of ileocolic resectable arterial length each standard point of a theoretical tumoral position on the right colic frame; **B.** 3D reconstruction CT shows measurement of the inferior mesenteric resectable arterial length to each standard point of a theoretical tumoral position on the left colic frame.

CT scan acquisition technique

The technical aspects of the imaging protocol for the patients scanned at the IRO was consistent with previous work published from our workgroup (16, 17). Patients had been scanned using a Bright Speed 16SL CT scanner with 16 detectors (GE Healthcare, Waukesha, WI, United States). The imaging station's protocol included a 16 mm, 1.25 mm increment, pitch value of 1.75, table speed of 35.0 mm per rotation, tube output of 120 kV and 260 mA. Arterial and venous phases were obtained using the automated bolus tracker

after injecting intravenously Iomeron 400® 816,5 mg/mL (Bracco Imaging, Milano, Italy) with an automated infusion pump at a rate of 2.50 mL/s. Data sets were reconstructed with a 1-3 mm section thickness and the reconstruction interval varied between 1 mm and 3 mm (idem). The CT scans had been originally ordered as for routine preoperative examination and not ordered for the purpose of this study.

Statistical analysis was conducted using the *IBM® SPSS® Statistics version 20.0* (IBM Corp) software. A p values lower than 0.05 was considered statistically significant.

RESULTS

The radio-pathological correlation of CT versus fresh specimen measurement of RAL was performed on 18 paired cases and the methods were similar with statistically significance: paired t-test showed a mean difference of 13.5 mm, p = 0.104.

After validating the CT measurement of RAL, we included 33 patients in our initial study group: 23 right colectomies and 10 left colectomies. The mean RAL (±SD) (range) for the ICA was 82.5 mm (±28.5) (20-127) and for the IMA 115.7 mm (±30.4) (70-170). The mean (± SD) LN number per entire specimen, was 39 (±12.6) for the right colectomies and 35 (±15.2) for the left colectomies. The *Pearson* correlation test between the RAL and LN count, did not reveal any statistical significance p = 0.822 for the whole group, neither when stratified for right (p = 0.372) and left colectomies (p = 0.183).

On the second study group we conducted 300 RAL measurements on 50 different patient computed topographies. Regardless of the hypothetical position of a tumor on the colic frame, the RAL vary significantly. Moreover, the RAL vary significantly within each of the 6 standardized points even if the level of arterial ligature is constant and the surgical gesture is standardized (figs. 5 and 6).

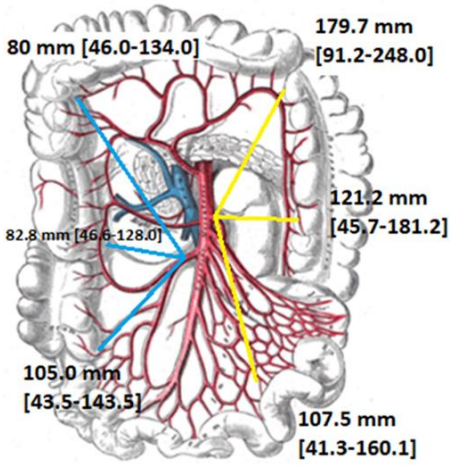

Fig. 5. Colon schematics with results of resectable arterial length to each of the 6 standardized points expressed as median mm (range).

The paired histograms below (fig. 7) show a statistically significant difference (p < 0.001) between the variances when *Levene's* test was performed between the ICA and IMA RAL compared to their respective D2-CVL lengths. This shows that although the length from the ICA origin to the right side of the superior mesenteric vein (*i.e.*, D2-CVL) is almost constant (15 mm ± 4 SD), whereas the D2CVL-right colon length varies greatly from 40 to 140 mm, mean 89.3, ±21.1 SD. Similarly, when comparing the left colon parameters, the length from the origin of the IMA to the emergence of the left colic artery (*i.e.*, D2-CVL) is almost constant (mean 40 mm ± 0.9 SD), whereas the D2CVL-left colon varies significantly (136 mm ±44.8 SD).

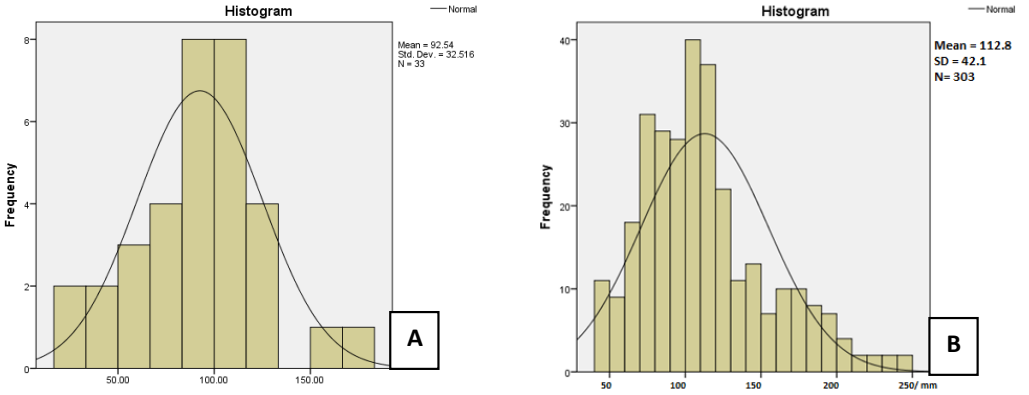

Fig. 6. A. Resectable arterial length from predicted D2-CVL to tumor margin – all 33 measurements showing very wide standard deviation ±32.5; **B.** Resectable arterial length from predicted D2-CVL to standard positions on colon – all 300 measurements showing very wide standard variation ±42.1.

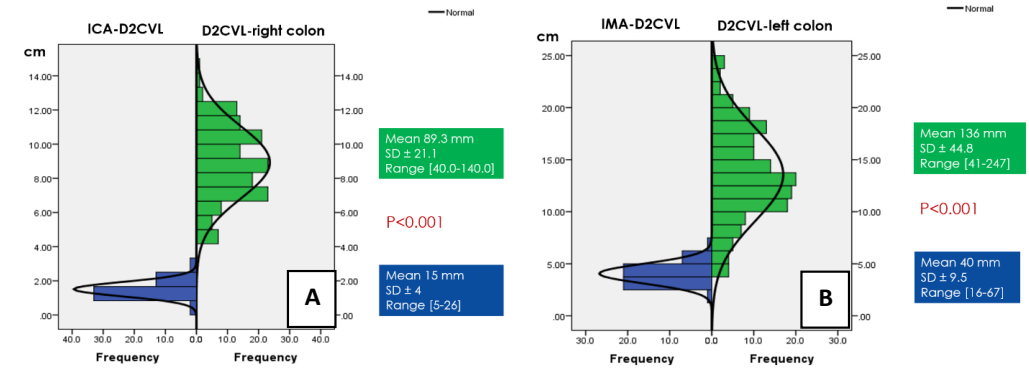

Fig. 7. Paired histograms showing the Levene's test for homogeneity of variances comparing the length from arterial origin to D2-CVL with the length from the D2 central vascular ligation to standard points on right colon (**A)** and left colon **(B)**.

DISCUSSION

In our study, the RAL varied significantly with regard to patient anatomy and tumor location and showed no correlation with the LN count. Nevertheless, it would be logical to consider that a longer colic arterial axis should be of important prognostic in some patients. A longer artery could represent a longer distance available for periarterial lymphatic drainage into the apical nodes. This could imply more ganglionic stations and would require more time for a sequential lymph node dissemination of cancer cells. However, this would be derived by local anatomy and not by surgical quality. This is also argued by several studies that have shown that that transverse colon cancer disseminates more frequently to the central LNs and extra mesocolic LNs and generally carries a worse prognosis (18).

As far as we know Solon *et al*. are the only ones to have had a similar study design. They reached the same conclusion as

us, stating that preoperative radiological calculation of pedicle length does not help predict the number of lymph nodes retrieved (14).

The mean RAL values for the ICA and the IMA in our study illustrated a high variation expressed as an increased standard deviation and a wide interval range both of which stand against a feasible indicator for surgical quality. The arterial length to tumor margin varies greatly dependent on patient anatomy (range 20-170 mm) and the pathologist cannot quantify the residual mesocolic/ arterial tissue remained in situ only by evaluating the pathology specimen.

Regarding limitations, a cohort of 50 patients is representative for the 2nd group, but larger cohort would strengthen the radio-pathological correlation of the 1st group. Only the ileocolic artery was chosen as representative for right colon cancer because of its anatomical constancy; the right colic artery has many anatomical variants and was excluded (19, 20).

Even after removing the effect of tumor location variation on the resulted length, the standard resectable arterial length to each of the 6 predetermined points on the colic frame is itself of significant variance, intrinsic to patient anatomy and hence it should not be used to discriminate between surgical specimens due to this intrinsic high variation.

Interestingly, the arterial length from origin to the vascular tie (i.e., arterial stump length) is significantly less variable compared with the entire RAL for all 6 hypothetical tumor positions on the colic frame (*Levene's* test, $p < 0.001$). Since the length of the arterial stump was proven to be the most constant vascular parameter after standardized surgery, our workgroup has recently published a validated postoperative quality assessment score that can select patients with high risk for locoregional recurrence in colon cancer based on the of colic the arterial stump length (17).

CONCLUSIONS

The resectable arterial length to tumor margin cannot be used as tool for surgical quality evaluation due to large variances.

Given the inconclusive correlation with a proven prognostic factor (LNs), we cannot consider the radiological resectable arterial length as a reliable prognostic tool.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest to disclose, and they received no specific funding regarding this scientific research.

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THE INFLUENCE OF THE OSTEOSYNTHESIS METHODS ON THERAPEUTIC MANAGEMENT OF THE PATIENTS WITH TIBIAL PLATEAU FRACTURES

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THE INFLUENCE OF THE OSSEOINTEGRATION METHODS ON THERAPEUTIC MANAGEMENT OF THE PATIENTS WITH TIBIAL PLATEAU FRACTURES - A RETROSPECTIVE STUDY (Abstract): **Aim:** The aim of study was to assess the influence of the osteosynthesis methods on the therapeutic management of the patients with tibial plateau fractures. **Material and methods:** The retrospective study was conducted on 63 patients (mean age 59.5 years; 37 males, 26 females) with tibial plateau fractures treated in Orthopedic Clinic - Traumatology Clinic Aschaffenburg-Alzenau (Germany) in the period 2015-2016. The mean values of pre-operatory interval, hospitalization time, surgical procedure duration, ablation time were calculated for each osteosynthesis method. Mann-Whitney tests were used for statistical comparisons between the implants systems in relation to the assessed parameters. Statistical significance was set up for $p<0.05$. **Results:** The pre-operative time was the highest for patients treated with rotating hinge (5.1 days), followed by plates + screws + Kirschner wires (4.0 days). The length of hospitalization was the highest for patients treated with rotating hinge (18.8 days), followed by plates (12.88 days). The duration of the surgical intervention was the highest for patients treated with plates + screws + Kirschner wires (95.0 minutes), followed by plates + screws (84.29 minutes). The ablation interval was the highest for patients treated with plates (12 days). Length of hospitalization was significantly higher ($p<0.05$) for patients treated with rotating hinge *vs.* alternative implants systems. **Conclusions:** It is very important to correlate the parameters related to the patients' management with implants and other relevant factors (systemic status, surgeon experience, soft tissues status, osteoporosis) to optimize the decision- making process and the long-term results of the treatment of the tibial plateau fractures. **Keywords:** TIBIAL PLATEAU FRACTURES, MANAGEMENT, IMPLANTS, OSTEOSYNTHESIS.

Tibial plateau fractures, accounting for 1-2% of the tibial fractures (1), and 1.7% of all human fractures (2), are found in a large range of lesions (OTA/AO classification). While teenagers and young adults has more frequently cominutive tibial plateau, unicondylar or bicondylar tibial plateau fractures are found most frequently in elderly people (2). The prognosis of these fractures is influenced mostly by the lesions' mechanism, soft tissues status, postoperatively complications, neurovascular

status of the involved area (3). Tibial plateau fractures are associated to a large range of lesions (OTA/AO classification), which require various surgical approaches (4). The main therapeutic goals in the treatment of tibial plateau fractures are the restoration of the articular stability, the axial and rotational alignment of the limb as well as the stability and early motion of the joint (5). The decision whether to operate or not on a specific fracture is based on the fracture morphology, the soft tissues, the patient's status, and the expected limb axis and articular surface restoration. Some of the conditions required for surgical management are as follows: intra-articular displacement over 2 mm; metaphyseal-diaphyseal translation over 1 cm; angular deformity of > 10° in the varus-valgus or sagittal plane; open fracture (6). Open Reduction and Internal Fixation (ORIF) concept is the gold-standard in complete/complex fractures (AO/OTA 41 C1, C2, C3/ Schatzker IV, V, VI) while a staged approach is recommended to prevent soft-tissues complications (6). Orthopedic surgeons have at their disposal a large range of implants and osteosynthesis methods. The selection of the proper surgical approach to optimize the therapeutic management of patients affected by tibial plateau fractures has raised interest along with the innovative improvement of the implants systems and osteosynthesis methods.

In this context, we aimed to assess the influence of the implants systems on the management of the patients with tibial plateau fractures.

MATERIAL AND METHODS

The retrospective study included patients with tibial plateau fractures who were treated at the Aschaffenburg-Alzenau Clinic, Germany, between 2015 to 2016. A total of 63 patients aged 20-99 years (mean age 59.5 years) who met the inclusion/exclusion criteria were assessed. Inclusion criteria: patients with surgically treated tibial plateau fractures (medial and / or lateral or posterior plates; Kirschner wires; screws; rotating hinge implant system). Exclusion criteria: age < 18 years; patients who attended the Emergency Unit of the Aschaffenburg-Alzenau Clinic, Germany but refused surgical treatment opting for functional treatment. We investigated the influence of the implants/osteosynthesis methods on the therapeutic management (pre-operatory interval, hospitalization time, duration of surgical intervention, ablation time) of the tibial plateau fractures. The definitions of the assessed parameters were as follows: pre-operatory time (days) - time between patient' admission and day of surgery; length of hospitalization time (days) - time between patient' admission and day when patient leaved the hospital; duration of surgical intervention (minutes)- time between the beginning and the end of the surgical intervention; ablation interval (days)- time between osteosynthesis and the moment when implant was taken out. Considering OTA/AO classification, the tibial plateau fractures had the following distribution: 7.9% fractures class A, 58.7% fractures class B (B1.1 22.2%, B3.1 15.9%, B1.2 11.1%), 33.3% fractures class C. The distribution of the implants systems and osteosynthesis methods used in the therapy of the tibial plateau fractures was as follows:

- Implants: plates (P) (57.13%), plates + additional screws (22.22%), plates + screws + Kirschner wires (4.76%), rotating hinge (15.9%);

• Osteosynthesis methods: multiplates (2.56%), external plate + medial plate (23.06%), external plate (30.76%), external plate + Kirschner wires (7.69%), medial plate (30.76%), posterior plate (5.12%).

Statistical analysis was performed using *Microsoft Office Excel 2013* and *SPSS 19.0* for calculation of mean values and standard deviation (SD). Mann-Whitney tests were used to compare the implant system regarding the mean values of the assessed therapeutic management parameters (statistical significance, $p<0.05$).

RESULTS

Tables I shows the mean values of the assessed parameters (preoperatory time; length of hospitalization; duration of surgical intervention; ablation interval) and the results of the statistical comparisons between the implants systems.

The Mann-Whitney tests applied to the parameters for all implants systems found a single significant statistical difference: length of hospitalization was significantly higher for patients treated by rotating hinge implants *versus* alternative implants systems. It was not found any significant statistical difference between osteosynthesis methods regarding pre-operative time, duration of surgical intervention, or ablation interval.

TABLE I

Mean values and statistical comparisons between implants systems

	YES		NO		p-Value§
	n	Mean ± SD	n	Mean ± SD	
***Rotating hinge vs.* alternative implants**					
Pre-operative time (days)	10	5.1 ± 4.433	53	3.72 ± 3.022	.513
Length of hospitalization (days)	10	18.8 ± 7.33	53	11.49 ± 7.531	**.002***
Duration of surgical intervention (minutes)	10	76.3 ± 17.77	53	80.91 ± 43.907	.504
Ablation interval (months)	1	7	23	10.48 ± 3.315	.417
***Plates vs.* alternative implants**					
Pre-operative time (days)	8	3.38 ± 3.159	55	4.02 ± 3.319	.625
Length of hospitalization (days)	8	12.88 ± 5.33	55	12.62 ± 8.259	.514
Duration of surgical intervention(minutes)	8	75.5 ± 38.199	55	80.85 ± 41.467	.804
Ablation interval (months)	2	12 ± 4.243	22	10.18 ± 3.304	.522
***Plates + additional Screws vs.* alternatives implants**					
Pre-operative time (days)	28	3.64 ± 2.934	35	4.17 ± 3.56	.717
Length of hospitalization (days)	28	11.43 ± 7.136	35	13.63 ± 8.458	.261
Duration of surgical intervention (minutes)	28	84.29 ± 47.679	35	76.89 ± 34.743	.890
Ablation interval (months)	12	10.17 ± 3.614	12	10.5 ± 3.148	1.000
Plates + additional Screws + Kirschner wires *vs.* alternative implants					
Pre-operative time (days)	3	4 ± 1	60	3.93 ± 3.359	.591
Length of hospitalization (days)	3	10.67 ± 3.215	60	12.75 ± 8.077	.915
Duration of surgical intervention (minutes)	3	95 ± 47.032	60	79.43 ± 40.787	.393
Ablation interval (months)	2	10 ± 2.828	22	10.36 ± 3.416	.797
§Mann-Whitney U test					

Table II shows the mean values for each parameter in relation to the implants category. The pre-operative time was higher for patients treated with rotating hinge (5.1

days), followed by plates + screws + Kirschner wires (4.0 days), plates + screws (3.64 days), and plates (3.38 days). The length of hospitalization was higher for patients treated with rotating hinge (18.8 days), followed by plates (12.88 days), plates + additional screws (11.43 days), and plates + additional screws + Kirschner wires (10.67 days). The duration of surgical intervention was higher for patients treated with plates + additional screws + Kirschner wires (95.0 minutes), followed by plates + additional screws (84.29 minutes), rotating hinge (76.3 minutes), and plates (75.5 minutes). The ablation interval was higher for patients treated with plates (12 days), followed by plates + additional screws (10.17 days), plates + additional screws + Kirschner wire (10 days), and plates + additional screws + Kirschner wires (7 days).

TABLE II

Mean values of the surgical management parameters related to implant category

Parameters	Implants category			
	Rotating hinge	Plates	Plates + additional screws	Plates + additional screws + Kirschner wires
Pre-operative time (days)	5.10	3.38	3.640	4.00
Length of hospitalization (days)	18.8	12.88	11.43	10.67
Duration of surgical intervention(minutes)	76.3	75.50	84.29	95.0
Ablation interval (months)	7.00	12.00	10.17	10.0

DISCUSSION

We aimed to gain an insight about how the implants category can change therapeutic management parameters (preoperatory time; duration of the surgical intervention; length of hospitalization; time from surgery to ablation of implant) of the patients with tibial plateau fractures. The most common categories of tibial plateau fractures in the group of patients investigated in our study were type B (58.7%); the most frequent type B fractures were B1.1 (22.2%), B3.1 (15.9%) and B1.2 (11.1%).

The proper selection of the implants and osteosynthesis method has a major importance in the treatment success of the tibial plateau fractures. In our study, the most frequent implants used in the treatment of the tibial plateau fractures were plates (57.13%), followed by plates + additional screws (22.22%), rotating hinge (15.9%), and plates + screws + Kirschner wires (4.76%). Different implants categories can reduce or lengthen the optimal values of these parameters as reported by literature data. For example, different preoperative time (time between injury and surgical intervention) of tibial plateau fractures are associated with different incidences of complications (skin ischemic necrosis, superficial infections, deep infections) while the optimal moment for surgical intervention for patients with closed complex tibial plateau fractures is between 5-8 days (7), and 6-8 days (8) post-injury. It was reported 92.5 ± 14.5 minutes (83-107 minutes) average duration of the surgical intervention when tibial plateau fractures were treated with proximal tibia locking compression plate (9). In our study, we noticed different mean values between the assessed parameters evaluated for various

categories of implants systems, but without significant statistical differences. The only exception was the length of hospitalization in patients treated with rotating hinge, significantly higher ($p<0.05$) when compared with the other alternative implants. The highest length of hospitalization was found in patients treated with rotating hinge (18.8 days), followed by plates (12.88 days). The highest pre-operative time was found in patients treated with rotating hinge (5.1 days), followed by plates + screws additional + Kirschner wires (4.0 days). The highest duration of the surgical intervention was found for patients treated with plates + additional screws + Kirschner wires (95.0 minutes), followed by plates + additional screws (84.29 minutes).

Considering the importance of the treatment of the complete/complex tibial plateau fractures for the post-operative life quality, the selection of the implants and osteosynthesis methods in ORIF concept depends on OTA/AO classification, location of fractures and various risk factors for the success of the surgical treatment. In posteromedial and posterolateral fractures anatomical reduction and stable fixation allow the prevention of the secondary malreductions. Many studies report the frequent use of the combination plates and screws due to their volume and easy-to-use features. Plates with 3.5 mm diameter and screws with diameter less than 3.5 mm can support bone fragments with reduced surfaces and allows the placement of screws closer to the joint surface. Sun *et al* (2017, 2018) support the use of multi-hole plates associated to parallel placement of screws near to joint surface to minimize the post-operative failure rate (10, 11). However, locking lateral proximal tibial plates (LISS), a proper posteromedial fragment fixation cannot be achieved by screw direction (12, 13). ORIF is recommended also in anterolateral or medial approach depending on the fracture type. In most cases, the use of medial plate for coronal fracture cannot provide fragment stability and the posterolateral and posteromedial fragments need to be buttress-plated and/or fixed from posterior to anterior. In posterolateral approach are preferred plates and screws that function as a non-slip tool allowing the resistance to shear forces; the screw must be placed near to the apex of fracture to ensure a fixed position of the plate (4). In bicondylar fractures are recommended the side plates and locking screws to withstand the axial, rotational and bending forces (4). Lateral platform fractures involving posterolateral column are approached by locking plate fixation (lateral + posterolateral) or locking plate fixation + 1/4 tubular plate edge fixation, Kirschner wire tension band fixation, and hollow nail fixation (for upper tibiofibular joint) (14). A research group reported high success rate for extended anterolateral approach with lateral tibial locking compression plate fixation for patients with Schatzker type II or extended anterolateral combined with a medial approach and lateral tibial locking compression plate + medial locking compression plate fixation for patients with Schatzker type V or VI (15). As proper fixation of the posterolateral and/or posteromedial fragments is difficult to achieve through conventional anterolateral and medial approaches, the three-column concept was introduced in the treatment strategy (16). A more recent therapeutic approach that decreases the rate of mechanical complications and loss of reduction is based on polyaxially variable-axis locking plate fixation devices (17, 18, 19).

CONCLUSIONS

It is very important to correlate the parameters related to the patients' management with implants and other relevant factors (systemic status, surgeon experience, soft tissues status, osteoporosis) to optimize the decision- making process and the long-term results of the treatment of the tibial plateau fractures. Integrated orthogeriatric treatment should be the standard approach to the multidimensional needs of senior patients with tibial plateau fracture. That is why it is necessary, for senior patients with tibial plateau fracture, who have associated osteoporosis, to use a rotating hinge prosthesis to be used as the first option of treatment, to regain and improve the quality of life.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

EFFECTS OF PROBIOTIC NVP-1704 ON MENTAL HEALTH AND SLEEP IN HEALTHY ADULTS: AN 8-WEEK RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

A direct link was shown between the gut microbiota and mental health via the gut-brain-microbiota (GBM) axis. The perturbation of this axis is involved in altered stress response and behavior and is believed to cause various psychiatric disorders, such as anxiety, depression and insomnia. The probiotic NVP-1704 consists of *Lactobacillus reuteri* NK33 and *Bifidobacterium adolescentis* NK98. Several studies have shown that NVP-1704 has a favorable effect on anxiety and depression in mice, therefore the aim of the study was to verify the outcome of administrating it to healthy adults. The study took place at the Seoul National University Bundang Hospital. The study group consisted of healthy adults in the group age 19 to 65 who have shown psychological stress and subclinical symptoms of depression or anxiety. The experimental group received 500 mg capsules of NVP-1704, which contained 2.5×10^9 colony-forming units (CFU) of microorganisms – 2.0×10^9 CFU of *Lactobacillus reuteri* NK33 and 0.5×10^9 CFU of *Bifidobacterium adolescentis* NK98, while de control group was given a placebo capsule, made of maltodextrin, created to look identical to the NVP-1704 capsule. Several questionnaires were applied throughout the study, regarding symptoms of stress, depression, and anxiety, as well as sleep quality and symptoms of insomnia: Stress Response Inventory (SRI), BDI-II (Beck Depression Inventory), BAI (Beck's Anxiety Inventory), Pittsburgh Sleep Quality Index (PSQI) and Insomnia Severity Index (ISI). A total of four hospital visits were necessary: the first one two weeks prior to administrating the capsule, the second one when the patients were divided into two groups, the experimental group and the control group and were instructed to take two capsules once a day, daily, for eight weeks, the third one after 4 weeks and the last one after 8 weeks. Participants taking NVP-1704 have shown a significant decrease in the BDI-II score compared to those on placebo, at both the third and the fourth visit. There was also a decrease in the BAI score in the experimental group in comparison to the control group at the third visit. The SRI score dropped significantly at the third visit and further reduction was observed at the final visit. Overall, the experimental group was shown to have noteworthy improvements in overall depression and anxiety symptoms, at both the third and last visit (Lee HJ, Hong JK, Kim JK, *et al.* Effects of Probiotic NVP-1704 on Mental Health and Sleep in Healthy Adults: An 8-Week Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients* 2021; 13(8): 2660).

THE EFFECT OF BILE DECOMPRESSION ON PRO- AND ANTIOXIDANT MARKERS IN THE COMPLICATIONS OF GALLSTONES ASSOCIATED WITH CHOLESTATIC JAUNDICE

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THE EFFECT OF BILE DECOMPRESSION ON PRO- AND ANTIOXIDANT MARKERS IN THE COMPLICATIONS OF GALLSTONES ASSOCIATED WITH CHOLESTATIC JAUNDICE (Abstract)**:** Argumentation of optimal terms for a staged surgical approach in the benign complications of gallstones associated with cholestatic jaundice by assessing the effect of biliary decompression on pro- and antioxidant markers. **Material and methods:** Prospective study, selected 105 patients with complicated gallstones and associated cholestatic jaundice, divided into three homogeneous subgroups, dependent on evolutionary complication. Control group - 35 patients with uncomplicated gallstones. All patients in the study group followed the staged treatment protocol in obstructive jaundice, with the primary prerogative - internal biliary decompression by endoscopic papillosphincterotomy. Pro- and antioxidant markers were assessed at hospitalization, on the 1st and 4th day after decompression. **Results:** Malonic dialdehyde at hospitalization marked in all subgroups with statistically significant results compared to the control group: I - 12.11±0.64 mol/l [$p<0.001$ ($t=10.7$)], II - 11.12±0.53mol/l [$p<0.001$ ($t=10.9$)]; III - 13.16±0.44 mol/l [$p<0.001$ ($t=16.5$)]. The activity of the antioxidant system evaluated by superoxide dismutase values [$p<0.001$($t=6.4$); ($t=4.1$); ($t=5.7$)] and catalase [$p<0.001$($t=6.7$); ($t=5.6$); ($t=7.6$)] shows a decrease in all subgroups compared to the control group. On the 1st day after drainage, malonic dialdehyde values did not show statistical significance compared to the before decompression state [I - $p>0.05$ ($t=0.11$); II - $p>0.05$ ($t=0.91$)]. An obvious reduction of its activity was found on the 4th after decompression day [I - $p<0.001$ ($t=5.15$; II - $p<0.01$ ($t=2.6$); III - $p<0.001$ ($t=3.99$)]. The return of the antioxidant system in all subgroups to values relatively similar to the control group: superoxide dismutase [I - $p<0.05$ ($t=2.21$); II - $p<0.05$ ($t=0.0008$); III - $p<0.05$ ($t=0.33$)] and catalase [I - $p<0.05$ ($t=2.39$); II - $p<0.05$ ($t=3.52$); III - $p<0.01$ ($t=2.87$)] was found more than 4 days after decompression. **Conclusions:** The study demonstrated the positive influence of biliary decompression in the complications of gallstones associated with cholestatic jaundice for the reduction of a massive lipid oxidation and the creation of conditions for increasing the antioxidant capacity with the restoration of the body's biological balance, which is more pronounced 4-5 days after decompression, serving as criterion for the possibility of definitive resolution of gallstones in these terms. **Keywords:** COMPLICATIONS OF GALLSTONES, CHOLESTATIC JAUNDICE, OXIDATIVE STRESS, ANTIOXIDANT SYSTEM.

From the range of surgical pathologies of the organs of the hepato-biliopancreatic region, the most difficult to approach are the diseases associated with mechanical jaundice syndrome, being accompanied by the partial or total stoppage of the bile passage. Biliary lithiasis is one of the pathologies located in this area, with an incidence of 10-15% in the adult population, which frequently evolves with complications associated with cholestatic jaundice (1). Despite the improvement of the population's access to medical assistance, the emergence of new non-invasive and informative methods in the diagnosis of gallstones, the rate of complications associated with cholestatic cholemia in pathology remains high-10-20% cases (1, 2). Studies show choledo-cholithiasis as the main cause of jaundice, but acute biliary pancreatitis, acute cholecystitis, cholangitis, are also mentioned (2, 3). It is known that, over time, obstructive cholestasis causes variable functional disorders in several systems and organs, with the most severe repercussions in the liver (3, 4, 5). Some authors, in studies aimed at suppressing the metabolic activity of the liver in mechanical jaundice, associate the severity of jaundice with blood bilirubin values, which of course is an important marker (2, 4, 5). However, taking into account a single indicator is not enough to evaluate the complex changes that occur when there is an interruption of the bile passage. Recently, papers have appeared that associate liver dysmetabolism in jaundice with the universal mechanism of cell injury - oxidative stress (OS) (6, 7, 8). The notion of oxidative stress, according to the opinion of several researchers, includes all the oxidative damage produced by oxygen free radicals. It is defined as the imbalance between the free radicals - the prooxidant and antioxidant systems, in the conditions where the oxidants are more and have a destructive potential on the human body (6). In other words, oxidative stress is triggered by the imbalance between the amount of reactive oxygen produced in the body and its ability to eliminate it. (8). The presence of marked lipid peroxidation, which occurs in endotoxemia associated with cholestasis, is known in the literature (9, 10). Previous studies on the issue report that the suppression of the metabolic activity of the liver present in jaundice contributes to the reduction of the barrier function of the gastrointestinal tract, the inhibition of the immune system and hemostasis mechanisms, appreciating the mechanical jaundice syndrome as a medical-surgical emergency, which requires solving the biliary obstruction as early as possible (6). However, the laborious surgical interventions performed at the peak of the “icterus” to restore the bile flow, demonstrated an evolution with a significant number of postoperative complications, and a mortality of 15-40% (2, 11, 12), thus opening a field larger for staged surgical treatment, which has as its main goal biliary decompression in the first stage and definitive resolution of gallstones in the second stage (5;11). Because it contributes to the reduction of complications through the use of miniinvasive methods, the treatment in stages is currently widely used, although the problem of assessing the times of surgical interventions in stages, continues to be a topic of dispute. Also, the accessible literature data do not contain information, which aims to use the deviations of oxidative stress in the assessment of the times of surgical intervention in stages. In this context, we set out to try to argue the optimal

terms for a staged surgical approach in the benign complications of gallstones associated with cholestatic jaundice by assessing the effect of biliary decompression on pro- and antioxidant markers.

MATERIAL AND METHODS

The clinical research was carried out within the Clinical Base II of the Department of Surgery no.1 of SUMF "Nicolae Testemițanu". In order to achieve the goal, 105 patients with complicated gallstones and associated cholestatic jaundice were investigated, followed the staged treatment protocol in obstructive jaundice, with the primary prerogative - internal biliary decompression through endoscopic papillosphincterotomy (PST) with or without removal of the stone. 35 patients with uncomplicated gallstones were included in the control group. Biliary decompression by PST with or without removal of the stone in the study subgroups was performed after confirming the etiological diagnosis and establishing the indications. In subgroups I and II, endoscopic PST was practiced within 24-96 days of hospitalization, in subgroup III, in the case of preserved bile flow - after 4-5 days of conservative treatment. Indication for internal drainage via PST in subgroups was persistent cholestatic jaundice. The age of the patients varied between 51-72 years. Female/male ratio - 3/1. The criteria for including patients in the basic group were: the presence of complications of gallstones associated with cholestatic jaundice, confirmed by positive clinical and paraclinical diagnostic parameters, which met:

- clinical criteria: history of gallstones or biliary colic at the onset, the presence of jaundice during hospitalization;
- biological syndrome: battery of characteristic tests: - elevated values of serum bilirubin on account of the direct fraction, elevated values of alkaline phosphatase, elevated values of leukocytes, serum fibrinogen, serum urea, serum amylase, marked ALAT values and imaging confirmation of gallstones and diameter of main bile duct > 0.8 cm.

Cases that met the stated criteria were included in the study. The research was started after obtaining approval from the Institutional Ethics Committee.

Depending on the evolutionary complications present: the patients from the base group were divided into 3 homogeneous subgroups: I - acute cholecystitis, cholangitis and cholestatic cholemia în gallstones (n=35); II - chronic gallstone disease and cholestatic cholemia (n=35) and III - acute biliary pancreatitis and cholestatic cholemia (n=35).

The comparative evaluation of SO in subgroups was carried out by assessing in the blood serum the correlation between the intensity of peroxidic oxidation of lipids (POL) and the antioxidant potential. The tests were taken during hospitalization, until the start of treatment, on the first day after biliary drainage, over 4 days after drainage. In the case of a preserved bile flow, in subgroup III, the primary testing was performed after 4 days of conservative treatment. The intensity of POL was determined by the values of the final product of lipoperoxidation - malonic dialdehyde (MDA), assessed according to the procedure modified by Gudumac V. *et al*. (2010) (13). The antioxidant potential was expressed by the values of superoxide dismutase (SOD) and catalase (CAT). SOD activity was estimated according to the procedures modified by Gudumac V., Tagadiuc O *et al*. (2010) (14). CAT activity

was determined according to the method modified by Baciu E. and Nastas I. (1996) (15). The data of each case were recorded in a standardized form and processed, using the MS Excel program. The obtained results were compared and the changes in dynamics were related to the values of the parameters of the control group. The statistical analysis was performed. The discrepancy with the margin of error < 5% was considered statistically significant ($p < 0.05$).

RESULTS

Survey data and results of laboratory parameters in the study subgroups and the control group at hospitalization are presented in table 1. Depending on age and concomitant pathologies, the control group and the study subgroups were selected homogeneously, with an insignificant difference in subgroup I (p c/1 >0.05 (t=1.10); p c/2 <0.05 (t=2.55); p c/3 <0.01 (t=2.84)). The homogeneity of the subgroups is also with insignificant deviations between subgroups I and II (p 1/2 >0.05 (t=1.64); p 1/3 <0.01 (t=3.31); p 2/3 <0.01 (t=4.55)). The data analysis shows that the association of an inflammatory, progressively painful component presents the patient earlier for hospitalization, reducing the duration of jaundice until hospitalization (p 1/2<0.001(t=3.5); p 1/3<0.001 (t=8.6); p 2/3<0.001(t=9.6)). Statistically significant difference in the levels of biochemical markers of cholestasis: alkaline phosphatase (p<0.001(t=10.6); (t=8.2); (t=7.27)), direct bilirubin (p<0.001 (t=12.3); (t=12.5); (t=14.9)) in all subgroups compared to the control group, reveals the severity of the liver disease, which is correlated with inflammation markers - elevated values of fibrinogen, blood leukocytosis, neutrophils - p<0.001 in subgroups I and III. The assessment at hospitalization of the DAM values as the final product of lipoperoxidation, confirms a marked lipoperoxidation in all subgroups, compared to the control group (p<0.001 (t=10.7); (t=10.9); (t=16.5)). The activity of the antioxidant system evaluated by superoxide dismutase and catalase values shows a decrease in both SOD values (p<0.001(t=6.4); (t=4.1); (t=5.7)) as well as CAT (p<0.001(t=6.7); (t=5.6); (t=7.6)) in all subgroups compared to the control group.

TABLE I.

Survey data and results of laboratory parameters at hospitalization

Markers	Control group n=35)	Subgroup I (n=35)	Subgroup II (n=35)	Subgroup III (n=35)	p c/1	p c/2	p c/3
Age	59.1±1.12	61.4±1.75****	64.2±1.65*	53.1±1.79**	p>0.05 t=1.1	p<0.05 t=2.55	p<0.01 t=2.84
Duration of jaundice	0	3.19±0.19	4.44±0.38	1.48±0.06			
Alkaline Phosphatase (U/l)	149.8±12.4	541.4±34.6***	881.14±78.3***	760.0±83.0***	p<0.001 (t=10.6)	p<0.001 (t=8.2)	p<0.001 (t=7.27)
Total bilirubin (mmol/l)	12.1±0.79	78.55±5.3***	119.31±8.7***	80.28±4.9***	p<0.001 (t=12.4)	p<0.001 (t=12.2)	p<0.001 (t=8.7)
Direct bilirubin (mmol/l)	4.2±0.06	56.2±4.2***	88.2±6.7***	54.8±3.4***	p<0.001 (t=12.3)	p<0.001 (t=12.5)	p<0.001 (t=14.9)

Markers	Control group n=35)	Subgroup I (n=35)	Subgroup II (n=35)	Subgroup III (n=35)	p c/1	p c/2	p c/3
ALAT (U/l)	19.57±0.97	207.1±12.1***	128.7±8.2***	289.62±9.4***	p<0.001 (t=15.4)	p<0.001 (t=13.2)	p<0.01 (t=28.5)
Blood amylase (U/l)	20.57±1.4	43.1±2.3***	37.9±1.9***	164.9±9.7***	p<0.001 (t=8.4)	p<0.001 (t=7.37)	p<0.001 (t=14.7)
Fibrinogen (U/L)	2.69±0.06	4.21±0.1***	3.52±0.1***	4.43±0.17***	p<0.001 (t=13.3)	p<0.001 (t=7.4))	p<0.01 (t=9.07)
Blood leuko-cytes(n)	6.23±0.23	12.12±0.52***	7.51±0.31**	10.24±0.43***	p<0.001 (t=10.35)	p<0.01 (t=3.31)	p<0.001 (t=8.2)
Neutrophil(n)	3.86±0.22	13.1±0.43***	7.5±0.39***	9.9±0.56***	p<0.001 (t=19.1)	p<0.001 (t=8.1)	p<0.001 (t=10.0)
Urea (U/l)	6.23±0.21	7.0±0.21*	6.56±0.24****	7.92±0.47**	p<0.05 (t=2.1)	p>0.05 (t=1.0)	p<0.01 (t=3.24)
Creatinine (U/l)	68.8±1.19	86.58±3.2**	81.0±3.5**	82.17±2.8**	p<0.01 (t=5.1)	p<0.01 (t=3.3)	p<0.01 (t=4.3)
DAM (mol/l)	4.62 ±0.27	12.11±0.64***	11.12±0.53***	13.16±0.44***	p<0.001 (t=10.7)	p<0.001 (t=10.9)	p<0.001 (t=16.5)
SOD (UC/ ml)	1230.74±16.39	1060.91± 21.05***	1096.97± 27.4***	1057.44± 25.2***	p<0.001 (t=6.4)	p<0.001 (t=4.1)	p<0.001 (t=5.7)
CAT (μmol/s)	15.79±0.33	13.27±0.14***	13.17±0.33***	12.62±0.25***	p<0.001 (t=6.7)	p<0.001 (t=5.6)	p<0.001 (t=7.6)

Caption *: p<0.05*; p<0.01**; p<0.001***; p>0.05****- compared to the values of the control group

According to the results of the study, depending on the time interval after drainage, biliary decompression differently influenced the activity of the pro- and antioxidant systems. On the first day after endoscopic PST, a decrease in the level of direct bilirubin was found in all subgroups (I - p<0.01 (t=5.4); II - p<0.01 (t=3.3); III - p<0.001 (t=7.6)), but the level of leukocytes and neutrophils in the blood increased (I - p<0.05 (t=3.2); II - p<0.01 (t=1.6); III - p<0.05 (t=5.2)), factor that influenced a massive continuation of lipid peroxidation in all subgroups. The evaluation of the influence of internal biliary decompression on the prooxidant system by assessing the MDA values showed, on the first day after decompression, the maintenance of high values compared to the before decompression parameters in all subgroups. Characteristic for subgroups I and II, the increased values of MDA did not show statistical significance compared to the before decompression state (I - p>0.05 (t=0.11); II - p>0.05 (t=0.91)), characteristic of subgroup III in which the patients were initially subjected to a complex drug treatment, which reduced the degree of endogenous intoxication and contributed to the decrease of before decompression MDA values (p< 0.001 (t=8.66)), elevated MDA values after drainage showed statistical significance (p<0.001 (t=6.4)). An obvious reduction in lipid oxidation activity was found on the 4th day after decompression, when MDA values decreased significantly (I - p<0.001 (t=5.15; II - p<0.01 (t=2, 6); III - p<0.001 (t=3.99)), but not up to control group values. The influence of time-dependent biliary decompression on MDA values is shown in first figure.

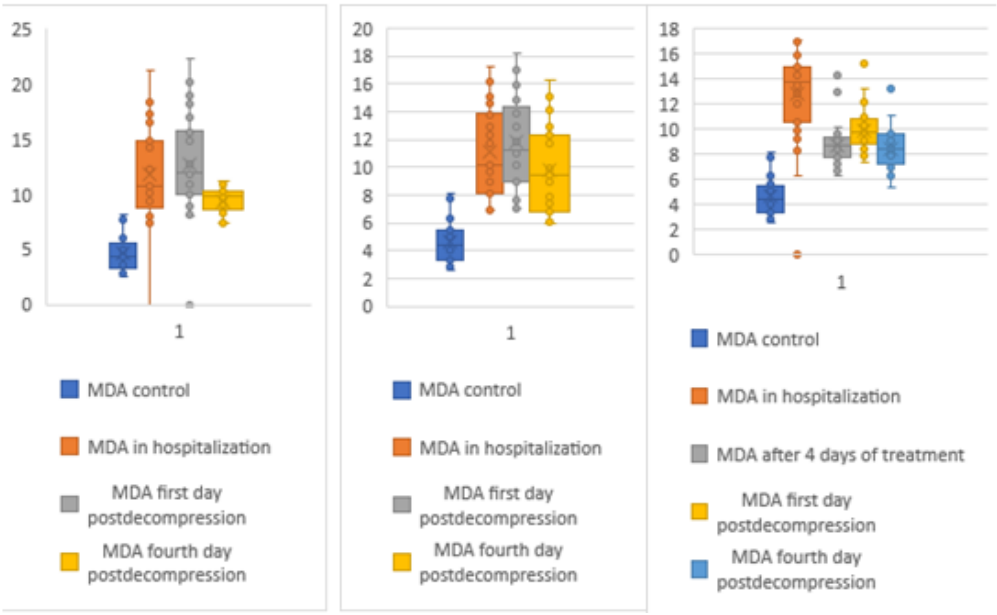

Fig. 1. Oscillation of DAM parameters in the complications of gallstones associated with cholestatic jaundice

The analysis of the influence of internal biliary drainage on the systemic antioxidant response in the case of endotoxemia with a marked lipoperoxidation in the complications of gallstones associated with cholestatic jaundice, also reported a variability in the values of SOD and CAT parameters depending on the elapsed time after decompression. Fluctuations in SOD and CAT values are shown in figures 2 and 3.

On the first day after PST, the values of SOD and CAT in groups I and II decreased insignificantly compared to before decompression values but showing a statistically significant decrease compared to the control group SOD (I - $p<0.001$ ($t=4.1$); II - $p<0.01$ ($t=4.8$)); CAT (I - $p<0.001$ ($t=4.1$); II - $p<0.01$ ($t=4.8$)), demonstrating the maintenance of an antioxidant response inability in this postoperative stage. In subgroup III, subjected preoperatively to standard complex therapy used in case of acute biliary pancreatitis, with potentiation of the antioxidant system through increased values of SOD (III - $p<0.001$ ($t=4.8$)) and CAT (III - $p<0.001$ ($t=7.4$)) before decompression, decreased values after drainage of SOD (III - $p<0.05$ ($t=3.04$)) and CAT (III - $p<0.05$ ($t=2.19$)), according to statistical significance, they showed a better antioxidant response capacity in these patients.

The increase in SOD and CAT values on day 4 after drainage exposes an increased potential of the antioxidant system at this postoperative stage (tab. II).

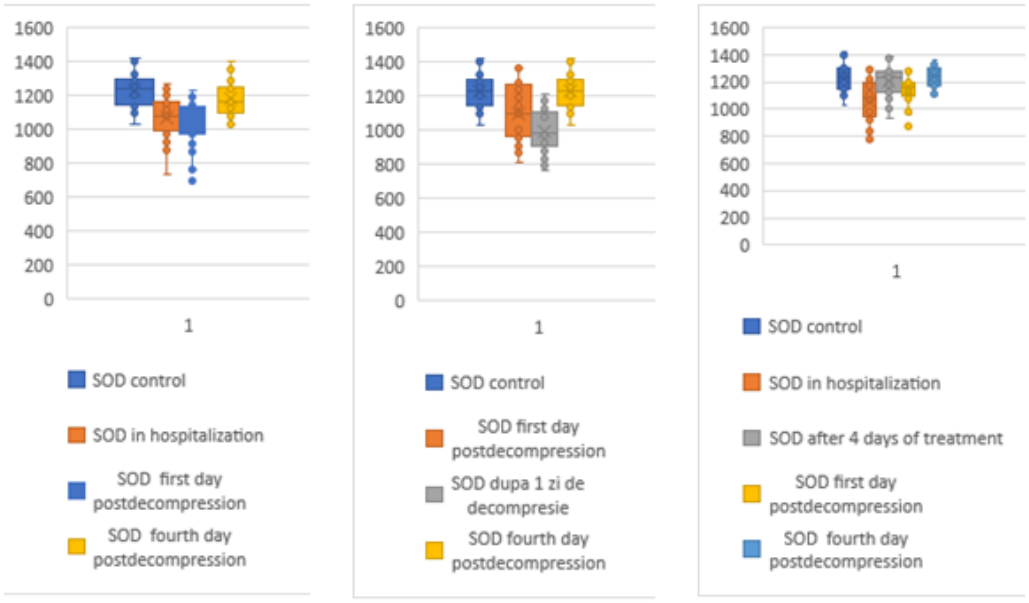

Fig. 2. Oscillation of SOD parameters in the complications of gallstones associated with cholestatic jaundice

TABLE II.

SOD and CAT parameters 4 days after decompression

Markers	Control group (n=35)	Subgroup I (n=35)	Subgroup II (n=35)	Subgroup III (n=35)	p c/1	p c/2	p c/3
SOD (UC/ ml)	1230.74±16.39	1178.7±16.77	1230.72±16.26	1237.6±12.2	p<0.05 (t=2.21)	p<0.05 (t=0.0008)	p<0.05 (t=0.33)
CAT (μmol/s)	15.79±0.33	14.94±0.13	13.99±0.39	14.77±0.13	p<0.05 (t=2.39)	p<0.05 (t=3.52)	p<0.01 (t=2.87)

DISCUSSION

According to the data accumulated in the literature, cholestatic jaundice, associated with various pathologies, is considered a complex surgical problem, presenting both diagnostic and therapeutic challenges (4, 5, 11). Early biliary decompression is recognized as the optimal method of treatment in avoiding functional and organic alteration of the liver. The ideal for the patient would be to resolve both the cholestatic jaundice and the etiological factor triggering cholestasis at the same time, an argument supported by some authors (17). However, practice has shown that these interventions are more often associated with complications and a slow postoperative evolution (16, 18). Staged treatment with the primary prerogative of biliary de-

compression is currently recognized and widely used in solving pathologies associated with cholestatic jaundice (4, 11, 19). However, the issue of operating times is presented controversially in the literature. Some authors, taking into account the high cost of diagnosis and treatment of these patients, claim that the definitive resolution of gallstones after decompression must be performed in the first 24-48 hours (18, 20). Others, focusing on the hepatic homeostatic disturbances, characteristic of lithiasis cholestasis, prefer for "late" cholecystectomy, in repeated hospitalization (4, 11,19), which according to studies can lead to a recurrence of complications (18, 20). The continuous study of the complex pathophysiological mechanism characteristic of the evolution of cholestatic jaundice requires solving the problem depending on the biological balance of the patient (11, 19).

It is known that the permeability of the biliary tree and the uncompromised drainage of bile into the intestine are important for a homeostatic balance of the body. Substances normally excreted in the bile, in the case of biliary obstructions, accumulate in the blood system and, together with the bile salts also accumulated, have systemic toxic effects, clinically manifested by prolonged infectious complications, coagulopathy, hypovolemia and endotoxemia, which can ultimately lead to to organ failure (2, 4, 21). Among the factors capable of modifying the normal functioning of biological membranes are reactive oxygen species, capable of inducing the phenomenon of lipoperoxidation, which contributes to the destruction of membrane lipid structures, consequently increasing the permeability of membranes with an unbalanced transport of ions (7, 9, 21). The attack of oxygen free radicals on cellular lipids results in the formation of aldehyde lipid hydroperoxide decomposition products, such as malonic dialdehyde, the increase in values denoting a pro-oxidant activity, which was also found to be significant in our study. MDA values 3 times higher compared to the control group were in all subgroups of the study before decompression. The pro-oxidant aggressiveness was also maintained on the first day after decompression with an involution 3-4 days after drainage, being correlated with the decrease in blood leukocytosis values ($p<0.01$ ($t=2.1$)). Analysis of the effect of internal biliary decompression on the prooxidant system demonstrated a decrease in lipid oxidation values after drainage, which is significant over time and is correlated with the decrease in bilirubin values and the inflammation syndrome.

The liver, as the body's metabolic center, has an important role in immunological processes and phagocytosis, thus being a source of antioxidants. It is known that serum bilirubin has the ability to inhibit lipid peroxidation, capturing singlet oxygen, thus being considered as a non-enzymatic liver antioxidant (7, 18, 22), a point that argues for an abundant production of all bilirubin fractions in all forms of jaundice. But the excessive increase in direct bilirubin values in an obstructive jaundice, turns it into a factor of aggression with the mobilization of other antioxidant defense mechanisms. According to studies, the decrease in SOD and CAT values correlates with the duration of jaundice and in prolonged cholestasis shows a late rebalancing (9, 18, 22). Our data reported in all study subgroups a no prolonged jaundice with a minimum duration of 1.48±0.06 and a maximum duration of 4.44±0.38 from onset to hospitalization, a moment that

correlates with direct bilirubin values, ranking all patients in the group with an average degree of severity (17, 21). The decrease in before decompression SOD ($p<0.001$ ($t=6.4$)) and CAT ($p<0.001$ ($t=6.7$)) is consistent with the severity of jaundice and the presence of inflammation. Comparative analysis of SOD results (I - $p<0.05$ ($t=2.21$), II - $p<0.05$ ($t=0.0008$), III - $p<0.05$ ($t=0.33$)) and CAT (I - $p<0.05$ ($t=2.39$), II - $p<0.05$ ($t=3.52$); III - $p<0.01$ ($t=2.87$)) with the values from the control group, time-dependent, reveals the return of the antioxidant system in all subgroups to values relatively similar to the control group 4 days after decompression.

Thus, our study demonstrates that in the case of the patient hospitalized early for complications of gallstones with a non-prolonged jaundice, classified in the medium severity group, the restoration of the capacities of the antioxidant system returns close to normal values 4-5 days after decompression, being a favorable predisposing factor in reducing intra- and postoperative complications, in case of a second stage surgical intervention in these terms. Therefore, the data-lot known in the literature about use of MDA, SOD and CAT values as diagnostic tests to evaluate the severity of jaundice (22, 23), we consider that these parameters and their correlations can be additionally recommended as indicator tests in determining the type of treatment, volume of surgical treatment and operative times.

CONCLUSIONS

Oxidative stress is an important pathogenetic element in the self-maintenance and exacerbation of the inflammatory response present in the complications of gallstones associated with cholestatic jaundice. Until the initiation of biliary decompression, in these patients, there is a significant intensification of the peroxidic oxidation of lipids, with a character of a chain oxidation process, and an increase in the level of the final product of lipid peroxidation-malonic dialdehyde, a fact that confirms the need for an immediate decompression. Antioxidant activity before decompression is also compromised, correlating with the degree of endotoxemia and severity of jaundice. It is obvious that, immediately after decompensation MDA maintains its values, yielding over time under a complex conservative treatment. Bile drainage, however, creates conditions for increasing the antioxidant capacity and restoring the body's biological balance, which is more pronounced 4-5 days after decompression, serving as a criterion for the possibility of definitive resolution of gallstones in these terms.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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TREATMENT OF HEPATIC HYDATID CYST

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TREATMENT OF HEPATIC HYDATID CYST (Abstract): Hydatid cysts of the liver are benign lesions which require a wide range of surgical strategies for their treatment. The complex treatment of the hepatic echinococcosis has made great progress to less invasive therapy for this category of patients. The multimodal therapy is represented by administration of antiparasitic agents, interventional radiology, interventional endoscopy, eco- or CT-guided puncture, laparoscopy and associations between those. There are still difficulties in the treatment of complex echinococcosis, especially of the cases with one or more complications. **Material and methods:** We analyzed retrospectively a group of 74 patients who underwent surgery for complex hydatid cysts over several years at General Second Surgical Clinic, Iasi, Romania, between 2005-2021, in order to determine the evolution of the surgical treatment. We recorded the following parameters: age, gender, place of origin, serological and paraclinical investigations relevant to liver function and *Echinococcus granulosus* infection, affected hepatic segment, surgical technique, postoperative evolution and days of hospitalization, complications, recurrences and chemotherapy. **Results:** From the total number of patients admitted in our clinic diagnosed with hepatic hydatid cyst, only 30.7 % had surgical indication. We performed the surgical treatment of the hydatid cyst by laparoscopic approach in 35.1% of cases and a classic approach 56.8%. The surgical technique used in the most cases was partial peri cystectomy (n=54, 73%), total cysto-peri cystectomy in 8.1 % of cases (n=6), and simple drainage of the remaining cavity after the inactivation of the parasite, in the rest of the cases (n=14, 18.9%). The post-operative biliary fistula was encountered in 19 cases (28.4%), with no significant correlation between the surgical approach and the apparition of biliary fistula, the percentage being similar in laparotomy and laparoscopy, with only a 3% difference towards the laparotomy. **Conclusions:** The biliary fistula remains rather frequent complication of the hepatic hydatic cyst and it's a challenge to predict the apparition of this complication. **Keywords:** HYDATID CYST, LIVER SURGERY, BILIARY COMMUNICATION.

The human hydatidosis is a potentially serious, sometimes fatal condition caused by cysts containing the larval stages of the *Echinococcus granulosus* (*E. granulosus*) tapeworm that infect dogs and other canines. The tapeworm eggs are excreted in

feces of these infected animals and people become infected by ingesting them. The contamination can occur via hand-to-mouth transfer after handling dogs or objects contaminated with the eggs, or from consuming contaminated food or water (1).

Liver localization is the most common (over 60% of all localizations), infestation by digestive tract. The liver is the first filter in the path of the parasite, and the lung is the second filter and therefore the second location (about 30%). In Romania, the incidence of this pathology is increasing, with 5-6 cases per 100,000 inhabitants each year (2).

The uncomplicated hydatid cyst, in most of the cases, is asymptomatic. The diagnostic is established by random abdominal echography or during the surgery performed for another pathology. There are cases where the hydatid cyst was first diagnosed in surgical intervention performed for a different pathology, or even during necropsy. The hydatid cyst can be asymptomatic for many years, the clinical manifestations appear in case of large dimensions, depending on the location of the cyst and its complications.

At this time, the World Health Organization classification sets the staging of hepatic hydatid cysts based on the ultrasound aspect (tab. I) (3).

TABLE I.

Classification of hepatic hydatid cyst

Stage	Echographic aspect according to WHO Classification
CL	Anechogenic uniloculated cyst, with no echoes or internal sepsis
CE 1	Anechogenic cyst, with fine echoes inside, representing the hydatic sand – active cyst
CE 2	Cyst with multiple septa at the interior, giving it a multivesicular aspect or "honeycomb" aspect, with a uniloculated primary cyst – active cyst
CE 3	Uniloculated cyst with decollated proligere membrane ("waterlily sign") (CE3a) or daughter vesicles associating hypo-/ hyperechogenic images (CE3b) -cyst in transition phase
CE 4	Cyst with mixed content, hypo/hyperechogenic, without daughter vesicles – "wool clew" aspect-cyst in the degenerative phase
CE 5	Cyst with partial or totally calcified wall – inactive cyst

The multimodal therapy is represented by administration of antiparasitic agents, interventional radiology, interventional endoscopy, ultrasound or CT-guided puncture, laparoscopy and associations between those.

The classical surgery procedures used for the treatment of the hepatic hydatid cyst are divided, according to their attitude towards the pericyst, into procedures that do not involve pericyst resection (cystectomy) and procedures involving pericyst resection (partial peri cystectomy, pericystoresection, hepatectomy). They are associated with procedures that should treat the remaining cavity: external drainage with a drain tube, bipolar drainage of the cavity and the main bile duct, padding, omental plombage, drainage of the cavity by anastomosis with the stomach/ jejunum, pericysto-biliar drainage.

Laparoscopic interventions are primarily suited for cysts located superficially on the anterior surface of the liver. The types of interventions that may be performed by laparoscopy are peri cystotomy with cys-

tectomy, partial or total peri cystectomy, hepatic segmentectomy. The contraindications of laparoscopy are cyst rupture in the biliary tree, central cyst localization, cystic dimensions over 15 cm, thickened or calcified cystic walls (4).

MATERIAL AND METHODS

We analyzed the patient admitted between January 2005 and July 2021, in the General Second Surgery Clinic of Emergency Hospital Saint Spiridon Iasi with the diagnostic of hepatic hydatid cyst with indication of surgical intervention for the treatment of this pathology. The information was obtained retrospectively from the register of the hospital registry and the observation sheets, in full accordance with GDPR regulations governing the medical act (5, 6).

The aim of this study was to retrospectively determine the evolution of the surgical treatment. We recorded the following parameters: age, gender, place of origin, symptomatology, serological and paraclinical investigations relevant to liver function and E. Granulosus infection, affected hepatic segment, surgical technique, chemotherapy use, postoperative evolution and days of hospitalization, complications, recurrences. The affected hepatic segment was assessed by the Couinaud classification, currently the most widely used system to describe functional liver anatomy.

At admission liver function was investigated in all patients. Hepatic cytolysis syndrome investigated through serum transaminases (AST and ALT). Excretory biliary syndrome or cholestatic syndrome was evaluated by measuring the serum alkaline phosphatase and serum bilirubin values. Inflammatory syndrome was investigated by number of white blood cell and The level of C-reactive protein, and plasma fibrinogen values.

All patients received an abdominal ultrasound scan. Additional Computed Tomography was performed to patients that required additional imaging. Many patients also required other imaging investigations, such as a chest X-ray, needed for the anesthesiologist's evaluation.

After the radiologic evaluation the cystic stage was assessed according to the WHO classification and also the liver segments affected by the cyst.

RESULTS

The total number of patients registered between January 2005 and July 2021 in Between January 2005 and July 2021 in the General Second Surgery Clinic of Emergency Hospital Saint Spiridon Iasi, with the diagnostic of hepatic hydatid cyst was 274. From this number of patients only 74 (30.7%) had indication of surgical intervention. The rest of the patient had hepatic hydatid cysts less than 5 cm diameter, and the drug treatment was administered with the recommendation of periodic revaluation. In this category were included also patient with inactive cysts.

Of the total number of patients enrolled in the study, 50 were female and 38 were male. The average age of the patients was of 46 years, and more than half of the patients lived in rural areas.

The most commonly reported symptom was spontaneous or palpatory pain in the right hypochondrium. Other frequent manifestations were asthenia and jaundice/slight jaundice.

All patients received an abdominal ultrasound scan, cysts range from 7 to 18 cm in diameter, with an average size of the cyst was 10 cm, most of the cysts were CE3 and CE1 stages.

The cases were solved by ultrasound guided puncture (n=1; 3.3%), laparoscopic (n= 26; 35.1%) and classic approach (n=42; 56.8%) (fig. 1).

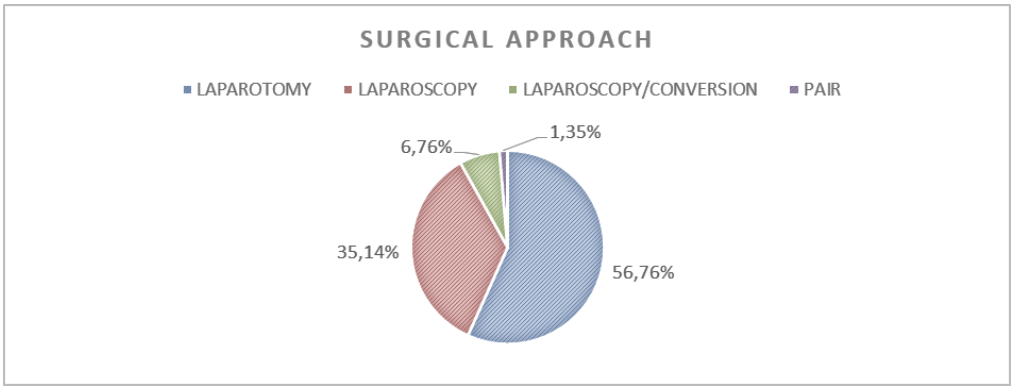

Fig. 1. Surgical approach of the hepatic hydatid cyst.

In the minim invasive approach, we performed inactivation-suction-drainage and partial perichistectomy. Using open approach, we did inactivation-suction-drainage, partial perichistectomy or total cystectomy. Laparoscopic treatment has significant correlation with type I-II (p=0.016) located in segments II, III and IV.

The surgical technique used in the most cases was partial peri cystectomy (n=54, 73%), total cysto-peri cystectomy in 8.1 % of cases (n=6), and simple drainage of the remaining cavity after the inactivation of the parasite, in the rest of the cases (n=14, 18.9%) (fig. 2).

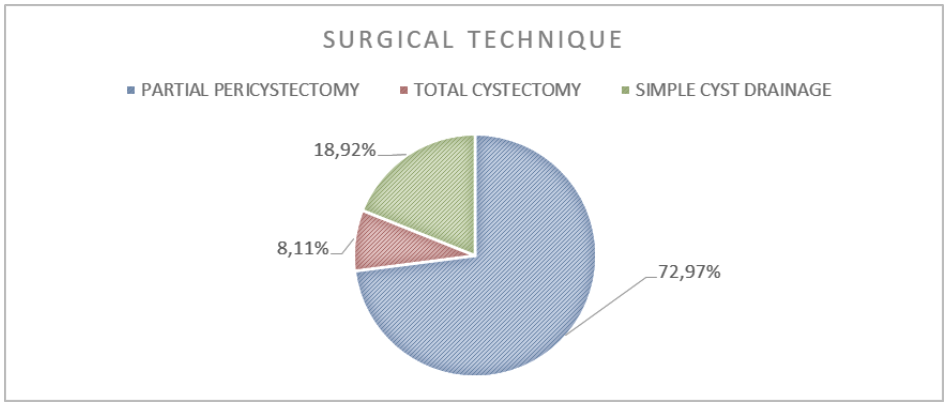

Fig. 2. The surgical technique

The post-operative biliary fistula was encountered in 19 cases (28.4%). In the graphic below we see than there is no significant correlation between the surgical approach and the apparition of biliary fistula, the percentage being similar in laparotomy and laparoscopy, with only a 3% difference towards the laparotomy (fig. 3).

There were no differences in age, sex, cyst type, alkaline phosphatase level, gamma-glutamyl transpeptidase level, alanine aminotransferase level, aspartate aminotransferase level, bilirubin level, and number of cysts and cyst locations between the patients with and without bile leakage. The treatment for the biliary fistula was conservatory, thus non-invasive procedure in 9.5% (n=7) and we did perform an Endoscopic Retrograde Cholangiopancreatography (ERCP) and sphincterotomy in 12.2% of the cases (n=9.5%) (fig. 4).

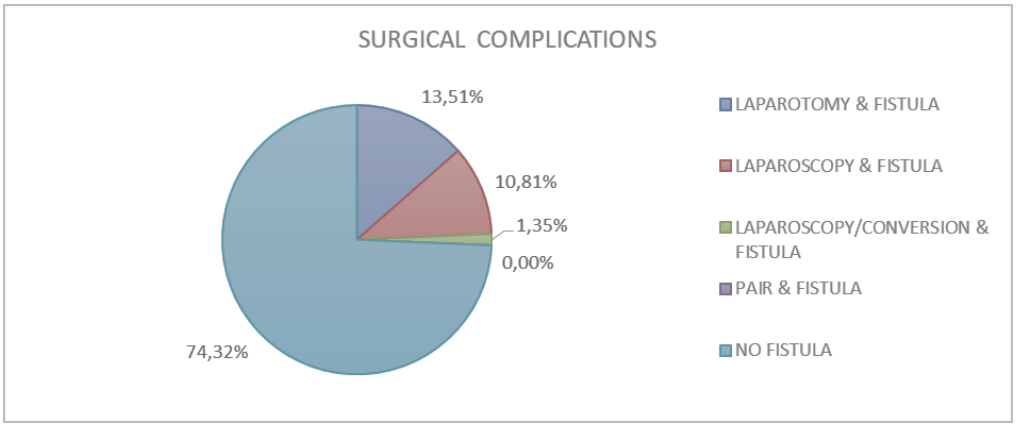

Fig. 3. Correlation between the surgical approach and the apparition of biliary fistula.

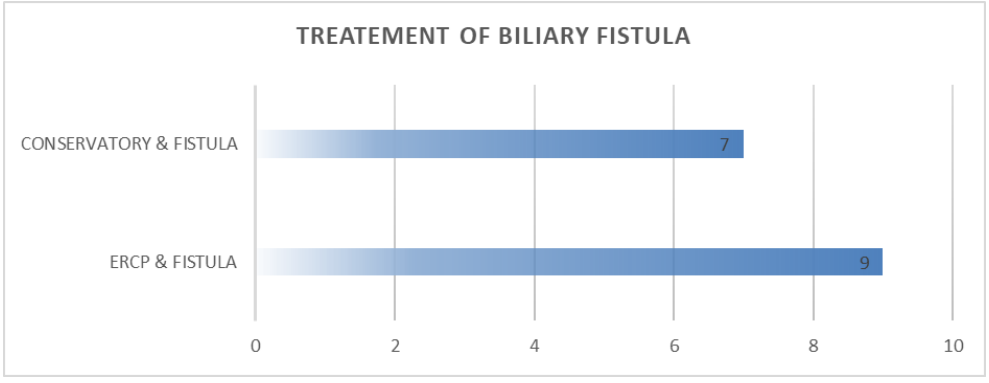

Fig. 4. The distribution of the biliary fistula treatment.

There was no significant difference in the postoperative evolution between laparoscopic procedures and open radical surgery (total perichistectomy).

We did not register significant correlations about recurrences and complications. Chemotherapy was used in 95% of cases, the election treatment was represented by Albendazol, administered in patient with weight of 60 kg or greater, the dose was 400 mg twice daily, for patients with a weight less than 60 kg we administered 15 mg / kg/ day given in divided doses twice daily with meals (maximum total daily dose 800 mg) for a 28-day cycle followed by a 14-day albendazole-free interval, for a total of 3

cycles. Monitoring of the blood counts and liver enzymes (transaminases) was done at the beginning of each 28-day cycle of therapy, with slightly increases values of liver enzymes in about 12 % of the patient, without significant secondary effects.

The most frequent hepatic segment localization of the hydatid cyst was in segment 4 (n=14, 18.9 %), in segment 7 of the liver in 8.1 % of cases for the single localization, and the multiple localizations most frequent hepatic segments were also 4 (22.9 %) and 7 (24.32%). The majority of the subjects have single-segment involvement 40.54 %.

In 6 cases the patient had other synchronous localization of the echinococcosis, with 2 cases of pulmonary hydatid cyst, one case of pulmonary and splenic localization, and one case of retroperitoneal localization, in the right subphrenic space and one case of localization in the abdominal wall in the preperitoneal space.

The post-treatment evolution was favorable in 72.6% of the cases, due to multiple therapeutic options and an ongoing development, with an average longer hospitalization period was obtained after open surgery treatment (18 days), a value of 15 days of hospitalization was obtained for minimally invasive techniques.

DISCUSSION

The liver is the most common location of the hydatid cyst, followed by the lung, with the approximate occurrence rates of 70% and 12%, respectively (7).

From 274 of patients with the diagnostic of hepatic hydatid cyst only a third had indication of surgical intervention. The rest of the patient, also included patient with inactive cysts, had drug treatment indication with the recommendation of periodic revaluation.

The drug of election was Albendazole, an anthelminthic medication with numerous indications such as cystic hydatid disease of the liver, lung, and peritoneum resulting from the larval form of the dog tapeworm, *Echinococcus granulosus* (8, 9). The most common adverse effects accompanying albendazole use are headaches and elevated liver enzymes because the drug is metabolized primarily by the liver, with 10% to 20% of patients experiencing elevated liver enzymes (12). Another adverse effect of this drug is induced myelosuppression, with pancytopenia and anemia (13). This is the reason we monitored of the blood counts and liver enzymes (transaminases) for each 28-day cycle of therapy.

Female patients were predominant in our group, with an age average age of 46 years, and more than half of the patients lived in rural areas. The usual hosts of E. granulosus is the domestic dog and livestock, have been hypothesized to be transmitted, at least partially, to humans through contaminated food. There is evidence that contaminated water is a potential disease determinant as well (12, 13). This transmission path explains the majority of patients infected from the rural area, because of the higher contact of this patients with the hosts of *E. granulosus*.

All patients received an abdominal ultrasound scan, cysts range from 7 to 18 cm in diameter, with an average size of the cyst was 10 cm. The smallest diameter is explained by the purpose of this study, with only surgical treated hydatid cysts because the indications for surgical therapy are cysts ≥7.5–10 cm (symptomatic or asymptomatic), cysts < 7.5–10 cm with complications such as compression to vasculo-biliary structures, fistulization to peritoneum, pleural cavity and adjacent organs (14).

The most commonly reported symptom

was spontaneous or palpatory pain in the right hypochondrium, a nonspecific symptom, the literature cites that this condition often remains asymptomatic for years before the cyst grows large enough to cause symptoms in affected organs.

Imaging, especially the ultrasound examination, was the basis for pre-surgical characterization of cysts. Most of the cysts were CE3 and CE1 stages and, in terms of size, between 7 and 10 cm diameter. Ultrasound examination is the easy, cheap and non-irradiation method the diagnosis of hepatic hydatid cyst (4).

The comparison between classic and minimally invasive surgical treatment revealed fewer complications and fewer necessary days of hospitalization in favor of the latter. There is no significant correlation between the surgical approach and the apparition of biliary fistula, the percentage being similar in laparotomy and laparoscopy, and no significant correlation between the other data registered and the bile leakage. Studies in the literature suggest that cyst diameter is an independent factor that is associated with a high risk of biliary-cyst communication in clinically asymptomatic patients (15-17). Our data didn't show a significant correlation between the diameter of the cyst and the apparition of the biliary fistula.

The treatment of election for the biliary fistula was Endoscopic Retrograde Cholangiopancreatography (ERCP) and sphincterotomy.

CONCLUSIONS

Hydatid disease has the benefits of a multimodal therapy. Chemotherapy, PAIR, laparoscopic surgery and open approach are completing themselves and not excluding each other. The biliary fistula remains relative frequent complication of the hepatic hydatic cyst and it's a challenge to predict the apparition of this complication. Endoscopic therapy is an effective mode of treatment for biliary fistulas complicating hepatic hydatid cyst.

CONFLICTS OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest to disclose, and they received no specific funding regarding this scientific research.

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NEWS

IT'S ALL IN THE RETINE LAYER: ASSOCIATION BETWEEN RETINAL NERV FIBER LAYER AND RISK OF COGNITIVE DECLINE IN PACIENTS WITH MULTIPLE SCLEROSIS

The multiple sclerosis and neurodegenerative process is present, and this is why there is a great desire to discover the perfect screening for multiple sclerosis consequence, for example cognitive decline. The researchers used the retinal study to explore the central nervous system. In this study, 64 patients underwent cognitive testing and optical coherence tomography imaging. The results showed that 36.0% participants were cognitively impaired. RLT of the macular retinal nerve fiber layer was associated with performance in TMT-B ($\beta = -0.259$). This study is a very exciting one, because it opens new directions for research in potential marker advocating further extensive neuropsychological examination (Sharon Jean Baetge, MSc, Michael Dietrich, PhD, Melanie Filser, *et al.* Association of Retinal Layer Thickness With Cognition in Patients With Multiple Sclerosis. *Neurol Neuroimmunol Neuroinflamm* 2021; 8: e1018).

A CHRONIC SCOPOLAMINE-INDUCED ANIMAL MODEL FOR COGNITIVE ALTERATIONS RESEARCH – ANATOMY AND METABOLISM

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A CHRONIC SCOPOLAMINE-INDUCED ANIMAL MODEL FOR COGNITIVE ALTERATIONS RESEARCH - ANATOMY AND METABOLISM (Abstract): As long as complete etiology and curative treatment of Alzheimer's disease (*AD*) are not available, numerous animal species have been tested, but the ideal *AD* model has not been yet found. Although wild-type rodents do not develop β amyloidplaques or neurofibrillary tangles, rat species present some advantages for anatomical and functional *AD* modeling. **Material and methods:** Memory impairment was induced by intraperitoneal injection of 2 mg/kg *scopolamine (Sco)* into Wistar male rats. A control group received a similar injection with 0.2 mL physiological saline. Hybrid imaging acquisitions *^{18}F-fluorodeoxyglucose PET/MRI (^{18}F-FDG PET/MRI)* were achieved after 14 days of administration and euthanasia for harvesting rat brains and cerebral samples preservation was accomplished after 28 consecutive days of treatment. Specific cerebral regions were visually evaluated and quantified using *standardized uptake values*, then were correlated with the anatomic regions harvested, comprising: the cerebral cortex, hippocampus, thalamus, and cerebellum, a separate sample being collected for each side. **Results:** In coronal view, 56 cerebral samples, 160 cerebral *MRI*, and over 800 *^{18}F-FDG PET* cerebral images were obtained. Although anatomical macroscopic differences were not found between rats cerebral brains, *in vivo* functional imaging provided an obvious distinction between the saline-treated and the *Sco*-induced animals, changes that persist in long-term administration. **Conclusions:** The present study proves that the cerebral metabolism increase is maintained during chronic administration of *Sco*, with intense metabolism in the amygdala, the entorhinal cortex, and other specific cerebral regions specialized in memory processing. We emphasize the functional impact of *Sco*-induction correlated with anatomical implications after tissue dissection. **Keywords:** SCOPOLAMINE-INDUCED AD ANIMAL MODEL, CHRONIC INDUCTION, METABOLIC QUANTIFICATION, RAT BRAIN HARVESTING.

Alzheimer's disease (AD), the most common form of dementia, causes gradual to irreversible cognitive loss, as the brain loads with prominent deposits of specific proteins. As long as the complete etiology and curative treatment of *AD* are unknown, many studies examine brain changes in rodent models of *AD* (1, 2, 3, 4, 5).

AD phenotypes involve cognitive decline, imaging changes, and biomarker accumulations (6). *Neurofibrillary tangles (NFT), β amyloid (βA)* plaques, and neuritic plaques precede symptomatology by more than a decade (7). In *AD,* the glycolysis with oxidative deterioration of specific enzymes, the tricarboxylic acid cycle, and the biosynthesis of *adenosine triphosphate (ATP)* cause cerebral sclerosis, particularly in the hippocampus and cingulate cortex (8, 9). Glucose metabolism is decreased, partly due to oxidative damage. In some preclinical models (2, 3, 8), antioxidant molecules exert protective properties for the brain.

Thus, biochemical and imaging markers of neurodegenerative disorders must be investigated on animal models (10), but the ideal *AD* model has yet to be discovered. All models have been tested, from the ease of breeding and genetic manipulation of fruit flies to the longevity and the ability to exhibit amyloidosis (but not tauopathy) of dolphins and non-human primates (1).

Although wild-type rodents usually do not develop *βA* plaques or *NFT*s, probably due to their relatively short lifespan for pre-symptomatic phase development and molecular differences to human genes, the rat species have some advantages for *AD* modeling: ease of human handling, little susceptibility for external influence in spatial learning strategies and a large brain size that allows brain surgery (11). Despite all shortcomings, the increased availability of gene-editing tools could develop a multitude of new rat genetic models, from the suitable but less complete model, obtained in a shorter time frame, to the best model obtained in a longer period (1).

Unlike humans, Wistar rats have a smooth cerebrum and no foramen of Magendie in the ventricular system. The literature describes environmental effects on rat cerebral anatomy (12, 13). Rodents' large brains are useful in structural and functional neuroimaging studies. Male Wistar rats are superior models in neuroanatomical experiments, especially in behavioral tests originally perfected for this breed (11).

Over time, hybrid imagistic tools, like functional *Positron Emission Tomography (PET)* and structural *Magnetic Resonance Imaging (MRI)* for localization, were developed for *in vivo* studies of neurocognitive disorders (14). A glucose analogue, ^{18}F *fluorodeoxyglucose* (^{18}F*-FDG),* the most widely used radiopharmaceutical in *PET* examinations of *AD*, shows hypometabolism in bilateral temporoparietal regions, precuneus and posterior cingulate regions in humans (15).

Scopolamine (Sco), a cholinergic receptor antagonist, helps induce reversible memory impairment in animals, resembling the cognitive and behavioral deficits of aging or *AD* onset by impairing attention maintenance, information processing, and knowledge acquisition (16).

Considering all the above-mentioned aspects, we aim to understand the framework of neuroanatomy and metabolism of *Sco*-treated rodents. In this article, we bring helpful information in degenerative diseases research, emphasizing on the functional impact of *Sco*-induction correlated with anatomical implications after rat brain removal and tissue dissection.

MATERIAL AND METHODS

Experimental protocols and animal treatments

Adult male Wistar rats (200 ± 8 g) purchased from "Cantacuzino" Institute (Bucharest, Romania), were maintained under controlled conditions: 24 ± 2 ºC, 50 ± 5% humidity, and a 12-hour-light/dark cycle, with *ad libitum* access to tap water and 10g per day standard rodent chow. The animal species and breed were chosen following the indications of Mazen Asaad and Jin Hyung Lee in their guide for studying Alzheimer's disease in animal models (10, 17).

Groups were formed: a *Negative Control group (NG)* received 0.2 mL vehicle (physiological saline), intraperitoneally and another *group(SG)* received 2 mg/kg bodyweight *Sco*, dissolved in 0.2 mL physiological saline, also intraperitoneally, for 28 consecutive days of the experiment.

The Committee on the Ethics of Animal Experiments of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania approved the involved protocols (permit number: 38/7.02.2021). All the procedures were in compliance with Directive 2010/63/EU published by the European Parliament and the Council of the European Union on September 22nd, 2010, regarding the protection of animals used for scientific purposes. For this study, we minimized the number of animals used and their potential suffering.

Hybrid PET/MRI cerebral imaging – data acquisition and processing

In the 3rdweek of the experiment, after 15 days of treatment, rats were scanned using a 1 Tesla NanoScan *PET/MRI* system (Mediso, Hungary), dedicated to small animals. The characteristics of this device were previously described (18). A dose of 32.04 ± 6.3 MBq of ^{18}*F-FDG* was injected in a tail vein, allowing the acquisition of *in vivo* hybrid images and metabolic analysis of the cerebral tissue. The animals were kept in a dark room before *Isoflurane* sedation (induction 4%, maintenance 2%). Data acquisition started 30 minutes after radiopharmaceutical administration with an *MRI* scout and an *MRI* localization scan and continued with the *PET* scan, 45 minutes after administration. Animals were deprived of food for about 12-16 hours before the ^{18}*F-FDG* injection. Visual data analysis and quantification were performed using InterView™ FUSION processing software (Mediso, Hungary). The *maximum standardized uptake value (SUVmax)* in g/mL of ^{18}*F-FDG* in cerebral tissue was calculated according to the formula:

$$\text{SUVmax} = \frac{\text{the decay} - \text{corrected maximum activity of tissue volume (Bq/mL)}}{\text{the injected radiotracer dose (Bq)}} * \text{body mass (g)}.$$

Data were collected from 20 specific brain tissue regions, right and left areas: cingulate cortex, entorhinal cortex, retro splenial cortex, temporal association cortex, hippocampal regions (anterodorsally, posterior, poster dorsal, subiculum and ventral) and amygdala, also from cerebellum cortex and the whole brain.

Brain removal and tissue dissection

Inhaled gaseous anesthetics are considered an adequate euthanasia method (19, 20) that we applied after *in vivo* assessments. All rats were terminally anesthetized in a plexiglass chamber, without pain or suffering, using 5% Isoflurane delivered at a rate of 40% volume per minute, to

induce rapid loss of consciousness. In accordance with American Veterinary Medical Association and European Commission guidelines for laboratory animal euthanasia, isoflurane was administered until 15 minutes after the respiratory arrest, then death was confirmed by physical examination (21, 22, 23).

Immediately after euthanasia, the procedure of whole brain collection was accomplished according to the steps described by Parkinson CM, and Kobeissy FH (24, 25). With the rat carcass in ventral recumbency on a clean dissection work surface, scissors and forceps are used to cut the skin over the calvaria along the skull midline. Bilateral cervical skin dissection removes posterior cervical muscles and flips skin over the eyes. The scissors cut the optical nerve beneath the eyes and remove eye bulbs. The midline surface of the skull can be scratched with sharp scissors or a knife, then a blade of small scissors inserted into the foramen magnum cuts the spinal cord below the cerebellum. With the forceps and the inferior blade of scissors inserted into the nasal cavity, both halves of the nasal bones and calvaria can be reflected, exposing the brain. Scissors inserted caudally into the interparietal bone can cut along the sagittal suture through the midline of the bone still holding together to remove occipital bone pieces. Gravity causes brain fallout when the carcass inverts. A curved forceps blade slid under the brain, cerebrum, and cerebellum from the olfactory lobes can pinch any connective tissue or nerves that prevent the brain from falling from the skull. The brain must be rinsed with sterile normal saline buffer and placed on ice to cool down immediately.

After euthanasia, whole brain removal, tissue dissection, probe collection, and labeling required 15-20 minutes per animal. According to the literature, samples were stored at -80°C, before tissue processing (26).

RESULTS

Visual and metabolic analysis

The hybrid ^{18}*F-FDG PET/MRI* imaging technique was used to obtain 160 *MRI* cerebral images and over 800 ^{18}*F-FDG PET* cerebral 2D images in coronal view.

In the first stage, by visual analysis, specific cerebral regions are identified on *MRI* images, like the cerebral cortex, hippocampus, and thalamic area. The *MRI* images did not highlight significant structural differences between the same regions of the two groups. Discreet functional differences are observed in *PET* images from *Sco*-induced brains in comparison with the *NG*. An example of a normal brain *MRI* image and one example of an ^{18}*F-FDG PET* brain image for each group were illustrated (fig. 1). On a gray color scale, brain metabolic activity is normal in the *NG*, represented by a moderate and relatively homogenous whitish-gray cerebral cortex and a homogenous discreetly grayish amygdala and thalamic area. The images of *Sco*-induced brains present homogeneous darker shades of gray in the cerebral cortex, thalamic area, and amygdala.

In the second stage of image analysis, data from the ^{18}*F-FDG PET* scan were expressed in *SUVmax* (g/mL). Overall, from the evaluation of right and left *AD* specific regions, we obtained a metabolic chart (fig. 2) that highlights a global increased metabolic activity in brain tissues of *Sco*-induced rats (a median *SUVmax* = 3.10 g/mL ± 1.13 *standard deviation (SD)*) compared to normal ones (a median *SUVmax* = 1.75 g/mL ± 0.48 *SD*). Also, we identified spike metabolic values (2.5 times bigger) in the amygdala (*SUVmax* = 5.06

g/mL ± 0.21 *SD*) and entorhinal cortex (*SUVmax* = 4.99 g/mL ± 0.11 *SD*) for *Sco*-treated rats, compared to *NG* values (*SUVmax* = 1.88g/mL ± 0.04 *SD*) in the amygdala and respectively (*SUVmax* = 1.88g/mL ± 0.02 *SD*) in the entorhinal cortex. These values exceed *SUVmax* = 3,39 g/mL ± 0.35 *SD*, the value of cerebellum cortex in *Sco*-induced rats. Other regions influenced by *Sco* administration were the subiculum and ventral hippocampus, with values that don't exceed the median value identified in the cerebellum cortex (*SUVmax* = 3.39g/mL ± 0.35 *SD*).

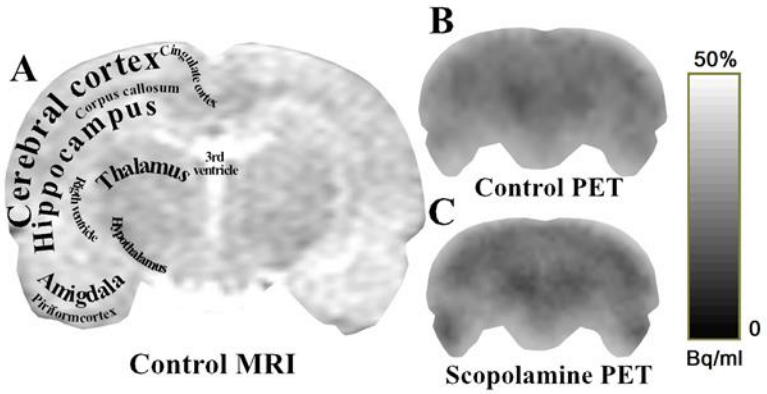

Fig. 1. Representative ^{18}*F-FDG PET/MRI* images of the Wistar rat brain, coronal sections. A, *in vivo MRI* or structural image of a normal rat brain; B, *in vivo PET* or functional image of a normal rat brain and C, *in vivo PET* or functional image of a *Sco*-induced rat brain.

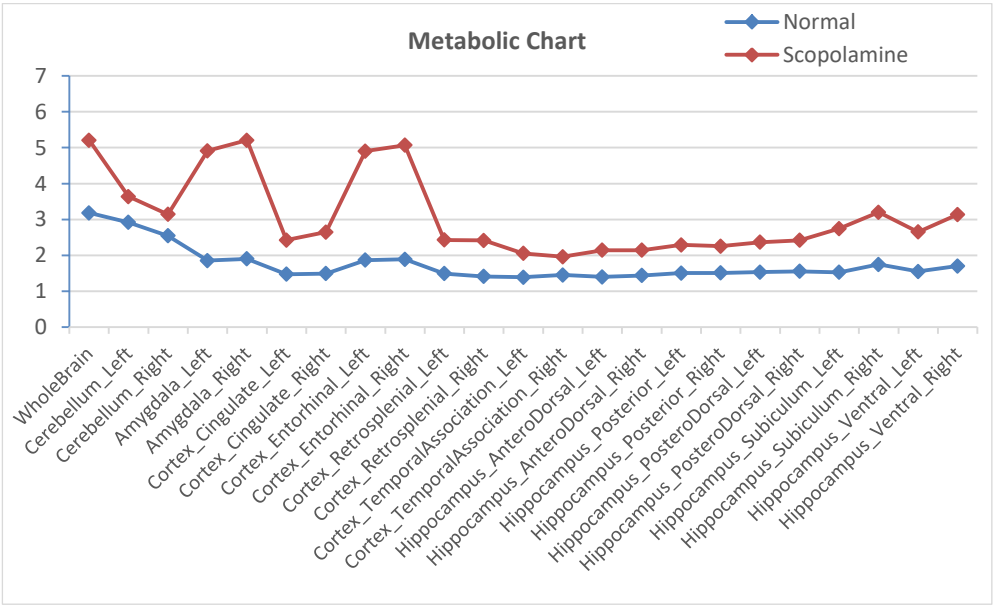

Fig. 2. Scopolamine effects in rat brain regions, compared with normal rats. Results are expressed as *SUVmax* (g/mL) related to body mass.

Brain tissue evaluation

For each rat, 8 different probes were obtained, separately collected and labeled, 4 for each side, right and left: cerebral hemispheres, hippocampus, thalamus, and cerebellum hemispheres. In total, 56 cerebral samples were preserved at low temperatures for homogenization, processing, and further examinations. Brain sections and anatomical regions of a normal Wistar rat from the control group are representative (fig. 3). By macroscopic analysis, no significant anatomic differences were highlighted between the two groups.

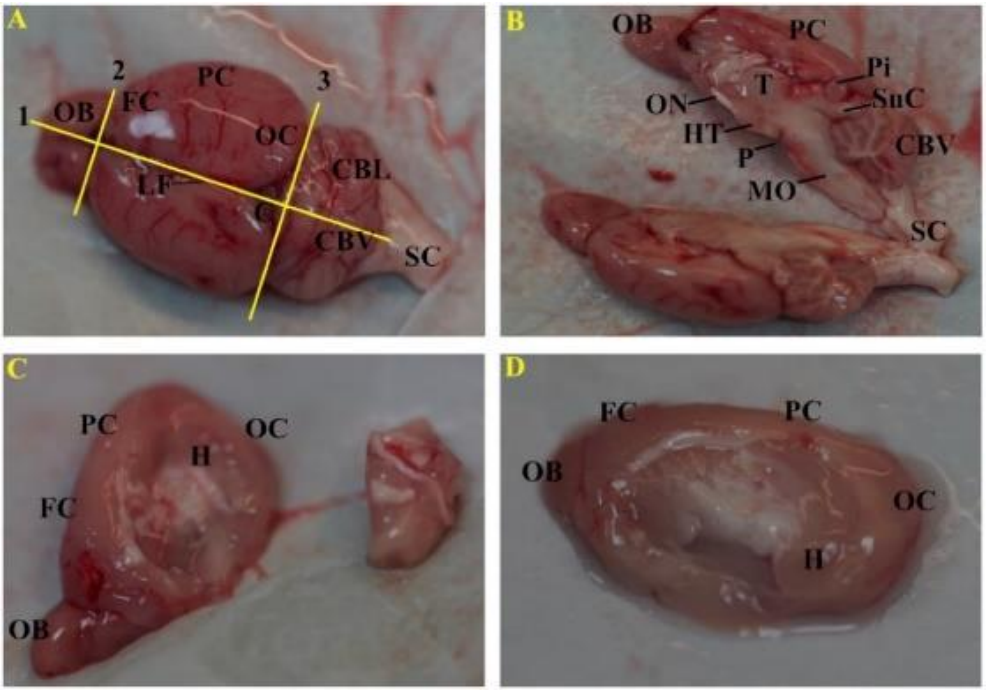

Fig. 3. Major features of the dorsal surfaces and cross sections of a whole brain from a normal Wistar rat (control group). Yellow lines: the number 1 level shown in *A (dorsal surfaces)* corresponds to the *sagittal section* shown in *B, C, and D;* the number 2 level corresponds to the cross-section for olfactory bulb removal; the number 3 level corresponds to the cross-section for cerebellum excision. OB, olfactory bulb; FC, frontal cortex; PC, parietal cortex; OC, occipital cortex; LF, longitudinal fissure; C, rostral and caudal colliculus; CBL, cerebellum-lateral hemisphere; CBV, cerebellum-vermis; SC, spinal cord; Pi, pineal gland; SuC, superior colliculi; ON, optic nerve; T, thalamus; HT, hypothalamus; P, pons; MO, medulla oblongata; H, hippocampus.

DISCUSSION

Sco-induced neurodegeneration in rats includes many karyopyknotic cells, neurons with low tigroid substance staining, increased protein peroxide oxidation, and 2-thiobarbituric acid in the cerebral cortex and hippocampus. Morphological alterations determined by *Sco* initiate along with sleep disorders. In rodents, *Sco* increases non-rapid eye movement sleep, rapid eye movement sleep, and waking electroencephalogram power in the 5.5-10.0 Hz

band. *Sco* also increases regional cerebral glucose metabolism, according to research (27-30). Low doses of anticholinergic drugs can cause non-cognitive effects like increased locomotor activity and increased blood-oxygen level changes in the orbital cortex (31). Long-term *Sco* treatment induces cognitive deficits after the first week, and neurobehavioral changes in rats, including hyperactivity, anxiety-like behavior, and decreased serotonin levels (17, 28). As far as we know, there is only one paper discussing about cerebral rat metabolism related to one dose *Sco*-induction (31), and no article about chronic administration of *Sco*.

In our study, functional ^{18}*F-FDG PET* images show quantifiable brain metabolism changes in *AD*-induced rats compared to the *NG*, even though there are no visible anatomical and structural differences between brain sections and *MRI* images (31, 32). The cerebral metabolism increase is maintained during chronic administration of *Sco*, sparing the cerebellar cortex. Intense metabolism (spike values) is observed in specific *Sco*-treated cerebral regions: the amygdala, with a primary role in the processing of memory decision-making and emotional responses, and the entorhinal cortex, a major player in memory processing of complex associations between experienced events. *Sco* administration discreetly affected the subiculum and ventral hippocampus, reducing hippocampal information sent to the amygdala, with fear conditioning alteration and recent memory deficits in these rats (29, 33, 34). Our study shows that long-term *Sco* treatment increases and maintains metabolic activity in *AD* brain regions. We measured over 100 image slices in both hemispheres for each rat, so data were quantifiable despite small group sizes. Our *AD* animal model study showed that cerebral tissue sampling was simple, fast, and effective if the sampling steps were followed.

Cerebrospinal fluid sampling is a more laborious, demanding, and time-consuming technique, especially for lumbar puncture and it was not discussed in this article.

Without claiming to have found a good *AD* model for reproducing biochemical, histological, and morpho-functional abnormalities of *AD* brain neurodegeneration, nor the best cerebral tissue sampling method, translatability and reproducibility of these techniques are essential keys. Animal research is essential for studying *AD*-specific molecular and cellular processes (1).

CONCLUSIONS

Despite many studies on rat models of *AD*, few describe the methods and necessary steps for brain tissue harvesting and further anatomo-pathological and biochemical analysis.

In conclusion, the anatomical and functional approach of *AD* with drug-induced animals has been shown to be useful in supplementing metabolic data of impaired brain. More precisely, we showed that chronic *Sco* administration maintains quantifiable effects in brain metabolism of *AD*-induced rats (whole cerebral metabolism increase, intense metabolism in amygdala and entorhinal cortex). Our study provides useful information, helps characterization of brain tissues and *AD* physio-pathological mechanisms, improving daily research practice.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest and no funding regarding this scientific research.

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ADVANCING PRETERM BIRTH PREDICTION: A PROSPECTIVE MULTICENTRIC STUDY INVESTIGATING THE PREDICTIVE PERFORMANCE OF PARTOSURE, FETAL FIBRONECTIN, AND CERVICAL LENGTH

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ADVANCING PRETERM BIRTH PREDICTION: A PROSPECTIVE MULTICENTRIC STUDY INVESTIGATING THE PREDICTIVE PERFORMANCE OF PARTOSURE, FETAL FIBRONECTIN, AND CERVICAL LENGTH (Abstract): The prediction of preterm birth (PTB) is still a controversial topic, and many efforts have been made to identify the best predictive markers. The **aim** of this study was to prospectively assess the predictive performance of placental alpha macroglobulin-1 (PAMG-1), fetal fibronectin (fFN), and cervical length, both individually and combined, for the prediction of spontaneous preterm birth at 7 and 14 days in a cohort of pregnant patients with threatened preterm labor. **Material and methods:** Between October 2022 and April 2023, we performed a prospective observational study that evaluated 80 pregnant patients with singleton pregnancies, between 24 and 32 completed weeks of gestation. We qualitatively determined PAMG-1 (PartoSure test) and fFN from the vaginal fluid. Clinical data along with cervical length measurements were also retrieved. The predictive performance of the evaluated markers was determined using logistic regression and ROC analysis. **Results:** PartoSure obtained the best results for the prediction of PTB in the next 7 or 14 days. On the other hand, cervical length, at an established cut-off of 19 mm, had the lowest predictive performance for the evaluated outcomes. A combined algorithm that comprised PartoSure, fFN, and cervical length measurements achieved the best predictive performance for the prediction of PTB at 14 days. **Conclusions:** The combined algorithm is superior in terms of predictive performance for the prediction of PTB in the next 7 or 14 days. The PartoSure test could be used individually for the prediction of PTB, but clinicians need to correlate its results with other clinical findings. **Keywords:** PRETERM BIRTH PREDICTION; PARTOSURE; PAMG-1; FETAL FIBRONECTIN; CERVICAL LENGTH.

Preterm birth (PTB), defined as delivery before 37 completed weeks of gestation, is one of the most important public health issues, associated with increased morbidity and mortality rates worldwide (1). According to one of the most recent studies published in the literature, the number of preterm births worldwide declined by 5.26% from 16.06 million in 1990 to 15.22 million in 2019, and the number of fatalities decreased by 47.71% from 1.27 million in 1990 to 0.66 million in 2019 (2).

Even though some significant improvements have been made for the prediction and prevention of this disorder, there is still no accurate test that will correctly identify the majority of patients who will deliver prematurely (3). Maternal risk factors such as personal history of preterm birth, cervical excisional procedures, smoking status, use of assisted reproductive techniques, vaginal and urinary tract infections, thrombotic disorders, or ischemic placental disease have been proposed as predictors of PTB (4-8). Moreover, sonographic markers such as short cervical length, uterocervical angle, uterine artery pulsatility index, as well as cervical and elastography parameters, have proven to be associated with increased risk of preterm birth and adverse pregnancy outcomes (9-12).

Placental alpha macroglobulin-1 (PAMG-1), which is comprised in the PartoSure test, is synthesized by decidual cells that line the uterus and is subsequently released into the amniotic fluid (13). Typically, the concentration of PAMG-1 in vaginal discharge is minimal. However, research has demonstrated that the detection of PAMG-1, either qualitatively or quantitatively, in vaginal discharge is indicative of impending delivery (14, 15).

Fetal fibronectin (fFN) is an important constituent of extracellular matrix, and it is located at the interface between the decidual and chorionic tissues (16). The release of fFN into cervicovaginal secretions occurs as a result of the disruption of the interface caused by inflammation, abruption, or uterine contractions (17). This phenomenon can serve as a foundation for predicting preterm birth.

It is estimated that approximately 65-75% of PTBs occur spontaneously, whereas the remaining cases are iatrogenic in nature (18). The occurrence of spontaneous PTB can be attributed to various pathophysiological mechanisms (19). Consequently, accurately predicting PTB has been a persistent obstacle, given that relying solely on clinical symptoms does not provide sufficient predictive precision (3, 20). In a recent meta-analysis by Melchor *et al.*, PAMG-1, fFN, and cervical length showed a variable predictive performance between studies, due to the influence of demographic and clinical factors (21).

Nikolova *et al.*, comparatively evaluated the predictive performance of PAMG-1, fFN, and cervical length for the prediction of preterm birth in a time-frame between 7-14 days in a cohort of patients 203 patients at risk (22). The authors concluded that PAMG-1 (Sensitivity- Se: 80%; Specificity- Sp: 95%) was the best predictor of PTB within 7 days compared to fFN (Se: 50%; Sp: 72%) and cervical length (Se: 57%; Sp: 73%).

The current literature data is reported heterogeneously, and the predictive performance of combined algorithms was not studied in prospective studies. Therefore, the aim of this multicentric study was to prospectively assess the predictive performance of PAMG-1, fFN, and cervical

length, both individually and combined, for the prediction of spontaneous preterm birth at 7 and 14 days in a cohort of pregnant patients with threatened preterm labor

MATERIAL AND METHODS

We performed a prospective observational study that evaluated pregnant patients with singleton pregnancies, between 24 and 32 completed weeks of gestation who self-reported pelvic pressure, uterine contractions with or without pain, intermittent lower abdominal pain, or any other sign, symptom, or complaint that might be indicative of imminence of preterm birth. Between October 2022 and April 2023, 80 pregnant patients who were evaluated at "Sf. Ioan cel Nou" Emergency Hospital, Suceava, and at "Cuza-Voda" Clinical Hospital of Obstetrics and Gynecology, Iasi, Romania, were enrolled.

Ethical approval for this study was obtained from the Institutional Ethics Committees of "Grigore T. Popa" University of Medicine and Pharmacy Iasi (No. 101/08.07.2021), and "Sf. Ioan cel Nou" Emergency Hospital, Suceava (No. 21/26.10.2022). Informed consent was obtained from all participants included in the study. All methods were conducted in accordance with relevant guidelines and regulations.

The speculum examination of the enrolled individuals revealed that they had cervical dilatation (less than 3 cm) and clinically intact amniotic membranes. Patients with overt fetal membrane rupture, advanced cervical dilatation (more than 3 cm), presence of blood on speculum examination, suspected placenta previa, cervical cerclage in place, patients less than 18 years old, or who were unable to offer their informed consent were excluded from the study.

Complete obstetrical and medical examinations were performed at admission. Before cervical examination, we collected vaginal fluid samples for PartoSure (Germantown, MD 20874, USA), and fetal fibronectin tests (Antagen Pharmaceuticals Inc, Boston, MA 02118, USA). The samples were processed according to the manufacturer's instructions, and both PAMG-1 and fFN were determined qualitatively (test and control line positives). We proceeded with digital and cervical examinations.

The patients underwent standard anatomy scans as well as transvaginal ultrasound performed by experienced obstetricians, with at least level 2 qualification in ultrasound examination, using an E10 scanner with a 4.8 MHz transabdominal probe, and a 5-15 MHz intravaginal probe (GE Medical Systems, Milwaukee, WI, USA).

The following data were recorded: gestational age, clinical symptoms, cervical length, presence of PAMG-1 and fFN, and obstetrical outcomes. The primary outcome was preterm birth in the next 7 days, while the secondary outcome was preterm birth in the next 14 days after admission.

In the first stage of the statistical analysis, descriptive statistics were used for the characterization of our cohort of patients. In the second stage of the analysis, we evaluated and compared the predictive performance of PAMG-1, fFN, and cervical length for the prediction of PTB in a 7-days and 14 time-frames using logistic regression and ROC analysis. Cut-off values, adjusted to gestational age at diagnosis, were identified according to the Youden index. The combined algorithm comprised all three markers and its predictive performance was also calculated. The statistical analyses were performed using *STATA SE*

(version 17, 2022, StataCorp LLC, College Station, TX, USA). A p value less than 0.05 was considered statistically significant.

RESULTS

This study enrolled 80 patients with imminence of preterm birth. Their demographic characteristics are presented in table I. The median age for this cohort of patients was 29.5 years (interquartile range, IQR: 24-35), while the median BMI was 26.43 kg/m^2 (IQR: 22.86- 28.48). The majority of these patients came from a rural background (52.5%), and their clinical characteristics revealed high rates of vaginal infections (22.5%) as well as a significant personal history of preterm birth (17.5%) and other adverse pregnancy outcomes such as preeclampsia and intrauterine growth restriction (13.75%).

The predictive performance of PartoSure, fFN, and cervical length for the prediction of PTB in a 7-days' time-frame was calculated, and it is presented in table II and figure 1. PartoSure obtained the best results when evaluated individually for the prediction of PTB in the next 7 days, with a sensitivity of 71.79%, specificity of 48.78% and an AUC value of 0.602. The combined model achieved a sensitivity of 74.89%, a specificity of 90.24%, and an AUC value of 0.769.

TABLE I.

Demographic and clinical characteristics of patients included in the study

Parameter	Median and IQR or n (%)
Age, years	29.5 (24- 35)
BMI, kg/m^2	26.43 (22.86- 28.48)
Medium	Rural= 42 (52.5%) Urban= 38 (47.5%)
Preterm birth history	14 (17.5%)
Confirmed urinary tract infection	7 (8.75%)
Confirmed vaginal infection	18 (22.5%)
Gestational hypertension	3 (3.75%)
Personal history of adverse pregnancy outcomes	11 (13.75%)
Gestational diabetes	2 (2.5%)
Autoimmune disorders	1 (1.25%)

IQR- interquartile range; BMI-body mass index.

The predictive performance of PartoSure, fFN, and cervical length for the prediction of PTB in a 14-days' time-frame was calculated and it is presented in table III and figure 2. PartoSure obtained the best results when evaluated individually for the prediction of PTB in the next 14 days, with a sensitivity of 53.66%, specificity of 87.18% and an AUC value of 0.704. The combined model achieved a sensitivity of 70.73%, a specificity of 94.87%, and an AUC value of 0.828.

TABLE II.

The predictive performance of individual and combined tests for the prediction of PTB at 7 days.

Test	Sensitivity	Specificity	LR+	LR-	AUC and 95% CI
PartoSure	71.79	48.78	1.40	0.57	0.602 (0.48- 0.70)
fFN	46.15	73.17	1.72	0.73	0.596 (0.48- 0.70)
Cervical length (cut-	38.46	17.07	0.463	3.604	0.204 (0.11- 0.30)
Combined	74.89	90.24	7.35	0.31	0.769 (0.66- 0.86)

LR+- positive likelihood ratio; LR- - negative likelihood ratio;
AUC- area under the receiver operating curve; CI- confidence interval; fFN- fetal fibronectin.

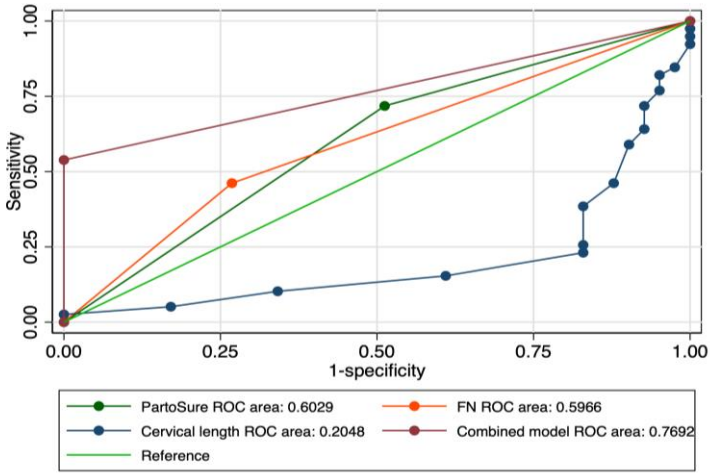

Fig. 1. Multiple ROC comparisons of individual and combined tests for the prediction of PTB in the next 7 days. (ROC- receiver operating characteristic curve; FN- fetal fibronectin)

TABLE III.

The predictive performance of individual and combined tests for the prediction of PTB at 14 days

Test	Sensitivity	Specificity	LR+	LR-	AUC and 95% CI
PartoSure	53.66	87.18	4.18	0.53	0.704 (0.61- 0.79)
fFN	43.90	79.49	2.14	0.70	0.616 (0.51- 0.71)
Cervical length	36.59	69.23	1.18	0.91	0.500 (0.37- 0.62)
Combined	70.73	94.87	13.79	0.30	0.828 (0.72- 0.90)

LR+- positive likelihood ratio; LR- - negative likelihood ratio;
AUC- area under the receiver operating curve; CI- confidence interval; fFN- fetal fibronectin.

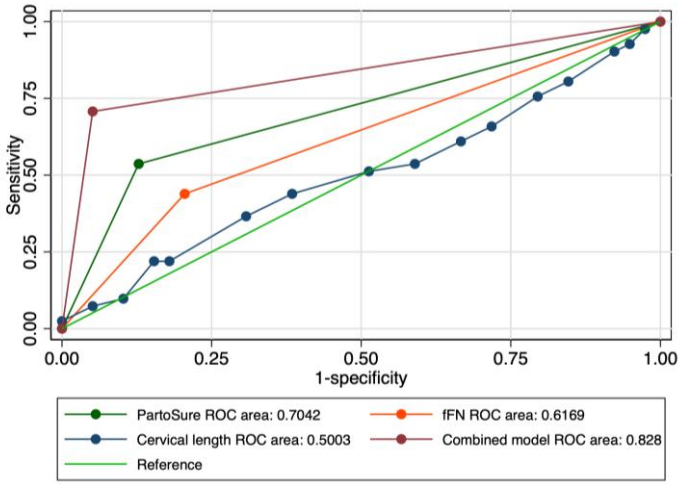

Fig. 2. Multiple ROC comparison of individual and combined tests for the prediction of PTB in the next 14 days. (ROC- receiver operating characteristic curve; fFN- fetal fibronectin)

DISCUSSION

The present study evaluated the predictive performance of PartoSure, fFN, and cervical length for the prediction of PTB in a 7-days and 14-days' time-frames. Our results indicated that PartoSure achieved the best results in terms of sensitivity and specificity for the prediction of PTB at both 7 days and 14 days intervals from its detection. Its sensitivity was higher for the evaluated outcome at 7 days, while its specificity increased when used to predict PTB at 14 days. On the other hand, cervical length, at an established cut-off of 19 mm, had the lowest predictive performance for PTB at 7-and 14-days intervals after its measurement.

Our results are similar to those reported in previous literature. Nikolova *et al.*, reported the following sensitivities for PartoSure, fFN, and cervical length for predicting imminent spontaneous PTB within 7 days: 80%, 50%, and 57%, along with their corresponding specificities: 95%, 72%, and 73% (22). Moreover, the authors indicated lower predictive performance of PartoSure (Se- 63%, Sp- 96%), fFN (Se-42%, Sp-72%), and cervical length (Se-54%, Sp-74%) for the prediction of imminent spontaneous PTB within 14 days.

A recent review by Rouholamin *et al.*, indicated that PartoSure test had the highest specificity, positive predictive value (PPV), and positive likelihood ratio (LR+) for the prediction of PTB in all categories of pregnant patients, but, at the same time, outlined the need to correlate its results with the clinical findings and other diagnostic procedures (15).

We also calculated the predictive performance of a combined algorithm that comprised PartoSure, fFN, and cervical length for the prediction of imminent spontaneous PTB within 7 and 14 days. Our results indicated relative similar sensitivities and specificities, although the overall accuracy was higher for the prediction of PTB at 14-days (AUC of combined algorithm: 0.828; 95%CI: 0.72- 0.90). As far as we know, this is the first study in the literature that evaluated the predictive performance of combined algorithms, so comparative data is not available.

PartoSure (PAMG-1) and fFN were both qualitatively determined in our study. Ravi *et al.*, evaluated the predictive performance of various cut-offs of fFN and compared the results with qualitative PAMG-1 for the prediction of PTB in the next 7 to 14 days (23). Their results indicated that PAMG-1 test was the best predictor for the evaluated out-comes, and reported a sensitivity of 67%, and a specificity of 96% for the prediction of PTB in the next 7 days. Moreover, the authors reported that a conventional cut-off for fFN of 50 ng/ml provided enough accuracy for the prediction of the evaluated outcomes (Se- 67%, and Sp-77%).

This study has several limitations: small cohort of patients and qualitative determination of PAMG-1 and fFN. On the other hand, this is the first study that evaluated the predictive performance of a combined algorithm in a prospective setting. Further studies, on larger cohorts of patients, could determine the cost-effectiveness of combined strategies for the prediction of PTB.

CONCLUSIONS

The results of this study indicated that PartoSure achieved the best results in terms of sensitivity and specificity for the prediction of PTB at both 7-days and 14-days intervals from its detection.

On the other hand, cervical length, at an established cut-off of 19 mm, had the lowest predictive performance for PTB at 7-

and 14-days intervals after its measurement.

A combined algorithm that comprised PartoSure, fFN and cervical length measurements achieved the best predictive performance for the prediction of PTB at 14 days.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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D. Nemescu, A. Carauleanu and Luiza-Maria Cobzeanu had equal contribution to the first and correspondent authors and should be regarded as main authors of the manuscript.

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CONVENTIONAL *VERSUS* ENDOVENOUS TREATMENT IN CHRONIC VENOUS DISEASE - A COMPARATIVE STUDY BASED ON QUALITY OF LIFE

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CONVENTIONAL VERSUS ENDOVENOUS TREATMENT IN CHRONIC VENOUS DISEASE - A COMPARATIVE STUDY BASED ON QUALITY OF LIFE (Abstract): Chronic venous disease (CVD) modern treatment has to meet efficiency criteria, minimal invasiveness, aesthetical criteria, has to be used in both primary and recurrent treatment and allows the patient to resume normal activities as soon as possible. Alongside the proven effectiveness of the endovascular technique using LASER thermal ablation, a constant concern remains the benefit of improving the patient's quality of life. The patient perceives the aggressiveness of the procedure and the post-procedural recovery differently when treated through conventional method than through endovenous procedures. **Material and methods:** In the present paper we resume a comparison between the surgical treatments of chronic venous disease, conventional versus endovenous LASER thermal ablation. A prospective study was performed on two groups with equal number of patients and similar clinical staging. VEINES and CIVIQ-20 questionnaires were applied pre- and postoperatively (10 days, one month and two months intervals). **Results:** The Global Score Index calculated for all patients preoperatively and postoperatively showed increases in each of the 3 postoperative assessments, proving the therapeutic indication and efficacy of both surgical procedures. **Conclusions:** The recording of higher values for patients treated with endovenous procedure compared to patients treated with the traditional procedure support the hypothesis of this study that quality of life is higher in the group treated with endovenous LASER therapy. **Keywords:** QUESTIONNAIRES, VENOUS DISEASE, CONVENTIONAL VERSUS ENDOVENOUS.

Chronic venous disease (CVD) is a common clinical problem which has a significant impact on patient quality of life (Qol) (1). Reflux in the great saphenous vein (GSV) is the most frequent causes of primary CVD. Saphenofemoral ligation and

stripping of the GSV is considered to be the standard procedure for GSV incompetence with high rate of success and the low rate of recurrence. Although efficient, the conventional procedure is associated with considerable morbidity (2). Among common complications relevant for the impact on the Qol are postoperative pain, paresthesia due to saphenous nerve injury, hematoma and bruising.

Modern CVD treatment must meet several criteria: to be minimally invasive, to be aesthetic, to be effective in the treating the reflux and its consequences, to be effective in recurrences treatment, to allows the resumption of the patient's usual activities in the shortest possible time.

New endovenous procedures have proved their efficacy. Among this procedures endovenous thermal ablation has comparable efficiency with conventional treatment (3, 4, 5).

MATERIAL AND METHODS

The aim of this prospective study was to compare the improvement in Qol in patients undergoing great saphenous vein (GSV) ligation and stripping versus endovenous LASER thermal ablation. Included in this study were 60 patients, divided into 2 equal groups. The patients, with primary varicose veins and GSV incompetence, coming from 2 different vascular surgery units (Department of Vascular Surgery of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital and Epimedica Clinic) were enrolled after informed consent. They already had surgical treatment indication and previously established type of procedure, either endovenous or conventional. Patients underwent Duplex evaluation. Patients with a tortuous GSV or GSV diameter exceeding 10 mm were not included. Each enrolled patient would have been suitable for both procedures and the choice between them belonged to the patient.

The groups included patients with similar demographic (sex distribution, age median, provenience) and medical characteristics (associated pathologies, clinical stages of CVD). All the patients had the same pre- and postoperative recommendations (medication and compressive stockings).

Qol was assessed prior to surgery and for several different intervals over 2 months postoperatively using 2 questionnaires, CIVIQ-20 and VEINES Qol/Sym (6, 7).

CIVIQ-20 questionnaire included 20 questions covering the patient evaluation on 4 aspects: pain characteristics, physical assessment, psychological assessment, social implications. Every item had 5 answer options resulting in 20 items. The final score ranges from 20 to 100 and it is used to calculate an index (GIS index) quantifying the Qol. GIS index stands for: the difference between the final score obtained and the minimum possible score divided by the difference between the maximum possible score and the minimum possible score, all multiplied by 100.

*GIS = 100 - [(final score - 20)/80] * 100*

The other used questionnaire was VEINES Qol/Sym, a specific questionnaire adapted to CVD. This included 26 items regarding CVD symptoms (10 items), CVD related limitations in daily activities (9 items), psychological impact (5 items), assessment of the CVD progression in the last year (1 item), period of the day when the pain intensifies (1 item) (8).

RESULTS

Patient general characteristics are summarized in table I.

Clinical aspects are shown in table II.

TABLE I.

Patient general information

Patient characteristics		Study group (60 ps)	Conventional procedure (30 ps)	Endovenous procedure (30 ps)
Sex				
	M	20%	0%	12 (40%)
	F	80%	30 (100%)	18 (60%)
Age				
	median	51 years	54 years	47 years
Provenience				
	R	24 (40%)	15 (50%)	9 (30%)
	U	36 (60%)	15 (50%)	21 (70%)
Affected lower limb				
	unilateral	33 (55%)	17 (56.67%)	16 (53.33%)
	bilateral	27 (45%)	13 (43.33%)	14 (46.67%)
Previous venotonic medication		45 (75%)	26 (86.66)	16 (53.33%)
Previous compression stockings wearing		35 (58.33%)	14 (46.67%)	11 (36.67%)

TABLE II.

Clinical classification

Clinical classification	Study group (60 ps)	Conventional procedure (30 ps)	Endovenous procedure (30 ps)
C0	0	0	0
C1	0	0	0
C2	9 (15.00%)	3 (10.00%)	6 (20.00%)
C3	31 (51.66 %)	17 (56.67%)	14 (46.67%)
C4	6 (10.00%)	3 (10.00%)	3 (10.00 %)
C5	8 (13.33%)	3 (10.00%)	5 (16.67%)
C6	6 (10.00%)	4 (13.33%)	2 (6.67%)

Majority of patients were female, with unilateral affected limb, in C3 clinical class, previously on venotonic medication (tabs. I, II).

For both groups we evaluated the CIVIQ-20 score and VEINES Qol/Sym at specific periods of time: before the procedure, after the procedure at 10 days, 1 and 2 months. The results are as following.

The average CIVIQ-20 score for the entire lot, calculated to evaluate the Qol before the procedures was 61.19 points. The score evaluated 10 days after the procedures dropped to 53.15, due to postprocedural discomfort. The postprocedural score range was 22.5-96.25 points. The higher the score, the better the patient perceived the Qol. A significant improvement was noticed at 1 and respective 2 months after the procedure (67.44 points, respective 75.59 points) (fig.1).

When we compared the improvement of Qol between groups, we noticed that the average GIS score at each postoperative evaluation timing was higher for the endovenous group (fig. 2). There was no significant difference between the preoperatory evaluation of the groups, certifying the

groups were comparable. Patients had similar perception over the preprocedural Qol. The comparison data that proved to differ significantly between groups were the scores calculated at 30 days and 2 months ($p<0.05$) (tab. III).

Fig. 1. GIS score on the study group

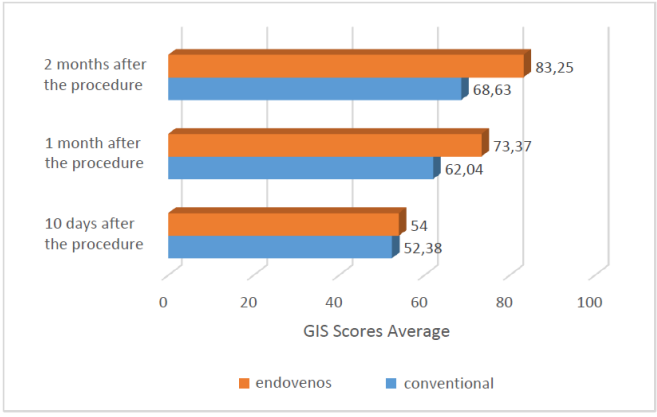

Fig. 2. Comparison between endovenous and conventional average GIS scores

TABLE II.

GIS score comparison between endovenous and conventional

	GIS score before the procedure		GIS score 10 days after the procedure		GIS score 1 month after the procedure		GIS score 1 months after the procedure	
	C	E	C	E	C	E	C	E
Average	55.90	67.00	52.38	54.00	62.04	73.37	68.63	83.25
Standard deviation	22.00	14.90	20.15	18.68	15.36	14.01	14.19	9.32
Maximum score	88.75	96.25	76.25	81.25	80.00	100.00	88.75	100.00
Minimum score	22.50	48.75	8.75	30.00	31.25	51.25	43.75	72.50
Chi test	p=0.098445		p=0.425792		p=0.047343		p=0.006269	

*C=conventional, E=endovenous LASER ablation

Similar to CIVIQ-20 score, the overall analysis of patient responses on VEINES Qol/Sym questionnaire indicates an improvement in postoperative quality of life by achieving higher scores (fig. 3).

The average VEINES Qol/Sym score before the procedures was 54.286 points and remained almost unchanged 10 days after the procedures (55.19 points). After 1 and respective 2 months the scores increased significantly (67.667 points, respective 71.33 points). Endovenous group patients scored higher also in VEINES Qol/Sym ($p<0.05$) (fig. 4).

If we compare the results in both types of questionnaire we find similarities in preprocedural evaluation and the postprocedural data trend (figs. 1, 2, 3, 4).

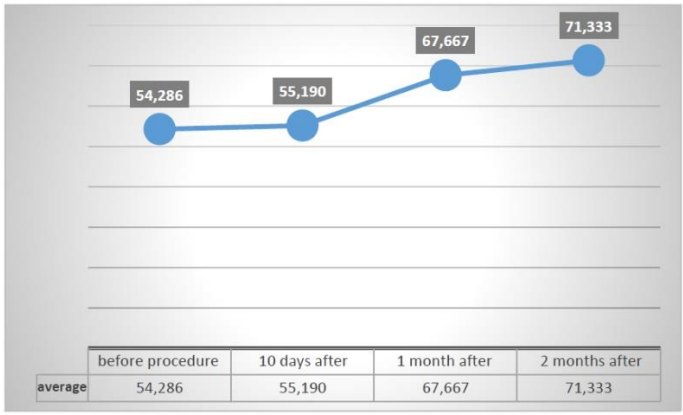

Fig. 3. VEINES Qol/Sym score on the study group

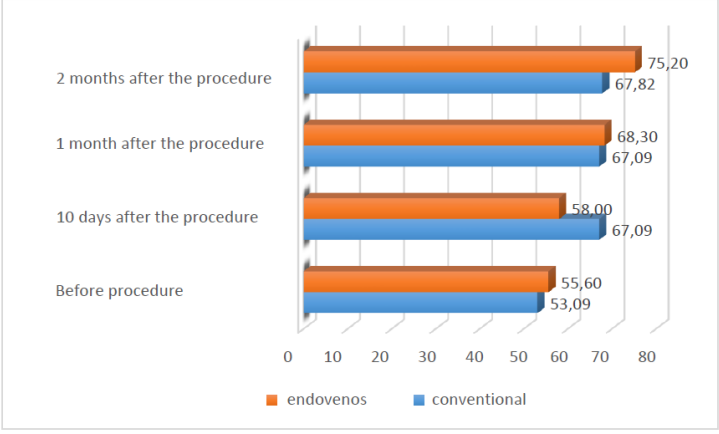

Fig. 4. Average VEINES Qol/Sym score-comparison between endovenous and conventional

DISCUSSION

Enrolling patients both from the private clinic and the hospital allowed us to form 2 comparable groups without taking into consideration the financial aspects that might influence the patients in deciding

between the types of procedures. Patients included in the study had previously established indication of treatment and were suitable for both procedures, conventional or endovenous. Groups characteristics were similar. According to CEAP classification, patient included in clinical class 3 were predominant in both groups.

Lower preoperatively scores are interpreted in the context of CVD symptoms. A correlation between the clinical class and the Qol remain to be investigated. Higher score recorded after any of the procedures prove the efficiency in relieving symptoms for both treatment techniques. A number of studies that refer to patients Qol, including a Cochrane review, concluded that both therapies led to improvement. The present study advocates the same. Recurrence of venous reflux, adverse effects, occurrence of hematomas, neo-vascularization, ulcers, persistence of ecchymosis, recorded lower occurrence rates in patients treated through endovenous therapy (9). This aspect supports the results of the study, which show a significant increase in Qol scores.

Endovenous self-assess higher postoperative scores, with faster reintegration into daily activities is supported through this study and was addressed by several other studies. A retrospective study involving 182 patients who underwent endovenous or conventional surgery, concluded that patients were more satisfied after endovenous procedures. Symptoms of CVD reappeared more often in those who underwent conventional surgery. Also, those who underwent endovenous intervention were shown to have their health condition improved (10).

Evaluating the Qol through questionnaires can be suspected of subjectivity, but by getting similar results through 2 different questionnaires validates the results.

It is important to continue the study after 1 year to see if different perception upon the Qol modifies with time. The presumption is that after a longer period the differences between the 2 procedures are no longer significant.

CONCLUSIONS

CVD is a frequent pathology, with significant impairment on the Qol. Interventions for reflux in the great saphenous vein result in significant clinical improvement, increases the Qol, and may be associated with economic benefits.

In this study, compared CVD treatment procedures proved efficient in improving the Qol. The improvement was obvious and significantly higher in the case of endovenous treated patients in comparison with conventional treated patients. This advocates for better results, with faster recovery, less intense postoperative pain, faster social reintegration for patients treated through endovenous procedure. Conventional surgical treatment is effective and improves the quality of life, but due to the invasiveness of the procedure and the long duration of postoperative recovery, it registers lower postoperative scores

In a rapidly evolving field, non-thermal, non-tumescent options such as mechanochemical ablation or cyanoacrylate adhesive closure are the latest advances and may improve the Qol even more.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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ENDOPHILIN A3 IMMUNOHISTOCHEMICAL HETEROGENEITY IN PANCREATIC DUCTAL ADENOCARCINOMA

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ENDOPHILIN A3 IMMUNOHISTOCHEMICAL HETEROGENEITY IN PANCREATIC DUCTAL ADENOCARCINOMA (abstract): **Background and aim**: Pancreatic ductal adenocarcinoma (PDAC) represents a malignant proliferation with minimally improved survival in last decades that presents systematic changes in classification framework based on new molecular and morphological data. Endophilin A3 (EndoA3), a protein involved in non-conventional endocytosis that was recently integrated in modulation of tumor cell adhesion molecule CD166/ALCAM, has not been evaluated in PDAC. **Materials and methods**: EndoA3 immunoreaction were qualitatively and semi-quantitatively analyzed in 87 cases of PDAC. Statically association between classic PDAC prognostic factors and Endophilin A3 immunohistochemical profile were evaluated. **Results:** Data analysis revealed that EndoA3 immunoprofile is associated with tumor degree of differentiation ($p = 0.01$). For the others microscopic prognostic factors there were no significant statistical correlations. Although not statistically significant, patients with high EndoA3 score presented the lowest survival. **Conclusions**: Endophilin A3 expressed in PDAC presents a cytoplasmic, diffuse or granular, tumor staining, but also a stromal staining. High EndoA3 immunoexpression appears to be associated with a negative prognosis in PDAC. **Keywords**: PANCREATIC DUCTAL ADENOCARCINOMA, ENDOPHILIN A3, IMMUNOHISTOCHEMISTRY.

The histopathological evaluation of pancreatic ductal adenocarcinoma (PDAC) directly impacts on the prognosis. According to the latest World Health Organization (WHO) classification, the confirmed prognostic clinicopathological factors for PDAC are: tumor stage, by tumor size and lymph node invasion, resection margin, degree of differentiation, mitotic index, cellular atypia, lympho-vascular and perineural invasion (1). Additionally, other parameters are also considered as prognostic factors: obesity, race, metastatic location, CA19-9 marker value and SMAD4 gene mutation status (1). Nowadays, the research focused on pancreatic malignant proliferation aims to identify new prognostic and predictive factors, defined histologically and at molecular level. These factors reflect the mechanism of carcinogenesis, being correlated with the progression of pancreatic malignant proliferations (2). Therefore, the prognostic factors panel confirmed by WHO is currently completed by the following histological and serological parameters with prognostic potential:

desmoplastic reaction, tumor budding, circulating tumor cells, Glypican positive exosomes, inflammatory profile - including cytokine levels, microRNA subtypes and non-coding RNA (2).

Endophilin A3 (EndoA3), also called SH3GL3 or EEN, is part of the endophilin family, acting in endocytosis and cell signaling processes (3). In cancer biology, information regarding EndoA3 immunoexpression is conflicting, being associated with negative prognosis in colon carcinogenesis (4), and with favorable prognosis, as a tumor suppressor, in lung cancer (5).

The contribution of clathrin-independent endocytosis in metastatic process particularly involves the migration and invasion step, in connection with retrograde trafficking and cell polarity (6, 7). The EndoA3 mediated endocytosis initiated by Galectin8, that operates through a possible glycolipid-lectin mechanism, represents a method of modulation for tumor marker CD166/ALCAM cell surface expression, consequently interfering with cell adhesion using this non-conventional clathrin-independent pathway (7, 8). In PDAC, the recently described endocytic machinery involvement in cellular trafficking is represented by the clathrin-dependent route, that reports also a potential use for extracellular vesicles as a potential chemotherapeutic transporter (9), without the intervention of the clathrin-independent pathway. In this general context of knowledge, the main stream publications do not include data on the immunoexpression and prognostic role of EndoA3 in pancreatic tumors.

Therefore, our study aimed to investigate the EndoA3 immunoprofile in PDAC, in correlation with the main clinicopathological characteristics and survival.

MATERIALS AND METHODS

The study group comprised 90 patients admitted at the “Sf. Spiridon” County Clinical Emergency Hospital Iasi during a period of ten years (2007-2017), clinically diagnosed with malignant pancreatic tumors. All patients underwent surgical treatment, and the histopathological exam revealed, in 88 cases, a specific morphology sustaining the diagnosis of PDAC. One of these cases was rule out because of partial clinicopathological information. Consequently, our final study group included 87 cases, of which 41 were men (47.13%) and 46 were women (52.87%), with an average age of 60.77 years. The research was approved by the Research Ethics Committees of the “Sf. Spiridon” County Clinical Emergency Hospital Iasi and the “Grigore T. Popa” University of Medicine and Pharmacy of Iasi, respectively.

The retrospective research was based on: (i) the clinicopathological information obtained from the Pathology Department; (ii) the survival data achieved by the clinical follow-up; (iii) the reassessment of all specimens accordingly with the diagnosis criteria recommended by the American Joint Committee of Cancer (10) and the last WHO classification of pancreatic malignancies (1); (iv) the immunohistochemical (IHC) exam for EndoA3 (anti-EndoA3, rabbit polyclonal, PA5-85413, dilution 1/1000, Thermofisher Invitrogen, Waltham, MA, SUA) made on archived paraffin-embedded blocks corresponding to each patient.

Clinicopathologically, the tumor stage was pT1 in 9 patients (10.34%), pT2 in 46 patients (52.87%), pT3 in 30 patients (34.48%), and pT4 in 2 patients (2.29%), the lymph node involvement revealed pN0 in 24 cases (27.58%), pN1 in 43 cases (49.42%)

and pN2 in 20 cases (22.98%), and the TNM stage was established as low for 23 patients (26.43%) and high for 64 patients (73.56%); 19 cases (21.83%) were well-differentiated, 60 cases (68.96%) – moderately differentiated and 8 cases (9.19%) – poorly differentiated; the lympho-vascular invasion was confirmed in 60 cases (68.96%), and the perineural tumor extension was present in 81 cases (93.10%). The clinical follow-up indicated a mean overall survival (OS) of 18.92 months.

The reassessment of specimens allowed us to complete the pathological diagnosis with supplementary data regarding the subtypes and patterns present in each case (11, 12), leading to the following two classifications of cases: conventional PDAC in 57 cases (65.51%) and non-conventional PDAC in 30 cases (34.48%), or glandular PDAC in 50 cases (57.47%) and non-glandular PDAC in 38 cases (43.67%). Several patterns (common in 75 cases, foamy cell in 23 cases, cribriform in 18 cases, large-duct in 12 cases, basal/composite in 13 cases, micropapillary in 7 cases) were identified, defining a homogeneous picture in 40 patients (45.97%) and a heterogeneous one in 47 patients (54.03%).

The IHC exam was validated only for 75 cases. The immunoexpression of EndoA3 was semi-quantitatively evaluated by adapting a scoring system (13), taking into consideration the intensity of immunostaining (I) and the percentage of tumor cells (P). The I assessment led to classification into 4 classes: 0 = no reaction; 1 (+) = weak reaction; 2 (++) = moderate reaction; 3(+++) = strong reaction. For the P assessment, thresholds of 33% and 66% were used, with the following correspondence in the scoring system: 0 = absent; 1-33% = score 1; 34-66% = score 2; 67-100% = score 3. The final score resulted as I x P product, with values between 0 and 9, grouped in the following classes: 0 - negative score; 1-3 – low score; 4-6 – medium score; 7-9 – high score. In addition to this scoring system, EndoA3 immunoexpression was defined as absent (completely negative) and present (not reported at the intensity level).

The statistical analysis was performed for the 75 cases with validated EndoA3 immunoexpression, by using *SPSS version 20* (IBM, Armonk, NY, USA) and Microsoft Excel 2016 (Microsoft, Redmond, WA, USA) and applying χ^2 or Fisher's tests for the clinicopathological correlations, log rank test and Kaplan-Meyer curves for the survival analysis and Cox univariate regression for the prognostic significance.

RESULTS

Qualitative assessment

Comparing the EndoA3 immunoreactivity present in the testis (fig. 1A), used for the validation of IHC exam, with that identified in normal pancreatic parenchyma (fig. 1B), we found acinar immunostaining (cytoplasmic, weak intensity, inconsistent), Langerhans islet immunostaining (cytoplasmic, strong intensity), as well as a positive immunoreaction in intralobular ducts (cytoplasmic, weak intensity, homogeneous). In the medium-sized extralobular ducts we noted a brush-border-like cytoplasmic condensation at the apical domain, of higher intensity than the cytoplasmic one (fig. 1C); the islet of Langerhans showed the highest intensity among the components of the normal pancreatic parenchyma, similar to the intensity of the neuronal bodies from peripheral nervous system (fig. 1B-D).

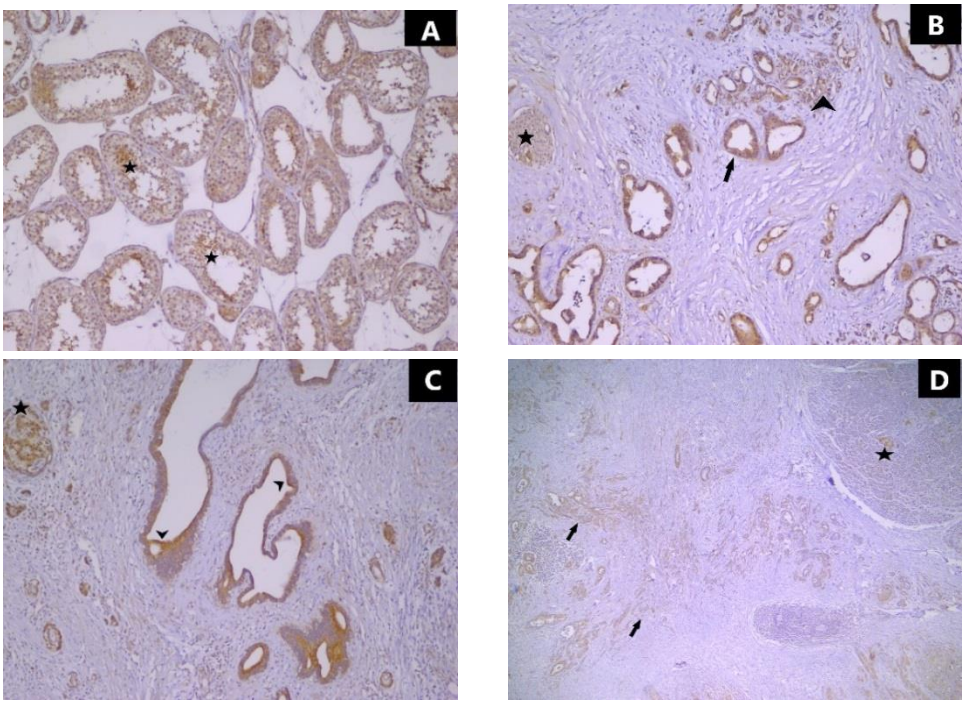

Fig. 1. EndoA3 immunostaining validation elements in testis and pancreatic parenchyma; stars – strong EndoA3 intensity in late spermatids (A), neuronal bodies (B), Langerhans islet (C, D); arrowheads - brush-border-like condensation at the apical domain; arrows – PDAC with homogeneous diffuse strong cytoplasmic EndoA3 immunostaining (B) and heterogeneous cytoplasmic with strong, moderate and weak intensity (D); IHC, anti-EndoA3, x10 (A-C), x4 (D).

The EndoA3 immunoreaction in tumor proliferation was positive in 59 cases (78.66%) and negative in 16 cases (21.33%) (fig. 2A-D). Beside epithelial compartment immuno-reactivity, EndoA3 immunoreaction was identified also in peritumoral stroma (fibroblasts with circular peri glandular distribution) (fig. 2B), being positive in 18 cases (24%), with the following particularities: in two cases, exclusively stromal expression (11.11% cases with stromal expression, 2.66% of the study group), while the other 16 cases presented dual expression, in tumor cells and in peritumoral stroma (88.88% cases with stromal expression, 21.33% of the study group).

The evaluation of EndoA3 in accordance with the histological subtype and morphological patterns identified in ductal proliferations revealed the following aspects: conventional/common areas showed predominantly diffuse, homogeneous (fig. 2A), and focal granular cytoplasmic immunolabeling (fig. 2B); foamy cell and large duct areas were predominantly negative (fig. 2C); cribriform and micropapillary areas showed medium intensity cytoplasmic immunolabeling; poorly differentiated areas showed heterogeneous cytoplasmic immunolabeling (fig. 1D).

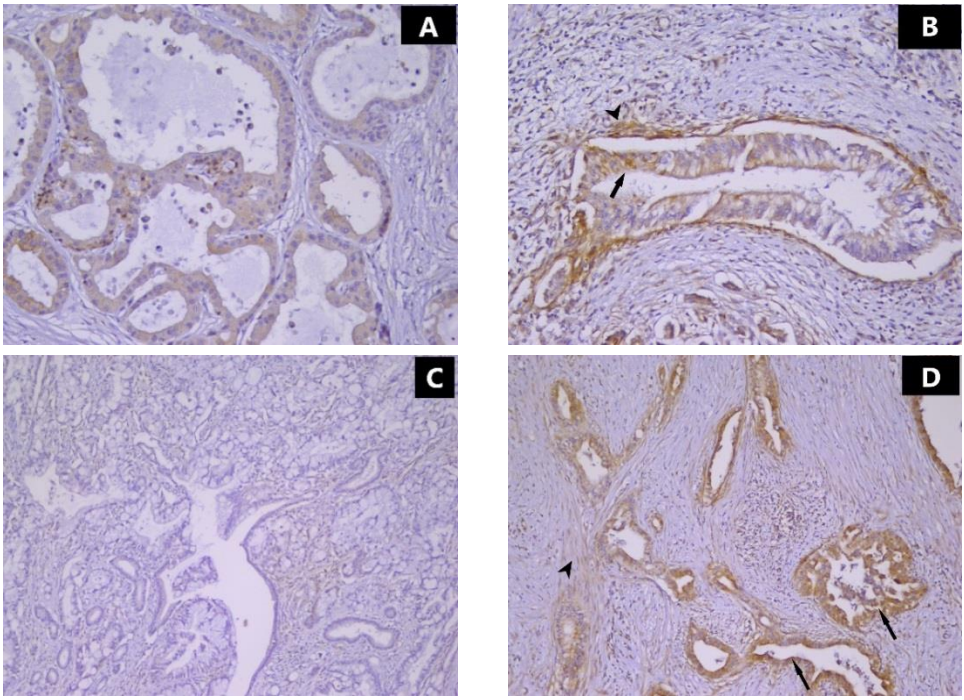

Fig. 2. EndoA3 immunostaining variability in PDAC: tumoral glands – weak diffuse cytoplasmic stain (A), dual stain with stromal – arrowheads, and moderate focal granular cytoplasmic – arrows (B), negative/absent immunoreaction (C), dual stain with stromal weak stromal stain – arrowheads and strong cytoplasmic diffuse and granular stain (D). IHC, anti-EndoA3, x20 (A, B), x10 (C, D).

Evaluation of EndoA3 immunoexpression in lymph node metastases associated with the primary tumor was performed for 36 cases (65.06% of cases with lymph node metastases), being similar to that identified in PDAC.

Semiquantitative assessment

The semiquantitative evaluation of EndoA3, using the scoring system based on immunoreaction intensity (I) and percentage of positive tumor cells (P), led to the stratification into 4 classes of immunoexpression: negative score (16 cases – 21.33%), low score (28 cases - 37.33%), medium score (13 cases – 17.33%) and high score (18 cases – 24%).

Clinicopathological correlations

The statistical analysis revealed statistically significant differences ($p = 0.01$) between the degree of tumor differentiation and EndoA3 profile, defined in 4 classes of immunoexpression by negative score, low score, medium score and high score (tab. I).

Analysis of the degree of tumor differentiation (G) in relation to EndoA3 immunoscore revealed the association between well-differentiated tumors and negative EndoA3 score (8 cases, 50% of well-differentiated tumors), between moderately differentiated tumors and low EndoA3 score (21 cases, 75%), medium EndoA3 score (9 cases, 69.23%) and high EndoA3

score (16 cases, 88.88%).

In addition, considering EndoA3 immunoexpression as absent versus present, statistical analysis also revealed significant association with the tumor differentiation ($p = 0.001$). No statistically significant correlations were obtained for other classical clinicopathological parameters ($p > 0.05$) (tab. I).

TABLE I.

Correlations between clinicopathological features and EndoA3 immunoexpression

Clinical pathological characteristics	EndoA3 #, %				p	EndoA3 #, %		p
	Negative score # total = 16	Low score # total = 28	Medium score # total = 13	High score # total = 18		absent # total = 16	present # total = 59	
Gender								
Female	8 (50%)	16 (57.14%)	5 (38.46%)	11 (61.11%)	0.60	8 (50%)	32 (54.23%)	0.76
Male	8 (50%)	12 (42.85%)	8 (61.53%)	7 (38.88%)		8 (50%)	27 (45.76%)	
Age (years)								
≤ 50	1 (6.25%)	4 (14,28%)	4 (30.76%)	1 (5,55%)	0.53	1 (6.25%)	9 (15.25%)	0.37
50-70	13 (81.25%)	16 (57.14%)	6 (46.15%)	15 (83.33%)		13 (81.25%)	37 (62.71%)	
>70	2 (12.5%)	8 (28.57%)	3 (23.07%)	2 (15.38%)		2 (12.5%)	13 (22.03%)	
PDAC size								
≤ 4 cm (pT1+ pT2)	11 (68.75%)	16 (57.14%)	5 (38.46%)	14 (77.77%)	0.13	11 (68.75%)	35 (59.32%)	0.49
>4 cm (pT3 + pT4)	5 (31.25%)	12 (42.85%)	8 (61.53%)	4 (22.22%)		5 (31.25%)	24 (40.67%)	
T Stage								
T1	4 (25%)	1 (3.57%)	2 (15.38%)	2 (11.11%)	0,60	4 (25%)	5 (8,47%)	0.19
T2	7 (43.75%)	15 (53.57%)	3 (23.07%)	12 (66.66%)		7 (43.75%)	30 (50.84%)	
T3	4 (25%)	11 (39.28%)	8 (61.53%)	4 (22.22%)		4 (25%)	23 (38.98%)	
T4	1 (6,25%)	1 (3.57%)	0 (0%)	0 (0%)		1 (6.25%)	1 (1.69%)	
N Stage								
N0	5 (31.25%)	9 (32.14%)	3 (23.07%)	1 (5.55%)	0.51	5 (31.25%)	11 (0.18%)	0.44
N1	7 (43.75%)	13 (46.42%)	7 (84.61%)	11 (61.11%)		7 (43.75%)	35 (59.32%)	
N2	4 (25%)	6 (21.42%)	3 (23.07%)	6 (33.33%)		4 (25%)	18 (30.50%)	

Clinical pathological characteristics	EndoA3 #, %				p	EndoA3 #, %		p
	Negative score # total = 16	Low score # total = 28	Medium score # total = 13	High score # total = 18		absent # total = 16	present # total = 59	
Tumor grade (G)								
G	8 (50%)	5 (17.85%)	4 (30.76%)	1 (5.55%)	0.001	8 (50%)	10 (16.94%)	0.001
G2	5 (31.25%)	21 (75%)	9 (69.23%)	16 (88.88%)		5 (31.25%)	46 (77.96%)	
G3	3 (18.75%)	2 (7.14%)	0 (0%)	1 (5.55%)		3 (18.75%)	3 (5.08%)	
Lympho-vascular (LV) and perineural (Pn) invasion								
Lv0	7 (43.75%)	8 (28.57%)	2 (15.38%)	4 (22.22%)	0.38	7 (43.75%)	14 (23.72%)	0.11
Lv1	9 (56.25%)	20 (71.42%)	11 (84.61%)	14 (77.77%)		9 (56.25%)	45 (76.27%)	
Pn0	1 (6.25%)	2 (7.14%)	2 (15.38%)	0 (0%)	0.37	1 (6.25%)	4 (6.77%)	0.93
Pn1	15 (93.75%)	26 (92.85%)	11 (84.61%)	18 (100%)		15 (93.75%)	55 (93.22%)	
Histopathological subtype								
PDAC conventional	11 (68.75%)	19 (67.85%)	6 (46.15%)	13 (72.22%)	0.44	11 (68.75%)	38 (64.40%)	0.29
PDAC non-conventional	5 (31.25%)	9 (32.14%)	7 (53.84%)	5 (27.77%)		5 (31.25%)	16 (35.59%)	
PDAC glandular type A	11 (68.75%)	16 (57.14%)	6 (46.15%)	10 (55.55%)	0.67	11 (68.75%)	32 (54.23%)	0.29
PDAC non-glandular type B	5 (31.25%)	12 (42.85%)	7 (53.84%)	8 (44.44%)		5 (31.25%)	27 (46.76%)	
TNM stage								
Low (Ia, Ib, IIa)	5 (31.25%)	8 (28.57%)	3 (23.07%)	1 (5.55%)	0,20	5 (31.25%)	12 (20.33%)	0.35
High (IIb, III, IV	11 (68.75%)	20 (71.42%)	10 (76.92%)	17 (94.44%)		11 (68.75%)	47 (79.66%)	

The mean OS for patients with negative EndoA3 score was 19.56 months, for patients with low EndoA3 score – 21.10 months, for patients with medium EndoA3 score – 22.92 months, and for patients with high EndoA3 score – 13.5 months.

The Kaplan-Meier analysis revealed lack of statistically significant differences between the survival of patients clustered into the 4 score classes ($p = 0.32$) (fig. 3A).

For cases divided according to the absence versus presence of EndoA3 immunoexpression, the mean OS in the EndoA3-negative group was 19.56 months, and for the EndoA3-present group – 19.18 months. The Kaplan-Meier analysis showed also lack of significant differences between survival of patients grouped in relation to EndoA3 immunoreactivity as absent versus present (p = 0.69) (fig. 3B).

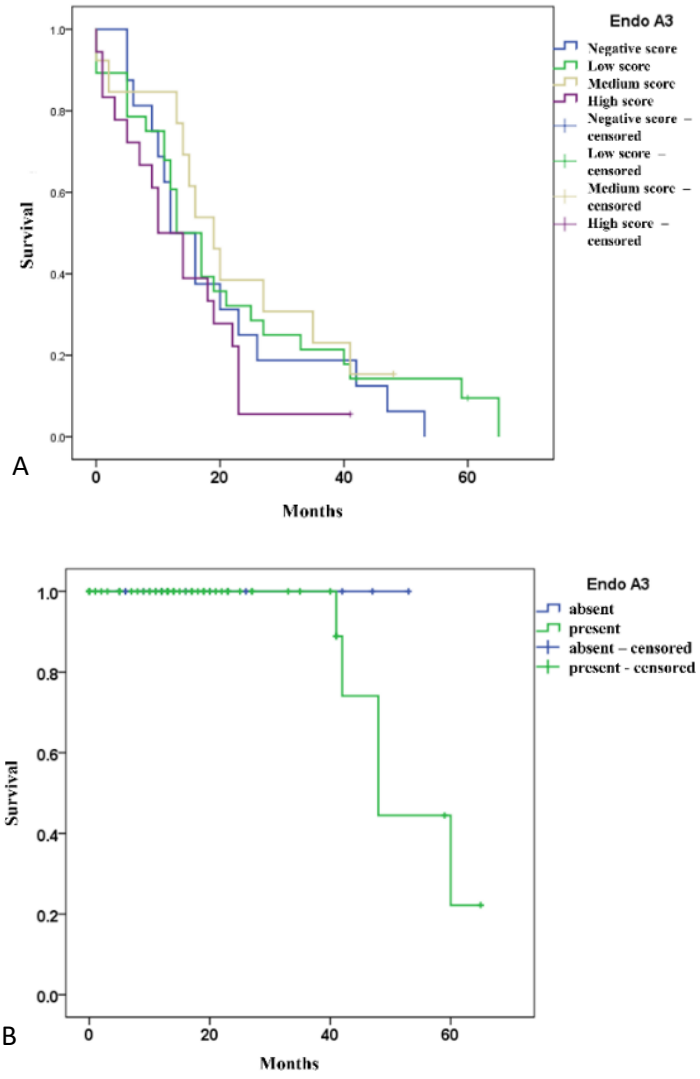

Fig. 3. Kaplan-Meyer survival curves based on EndoA3 immunoexpression assessed semiquantitative (A) and qualitatively (B).

DISCUSSION

Endophilin A3, part of the SH3-domain protein family, was originally described in the brain and testis (3). The endophilin A family includes 3 isoforms, A1, A2 and A3, which are encoded by similar genes and are

associated with the endocytosis process (3, 14). All endophilin isoforms are involved in carcinogenesis, the most studied being A1 and A2 - considered tumor suppressors as they are considerably reduced in metastatic tumors. EndoA3 is the least studied isoform (3). Data available on EndoA3 in the tumoral context reveal different functions depending on the domain involved (4, 5).

In tumor biology, EndoA3 expression has been reported both as a negative prognostic factor (4), being involved in cell proliferation and migration (8, 15), and as a positive prognostic factor, acting as a tumor suppressor, loss of EndoA3 immunoexpression being correlated with advanced tumor stages (5). In oral squamous cell carcinoma, low EndoA3 expression was correlated with the presence of lymph node metastasis (3). EndoA3 involvement in carcinogenesis appears to be dependent on tumor type, with changes at both the gene and protein level (4, 16).

Based on the limited data about the EndoA3 IHC profile in digestive tumor pathology, the relationship between EndoA3 and pancreatic ductal carcinogenesis is being virtually unaddressed. To the best of our knowledge, this is the first study to report EndoA3 immunoexpression pattern and clinicopathological correlations in PDAC.

Subcellular localization for endophilin A indicates cytoplasmic variability with diffuse or punctiform pattern, but also membrane recruitment (3). In colorectal carcinoma, the connection between EndoA3 and endocytic activity analyzed by immunofluorescence revealed a punctiform membrane expression pattern that was associated with the presence of EndoA3 in the cell membrane and was correlated with proliferation process, while the diffuse cytoplasmic expression pattern was associated with loss of EndoA3 from the cell membrane and correlated with a higher cell migration rate (4). In line with this, in our study, we observed heterogeneous immunoexpression in normal pancreatic tissue, with immunolabeling differences in intralobular ducts, which showed homogeneous immunoreactivity of low intensity, whereas in middle extralobular ducts cytoplasmic immunoreactivity was coupled with a brush-border-like condensation at the apical domain, with higher intensity than cytoplasmic. We point out that the standardization of the IHC reaction was performed according to existing literature data on immunolabeling present in testicular germline spermatids (3), in parallel with normal pancreatic tissue resulting internal controls, represented by the islet of Langerhans and neuronal bodies, considered also baseline or standard for the strong intensity of the reaction.

In PDAC, immunolabelling of positive cases (approximately 80% of the study group) was identified both in the epithelial (glandular) component and in the stromal compartment, in the immediate vicinity of the glandular tumor elements. Lymph node metastases showed a similar EndoA3 immunolabel as in the primary tumor, which in our opinion suggests that this molecule does not influence the extent of metastatic process.

At the tumor epithelial compartment level, based on the morphological heterogeneity of PDAC defined by patterns, En-

doA3 immunoexpression was heterogeneous, with subtle subcellular localization characteristics, being identified in homogeneous cytoplasmic variant for cribriform, common conventional or poorly differentiated areas, but also granular or punctiform cytoplasmic variant in common conventional and poorly differentiated areas. Foamy cell and large duct patterns were predominantly negative.

The overlap of EndoA3 immunoexpression with morphological patterns is confirmed by correlation with the degree of tumor differentiation (G), both in the semi-quantitative ($p = 0.01$) and qualitative (present/absent) ($p = 0.001$) assessment variants. According to the obtained results, the absence of Endo A3 is associated with well-differentiated G1 tumors, while the presence, respectively the high EndoA3 score class is associated with less differentiated G2-G3 tumors.

Although associated with tumor grade, a classic prognostic factor in PDAC, in our study EndoA3 immunoexpression was not associated with survival. However, the results for mean OS of patients in relation to semi-quantitative assessment indicate a possible involvement in the development of subtypes with poor prognosis. Patients with high EndoA3 score had the lowest overall survival (13.5 months), patients with negative score had about 5 months longer survival, while patients with low and medium score had better survival, about 9 months longer (22 months). A possible explanation for the lack of significant statistical correlations could be the relatively small number of patients included in the study group unevenly distributed in classes. However, the differences in survival may represent a clue supporting the involvement of EndoA3 in PDAC carcinogenesis, most likely in association with other molecules, possibly the already stated PDAC prognostic marker CD166/ALCAM (17, 18, 19).

CONCLUSIONS

Our study demonstrates the EndoA3 heterogeneity in PDAC, with cytoplasmic, diffuse or granular tumor staining, but also stromal staining. High EndoA3 immunoexpression appears to be associated with a negative prognosis.

CONFLICT OF INTEREST AND FUNDING

The authors declare that they have no conflict of interest. This research received no funding.

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EFFICACY AND SAFETY OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY (ESWL) IN PIELIC STONES AND INFECTED HYDRONEPHROSIS AFTER DOUBLE-J STENT INSERTION

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EFFICACY AND SAFETY OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY (ESWL) IN PIELIC STONES AND INFECTED HYDRONEPHROSIS AFTER DOUBLE-J STENT INSERTION (Abstract): Extracorporeal shockwave lithotripsy (ESWL), along with flexible retrograde ureteroscopy (RIRS), is the treatment of first choice for pelvic stones up to 10 mm, and double-J catheters are not routinely inserted prior to the procedure. However, their insertion is mandatory in patients with infected secondary hydronephrosis. The **aim** of the study was to evaluate the efficacy and safety of ESWL in this particular group of patients compared to patients without double-J ureteral catheters. **Materials and methods:** We conducted a retrospective case-control study of patients with pelvic stones up to 10 mm in whom a double-J ureteral catheter was inserted due to infected hydronephrosis and subsequently underwent ESWL between January 1, 2018 and December 31, 2023. As a control group, we included the patients with pelvic stones without a double-J catheter who underwent ESWL during the same period. We analyzed the demographic data, the stone-free rate and the occurrence of complications in the two patient groups. **Results:** We identified a number of 46 patients with double-J catheters and a number of 118 patients without double-J catheters who underwent ESWL for pelvic stones up to 10 mm. Patients in both groups were over 46 years of age, were predominantly male and had a body mass index (BMI) between 25 and 29.9. There were no statistically significant differences between the 2 groups in terms of stone-free rate, both after the first (78.3% *vs.* 73.7%, p=0.866), second (89.58% *vs.* 89.8%) and third ESWL sessions (93.7% *vs.* 95.7%, p=0.583). The complication rate was reduced in both groups, with no statistically significant differences (14.6% *vs.* 6.77%, p=0.324). **Conclusions:** The presence of double-J ureteral catheters inserted due to infected hydronephrosis did not reduce the efficacy of ESWL in patients with pelvic stones up to 10 mm. In this patient group, ESWL can be recommended as a first-line treatment alongside RIRS. **Keywords:** PIELIC STONES, INFECTED HYDRONEPHROSIS, ESWL, DOUBLE-J STENT SYNDROME.

According to European practice guidelines (1), extracorporeal shock wave lithotripsy and flexible retrograde ureteroscopy are the treatment methods of first choice

for pelvic stones smaller than 10 mm, with an almost stone-free rate of over 90% (2). Some studies show that prior insertion of a double-J catheter before ESWL would reduce the risk of complications (3, 4) or increase the efficacy (5), others show an equal stone-free rate (6) and even a decrease in the efficacy of the procedure (7), both after the first (8) and after several sessions (9, 10), especially for ureteral stones.

In the case of pelvic stones, prior insertion of the double-J catheter is only indicated for large stones (over 2 cm) with post-procedural risk of renal colic, *stein-strasse*, and ureteroscopy (1). Ureteral stenting prior to ESWL is generally contraindicated in patients with pelvic stones smaller than 10 mm, as the stone-free rate in these patients is high (2). In practice, however, some patients with pelvic stones in the pyeloureteral junction or with obstructive ureteral stones present to the emergency room with infected hydronephrosis. Insertion of a double-J catheter is then a necessity to prevent progression of urosepsis and to cure the additional urinary tract infection associated with antibiotic therapy.

After the obstruction and infection are resolved, patients present with localized pelvic stones and a double-J catheter. In this particular case, it is not known whether ESWL can be an alternative to flexible ureteroscopy, as in the case of patients without a double-J catheter. Therefore, we conducted a retrospective comparative study in which we compared the efficacy of ESWL in patients with pelvic stones less than 10 mm without double-J catheters with patients in whom double-J catheters were inserted due to infected hydronephrosis. We mention that in our clinic double-J catheters are not routinely inserted before ESWL to increase the efficiency of the procedure. As a secondary aim of our research, we analyzed the complications that occurred in patients with double-J catheters, especially since in some of them the catheters were inserted over a period of up to 3 months because 2 or even 3 sessions of ESWL were required to achieve stone-free status.

MATERIALS AND METHODS

We conducted a comparative, retrospective study over a 5-year period in patients admitted to Elytis Hospital between January 1st, 2018 and December 31st, 2023 with pelvic stones up to and including 10 mm, with or without double-J catheters, in whom we performed extracorporeal shock wave lithotripsy. The study was approved by the hospital ethics committee. We invite all patients to sign a general consent allowing us to register healthcare data for retrospective studies (laboratory, demographics, therapy and comorbidities). The patients' data were obtained from the observation forms, surgical records and the hospital's electronic registry. Patients were followed until they reached stone-free status or up to one month after the last ESWL session.

The study group consisted of all patients with radiopaque pelvic stones between 5 and 10 mm inclusive who had double-J catheters inserted at least 3 weeks ago, namely 56 patients. All double-J catheters inserted were 7CH, with a length between 24 and 28 cm. We mention that there were 10 patients who originally had ureteral stones and in whom these were pushed back into the renal pelvis by the insertion of the double-J catheter. All patients had negative urine cultures prior to the ESWL procedure.

This explains why the procedure was

performed 3 weeks after insertion of the double-J catheter to allow resolution of the pyelonephritic process. Patients with radiolucent stones, patients with multiple pelvic or pyelocaliceal lithiasis and patients in whom the double-J catheter was inserted because of hyperalgic renal colic or non-functioning kidneys detected by urography or contrast CT were excluded from the study.

Patients with double-J stent syndrome were treated with tolterodine 2 mg twice daily until the catheter was removed. We defined double-J stent syndrome as the presence of lower urinary tract symptoms (LUTS) and/or the presence of macroscopic hematuria. In the absence of improvement of symptoms, flexible retrograde ureteroscopy was proposed to patients and performed, considering the failure of the ESWL procedure, even if the 3 sessions of ESWL were not performed. All patients in this group received prophylactic antibiotic treatment with one tablet of cefuroxime 0.5 g before the procedure.

To form the control group, all patients with pelvic stones between 5 and 10 mm who were treated during the same period were included in the study. This resulted in a total of 126 patients. Patients with secondary 2nd, 3rd and 4th degree hydronephrosis according to the Onen classification (11) and patients with radiolucent pelvic stones were excluded from the study. We excluded patients with significant secondary hydronephrosis because we assumed that obstruction of the pyeloureteral junction may affect the efficacy of the ESWL procedure and because patients in the double-J catheter group did not have this degree of hydronephrosis. We included patients in the study who had secondary 1st grade hydronephrosis because some patients in the study group also had this grade of hydronephrosis.

The ESWL procedure was performed with a Siemens Sonolith device by 3 urologists using the same technique of shock wave application by the ramping method and the same protocol for patient follow-up. Each patient had a maximum of 3 ESWL sessions 4 weeks apart. This interval was chosen to allow for spontaneous elimination of the fragments (12) and recovery of the renal parenchyma after exposure to the shock waves. After each session, the stone-free status was assessed after 4 weeks. In the presence of stones or remaining fragments larger than 4 mm, ESWL procedure was continued.

For fragments less than 4 mm, the double-J catheter was suppressed in the patients in the study group, and the elimination of all fragments was monitored in all patients studied up to an interval of one month. If they had no remaining fragments one month after the last ESWL session, the procedure was considered successful. In the absence of fragmentation or the presence of fragments of any size still present, the procedure was considered a failure and patients underwent an active follow-up program every 6 months.

For all patients in the 2 groups, we collected demographic data and information related to the diagnosis, including age, gender, BMI, location and size of the stones. Their analysis was necessary because we knew that they could influence the stone-free rate of stones, so if there were bias factors in the comparison analyzed. We compared the ESWL parameters, i.e. the number of sessions, the total number of shock waves applied per patient, the average and maximum energy of the shock waves applied and the total energy applied

to each patient regardless of the number of sessions. We analyzed the stone-free rate in the 2 groups, the type of complications and the total number of complications in the 2 groups.

Statistical analysis. Quantitative variables were described by mean and standard deviation and compared using the t-Student test after analyzing the homogeneity of the batches using the Kolmogorov-Smirnov test. Qualitative variables were described by percentages and compared using the chi-square test or the Fischer test if the values of the variables were less than 5. Stone-free rates were compared after each session by calculating the odds ratio and confidence interval.

RESULTS

During the study period, a total of 182 patients with pelvic stones between 5 and 10 mm underwent ESWL. Of these, 56 patients had a double-J catheter and 126 were without a double-J catheter. From the group of patients with double-J catheters, 4 patients with radiolucent stones, 3 patients in whom the double-J catheter was inserted due to hyperalgic renal colic and 3 patients with multiple pyelocaliceal lithiasis were excluded. The result was a number of 46 patients with double-J catheters who formed the study group. Of the 126 patients without double-J catheters, we excluded 3 patients who had concomitant secondary hydronephrosis of the second, third or fourth degree and 5 patients with radiolucent stones. The result was a number of 118 patients without double-J catheters, which constituted the control group.

The demographic characteristics and information on the size and location of the stones are shown in the first table.

TABLE I.
The characteristics of the two groups

		Double-J catheter group (n=46)	Non-Double-J catheter group (n=118)	Statistical test	*p* value
Age, mean ± SD (median/limits)		49.33±15.0 (49/19-79)	46.24±12.59 (45/19-84)	t-Student	0.183
Gender	Male (No., %)	21 (45.7)	67 (56.8)	Chi2	0.201
	Female (No., %)	25 (21.3)	51 (43.2)	Chi2	0.201
BMI	< 24.9	14 (30.4)	14 (11.9)	Chi2	0.005
	25-29.9	29 (63.0)	102 (86.4)	Chi2	0.001
	> 30	3 (6.5)	2 (1.7)	Chi2	0.107
Size of the calculi (mm), mean ± SD (median/limits)		9.22±1.09 (10/6-10)	9.14±1.23 (10/6-10)	t-Student	0.694
	Right side (No., %)	20 (43.5)	59 (50.0)	Chi2	0.452
	Left side (No., %)	26 (56.5)	59 (50.0)	Chi2	0.452

There were no differences between the two groups in terms of average age at the time of ESWL, gender, size and location of the calculi. Regarding BMI, overweight patients were more numerous in the group without double-J catheter (p=0.001). In both groups, the average age was over 46 years, with men predominating and the majority of patients being overweight (63% and 86.4% respectively). The stones were

on average between 9 and 10 mm in size and were predominantly located on the right side in the study group, while they were the same size in the control group, with no significant differences between the two groups (p=0.452)

The parameters of ESWL in the 2 groups are presented in table II.

TABLE II.
The parameters of ESWL in the 2 groups

		Double-J catheter group (n=46)	Non-Double-J catheter group (n=118)	*p* value for t-Student
Mean no. of sessions ± SD (median/limits)		1.26±0.54 (1/1-3)	1.32±0.58 (1/1-3)	0.538
Total no. of shockwaves, Mean ± SD (median/limits)		2218±306 (2300/1180-2626)	2193±409 (2300/765-3203)	0.707
Energy level	Mean, mean ± SD (median/limits)	0.73±0.19 (0.75/0.20-1.10)	0.72±0.21 (0.75/0.25-1.25)	0.699
	Maximum, mean ± SD (median/limits)	1.22±1.29 (1.0/0.50-1.50)	1.02±0.33 (1.0/0.10-2.00)	0.119
Total delivered energy, Mean ± SD (median/limits)		27.36±6.82 (27.82/8.90-47.39)	26.30±8.40 (26.34/3.04-68.00)	0.447

There were no statistically significant differences between the two groups in terms of the average number of sessions required to achieve stone-free status (p=0.538), the total number of shock waves applied (p=0.707), the average and maximum energy levels and the total energy dose (p=0.447). In both groups, between 1 and 2 ESWL sessions were required, over 2000 shock waves, with an average intensity between 0.7 and 0.8 and with a total dose of energy below 30 joules. However, the total dose of applied energy varied in both groups fluctuated in wide intervals, with large differences between minimum and maximum.

The stone-free rate after each ESWL session and the complication rate are presented in table III.

There were no statistically significant differences between the 2 groups in terms of stone-free rate after each of the 3 ESWL sessions. In both groups, the stone-free rate was 70% after the first session, 80% after the second session and 90% after the third session. In the group with double-J catheters, 2 cases developed reflux nephropathy after the second session, necessitating the abandonment of the procedure. In addition, there were 5 cases with double-J stent syndrome, of which 1 case required abandoning the procedure after the first ESWL session. In the group without double-J catheters, there were 6 patients (5.1%) who presented with post-procedural renal colic that responded to analgesic and anti-inflammatory medications and did not require insertion of a double-J stent. In addition, two patients (1.69%) developed infected hydronephrosis after the first session, which necessitated the abandonment of the procedure and insertion of a double-J catheter.

There were no differences in the overall rate of complications between the two groups.

TABLE III.

Stone free rate and the complications incidence in the 2 groups

		Double-J catheter group (n=46)	Non-Double-J catheter group (n=118)	OR C.I.95%	*p* (chi square)
Stone free (No., %)	After 1st session	34 (73.9%)	87 (73.7%)		0.862
	After 2nd session	34+4=38 (82.60%)	87+19=106(89.8%)	0.97 0.32-2.93	0.315
	After 3rd session	38+4 = 42 (91.30%)	106+7=113 (95.7%)	0.66 0.15-2.89	0.269*
Complications (No., %)	Reflux nephropathy	2 (4.3%)	0 (0.0%)	13.31 0.62-282.80	0.023*
	Double-J stent syndrome	5 (10.9%)	0 (0.0%)		0.001*
	Renal colic	0 (0.0%)	6 (5.1%)		0.044*
	Infected hydronephrosis	0 (0.0%)	2(1.69%)		1*
Total number of complications		7 (14.6%)	8(6,77%)		0.324

*Fischer Exact Test Calculator for 2X2 contingency table

DISCUSSION

The results of the study show that ESWL has a similar stone-free rate and the same complication rate for pelvic stones between 5 and 10 mm in patients with double-J catheters inserted for infected hydronephrosis. The main limitations of our study were the retrospective nature and the relatively small number of the study group. The number was limited by the inclusion criteria of patients with infected hydronephrosis due to stones up to 10 mm. A further limitation of the study was the lack of measurement of the density and all 3 dimensions of the stones on native CT, as this is known to influence the rate of fragmentation and elimination of the stones. However, for stones up to 10 mm this has only a minor influence compared to stones over 10 mm.

While in ureteral lithiasis the insertion of a double-J catheter could create an expansion chamber for the fragments that occurred after ESWL (13), in pelvic stones the pyelocaliceal system takes over this role, so that the insertion of the double-J catheter would be useless, there is even the disadvantage of creating an obstacle in the path of the shock waves (13), thus reducing the efficiency of ESWL.

The two study groups had the same values for stone location and size, which allowed comparison of stone-free rates according to the presence or absence of double-J catheters with minimal error factors. In both groups, the age was under 50 years, younger than in other local studies (14), where the average age was over 50 years. This can be explained by the fact that in recent years more and more patients are having routine ultrasound examinations performed, or when symptoms are minimal, which allows diagnosis in the case of some still relatively small stones. As in previous studies, the majority of patients were overweight, with a predominance of males (14).

In both groups, the size of the stones was between 9 and 10 mm, a size at which spontaneous elimination is unlikely. It is likely that many patients with smaller

stones had eliminated the stones spontaneously without the need for ESWL.

If we analyze the stone-free rate in the two groups, we see a good result, the same in both groups as in other studies (15), over 70% even after the first ESWL session, much higher than the rate below 50% in the case of ureteral stones performed with the same technique and device (14). In both groups, the application of the second and third sessions proved useful, increasing the efficiency of the procedure to over 80% and 90%, respectively. Even if we did not obtained results of 98-100% as in flexible retrograde ureteroscopy (RIRS) (2), we consider that the difference of a few percent is not clinically relevant to contraindicate the ESWL procedure, even in patients who wear double-J catheters. In addition, there are patients who do not want surgery and local or general anesthesia, which is necessary to perform RIRS, and who request the non-invasive ESWL procedure, even if stone-free status is achieved faster after RIRS (16). We also see that the presence of the double-J catheters did not lead to an increase but also not to a decrease in stone-free status. We used a combined radiologic and ultrasound guide to focus the calculi, and we changed the position of the patient on the treatment table so that the double-J catheter did not interfere with the stone and shock waves, so that higher energy doses were not required to fragment the calculi in the presence of double-J catheters.

Comparing ESWL parameters, we note that the number of sessions required for ESWL success was low, using lower energy doses than for ureteral stones (14), reducing the risk of renal hematoma, a reducible complication that otherwise did not occur in any treated case.

In terms of complications, although wearing the double-J catheters eliminated the risk of renal colic, it did not reduce the risk of infection. Instead, complications related to the presence of the catheters occurred, such as double-J stent syndrome, which has been reported in high percentages in some studies (17, 18), but only in a low percentage in our study, similar to other local studies (19). However, the presence of double-J stent syndrome could be treated with medication. Only in one case ESWL treatment had to be abandoned, which proves that the presence of double-J stent syndrome does not significantly affect the efficacy of ESWL treatment. It is possible that double-J stent syndrome occurred immediately after catheter insertion, so some of them could not undergo ESWL and opted for RIRS, so the actual number of patients with double-J stent syndrome is higher than reported in our study.

The presence of double-J catheter did not increase the risk of post-procedural infection (20), as reported in some studies (21, 22). There were 2 patients in each group who had high UTIs. Double-J catheter carriers experienced reflux pyelonephritis, which has also been reported in other studies (22, 23), with possible progression to sepsis (22), even in the absence of an obvious inflammatory syndrome (24). This risk was probably reduced in the patients with double-J catheters by the fact that all of them received antibiotic prophylaxis with cefuroxime, one tablet 30-60 minutes before the procedure, and especially because all patients with double-J catheters underwent ESWL 3 weeks after stent insertion for infected hydronephrosis, which allowed the pyelonephritic process to resolve, and also only after a negative urine culture.

The advantage of having double-J catheters in our study, in contrast to other studies (25), was the elimination of the risk of

renal colic and the need to visit an emergency service (26), which can be a problem in patients in remote areas.

The fact that the fragmentation rate was already high after the first procedure made it possible in most cases to remove the double-J stents earlier than 2 months and thus minimize the risk of calcification of the catheters. Although some studies showed a higher recurrence rate in the first 5-10 years after ESWL compared to RIRS (27, 28), we did not find a high rate of residual fragments that would suggest this. In our study group, we found no patients with significant calcifications that required additional maneuvers to remove them, such as endoscopic lithotripsy or retrograde ureteroscopy. Further prospective studies in larger patient groups are needed to validate our results.

CONCLUSIONS

Our study demonstrated that ESWL for pelvic stones up to 10 mm, in patients with double-J catheters inserted for infected hydronephrosis, is a safe and effective method, with minimal side effects, just like ESWL in patients without double-J catheters. In these patients, ESWL can be recommended as a first-line treatment along with RIRS.

CONFLICT OF INTEREST AND FUNDING

Patient consent was obtained for presenting this case in a medical publication to contribute to the expansion of the database on this pathology.

The ethics committee of the medical unit has approved the presentation of the case in a medical article in a medical publication while respecting personal data.

Regarding conflicts of interest, all authors have declared that they have not received any financial support from any organizations related to the published work.

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A COMPARISON BETWEEN RISK ASSESSMENT SCORES IN PROSTATE CANCER

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A COMPARISON BETWEEN RISK ASSESSMENT SCORES IN PROSTATE CANCER (Abstract): **Background and aim**: Prostate cancer remains a prevalent cause of malignancy in men worldwide, representing the fifth leading cause of death globally. Oncologic treatment and monitoring of these patients are conducted using multiple scores that assess the risk of biochemical recurrence, with the CAPRA score and D'Amico risk stratification scheme currently being the most widely used. The study aims to compare the prognostic classification of prostate cancer patients using two different scores, identifying similarities and differences that may arise. **Materials and methods:** This retrospective study evaluated both the D'Amico and CAPRA scores in 54 men diagnosed with non-metastatic prostate cancer at the Regional Institute of Oncology between 2017-2021. Transrectal ultrasound-guided biopsy was performed on all patients for diagnostic purposes. The Gleason system was used to grade biopsy cores, and the 8th Edition of AJCC Cancer Staging Manual was used to evaluate clinical stage. **Results:** Following the evaluation of patients with CAPRA scores, out of the total of 54 patients, 12.96% (n=7) had low risk, 42.59% (n=23) had intermediate risk, and 44.44% (n=24) had high risk. Applying the D'Amico score stratified patients into the following risk groups: 5 patients (9.26%) in the low-risk group, 9 patients (16.67%) in the intermediate-risk group, and 40 patients (74.07%) in the high-risk group. In our study, we observed that after overlapping the stratification of patients with the D'Amico and CAPRA scores, out of the seven patients classified as low risk according to the D'Amico stratification, two of them were considered intermediate risk. Furthermore, D'Amico's score considers that out of the 23 patients with intermediate scores, 69.57% (n=16) of them have a high risk. **Conclusions:** The D'Amico stratification tends to categorize patients into a less favorable prognostic group, necessitating a different management approach and active oncological monitoring. On the other hand, the CAPRA score, due to its multitude of composing criteria, individualizes each case, especially for patients classified in the low and intermediate risk groups, thus completing the D'Amico score. Therefore, it beneficial to utilize both scores in evaluating prostate cancer patients for an accurate prognostic assessment. **Keywords**: PROSTATE CANCER, CAPRA SCORE, D'AMICO RISK STRATIFICATION, BIOCHEMICAL RECURRENCE, PREDICTIVE MODELS.

INTRODUCTION

Prostate cancer represents the second most common malignancy in men worldwide, after lung cancer, being the fifth leading cause of death globally (1). The incidence of prostate cancer increases with age (2), and although only one in 350 men under the age of 50 will be diagnosed with this type of cancer (3), in men over 65 years old the incidence rate approaches 60% (4).

Treatment and monitoring of patients depend on the following clinic-pathological parameters found in many models for predicting recurrence after curative treatment: prostate-specific antigen (PSA) level, Gleason score, clinical tumor (cT) stage, various biopsy core characteristics (percentage of positive biopsies), or age (5). Prognostic scores, calculated based on these parameter values, are valuable tools in the oncological approach.

The trend in oncology is to optimize and refine these scores to provide the most accurate assessment of the patient's oncological status and, consequently, optimal therapeutic management. Currently, there are three widely used models (6) to stratify biochemical recurrence after radical prostatectomy: the D'Amico risk stratification scheme (7), the CAPRA score (8), and the Stephenson nomogram (9).

In an attempt to optimize disease progression prediction, in 1998, D'Amico *et al*. developed a combined modality staging system to stratify patients into three risk groups: low, intermediate, or high risk of biochemical recurrence after surgical and radio therapeutic treatment (7). In the D'Amico model, there are three variables categorized as follows: (i) prostate-specific antigen (PSA) levels ≤ 10, 10.1-20, and ≥ 20 ng/mL; (ii) clinical stage categories were cT1/2a, cT2b, and cT2c/T3 or higher; and (iii) biopsy Gleason score categorized as ≤ 6, 7, and ≥ 8.

Several years later, in 2005, the Cancer of the Prostate Risk Assessment (CAPRA) score was published (8). The CAPRA score is based on five variables categorized as follows: (i) PSA levels of ≤ 6, 6.1-10.0, 10.1-20.0, 20.1-30, or ≥ 30 ng/mL; (ii) clinical stage of cT1/2 or cT3; (iii) biopsy Gleason score of no Gleason pattern 4, secondary Gleason pattern 4/5, or primary Gleason pattern 4/5; (iv) age of <50 or ≥ 50 year, and (v) percentage of cancer in the biopsy <34% or ≥ 34%. This score was designed as a predictive stratification tool that could be easily calculated. Subsequently, the CAPRA score was upgraded to the post prostatectomy score (CAPRA-S) and Japanese primary androgen-deprivation therapy (J-CAPRA) (10).

Both the D'Amico and the CAPRA score have been externally validated, and their ability to predict biochemical recurrence has been confirmed over time (11–14).

The aim of our study was to compare the prognostic classification of prostate cancer patients using both the D'Amico score and the CAPRA score to identify similarities and differences that may arise. The analysis of these similarities and differences may lead the oncologist to use one of these two scores in daily practice, considering that it provides beneficial risk stratification for patient monitoring.

MATERIALS AND METHODS

The study population consisted of 54 men diagnosed with prostate cancer (without distant metastases) at the Regional Institute of Oncology between January 1st, 2017, and December 31st, 2021. All patients underwent multicore transrectal ultrasound-guided biopsy for diagnosis. Biopsy cores were graded according to the Gleason system (15). PSA values were measured before digital rectal examination or biopsy. Clinical stage was assigned by the attending urologist / oncologist follow-

ing the American Joint Committee on Cancer TNM guidelines (16, 17). The variables we tracked were: (i) patient age, (ii) PSA value, (iii) Gleason score, (iv) TNM staging and (v) percentage of cancer in the biopsy. All patients were evaluated using both the D'Amico and CAPRA scores, and the results were compared.

RESULTS

The mean age of patients was 68.75 years (range: 48-84 years); one patient was under 50 years old (1.85%) and 53 were over 50 years old (98.15%). The median follow-up time among survivors in our patient cohort was 2 years Out of the 54 patients, 6 (11.11%) registered disease progression and 3(5.56%) have deceased.

In the study group, PSA values were as follows: < 6 ng/mL – 6 patients (11.11%), ≥ 6 – 10 ng/mL – 12 patients (22.22%), ≥ 10 – 20 ng/mL – 22 patients (40.74%), ≥ 20 – 30 ng/mL – 6 patients (11.11%), 8 patients (14.81%) > 30 ng/mL (tab. I).

TABLE I.

Comparison between the distribution of patients according to specific criteria in CAPRA and D'Amico scores

CAPRA score			D'Amico score		
Criteria		Cases # (%)	Criteria		Cases # (%)
Age	<50 y.o.	1 (1.85%)			
	>50 y.o.	53 (98.15%)			
PSA	< 6 ng/mL	6 (11,11%)	PSA	< 10 ng/mL	18 (33.33%)
	≥ 6-10 ng/mL	12 (22.22%)			
	≥ 10-20 ng/mL	22 (40.74%)		10-20 ng/mL	22 (40.74%)
	≥ 20 - 30 ng/mL	6 (11.11%)			
	> 30 ng/mL	8 (14.81%)		>20 ng/mL	14 (25.93%)
Gleason score	4 or 5 patterns	16 (29.63%)	Gleason score	≤ 6	16 (29.63%)
	4 or 5 secondary patterns	22 (40.74%)		7	26 (48.15%)
	4 or 5 primary patterns	16 (29.63%)		≥ 8	12 (22.22%)
Stage	cT1 or cT2	25 (46.30%)	Stage	cT1 – cT2a	5 (9.26%)
				cT2b	12 (22.22%)
	cT3a	29 (53.70%)		≥ cT2c	37 (68.52%)
Cancer in core biopsy	< 34%	16 (29.63%)			
	> 34%	38 (70.37%)			

- number; % - percentage

Gleason scores provided by the histopathological exam of prostate biopsy showed the subsequent distribution: 6 (3 + 3) in 16 patients (29.63%), 7 (3 + 4) in 21 patients (38.89%), 7 (4 + 3) in 5 patients (9.26%), 8 (4 + 4) in4 patients (7.41%), 8 (3 + 5) and 8 (5 + 3) – each in 1 patient (1.85%), 9 (4 + 5) and (5 + 4) – each in 3 patients (5.56%) (tab. I).

The classification/setting of patients in clinical stages revealed: 5 patients (9.26%) in cT1-cT2a, 12 patients (22.22%) in cT2b, 8 patients (14.81%) in cT2c and 29 patients (53.70%) in cT3a (tab. I).

According to the results of the histopathological exam, the percentage of positive cores in prostate biopsy was < 34% in 16 patients (28.63%) and >34% in 38 patients (70.37%) (tab. I).

Based on the evaluated clinical and

pathological parameters, the application of the D'Amico score led to the following patient stratification: 5 patients (9.26%) in the low-risk group, 9 patients (16.67%) in the intermediate-risk group, and 40 patients (74.07%) in the high-risk group. Similarly, following the assessment of patients using the CAPRA score, out of the total of 54 patients, 7 (12.96%) had low risk, 23 (42.59%) had intermediate risk, and 24 (44.44%) had high risk (tab. II).

Interestingly, upon evaluating the same patients using the D'Amico score, out of the 7 patients classified as low risk according to CAPRA score, two of them (28.57%) were considered intermediate risk. Furthermore, the D'Amico score indicated that out of the 23 patients classified as intermediate risk, 16 of them (69.57%) were categorized as high risk. Regarding patients classified as high risk according to the D'Amico classification, all 24 corresponded to the high-risk category in the CAPRA classification (tab. III).

TABLE II.

Distribution of patients in risk groups according to CAPRA and D'Amico scores

CAPRA score			D'Amico score		
Risk	**Score**	**Cases # (%)**	**Risk**	**Score**	**Cases # (%)**
Low	1	3 (5.56%)	low	3	5 (9.26%)
	2	4 (7.41%)			
Intermediate	3	5 (9.26%)	intermediate	4	2 (3.7%)
	4	6 (11.11%)		5	3 (5.56%)
	5	12 (22.22%)		6	4 (7.41%)
High	6	8 (14.81%)	high	5	2 (3.7%)
	7	4 (7.41%)		6	9 (16.67%)
	8	4 (7.41%)		7	14 (25.93%)
	9	3 (5.56%)		8	11 (20.37%)
	10	5 (9.26%)		9	4 (7.41%)

- number; % - percentage

TABLE III.

Differences in distribution of patients in risk groups according to CAPRA and D'Amico scores

CAPRA score		Correspondence to D'Amico score	
Risk group	**# (%)**	**Risk group**	**# (%)**
Low	7 (12.96%)	Low	5 (9.26%)
		Intermediate	2 (3.7%)
Intermediate	23 (42.59%)	Intermediate	7 (12.96%)
		High	16 (29.63%)
High	24 (44.44%)	High	24 (44.44%)

- number; % - percentage

Regarding patient survival, the 3 patients who deceased were categorized in the high-risk group in both the D'Amico score and the CAPRA score (tab. IV). Furthermore, concerning survival without progression, out of the 6 patients who experienced tumor recurrence over a 2-year follow-up period, the CAPRA score classified 2 patients in the intermediate-risk group and 4 in the high-risk group.

On the other hand, the D'Amico score categorized 5 patients in the high-risk group and 1 in the intermediate-risk group (tab. V).

TABLE IV.

Correlation between risk groups in CAPRA and D'Amico scores and overall survival

Overall survival – CAPRA and D'Amico scores				
RISK GROUP	Survival	Death	Survival	Death
	# (%)	# (%)	# (%)	# (%)
	CAPRA SCORE		d'AMICO SCORE	
Low	7 (100%)	0 (0.0%)	5 (100%)	0 (0.0%)
Intermediate	23 (100%)	0 (0.0%)	9 (100%)	0 (0.0%)
High	21 (87.5%)	3 (12.5%)	37 (92.5%)	3 (7.5%)

- number; % - percentage

TABLE V.

Correlation between risk groups in CAPRA and D'Amico scores and progression free survival

Progression free survival – CAPRA and D'Amico scores				
RISK GROUP	Recurrence absent	Recurrence present	Recurrence absent	Recurrence present
	# (%)	# (%)	# (%)	# (%)
	CAPRA SCORE		D'Amico	
Low	7 (100%)	0 (0.0%)	5 (100%)	0 (0.0%)
Intermediate	21 (91.3%)	2 (8.7%)	8 (88.9%)	1 (11.1%)
High	20 (83.3%)	4 (16.7%)	35 (87.5%)	5 (12.5%)

- number; % - percentage

DISCUSSION

Over time, several studies have demonstrated that the risk of biochemical recurrence after prostate cancer treatment is heterogeneous and depends on factors such as PSA level, clinical stage of the disease, biopsy Gleason score, and characteristics of the core biopsy variables (7–9, 18). Due to this heterogeneity, different authors have proposed various risk classification schemes to quantify the individual rate of biochemical recurrence more accurately after prostate cancer treatment (7–9, 18). In this context, the D'Amico (7) and CAPRA (8) models are the most widely used prediction tools for tumor recurrence risk. However, there have been relatively few studies directly comparing these two stratification models (6, 19).

Among these, the most important are those by Lughezzani *et al.* (19), which directly compare the CAPRA score with the D'Amico risk stratification scheme, and that of Boehm *et al*., which compares the ability of the scores to predict cancer-specific mortality in a large European cohort of patients (6). The study of Lughezzani *et al.* included 1,976 prostate cancer patients treated by open radical prostatectomy. The conclusion was that the CAPRA score outperformed the other scoring models when calibration and decision curve analysis were used as benchmarks (19). Interestingly, they observed that the predictions of the D'Amico model showed limited ability to distinguish high rates of biochemical recurrence in patients. On the other hand, the CAPRA model could discriminate between a much wider varieties of biochemical recurrence risks and showed

the greatest benefit in predicting biochemical recurrence at 3 and 5 years, being the best tool in the cohort (19).

Another study that aimed to identify the most informative prediction tool for cancer-specific mortality was conducted by Boehm *et al.* (6). On a cohort of 2,485 prostate cancer patients who underwent radical prostatectomy, the investigators analyzed three preoperative models (D'Amico, CAPRA, and Stephenson), comparing their ability to predict 10-year cancer-specific mortality. For the 10-year prediction, the CAPRA score showed the highest net benefit. In contrast, the D'Amico model showed underestimation of cancer-specific mortality (6).

In our study we observed that after overlapping the evaluation of patients with the D'Amico and CAPRA scores, out of the 7 patients classified as low risk according to the D'Amico stratification, 2 of them were considered intermediate risk. Furthermore, the D'Amico score categorized 16 (69.57%) out of the 23 patients classified as intermediate risk as high risk. As a result of the overlapping of the two scores, we noticed that the D'Amico risk stratification scheme tends to place patients in a higher risk group.

In our practice as oncologists, we commonly utilize the NCCN (National Comprehensive Cancer Network) guidelines (20), which employ risk prognostic groups ranging from very low to very high (6 groups), as well as the D'Amico classification, for prognostic scoring in prostate cancer. The CAPRA score tends to be more commonly used by urologists, primarily as a prognostic score for patients undergoing surgical intervention for curative purposes. Currently, there is a trend towards a less invasive therapeutic approach, specifically radiotherapy as a method of curative treatment, which is increasingly preferred by patients. As our study results have also shown, the D'Amico stratification tends to categorize patients into a less favorable prognostic group, necessitating a different management approach and active oncological monitoring. On the other hand, the CAPRA score, due to its multitude of composing criteria, individualizes each case, especially for patients classified in the low and intermediate risk groups, thus completing the D'Amico score.

CONCLUSIONS

The assessment of the risk of recurrence should be done on an individual basis, considering the specific characteristics of each patient. The CAPRA score tends to be more comprehensive due to the complexity of the criteria it encompasses, including age (which is absent in the D'Amico score calculation), better stratification of PSA values, and especially the Gleason score, considering each Gleason pattern separately and implicitly each ISUP (International Society of Urological Pathology) prognostic grading group. Therefore, we find it beneficial to utilize both scores in evaluating prostate cancer patients for an accurate prognostic assessment. However, individualization is crucial; placing a patient in a higher prognostic group may benefit them by allowing the application of all necessary therapeutic measures. At the same time, overtreatment should be avoided as much as possible to preserve the patient's quality of life.

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REAL LIFE RETROGRADE FLEXIBLE URETERORENOSCOPY *VERSUS* EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY IN PYELOCALICEAL STONES UNDER 20 MM

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REAL LIFE RETROGRADE FLEXIBLE URETEROSCOPY VERSUS EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY IN PYELOCALICEAL STONES UNDER 20MM (Abstract): The **aim** of our study was to highlight groups of patients with single pyelocaliceal stones less than 20 mm in whom flexible retrograde ureterorenoscopy (FURS) or extracorporeal shock wave lithotripsy (ESWL) are superior in terms of stone-free rate and complications. **Materials and methods:** We formed two groups of patients, consisting of patients with single kidney stones under 20 mm in whom FURS was applied (the study group) and another in whom ESWL was performed (the control group). We compared the profile of the patients, the presence of double-J catheters before the procedure, the stone-free rate and the occurrence of complications. **Results:** Patients in the study group were older ($p<0.001$), had larger stones ($p<0.001$) and were more likely to have concomitant diseases ($p<0.001$). The stone-free rate in patients with stones up to 10 mm was similar in both groups (81.25% vs 78.94%, $p=0.777$), while for stones between 11 and 19 mm the stone-free rate was higher in the study group (77.77% vs 45.45%, $p=0.008$). The rate of complications was similar in both groups ($p=0.555$). **Conclusions:** FURS has superior efficacy compared to ESWL in patients with single kidney stones between 11 and 19 mm and similar efficacy in patients with single kidney stones up to 10 mm. The complication rate is low and similar in both groups.
Keywords: FURS, ESWL, STONE-FREE RATE.

According to European guidelines, *flexible retrograde ureterorenoscopy* (FURS) and *extracorporeal shock wave lithotripsy* (ESWL) are the treatment of first choice for single pyelocaliceal stones smaller than 20 mm (1), with the choice of one or the other method being at the discretion of the urologist or the patient, with no clear criteria in favor of one or the other method. In recent years, FURS has taken on an important role alongside ESWL and percutaneous nephrolithotomy (PCNL) among the treatment methods for renoureteral lithiasis (2). However, it has not succeeded in replacing ESWL for stones smaller than 20 mm or PCNL for stones larger than 20 mm

(1). Previous studies have shown that the efficacy of FURS is slightly higher compared to ESWL at over 90% (3, 4, 5) and the complication rate is relatively low for both methods (6, 7, 8). Although there are many studies comparing FURS with ESWL (9, 10, 6), they were performed on homogeneous groups, on selected patients, under ideal conditions, without previous presence of urinary tract obstruction with or without infection, i.e. without possible inflammation in the context, without the presence of ureteral catheters inserted for more than 2 weeks , and without other urological procedures previously performed for the same stones. Also, the surgical limitations due to patients' comorbidities, which can sometimes be important, were not considered when calculating the efficiency of FURS.

Some studies on the efficacy of FURS have focused on the types of lasers used (11) and not on patient characteristics, with the exception of inferior calyx stones, where the infundibulopelvic angle seems to play an important role in the efficacy of FURS (1). For this reason, we conducted a study to determine the efficacy of FURS in terms of stone extraction rate (stone-free rate) and the incidence of complications compared to ESWL under the conditions of daily practice in patients with the above-mentioned characteristics. In this way, we have attempted to specify the indications for the two methods by identifying groups of patients with single pyelocaliceal stones less than 20 mm in which a particular treatment would have a higher success rate and a lower complication rate.

MATERIALS AND METHODS

We conducted a case-control study on patients who underwent FURS for pyelocaliceal stones between 01.04. 2022 and 31.03.2024 at the Urology Clinic of the "Dr. C.I. Parhon" Teaching Hospital in Iasi, Romania, and who constituted the study group. The control group consisted of patients hospitalized at Elytis Hope Hospital in Iasi, Romania, where ESWL was performed for pyolocaliceal stones, between 01.04. 2023-31.03.2024. In this group, we analyzed only the patients who were hospitalized during one year in , as we achieved a ratio of more than one to one compared to the study group. In this way, increasing the number of patients in the control group would not have increased the significance of the statistical tests used. The study was approved by the ethics committees of both hospitals. The patient data were taken from the electronic patient records. We included all patients with pelvic or calyx stones, single, up to 20 mm in size, whether or not they had a double-J ureteral catheter.

We excluded patients under 18 years of age, patients with multiple stones, single stones over 20 mm, stones associated with various renal or pyeloureteral malformations and patients with incomplete data in the electronic registries from the study. Among the patients in the study group, we also excluded patients who underwent FURS due to tumor pathology or exploratory.

The diagnosis of lithiasis was based on an ultrasound examination and a simple kidney X-ray. Computed tomography, which can accurately determine the size of the stones, was not used for diagnosis. We therefore divided the patients into two groups only, according to the size of the stones, and not in many groups, due to relative inaccuracy of the measurement methods. Patients with stones up to and including 10 mm and patients with stones between 11 and 19 mm. When performing

FURS, a 7Ch double J-ureter catheter (24-28 cm) was routinely inserted at least two weeks before the procedure. The reusable flexible ureteroscope (Olympus Comp. Germany) was used. ESWL was performed in the same way by all urologists, using the ramping method, with maximum intensity achieved after a minimum of 500 and a maximum of 3000 shock waves administered. A maximum of 2 procedures were performed one month apart. If fragments of more than 4 mm were still present, the procedure was considered a failure. The Siemens Modulith Lithotripter (Siemens Comp. Germany) was used.

We recorded the demographic data of the patients from the two groups, namely age, sex, location of stones, size of stones, and the presence of comorbidities, DM, obesity, renal failure, anemia, and cardiac diseases such as hypertension and ischemic heart disease. We then comparatively analyzed the frequency and reasons for previous double-J catheter insertion, presence of previous urinary tract infections and type of germs, history of lithiasis, duration of double-J catheter wear until the procedure, and history of urological maneuvers performed for the same stone. In terms of efficacy, we compared the stone-free rate and the occurrence of immediate complications (occurring in the first 7 days after the procedure). These included urinary tract infection, pain and hematuria. We considered "stone-free" status if, after the FURS or ESWL procedure, no remaining fragments were detectable at the 14 day follow-up or if the remaining fragments were less than 4 mm in size, as determined sonographically or radiologically. We considered urinary tract infection if a positive urine culture was highlighted after the procedure, up to 2 weeks, performed after the onset of febrile syndrome or low urinary symptoms. We considered the urine culture positive if more than 10^5 UFC/ml was highlighted in the culture by the qualitative technique.

Of the patients in the study group who were admitted to "Dr. C.I. Parhon" Hospital during the 2-year period, there were 78 patients. Among them, there were 10 patients who underwent FURS for multiple calyceal lithiasis, 4 patients with stones larger than 20 mm, 2 patients with associated renal or pyeloureteral malformations, and one patient who underwent FURS to investigate hematuria diagnosing an upper urothelial tumor. As for the control group, there were a number of 128 patients with kidney stones who underwent ESWL at Elytis Hospital during the above-mentioned period. Of these, we excluded 2 patients under 18 years of age, 4 patients with stones larger than 20 mm, 16 patients with multiple stones and 27 patients with incomplete data in the electronic registries.

Statistical analysis.

The quantitative variables were described by the mean and standard deviation and compared with the t-student test. Due to the large number of patients included in the study, it was not necessary to perform homogeneity tests of the two groups. Qualitative variables were described by percentages and compared using the chi-square test. When we compared qualitative variables that occurred in rare cases (< 5), we applied the Fischer Exact Test for Contingency Table. The data was first entered into an Excel spreadsheet and then transferred to the *SPSS 22.0* program. We considered the difference to be statistically significant if the statistical p-value < 0.05.

RESULTS

During the study periods mentioned, there were 61 patients in the study group who were treated with FURS and 79 patients in the control group who were treated with ESWL. The demographic characteristics of the patients in the 2 groups are listed in first table.

TABLE I.
The characteristics of the 2 groups.

		FURS group (n=61)	ESWL group (n=79)	*p* value chi square
Age (years) mean ± SD		55.87±13.96	46.3±14.66	**<0.001** t-student
Gender	Male (no., %)	21 (34.42%)	47 (59.49%)	**0.003**
	Female (no., %)	40 (65.58%)	32 (40.51%)	**0.003**
Affected kidney	Right kidney	28 (45.9%)	42 (53.16%)	0.394
	Left Kidney	33 (54.1%)	37 (46.84%)	0.394
Stone localization	Pelvis	30 (49.2%)	35 (44.30%)	0.566
	Upper + middle calyx	19 (31.1%)	24 (30.38%)	0.922
	Lower calyx	12 (19.7%)	24 (25.32%)	0.430
Stone size (mm) mean ± SD		13.26±3.84	0.98± 4.82	**<0.001** t-student
≤ 10mm		16 (26.2%)	57 (72.15%)	**<0.001**
11-19 mm		45 (73.8%)	22 (27.85%)	**<0.001**
Comorbidities	Diabetes mellitus (DM)	16 (26.22%)	4 (5.06%)	**<0.001**
	Obesity	18 (29.5%)	10 (12.66%)	**0.013**
	Kidney failure	11 (18.03%)	2 (2.53%)	**0.001** Fisher test
	Anemia	11 (18.03%)	1 (1.26%)	**<0.001** Fisher test
	Heart disease (HBP. ischemic cardiomyopathy)	31 (50.81%)	12 (15.19%)	**<0.001**

The patients in the study group were older ($p<0.001$), the female gender predominated ($p=0.003$) and they had larger stones ($p<0.001$) than the control group. On the other hand, there were no statistically significant differences between the two groups in terms of right/left location ($p=0.394$), pelvic level location ($p=0.566$), upper and middle calyx ($p=0.922$) and lower calyx ($p=0.43$).

In terms of comorbidities, patients in the study group had a statistically significantly higher proportion of DM ($p<0.001$), obesity ($p=0.013$), chronic kidney disease

(p=0.001), anemia (p<0.001), and heart disease (p<0.001).

Table II shows a comparison of the proportion of the presence of preoperative double-J catheter according to their indication and indwelling time, the presence of stones, and the frequency of other urological procedures performed for the same stones that failed to completely solve the stones, requiring FURS or ESWL as a secondary "rescue" treatment for the elimination of remaining stone fragments over 5 mm.

TABLE II.

The presence of double J catheters, history of lithiasis and previous urological procedures for the same stones.

		FURS group (n=61)	ESWL group (n=79)	*p* value for chi-square
Double-J stent inserted for infected hydronephrosis		15 (24.59%)	17 (21.52%)	0.667
Double-J stent inserted for hydronephrosis		35 (57.37%)	5 (6.33%)	**<0.001**
Double-J stent inserted without previous hydronephrosis/infection		10 (16.39%)	0 (0%)	**<0.001** Fisher test
Personal history of lithiasis		30 (49.18%)	14 (17.72%)	**<0.001**
Total nr. of patients with preprocedural double-J catheters		60 (98.5%)	22 (27.85%)	**<0.001**
Preoperatory indwelling time of ureteral double-J catheters	< 1 month	13 (21.31%)	15 (18.99%)	0.733
	1-2 months	18 (29.50%)	7 (8.86%)	**0.001**
	> 2 months	29 (47.54%)	0 (0%)	**<0.001** Fisher test
Interventions after other procedures for the same stone	PCNL	4 (6.56%)	2 (2.53%)	0.402
	URS	11 (18.03%)	2 (2.53%)	**0.002**
	ESWL	6 (9,48%)	0 (0%)	**<0.001**

URS-semirigid retrograde ureteroscopy

Patients in the study group had a higher proportion of double-J catheters inserted preoperatively for hydronephrosis (p<0.001) and to dilate the ureter and facilitate access of the flexible ureteroscope (p<0.001) compared to the control group. They also had a higher proportion of patients with a history of lithiasis (p<0.001), patients in whom the double-J was inserted earlier than two months (p=0.001) and more than 2 months (p<0.001), and more patients who had previously undergone ESWL for the same stone (p<0.001) or semi-rigid retrograde ureteroscopy (p=0.002). On the other hand, there was no statistically significant difference between the two groups in the proportion of patients in whom the double-J catheter had been previously inserted for infected hydronephrosis (p=0.667), in whom the double-J catheter had been inserted earlier than one month preoperatively (0.733) and in patients with a history of PCNL treatment for the same stones (p=0.402).

Table III shows the incidence of urinary tract infections in the two groups, divided by type of germs.

TABLE III.

The frequency of preoperative urinary tract infections by type of germs.

Previous UTIs (No., %)	FURS group (n=61)	ESWL group (n=79)	P Fisher Exact Test for 2x2 Contingency Table
E. coli	9 (14,76%)	2 (2,53%)	**0.010**
P. mirabilis	3 (4,92%)	0 (0%)	0.080
Enterococcus spp.	3 (4,92%)	0 (0%)	0.080
Klebsiella pn.	3 (4,92%)	1 (1,26%)	0.317
S. agalactie	1 (1,63%)	0 (0%)	0.435
P.s aeruginosa	1 (1,63%)	0 (0%)	0.435
Total	20 (32,78%)	3 (3,79%)	**<0.001**

The study group had a higher incidence of preoperative urinary tract infections ($p<0.001$) and urinary tract infections with *E. coli* ($p=0.010$).

For comparisons of stone-free rates in the two groups, including separate comparisons for stones less than 10 mm and greater than 10 mm, as well as the incidence of immediate complications (tab. V).

TABLE IV.

Stone-free rate and the complications incidence in the 2 groups.

		FURS group (n=61)	ESWL group (n=79)	*P* Chi-square
Stone-free		48 (78.69%)	58 (73.41%)	0.47
Stone-free rate for stones ≤ 10mm		13 (81.25%)	48 (78.94%)	0.777
Stone-free rate for stones > 10mm		35 (77.77%)	10 (45.45%)	**0.008**
Complications	Reflux pyelonephritis	2 (3.28%)	1 (1.26%)	0.580 Fisher test
	Clostridium difficile infection	1 (1.63%)	0 (0%)	0.435 Fisher test
	Renal colic	1 (1.63%)	5 (6.32%)	0.232 Fisher test
	Hematuria > 24h	5 (8.19%)	3 (3.79%)	0.295 Fisher test
Total no. of complications		9 (14.75%)	9 (11.39%)	0.555

The overall stone-free rate and the stone-free rate for stones smaller than 10 mm was similar in both groups ($p=0.47$ and $p=0.777$, respectively). However, the stone-free rate for stones larger than 10 mm was higher in the study group than in the control group ($p=0.008$). The incidence of complications was lower in both groups, with no significant differences in any of the complications examined ($p=0.555$). Clostridium difficile digestive infection appeared in one case in the study group.The stone-free rate in patients who underwent FURS surgery was similar regardless of stone size ($p=0.08$), while in the group of patients who underwent ESWL, the stone-

free rate was statistically significantly higher in patients with stones smaller than 10 mm compared to stones larger than 10 mm ($p<0.001$).

DISCUSSION

Our study showed similar efficacy of FURS and ESWL for pyelocaliceal stones up to 10 mm. In contrast, the efficiency of FURS proved to be superior to ESWL for pyelocaliceal stones between 11 and 19 mm. The rate of complications was reduced in both patient groups, with no statistically significant differences.

The main limitation of our study is the retrospective nature and the fact that the procedures were performed by different urologists, leading to some variability in the results, although the same surgical technique was used for FURS and the same method of shock wave administration was used for ESWL.

However, to our knowledge, there is little data in the literature comparing the two procedures and considering special patient groups, such as patients with double J ureteral probes inserted for hyperalgesic renal colic or infected hydronephrosis, as well as patients with comorbidities.

When analyzing the results obtained, we note that the average age of the patients in the study group was higher than in the control group. This could be due to certain treatments of the patients, such as treatment with anticoagulants, which contraindicate ESWL, and perhaps also to the preference of some surgeons who considered a continuous hospitalization in case of FURS to be safer for the patients, as opposed to ESWL, which is performed on an outpatient basis, with the patient being monitored only for a few hours after the procedure and returning for control in a maximum of 2 weeks or in case of complications. We attribute the fact that the female sex predominated in the study group and the male sex in the control group to a coincidence, without a physio pathological explanation.

As was to be expected, the frequency of stones on the right and left side was the same, as there are no anatomical features that lead to an increased frequency on a particular side. The location of stones in the pyelocaliceal system was also similar in both groups, which increases the reliability of the statistical tests, as we know that the stone-free rate is lower for stones in the lower calyx than for stones in the renal pelvis or upper/middle calyx, for both FURS and ESWL.

We found that patients in the FURS-treated group had significantly larger stones than patients in the control group. Stones between 11 and 19 mm predominated, while stones up to 10 mm predominated in the control group. This can be explained by the preferences of the surgeons, who perhaps intuitively recognized that FURS could provide better results compared to ESWL.

As far as comorbidities are concerned, we observe an increased incidence in the study group, which is probably due to the older age. An interesting aspect is that the FURS was performed after the patients had previously been admitted to the emergency room due to recurrent renal colic or infected hydronephrosis and were only later scheduled for FURS. However, due to the large volume of patients in our clinic, they waited over 1-2 months until the procedure, which increases the risk of double-J catheter carriers for the occurrence of urinary tract infections or double-J catheters intolerance. On the other hand, patients for ESWL had no previous emergency room procedures, and they went to an outpatient

ESWL service. The fact that the patients in the study group had larger stones may have caused the more frequent occurrence of complications, obstructions with and without infection.

Another interesting finding of our study was the fact that FURS was performed in a significant percentage as a second-line treatment after the failure of semi-rigid URS or ESWL, whereas the ESWL procedure was predominantly performed as a first-line treatment.

In addition to the prolonged preoperative presence of double-J probes (12), another factor that may have compromised the efficacy of FURS was the presence of urinary tract infections, which can cause inflammation and make advancement of the FURS access sheath difficult. In the case of ESWL, this procedure was performed under ideal conditions, without double-J probes and without preoperative infections. We mention that all ESWL sessions were performed after confirming the absence of UTIs by urine culture, while FURS was also performed during antibiotic treatment for UTIs, without expecting the negative urine culture, which was, however, illusory in the presence of double-J catheters. In contrast to other studies (13, 14), in which many patients did not have double-J catheters in place before the FURS procedure, all but one patient in our study had a stent in place preoperatively. Double J catheters are widely used in urology, not only for drainage of hydronephrosis, but also in open procedures (15, 16), helping to protect the ureter or favoring the healing of some ureteral sutures. However, if you keep them for longer than a month, they can cause irritating urinary tract symptoms (12), which may even require premature suppression.

It is somewhat surprising that the stone-free rate was also below 80% with FURS, not only with ESWL. If ESWL was to be expected according to other studies (17, 18, 19), the lower efficiency of FURS compared to other studies (20) is explained by the fact that it was not performed under ideal conditions, but in patients who had double-J catheters for more than 1-2 months, with associated infections, with comorbidities. Another element to remember is that in 2 patients the technical problems of the equipment forced the abandonment of the procedure, perhaps unjustifiably reducing the efficiency of the method in our study. However, in patients with stones between 11 and 19 mm, the efficiency of FURS was clearly superior, despite all the limitations in terms of patients and equipment.

Compared to other studies (6, 21-23), the rate of complications was reduced in both groups, although there were specific characteristics for each procedure. For example, reflux pyelonephritis and one case of digestive infection with *Clostridium difficile* occurred after FURS, while renal colic occurred after ESWL. Macroscopic hematuria over 24 hours was rare, proving that with adequate technique the risk of hemorrhage is low in both procedures. On the other hand, we had no cases of ureteral lesions or sepsis after FURS, as in other studies (24, 25). The low rate of postoperative infections was consistent with other studies (26), but the development of sepsis should not be neglected (27). The rate of complications in ESWL was also lower in our study than in other studies (23, 28).

Our study is useful for urologists treating such cases as it helps them to make the best decision for the procedure depending on the specifics of the patients and not on

subjective personal preferences.

CONCLUSIONS

FURS and ESWL have equal effectiveness in treating patients with single pyelocaliceal stones up to 10 mm. For patients with single pyelocaliceal stones between 11 and 19 mm, FURS has a superior efficacy. The complication rate is low, both procedures proving high safety. Future prospective studies will be necessary to validate our data.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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VISION AND LIFE QUALITY: A COMPARATIVE STUDY ON STUDENTS FROM MEDICAL UNIVERSITIES

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VISION AND LIFE QUALITY: A COMPARATIVE ANALYSIS ON ROMANIAN, ENGLISH AND FRENCH STUDENTS FROM MEDICAL UNIVERSITIES (Abstract). Computer Vision Syndrome (*CVS*) is a significant health issue, especially among medical students due to their extensive use of digital devices. This study aims to examine the relationship between *CVS* and the use of various gadgets among Romanian, English, and French medical students, while also assessing differences between these groups. **Materials and methods:** A cross-sectional, questionnaire-based study was conducted with 292 Romanian, 62 English, and 61 French medical students from universities in Iași and Paris. An anonymous survey, distributed via Google Forms, gathered data on *CVS* symptoms. **Results:** Female students predominated across all groups (64.7% of Romanians, 69% of French, and 54.8% of English). The most common eye symptoms reported were dry eyes, focusing difficulties, and eye fatigue. Romanian students experienced dry eyes (73.3%) and blurred vision (68.9%); English students reported eye fatigue (66.1%) and dry eyes (58.1%); while French students primarily faced eye fatigue (91.8%). Neck, shoulder, and back pain were common non-ocular symptoms across all groups. **Conclusions:** Ocular symptoms like dry eyes, eye fatigue, and blurred vision were similar among the groups, with widespread recognition of the health impacts of prolonged screen use. **Keywords:** LIFE QUALITY, VISION QUALITY, STUDENTS, MEDICINE.

INTRODUCTION

Nowadays, the lifestyle has forced the whole world to use modern technology. Smartphones have become so popular that almost all teenagers and adults use them. In the last decade, the social networks and applications have revolutionized the lifestyle of all mankind. Unfortunately, free time is increasingly occupied by the use of various gadgets. Various studies show that the use of gadgets, even for 3 h/day, can represent a health risk of developing Computer Vision Syndrome (*CVS*) (1-3).

Computer vision syndrome also known as digital eye strain (DES), represents a pathology of the modern world characterized by the presence of numerous oculars, musculoskeletal, and behavioral signs and symptoms caused by the prolonged use of electronic devices with digital screens. The first symptoms that appear are: headaches, stinging and redness of the eyes, blurred vision, neck and back pain, eye fatigue, visual accommodation difficulties and

attention disorders (4).

Prevalence of *CVS* ranges from 64% to 90% among computer users (5) and nearly 60 million people suffer from *CVS* globally. Moreover, a million new cases of *CVS* occur each year (1).

Computer Vision Syndrome is a significant health concern, particularly among medical students due to their frequent use of digital devices (4, 6).

This study aims to examine the relationship between *CVS* and gadget usage among Romanian, English, and French medical students, and to identify any differences between these groups.

MATERIALS AND METHODS

Cross-sectional, questionnaire-based study that aims to assess ocular and systemic changes associated with prolonged use of gadgets such as computers, smartphones, laptops and tablets. The anonymous survey was distributed via Google Forms to medical students in Romanian, English, and French. Invitations were sent to students from "Grigore T. Popa" University of Medicine and Pharmacy Iași, Romania, and Sorbonne University Paris 13, France. The survey was available from December 2021 to March 2022. All participants were informed about the study and provided informed consent. The study protocol was approved by the Research Ethics Committee of "Grigore T. Popa" University before enrollment.

The inclusion criteria are represented by the status of a student in a Medical University starting from the 1st year to the 6th year of study. After selection, the following student samples were deemed eligible for analysis:

• 292 Romanian language students from "Grigore T. Popa" University of Medicine and Pharmacy, Iași;

• 62 English language students from "Grigore T. Popa" University of Medicine and Pharmacy, Iasi;

• 61 French students from the Sorbonne University Paris 13.

Participants who did not understand the instructions or did not sign the informed consent form were excluded from the analysis.

The questionnaire entitled "Computer Vision Syndrome" was drafted in Romanian, English and French languages and was published using the Google Forms platform. The survey included 32 questions that collected the following categories of data:

• Socio-demographic data: sex, age, country of origin;

• Data on student status, field and year of study;

• The number of hours spent in front of a digital screen during a 24-hour period;

• Number of hours spent in front of a digital screen separately day and night;

• Data on the type of digital screen devices used and the frequency of their use (PC screen, laptop, tablet, smartphone);

• The degree of brightness of the screen used, the type of ambient light during the use of a digital device and its intensity;

• Ocular and extraocular symptoms noted following the use of a digital screen and their frequency;

• Pre-existing and diagnosed eye pathologies or the use of glasses/contact lenses;

• The purpose for which a medical student predominantly uses a digital screen device;

The data were systematized and centralized in an SPSS 18.0 database and were processed with the statistical functions to

which they lend themselves. Confidence intervals at the 95% significance threshold were used in the data presentation.

For the descriptive statistical analysis, the ANOVA test was used, which highlighted the following indicators:

• primary indicators: minimum, maximum, frequency:

• average value indicators: simple arithmetic mean, median;

• dispersion indicators: standard deviation, standard error, confidence interval of the mean;

• the Skewness test ($-2 < p < 2$) validates the normality of the series of values, it is used when the examined variable has continuous values.

RESULTS

Among French students, 31% were in the 6th year, 28% in the 5th year, 25% in the 4th year, 11% in the 3rd year and 5% were students in the 1st and 2nd year. All French participants were studying general medicine.

Among Romanian students, 44.5% were studying general medicine, 39.1% nursing, 7% dentistry and 4.7% pharmacy and majority of them (44.3%) were in the 2nd and 3rd years of study. Among English students, all of them were in the 5th year and were studying general medicine. In all 3 groups of students, females predominate (64.7% *vs.* 35.3%; p=0.019 – Romanian students, 69% *vs.* 31% - French students, 54.8% *vs.* 45.2%, the ratio being 1.2/1 – English students).

The average daily use of gadgets was 6.18 hours for Romanian students. The average number of hours of use of gadgets during the day was 4.41 hours, and 2.43 hours at night. In the case of English students, the average daily use of gadgets was 5.98 hours. The average number of hours of gadgets use during the day was 3.73 hours, and 2.89 hours at night (tab. I). In the case of French students, the average daily use of gadgets was 6 hours, during the day 4.01 hours, and during the night 2.55 hours.

TABLE I.

Descriptive data regarding number of hours of gadgets use in English students

N		English students		
		total	day	night
		62	62	62
Media		5.98	3.73	2.89
Median		6	3	3
Standard deviation		1.82	1.74	1.71
Variance		3.30	3.02	2.92
Skewness Test		0.654	0.593	1.647
Standard error Skewness		0.304	0.304	0.304
Minimum		3	1	1
Maximum		10	8	9
Percentile	25	5	3	2
	50	6	3	3
	75	7	5	3

Compared by gender, it is noted that a higher percentage of males spend more than 5 hours on electronic devices daily than females, for all 3 groups of students

(82.4% *vs.* 78.8% – English students, 75% *vs.* 67 % - French students, 70.8% *vs.* 66.8% - Romanian students).

In the case of all 3 groups, the most used gadgets were smartphones and laptops (figs. 1, 2, 3).

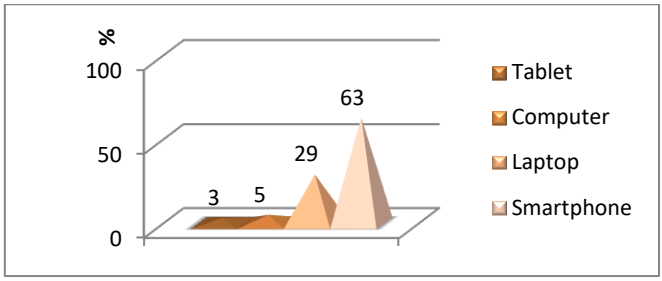

Fig. 1. Frequency of most used gadgets in English students

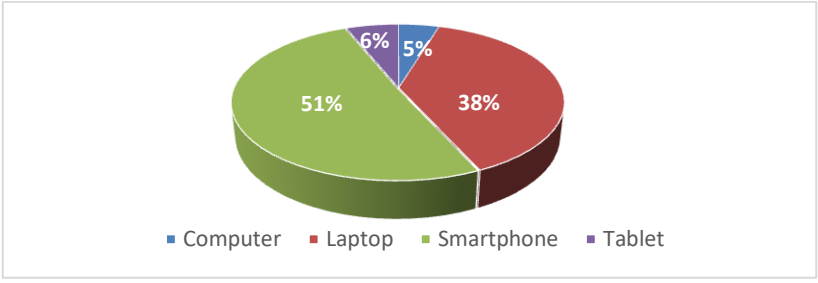

Fig. 2. Frequency of most used gadgets in French students

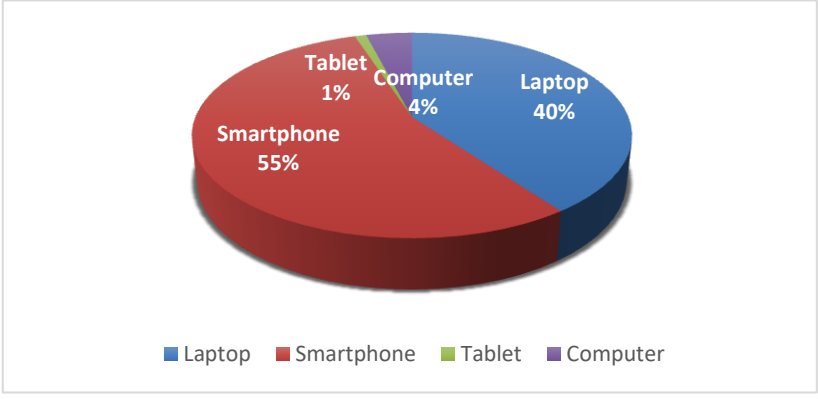

Fig. 3. Frequency of most used gadgets in Romanian students

Out of the total of 292 students from the Romanian language teaching series, 65.4% declared that they wear glasses and 3.9% wear contact lenses. In the case of the 62 medical students in English, 48.8% declared that they wear glasses and 21% wear contact lenses and in the case of the 61 medical students in French, 52% wear glasses and 15% wear contact lenses.

Regarding the most frequently ophthal-

mological symptomatology associated with the prolonged use of electronic devices with digital screens, Romanian language students declared that the most common eye symptoms are dry eyes (73.3%), focusing difficulties (72%), eye fatigue (69.3%), blurred vision (68.9). English language students declared that they most often associate eye fatigue (66.1%) and eye dryness (58.1%), and in the case of French language students, the most common ophthalmological symptoms are eye fatigue (91.8%), blurred vision (34.4%) and dry eyes (34.4%) (fig. 4).

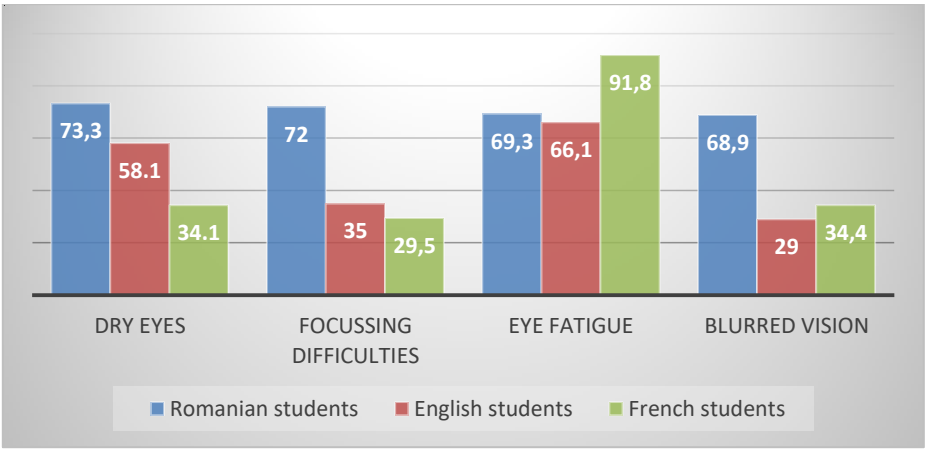

Fig. 4. The frequency of most associated ocular symptoms while using gadgets in all 3 groups of students

Neck, shoulder, and back pain were the most common extraocular symptoms across all groups: 78.7% of Romanian students, about 75% of English students, and 55.1% of French students reported these issues.

There is an awareness of the fact that the use of electronic devices for a long time affects health and lifestyle by all students:

- 229 out of 282 Romanian language students believe that digital screens affect their health and lifestyle;
- 52 out of 62 English language students believe that digital screens affect their health and lifestyle;
- 55 out of 61 French language students believe that digital screens affect their health and lifestyle;

There is also a desire to reduce the time of using electronic devices: 235 out of 282 Romanian students, 50 out of 62 English students and 50 out of 61 French students.

DISCUSSION

It is difficult to find information in the literature about Computer Vision Syndrome among medical students from different languages teaching series or from different universities. A study published in 2021 which used *CVS* Questionnaire in Chinese medical students who took classroom lectures and the same-grade foreign students from a Bachelor of Medicine and Bachelor of Surgery (MBBS) program who took online lectures, showed that online study may increase the risk of *CVS* among university students (7). Another study which was conducted on medical students and employers of College of Health and Medical Technology from province of Al-

Najaf, concluded that there were ocular symptoms in both employers and students (8). Another study analyzed the prevalence of symptoms of *CVS* in students who were studying business and medicine in Saudi Arabia, concluded that the prevalence of *CVS* symptoms was significantly higher among business students (9). Moreover, a recent study conducted on medical students and healthcare workers found that the most common symptoms associated with gadget use were dry eyes found in 73.3% of students, blurred vision in 68.9%, and neck, shoulder and back pain were declared by 65.2% of the students (10). There are also various studies that analyze the effect of *CVS* among medical students, but there is no comparison between *CVS* among medical students from different languages teaching series (11, 12).

Various studies showed that the most used gadget among medical students is the smartphone (13, 14), these results being similar to those of the present study in which the most used gadget was all the smartphone, being used by 55% from Romanian students, 63% from English students and 51% of French students.

In our study, the average daily gadget use was 6.18 hours for Romanian students, 5.98 hours for English students, and 6 hours for French students. These findings are consistent with Ganganahalli *et al.*'s study, which reported average usage of 6.5 hours for males and 6.2 hours for females among medical students in Western Maharashtra, India (15).

Dry eye disease (DED) is a major public health issue that causes visual discomfort and impacts quality of life (16). A study of 100 medical students found dry eyes, blurred vision, and neck, shoulder, and back pain to be the most common symptoms from prolonged screen use (17). Another study on 80 medical students reported headaches in 50% and dry eyes in 45% of participants (18). Similarly, in our study, the most common symptoms among Romanian students were dry eyes (73.3%), focusing difficulties (72%), and blurred vision (68.9%). English students reported eye fatigue (66.1%) and dry eyes (58.1%), while French students mainly experienced eye fatigue (91.8%), blurred vision (34.4%), and dry eyes (34.4%).

A 2022 study on healthcare workers found that prolonged screen time increased the risk of muscle pain, headaches, and stress, highlighting the need to reduce gadget use for doctors and nurses (19). Another 2022 study reported a high prevalence of *CVS* among healthcare workers (20). A study on medical students in Central India showed that 54% had eye-related symptoms, while 46.5% felt anxious or restless without their devices. Common health issues included headaches, back pain, and joint pain (21). In our study, the majority of students, 229 of 282 Romanian, 52 of 62 English, and 55 of 61 French, believed digital screens negatively impacted their health and lifestyle.

Numerous studies have evaluated the disadvantages of *CVS* on health and quality of life. Studies have shown that *CVS* represents a major cause affecting visual function, health and quality of life. Therefore, these studies highlighted that the use of special campaigns and advertisements in schools, universities and mass media are useful and necessary to urge the public to be aware of the risks and disadvantages of *CVS* on health, work and life style (22-25).

In our research, 235 out of 282 (83.33%) Romanian students, 50 out of 62 English students (80.64%) and 50 out of 61

French students (81.96%) are willing to reduce the time spent in front of gadgets. These results are better than those in the study conducted by Gammoh *et al.* which showed that 69.2% of students believe that gadgets affect their health and quality of life, and only 59.2% are willing to reduce time using gadgets (13). Moreover, in the study conducted by Iqbal et al., 76% of the students were willing to decrease their screen hours to avoid *CVS* (17). In our study, more than 80% of the participants are willing to reduce the time spent in front of gadgets, therefore the results of our research being more positive than the two other studies mentioned.

Several limitations should be considered when interpreting the results from our work. The students from English and French series were studying only general medicine, while Romanian students were studying general medicine, nursing, dentistry and pharmacy and therefore the number of Romanian students included in the study was significantly higher than the other 2 groups of students. Moreover, information on *CVS* was obtained through questionnaires, which are not always accurate. Further studies are needed to analyze the effect of gadget use on health and quality of life among medical students and to provide advice and clarification on appropriate gadget use and working time.

CONCLUSIONS

This study's findings, based on questionnaires, reveal that gadgets play a significant role in the daily lives of medical students. Ocular symptoms such as dry eyes, eye fatigue, and blurred vision were common across all three groups. Musculoskeletal issues, including neck, shoulder, and back pain, were also frequently reported. Students in all groups are aware that prolonged gadget use negatively impacts health and lifestyle. Most expressed a willingness to reduce screen time, indicating their recognition of the harmful effects of Computer Vision Syndrome and their desire to improve their quality of life.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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A PRELIMINARY MACHINE LEARNING APPROACH TO UNDERSTANDING THE RELATIONSHIP BETWEEN GLYCEMIC DYSREGULATION, BIOCHEMICAL MARKERS, AND SCHIZOPHRENIA SEVERITY SCORES

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A PRELIMINARY MACHINE LEARNING APPROACH TO UNDERSTANDING THE RELATIONSHIP BETWEEN GLYCEMIC DYSREGULATION, BIOCHEMICAL MARKERS, AND SCHIZOPHRENIA SEVERITY SCORES (Abstract): Schizophrenia is a multifaceted psychiatric disorder associated with significant metabolic disturbances, including glycemic dysregulation and systemic inflammation. This study **aims** to explore the relationship between glycemic control, biochemical markers, and schizophrenia severity using a machine learning approach. **Materials and methods:** A cross-sectional study was conducted on 70 patients diagnosed with schizophrenia, evaluating fasting glucose levels, lipid profiles, inflammatory markers (CRP, ESR), and clinical severity scores (BPRS, PANSS). Statistical and machine learning models, including regression and random forest algorithms, were employed to identify key predictors of psychiatric severity. **Results:** Results indicated that 18.6% of participants exhibited hyperglycemia, which was significantly correlated with higher BPRS and PANSS scores ($p<0.05$). Elevated CRP levels were detected in 45% of participants with moderate-to-severe symptoms, reinforcing the role of systemic inflammation in schizophrenia pathophysiology. Machine learning models identified glycemic status and systemic inflammation as key predictors of psychiatric severity, with behavioral dysregulation strongly linked to hyperglycemia. Furthermore, psychiatric symptom severity was positively associated with metabolic disturbances, particularly among individuals exhibiting irritability and agitation. **Conclusions:** These findings highlight a bidirectional relationship between metabolic dysfunction and schizophrenia severity, suggesting that glycemic control and inflammation may serve as important clinical biomarkers. The integration of machine learning techniques offers a novel approach to identifying metabolic predictors of psychiatric severity, potentially informing personalized treatment strategies. **Keywords:** SCHIZOPHRENIA, PSYCHOTIC DISORDER, METABOLIC DYS-REGULATION, GLYCEMIC VARIATIONS, BPRS, MACHINE LEARNING, AI TECHNIQUES.

INTRODUCTION

Schizophrenia (SCZ) is a complex neuropsychiatric disorder characterized by cognitive, emotional, and behavioral dysfunction. Despite extensive research, its pathophysiology remains incompletely understood, with growing evidence highlighting a crucial link between metabolic

dysregulation and symptom severity (1, 2). Glycemic abnormalities, including insulin resistance and hyperglycemia, are disproportionately prevalent among individuals with schizophrenia, independent of antipsychotic treatment (3, 4). Patients with schizophrenia have a two- to threefold increased risk of developing type 2 diabetes, suggesting an intrinsic metabolic vulnerability mediated by hypothalamic-pituitary-adrenal (HPA) axis dysfunction, chronic inflammation, and oxidative stress (4, 5, 6).

Emerging studies indicate that metabolic disturbances may exacerbate psychiatric symptoms. Elevated fasting glucose levels and insulin resistance have been linked to increased symptom severity, cognitive impairment, and poor treatment response (7, 8). Chronic hyperglycemia and neuroinflammation may alter brain function through mechanisms such as vascular damage, impaired neurotransmission, and oxidative stress, potentially worsening psychotic symptoms (9, 10). Furthermore, systemic inflammation, marked by elevated C-reactive protein (CRP) and interleukin-6 (IL-6), is commonly observed in schizophrenia and correlates with both metabolic syndrome and psychiatric burden (11, 12, 13).

The rapid advancement of machine learning (ML) techniques has opened new avenues for analyzing complex relationships between metabolic biomarkers and psychiatric symptoms. ML models can identify nonlinear patterns in biochemical and clinical data, potentially enhancing our understanding of metabolic contributions to schizophrenia severity (14, 15). However, research integrating ML with biochemical markers and psychiatric scales remains limited.

This study aims to explore the relationship between glycemic dysregulation, biochemical markers, and schizophrenia severity using a preliminary machine learning approach. Specifically, we seek to identify key metabolic markers associated with psychiatric symptom severity using clinical scales such as the Brief Psychiatric Rating Scale (BPRS) and the Positive and Negative Syndrome Scale (PANSS), while developing and testing machine learning models to predict schizophrenia severity based on biochemical and psychometric data. By integrating biochemical assessments with advanced data analytics, this research contributes to precision psychiatry by investigating metabolic predictors that could inform individualized treatment strategies, ultimately improving clinical outcomes for individuals with schizophrenia.

MATERIALS AND METHODS

Study design. This study utilized a cross-sectional design to examine the relationship between glycemic dysregulation, other biochemical parameters, and schizophrenia symptom severity. Data were collected from patients diagnosed with schizophrenia or psychotic disorders according to ICD-10 criteria at the largest psychiatric hospital in northeastern Romania. Ethical approval was secured from the institutional review board, and all participants provided written informed consent prior to enrollment.

Participants. A total of 70 adult participants (18–65 years old) with a confirmed schizophrenia or psychotic disorder diagnosis were included. Inclusion criteria required recent biochemical profiles and willingness to participate. Exclusion criteria comprised comorbid neurological or metabolic disorders unrelated to schizo-

phrenia, current substance use disorders, and history of traumatic brain injury. Clinical severity was assessed using the Brief Psychiatric Rating Scale (BPRS) and the Positive and Negative Syndrome Scale (PANSS), ensuring standardized evaluation. Sociodemographic data, including age, gender, and residential background, were collected for context.

Data collection. Biochemical assessments were performed using fasting blood samples to measure glucose levels, lipid profiles (HDL, LDL, triglycerides), and inflammatory markers (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]). Psychiatric symptom severity was evaluated with the BPRS and PANSS scales, administered by trained clinicians to ensure reliability. Anthropometric measurements (weight, height, waist circumference) were recorded to calculate body mass index (BMI), following World Health Organization (WHO) guidelines.

Statistical analysis. Data integrity was ensured by handling missing values through multiple imputation and excluding clinically irrelevant outliers. Pearson's and Spearman's correlation analyses were applied to examine relationships between biochemical markers and psychiatric severity scores. Regression models were utilized to identify predictors of glycemic levels and their association with symptom severity.

For machine learning analysis, models including random forest regression and support vector machines (SVM) were implemented to identify nonlinear relationships between biochemical and psychometric data. Performance was evaluated using R-squared (R^2), root-mean-square error (RMSE), and mean absolute error (MAE). K-fold cross-validation was applied to enhance generalizability, and feature importance rankings were generated to highlight the most significant predictors.

Ethical considerations. The study adhered to the principles of the Declaration of Helsinki, ensuring the ethical treatment of all participants. Data confidentiality was maintained by anonymizing all identifying information. Participants retained the right to withdraw from the study at any time without consequence.

RESULTS

Participant characteristics

The study included 70 participants diagnosed with schizophrenia, evenly distributed across age and gender categories. The cohort consisted of 40 males (57.1%) and 30 females (42.9%), with a balanced representation of urban (57.1%) and rural (42.9%) backgrounds. Participants had varying educational and occupational statuses, reflecting a heterogeneous sample.

Glycemic and metabolic profiles

Biochemical assessments revealed a high prevalence of metabolic disturbances. Hyperglycemia was observed in 18.6% of participants, while 81.4% had normal fasting glucose levels. Body mass index (BMI) analysis showed that 47.1% were overweight or obese, with 37 participants classified as normal weight, 22 as overweight, and 11 as obese. Dyslipidemia, including hypercholesterolemia (20%) and hypertriglyceridemia (10%), was present in a significant subset of patients.

Inflammatory markers were elevated in a large proportion of participants. CRP levels above 10 mg/L were found in 45% of participants, and elevated erythrocyte sedimentation rate (ESR) was detected in 60%, supporting previous findings on the inflammatory component of schizophrenia.

Behavioral dysregulation cluster

Behavioral symptoms such as irritability, dysphoric mood, agitation, and reduced frustration tolerance were among the most prevalent and clinically impactful features within the cohort. Irritability was observed in 61.4% of participants, making it the most frequently reported behavioral symptom, followed by agitation, which had an incidence rate of 17.1% over a 12-month period.

The relationship between behavioral dysregulation and metabolic disturbances was striking. Individuals with irritability were nearly five times more likely to experience hyperglycemia compared to those without this symptom, with a relative risk (RR) of 4.72. The odds ratio (OR) further highlighted the strength of this association, showing that participants with irritability had a 6.75-fold higher likelihood of hyperglycemia (fig. 1). From a diagnostic perspective, the sensitivity of irritability as a predictor of hyperglycemia was exceptionally high at 88.2%, though its specificity was moderate at 47.2%. This indicates that while irritability effectively identifies cases of hyperglycemia, it may also yield false positives among those without metabolic disturbances.

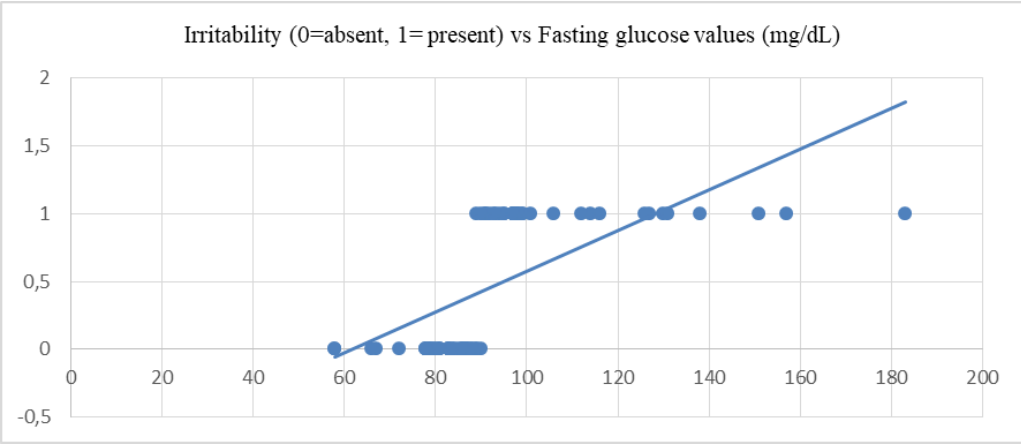

Fig. 1. Correlation graph between Irritability and Fasting glucose values (mg/dL)

Elevated inflammatory markers further linked behavioral symptoms to metabolic dysregulation. Participants with irritability frequently exhibited C-reactive protein (CRP) levels above 10 mg/L (45%), reinforcing the role of systemic inflammation in the pathophysiology of this cluster. Neuroinflammatory processes likely activate the hypothalamic-pituitary-adrenal (HPA) axis, contributing to both psychiatric and glycemic disturbances. Moreover, CRP and leukocytosis collectively explained 12.3% of glycemic prediction variance in machine learning models, highlighting their significance in the inflammatory-metabolic axis.

Psychiatric symptom severity

BPRS scores ranged from 18 to 77, with a mean score of 41.8 (SD = 7.6), placing most participants in the mild-to-moderate severity category. Within this cohort, 44.3% had mild symptoms (BPRS: 32–41), 40% had moderate symptoms (BPRS: 42–53), and 1.4% had severe symptoms (>65).

A statistical analysis revealed that par-

ticipants with BPRS scores >45 were 1.79 times more likely to have hyperglycemia, suggesting a link between psychiatric burden and metabolic dysfunction. Dyslipidemia, particularly hypertriglyceridemia (OR = 2.1, p = 0.036), was also more prevalent in those with severe symptoms. Systemic inflammation appeared to play a crucial role, with leukocytosis observed in 8.5% of high BPRS participants vs. 3.2% in lower-scoring individuals (RR = 2.65, p = 0.017). These findings indicate that psychiatric severity may contribute to metabolic disturbances via inflammatory or endocrine pathways.

A random forest model ranked BPRS scores as the second most influential predictor of hyperglycemia, after CRP levels. A positive correlation between fasting glucose and BMI suggests that metabolic dysregulation may be associated with psychiatric symptom severity (fig. 2). Including BPRS in the model improved prediction accuracy by 9%, reinforcing the role of psychiatric severity in metabolic risk assessment.

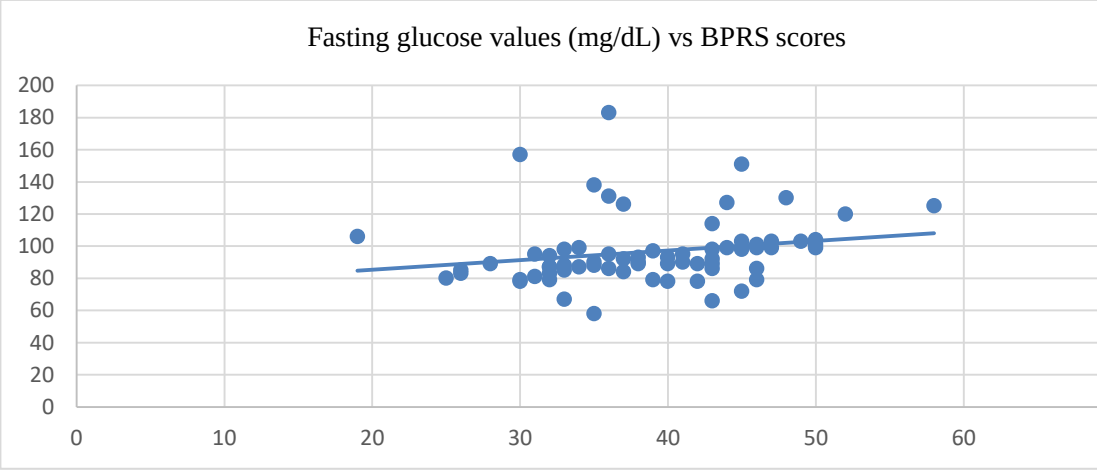

Fig. 2. Correlation graph between Fasting glucose levels (mg/dL) and BMI values

The Positive PANSS scores have a mean of 20.6 with a standard deviation of 6.45, indicating moderate variability in symptoms related to hallucinations, delusions, and other positive schizophrenia symptoms. The Negative PANSS scores average 21.15, with a lower standard deviation (4.29), suggesting more consistency in symptoms such as emotional withdrawal and apathy. The General PANSS scores have a mean of 39.1 with a wider spread (std = 6.65), indicating that general psychopathology symptoms vary more significantly across participants.

In terms of correlations, a moderate positive correlation (0.70) was found between Positive PANSS symptoms and General PANSS scores, suggesting that patients with more severe positive symptoms tend to have higher overall psychopathology severity. Similarly, Negative PANSS symptoms moderately correlated (0.47) with General PANSS, reinforcing the idea that both positive and negative symptoms contribute significantly to the overall illness burden.

Fasting glucose levels (mg/dL) show a positive correlation with both PANSS Gen-

eral and Negative symptom scores (0.54 and 0.39, p< 0.05), suggesting that higher glycemic values may be associated with greater severity of overall psychopathology and specific negative symptoms such as emotional withdrawal and apathy (figs. 3, 4).

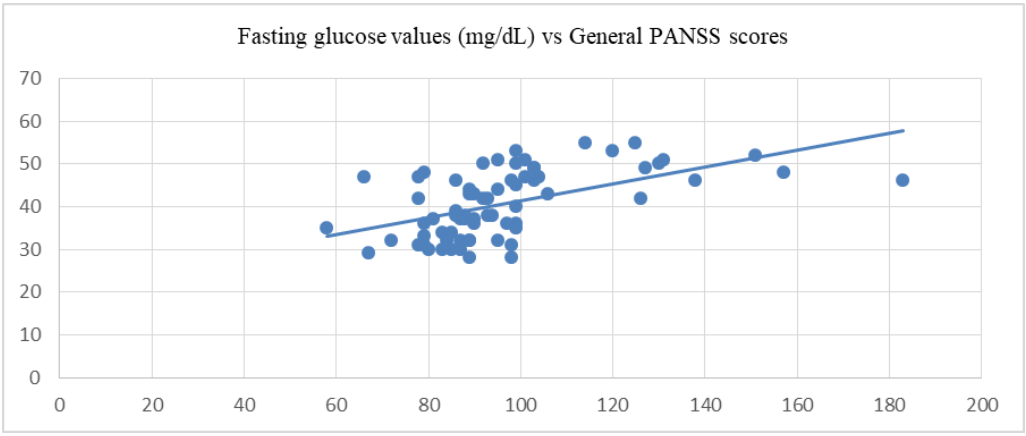

Fig. 3. Correlation graph between Fasting glucose values (mg/dL) and General PANSS scores

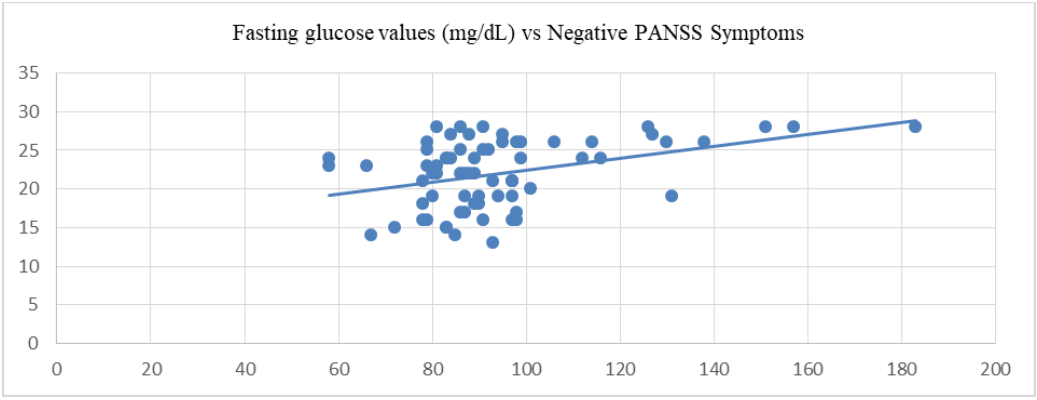

Fig. 4. Correlation graph between Fasting glucose values (mg/dL) and Negative PANSS scores

Body Mass Index (BMI) displayed a positive correlation with Negative PANSS (0.19) and General PANSS (0.29), indicating that higher BMI might be associated with more pronounced negative symptoms such as emotional withdrawal or blunted affect or general symptoms such as unusual thought content, tension or poor impulse control (fig. 5).

Regarding inflammatory markers, CRP (C-reactive protein) showed a significant positive correlation (0.8) with General PANSS, potentially suggesting that higher systemic inflammation may be associated with increased severity of general psychopathological symptoms in schizophrenia or psychotic disorders (fig. 6). Meanwhile, VSH (another inflamma-

tion marker) showed only weak correlations with all PANSS dimensions, suggesting minimal influence on symptom severity.

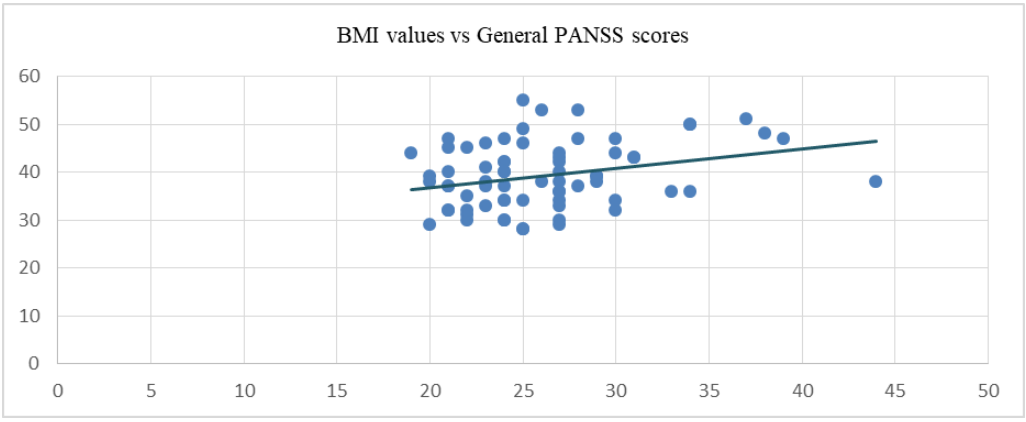

Fig. 5. Correlation graph between BMI values and General PANSS scores.

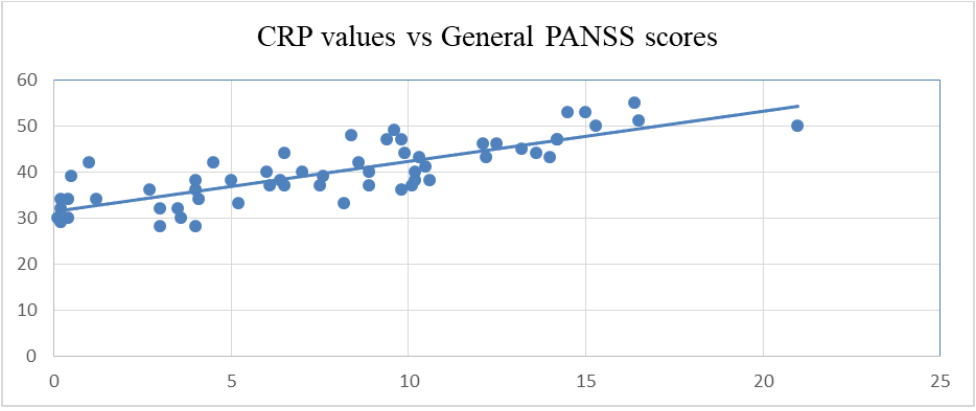

Fig. 6. Correlation graph between CRP values and General PANSS scores

Psychotic symptoms cluster

The psychotic symptom cluster, encompassing delusions, paranoia, and hallucinations, was less frequent but offered unique insights. Delusions, the most prevalent symptom in this cluster, affected 10% of participants. Paranoia and hallucinations were observed in approximately 7% of cases, highlighting their relatively lower incidence within the cohort.

The metabolic impact of psychotic symptoms was subtler compared to behavioral dysregulation. he correlation between delusions and hyperglycemia was weak, suggesting that metabolic dysregulation in this cluster may not be as pronounced. However, psychotic symptoms were significantly associated with inflammatory markers. Participants with delusions were 3.8 times more likely to exhibit elevated CRP levels compared to their non-delusional counterparts. This finding aligns with the broader hypothesis that systemic inflammation, potentially exacerbated by neuro-

chemical imbalances such as dopamine hyperactivity, influence psychotic symptomatology.

Biochemical / hematological markers

Participants with elevated CRP levels (>10 mg/L) and leukocytosis (5.7%) were more likely to exhibit higher schizophrenia severity scores, particularly in the general psychopathology subscale of the Positive and Negative Syndrome Scale (PANSS).

Elevated creatine kinase (CK-NAC), observed in 22.9% of participants, correlated with mild hyperglycemia, suggesting muscle stress or catabolism as potential contributors to glycemic imbalance. Dyslipidemia was common, with 20% and 10% of participants exhibiting hypercholesterolemia and hypertriglyceridemia, respectively. These lipid abnormalities were more frequent among participants with moderate-to-severe schizophrenia symptoms.

Hematological abnormalities, such as neutropenia (11%) and thrombocytopenia (2.9%), were associated with hyperglycemia and higher psychiatric severity scores. Neutropenia, in particular, was correlated with reduced BPRS scores ($\beta=-0.27$, $p=0.042$), likely reflecting the impact of medication-induced immune suppression, particularly from clozapine.

Interactions between symptom clusters and biochemical parameters

Cross-cluster analyses revealed significant interactions. Behavioral symptoms like irritability and agitation exhibited a moderate correlation with flattened affect, suggesting shared neurobiological mechanisms, potentially involving dopamine and serotonin dysregulation. Similarly, agitation showed a modest association with delusions, hinting at overlapping pathways in psychomotor hyperactivity and psychosis.

Machine learning models further enriched these findings. Behavioral symptoms emerged as the strongest predictors of glycemic variability, accounting for 35% of the variance in hyperglycemia prediction. Negative symptoms contributed an additional 18%, while psychotic symptoms added 11%. These models, including Random Forest and Gradient Boosting, achieved robust predictive performance, with R2R^2R2 values exceeding 0.4 and mean absolute errors (MAE) as low as 4.2 mg/dL.

DISCUSSION

This study highlights the strong association between metabolic dysregulation, systemic inflammation, and schizophrenia severity, reinforcing the growing recognition of schizophrenia as a multisystemic disorder. Our findings align with previous research indicating that patients with schizophrenia are at increased risk for diabetes mellitus, independent of antipsychotic medication use (3, 6, 12). The observed prevalence of hyperglycemia (18.6%) and its association with higher BPRS and PANSS scores supports the hypothesis that glycemic abnormalities contribute to worsening psychiatric symptoms (7,9,13). Metabolic disturbances, particularly insulin resistance and chronic hyperglycemia, have been shown to impair cognitive function and exacerbate oxidative stress, both of which contribute to schizophrenia's neuroprogressive course (14, 15, 16). Our results reinforce this link, demonstrating that individuals with higher BPRS scores were nearly twice as likely to exhibit hyperglycemia, while those with elevated inflammatory markers (CRP, ESR) displayed greater psychiatric symptom severity. These find-

ings suggest that metabolic and inflammatory pathways play a crucial role in schizophrenia symptomatology.

The integration of machine learning models provided additional insights into the metabolic-psychiatric connection. BPRS scores and CRP levels emerged as key predictors of hyperglycemia, highlighting the relevance of psychiatric severity and inflammation in metabolic risk assessment. The random forest model improved prediction accuracy by 9% when psychiatric variables were included, reinforcing the role of psychiatric burden in metabolic dysfunction. These findings align with emerging studies demonstrating that AI-driven models can effectively identify schizophrenia subtypes and predict treatment response based on biochemical markers (17, 18, 19, 20).

One of the most striking findings was the strong association between behavioral dysregulation (irritability, agitation) and metabolic abnormalities. Participants exhibiting irritability had a 4.72-fold increased risk of hyperglycemia, suggesting that emotional distress may exacerbate metabolic disturbances via neuroinflammatory mechanisms. Elevated CRP levels in these individuals further support the role of systemic inflammation in linking psychiatric and metabolic dysfunction. These results emphasize the need for integrated screening of metabolic health in patients with severe behavioral symptoms.

Evidence suggests that glucose metabolism alterations are not merely side effects of antipsychotic medication but may be intrinsic to schizophrenia pathophysiology (3). Lee *et al.* (2023) identified impaired glucose metabolism as a potential biomarker for psychiatric disorders, with machine learning models achieving high predictive accuracy in stratifying patient risk (21). Similarly, Zaki *et al.* (2022) developed diagnostic models using peripheral blood markers, reinforcing the role of metabolic parameters in schizophrenia symptoma tology (22).

Our findings support a multidisciplinary approach to schizophrenia management, incorporating metabolic and inflammatory assessments alongside psychiatric evaluations. Routine screening of fasting glucose, lipid profiles, and inflammatory markers may help identify high-risk patients and enable early interventions. Future research should explore longitudinal models to assess whether improving metabolic health leads to reductions in psychiatric symptom severity.

This study has several limitations. The cross-sectional design prevents causal conclusions regarding the relationship between glycemic dysregulation and psychiatric severity. The sample size (N=70) is relatively small, limiting generalizability. Additionally, while machine learning models demonstrated moderate predictive performance ($R^2 > 0.4$), further optimization and validation are needed before clinical application. Future studies should incorporate neuroimaging and multi-omics approaches to improve model accuracy and enhance precision psychiatry strategies.

CONCLUSIONS

This study reinforces schizophrenia as a multisystemic disorder, highlighting the link between metabolic dysregulation, systemic inflammation, and psychiatric severity. Glycemic abnormalities and inflammatory markers correlated with higher PANSS and BPRS scores, suggesting a metabolic-neuroimmune axis in symptom progression. Machine learning models identified glyce-

mic status and inflammation as key predictors, with behavioral dysregulation (irritability, agitation) strongly linked to hyperglycemia.

These findings support integrating metabolic screening into schizophrenia management, as targeted metabolic interventions may improve psychiatric outcomes. Despite limitations, future research should adopt longitudinal and multi-omics approaches to refine predictive models. Leveraging AI-driven biomarker discovery can advance precision psychiatry, leading to personalized, data-driven interventions for schizophrenia patients.

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IS OPEN SURGICAL REVASCULARIZATION STILL AN OPTION FOR TREATING CHRONIC MESENTERIC ISCHEMIA?

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IS OPEN SURGICAL REVASCULARIZATION STILL AN OPTION FOR TREATING CHRONIC MESENTERIC ISCHEMIA? (Abstract): Chronic mesenteric ischemia (CMI) is a condition caused by inadequate blood supply to the gastrointestinal tract for at least 3 months due to atherosclerosis. **Materials and methods:** Diagnosis is often delayed until the patient is referred to a vascular surgery unit. In patients requiring revascularization for CMI, the superior mesenteric artery (SMA) is the primary target vessel using either open or endovascular (EV) techniques. While EV therapy has become the first-line treatment, open surgical re-vascularization remains relevant for selected cases. The revascularization procedure consists of aortomesenteric bypass reconstructing the visceral arteries. **Results:** This paper presents six consecutive cases of CMI treated at the Vascular Surgery Unit of the "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania, between 2014 and 2024. All patients had symptoms suggestive of mesenteric disease, two cases were associated with aorto-iliac disease, one case had *thromboangiitis obliterans*. CT angiography revealed stenotic or occlusive lesions in the celiac trunk and mesenteric arteries in five cases and a fusiform partial thrombosed aneurysm of the SMA in one case. Surgically revascularized mesenteric arteries were SMA and IMA in one case and SMA in four cases. Venous grafts were used for revascularization in three cases and synthetic grafts in two cases. EV treatment consisted of double stenting of the SMA in one case. Primary patency in the surgical group was 60%, and secondary patency was 100%. Symptomatic resolution was observed in all cases. **Conclusions:** Open surgical revascularization remains an important option, especially for patients with extensive mesenteric artery disease. **Keywords:** CHRONIC MESENTERIC ISCHEMIA, MESENTERIC BYPASS.

INTRODUCTION

Chronic mesenteric ischemia (CMI) is defined as ischemic symptoms caused by inadequate blood supply to the gastrointestinal tract, lasting at least 3 months. It is a progressive disease that, if left untreated, can lead to severe complications and death from either starvation or entero-mesenteric infarction (1). With increasing rates of atherosclerotic disease and obesity worldwide, the prevalence of CMI has progressively increased.

The diagnosis of CMI remains challenging due to its non-specific symptoms and the need for advanced imaging techniques. The typical presentation includes postprandial pain, weight loss due to fear of eating, unexplained diarrhea or constipation (2). Diagnosis is often delayed until the patient is referred to a vascular surgeon. The incidence of CMI is less than 1 per 100,000 hospital admissions (3).

CMI is primarily caused by atherosclerosis leading to progressive stenosis of the mesenteric arteries, including the celiac artery (CA), superior mesenteric artery (SMA), and inferior mesenteric artery (IMA).

Typically, significant occlusion of 2 or 3 visceral vessels is required for the patient to become symptomatic. Isolated occlusion of the celiac trunk or superior mesenteric artery alone can lead to symptoms if the collateral network is inefficient (3).

Diagnostic imaging plays a critical role in the confirmation of CMI. In patients with suspected CMI, duplex ultrasound (DUS) of the mesenteric arteries is recommended as a first-line test (2). DUS is also useful for intraoperative assessment of open surgical procedures on the mesenteric arteries. In patients with moderate to high suspicion of CMI, computed tomography angiography (CTA) is recommended to map the occlusive disease and to detect or exclude other intra-abdominal pathology. With 3D reformatting, it can provide excellent reconstructions of the mesenteric arteries (2).

Revascularization is indicated in patients who develop symptoms of CMI. The goals of mesenteric revascularization include relief of symptoms, improvement of quality of life, restoration of normal weight, and improvement of survival by preventing bowel infarction. Once the diagnosis of symptomatic CMI is made, revascularization within a short delay is mandatory to avoid acute exacerbation and bowel infarction by "acute-on-chronic" ischemia (7, 8, 9). Revascularization is the mainstay of treatment, after which the patient can resume oral nutrition.

In patients requiring revascularization for CMI, the SMA is the main target vessel, using either open or EV techniques (4, 5, 6). Characteristics of the SMA that influence treatment selection include vessel diameter, degree of stenosis or occlusion, presence of tandem lesions, degree of calcification, and the extent of collateralization. Open surgical techniques include antegrade bypass (the distal thoracic or supracolic aorta is selected as the inflow source), retrograde bypass (the infrarenal aorta, a previous aortic graft, or the iliac arteries are selected as the inflow source), retrograde open mesenteric stenting (hybrid approach via midline laparotomy to expose the SMA or CA combined with EV retrograde stenting), and endarterectomy (trans-aortic or direct mesenteric endarterectomy) (2).

MATERIALS AND METHODS

This retrospective study presents six consecutive cases of CMI treated at the Vascular Surgery Department of "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania, during 2014-2024. Five patients were male and one patient was female, the mean age was 54.33 years. Two cases were associated with aorto-iliac disease, one case had thromboangiitis obliterans and one case had partial thrombosed aneurysm of SMA with "acute-on-chronic" mesenteric ischemia. All patients presented with postprandial abdominal pain, four patients had significant weight loss, two patients had intermittent claudication and

decreased walking distance (< 50 m). Laboratory tests revealed hypoproteinemia and hypoalbuminemia in four cases. Patients' characteristics are showed in tab. I and the symptoms and anatomical characteristics are resumed in second table.

TABLE I
Patients' characteristics

Patient	1	2	3	4	5	6
Gender	M	M	W	M	M	M
Age	51	60	73	54	45	43
Smoking	Yes	Yes	No	Yes	Yes	Yes
Diabetes Mellitus	No	No	Yes	No	No	No
Dyslipidemia	No	Yes	Yes	Yes	No	No
Hypoproteinemia	Yes	Yes	Yes	Yes	No	No

TABLE II.
Patients' symptoms and anatomical characteristics

Patient	1	2	3	4	5	6
Weight loss	Yes	Yes	Yes	Yes	No	No
Post-prandial pain	Yes	Yes	Yes	Yes	Yes	Yes
Arterial disease	PAD, CVD	PAD	COD	PAD	TAO, COD	Aneurysm SMA
Mesenteric arteries	CA thrombosis at the origin, SMA occlusion	CA thrombosis at the origin, SMA stenosis at the origin	SMA atheromatosis, undetectable flow on the first 3 cm	SMA atheromatosis, critical stenosis on the first 25 mm	SMA stenosis 85-90% at 20mm from the origin	Fusiform aneurysm of the SMA, partially thrombosed, located at 40 mm from the origin

CA-celiac artery, COD- coronary obstructive disease, CVD- cerebrovascular disease,
PAD -peripheral arterial disease, SMA- superior mesenteric artery, TAO-thromboangiitis obliterans

Preoperatively, CT angiography revealed severe CA and SMA stenosis in two cases (cases 1 and 2) with atheromatosis of the abdominal aorta, iliac arteries, and abdominal aortic branches. In three cases (case 3, 4, 5) CTA showed severe SMA atheromatosis at 20 mm from the origin, with flow at the level of the peripheral branches. In one patient (case 6), CT angiography showed a partially thrombosed aneurysm of the SMA located at a distance of 40 mm from the origin, with a maximum diameter of 32 mm and a length of 48 mm.

The surgical procedure for the two cases with aorto-iliac disease consisted of aorto-bifemoral bypass with Dacron graft associated with aorto-SMA and aorto-IMA bypass with autologous graft (GSV) in the first case (fig. 1), aorto-bifemoral bypass with Dacron graft associated with aorto-SMA bypass with venous graft (GSV) in the second case (fig. 2).

TABLE III
Details of surgical procedures

Patient	1	2	3	4	5	6
Procedure	Ao2F bypass (synthetic graft), Aorto-IMA and Aorto-SMA retrograde bypass (venous graft)	Ao2F bypass (synthetic graft), Aorto-SMA retrograde bypass (venous graft)	1. Aorto-SMA bypass; 2. Aorto-SMA bypass (reinforced PTFE graft)	Aorto-SMA bypass (reinforced PTFE graft and distal Miller cuff)	EV treatment double stenting of the SMA (BMS 6x40 mm and 7x60mm)	1. Aorto-SMA bypass (reinforced PTFE graft and Miller cuff), partial aneurysmectomy, SMA ligation distal to the aneurysm; 2. Aorto-SMA bypass (venous graft), right ileum resection, jejune-transverse anastomosis

EV - endovascular, IMA-inferior mesenteric artery,
PTFE-polytetrafluoroethylene, SMA- superior mesenteric artery

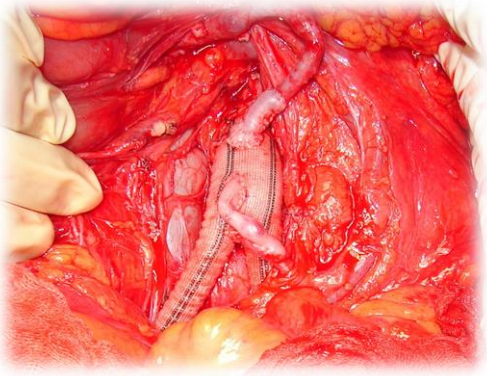

Fig. 1. Aorto-SMA bypass and aorto-IMA bypass- case 1

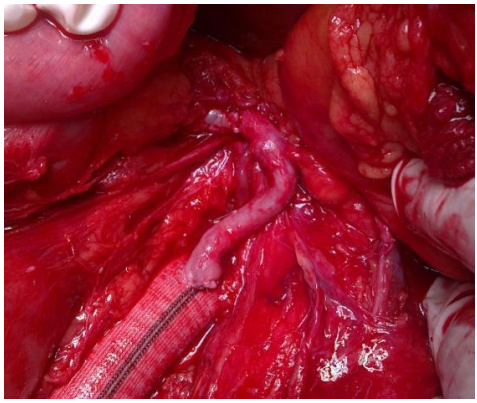

Fig. 2. Aorto-SMA bypass - case 2

The omentum was brought into the aorto-mesenteric space by coloepiploic dissection. In this way, the omentum was used as an anchoring fringe.

The case with a critical stenosis in the first 25 mm of the SMA (case 4) benefited from an aorto-SMA retrograde bypass with a synthetic graft (reinforced PTFE) and a distal Miller cuff (GSV) (fig. 3).

Postoperative CT angiography at 1 month revealed graft patency in these cases.

One of the patients (case 3) was treated surgically in two sequences: aorto-SMA retrograde bypass with venous graft (GSV) followed by aorto-SMA retrograde bypass with synthetic graft (reinforced PTFE) due to venous graft thrombosis. In this case, long-term patency could not be assessed, and the patient was lost to follow-up.

The young patient with thromboangiitis obliterans and critical stenosis of the SMA at 20 mm from the origin (case 5) benefited from EV treatment - balloon angioplasty and double SMA stenting (BMS 6x40 mm

and 7x60 mm) (fig.4).

Fig. 3. Aorto-SMA retrograde bypass with PTFE graft and distal Miller cuff-case 4

Fig. 4. Angiography after stenting-case 5

The case with a fusiform aneurysm of the SMA (case 6) underwent surgery, performing aorto- SMA bypass with reinforced PTFE graft and Miller cuff (GSV), partial aneurysmectomy, SMA ligation distal to the aneurysm. After 24 hours, CT angiography showed graft thrombosis, which required re-intervention with aorto-mesenteric bypass with venous graft, right ileum resection, jejune-transverse anastomosis. CT angio-graphy at 2 and 10 months after revascularization showed graft patency.

After surgery, the patients received the *Triple "A"* therapy (*antibiotic, anticoagulant, analgesic*), combined with vasodilator and hypolipemic agents.

The purpose of this study was to analyze the perioperative outcomes and follow-up results of patients with CMI who were managed in our center.

RESULTS

The five cases treated surgically benefited from CT angiographic control along with DUS follow-up at 1, 2, 3, 6, 12 months and then annually, which showed the patency of the bypass. The case treated EV was followed up with post-procedure control angiography along with periodic DUS at 1, 3, 6, 12 months.

TABLE IV.

Patients' outcomes and complications

Patient	1	2	3	4	5	6
Re-exploration	No	No	Yes	No	No	Yes
Resolution of symptoms	Yes	Yes	Yes	Yes	Yes	Yes
Body weight recovery	Yes	Yes	Yes	Yes	No	No
Imaging follow-up (years)	10	10	2	5	1	1
Imaging modality	DUS/CTA	DUS/CTA	DUS	DUS/CTA	DUS	CTA

Immediate and long-term results were favorable: resumption of physiological intestinal transit (in all cases) and presence of pulse in the peripheral arteries of the

lower limb (in the two cases with aortic and mesenteric revascularization). Remission of symptoms was achieved in 100% of cases. Primary patency in the surgical group was 60% and secondary patency was 100%. Primary patency at 1 year in the endovascular group was 100%.

Antiplatelet monotherapy was used in five cases and dual antiplatelet therapy in one case. With appropriate surgical or EV procedures, problems were resolved. Patients were reintegrated into their families and communities.

DISCUSSION

In daily practice, the clinical decision making for the optimal mesenteric revascularization procedure, remains challenging. In patients with CMI requiring revascularization, the superior long-term outcomes of open surgery must be weighed against the potential early benefit of EV intervention in terms of peri-procedural mortality and morbidity. Several studies have reported that patients with CMI treated with EV techniques appeared to have a higher incidence of symptom recurrence and have recommended EV treatment as a bridge to open surgical revascularization in nutritionally depleted patients with CMI (5). For surgical candidates, prompt surgical intervention is critical, as untreated symptomatic CMI has a 5-year mortality rate approaching 100% (5). Immediate symptom relief is reported in 90-98% of surgically treated patients and remains excellent at 5 years with 89-92% (8).

Mesenteric bypass offers improved patency with lower re-intervention rates and better freedom from recurrent symptoms. A mortality of <3% has been reported for mesenteric bypass performed in large tertiary care centers, although this may be higher in patients presenting late with established bowel infarction (2).

In low-risk patients aged 50 years or younger, open mesenteric revascularization offers the most durable vascular repair for the patient, with a 10-year primary patency rate of 85% (9). Other indications for open mesenteric revascularization include failed EV attempts, stenotic lesions unsuitable for stenting, such as long (>2 cm) stenoses or occlusions, flush ostial occlusions, small (<4 mm diameter) SMA and severely calcified stenosis incorporated into a porcelain aorta (9), and associated aorto-iliac disease (9, 10, 11).

There is no consensus on the best method of mesenteric surgical reconstruction for the treatment of CMI. A variety of open surgical techniques have been described for mesenteric artery reconstruction. The choice includes the type of incision (trans peritoneal vs retroperitoneal), conduit (vein vs prosthetic), graft configuration (antegrade vs retrograde), source of inflow (aortic vs iliac), and number of vessels to be reconstructed (single vs multiple). The type of open reconstruction is selected based on the anatomy and clinical risk assessment of the patient (4). In patients where bowel integrity may be compromised, the bowel should be examined and resected if necessary (12). This was the case of the patient with SMA aneurysm who underwent reintervention (case 6).

In open revascularization, the literature shows good results with revascularization of the SMA alone, with long-term results similar to those with multi-vessel grafts (11). Multivessel bypass reduces the risk of symptomatic recurrence and provides some protection against bowel infarction in the event of single graft occlusion (4). This was our choice in one of the patients (case

2). Autologous revascularization techniques are the first choice, but a prosthetic conduit can also be used as a single or multi-vessel bypass (8). Reports suggest that retrograde grafts perform as well as antegrade grafts (2). All patients operated on in our clinic benefited from retrograde aorto-mesenteric bypass. Permeability is higher with open surgery than with EV therapy, with an average of 70% for both, similar to that of bypasses for peripheral revascularization (3). Because of better long-term and reintervention-free patency, open revascularization should be considered in patients who are fit for surgery, especially those with long segment mesenteric artery occlusion (13). However, in patients deemed fit for open surgery, open surgery is preferred to EV revascularization in patients with long length SMA occlusive disease, flush occlusion, bulky calcification of origin, or dual SMA and celiac occlusion (14, 15).

The largest retrospective, propensity-matched single-center cohort analyzing long-term survival after intervention for CMI concluded that long-term mortality was independent of treatment modality and was associated with patient comorbidities. Therefore, the choice of treatment should be based on anatomic considerations (16).

In terms of secondary patency at 5 years, the surgical approach retains an advantage over the EV approach, with patency of 96-98% for the surgical approach versus 79-88% for the EV approach (17). In the present study, the clinical outcomes were satisfying and similar to the published experiences, secondary patency in surgical group in our series was 100%.

Monjur reported that open surgical revascularization is highly successful in improving symptoms, but is associated with significant 30-day morbidity (21.1%) and mortality (5.8%) (18). Kruger *et al*. presented 12% perioperative morbidity and 2.5% perioperative mortality. Higher values were observed for simultaneous aortic reconstruction, complete revascularization (three branches) and preoperative renal failure (19). Mesenteric revascularization during other concomitant aortic reconstructions remains controversial, as combined reconstructions have higher morbidity and mortality rates. It is also reasonable to perform repair of a single but severe, mesenteric artery stenosis concomitantly with open aortic reconstruction. In the hands of experienced surgeons familiar with mesenteric revascularization, open repair can be performed with low morbidity and mortality. In specialized centers, surgery remains a viable and sometimes preferred option for durable revascularization. Despite the switch to endovascular mesenteric revascularization in most centers since 2002, open revascularization has still its place in selected cases. Appropriate selection of patients is the best guarantee to treatment success and durability (9).

CONCLUSIONS

Despite the advantages of EV therapy, open surgical revascularization retains an essential role, particularly in patients with extensive mesenteric artery disease, associated aorto-iliac disease or failed EV interventions. Surgical intervention is considered in patients with good clinical status and acceptable surgical risk, considering the lower chance of recurrence (16, 20). Open revascularization procedures achieved good results with low procedural mortality, as reported in the literature for selected patients, confirming the utmost importance of open revascularization in

CMI, even in the endovascular era.

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The current study group points out that SMA should be the main target for surgical revascularization in patients with CMI.

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QUALITY OF LIFE AND MUSCULOSKELETAL DISORDERS AMONG MEDICAL STUDENTS

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QUALITY OF LIFE AND MUSCULOSKELETAL DISORDERS AMONG MEDICAL STUDENTS (Abstract): **The aim** of the study is to evaluate the presence of musculoskeletal disorders and to assess the quality of life among medical students. **Materials and methods**: A total of 105 medical students participated in the study. Data about their posture, the number of sleep hours, body mass index, physical activity, and strategies used to cope with stress were collected, and to evaluate their quality of life, several psychological instruments were used: Perceived Stress Scale (Cohen, Kamarch, & Mermelstein, 1983), Inventory of College Students' Recent Life Experiences (ICSRLE – Kohn, Lafreniere, & Gurevich, 1990), Stress Vulnerability Questionnaire (Sharon & Werner Hoeger, 2021), and The Brief Symptom Inventory (BSI-Derogatis, 1995). The statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) Statistics for Windows, *version 24* (*SPSS* Inc., Chicago, IL, USA). **Results:** More than half of the students (61.0%, N = 64) were found to have a moderate level of stress. Women scored higher on this instrument (Mean = 21.93 ± 7.29) compared to men (Mean = 17.08 ± 7.89). More than half of the students scored average or high on this scale, indicating that they are vulnerable to stress (34.3%, N = 36) or even have a high vulnerability to stress (39.0%, N = 31). The number of hours of sleep correlated negatively with the BSI scale (r = -0.29, p = 0.003). The level of physical activity correlates negatively with the PSS scale (r = -0.27, p = 0.004), meaning that the more sports students do, the lower their stress levels. **Conclusions**: The quality of life of participants changes throughout their student life, and many students find various strategies to cope with stress. The study highlights the role of sleep and exercise for a better quality of life. **Keywords:** QUALITY OF LIFE AND MUSCULOSKELETAL DISORDERS, MEDICAL STUDENTS.

INTRODUCTION

Quality of life (QoL) is increasingly recognized as a critical dimension in medical education and healthcare research. The World Health Organization (WHO) defines QoL as an individual's perception of their position in life, shaped by cultural context, value systems, goals, expectations, and concerns (1). Within health sciences, this has been further refined into the concept of health-related quality of life (HRQoL), encompassing physical, psychological, and social domains. Among medical students, QoL is of particular concern, as the demanding academic environment, long study hours, and constant performance pressure

are well-documented risk factors for diminished well-being. Numerous studies have shown that medical students often report poorer psychological health and social relationships compared to their non-medical peers, with high rates of anxiety, stress, and depression. These issues not only undermine academic performance but also have long-term implications for professional competency and patient care.

Musculoskeletal disorders (MSDs) represent an additional yet frequently overlooked challenge to the well-being of medical students. MSDs include a spectrum of conditions affecting muscles, tendons, ligaments, and joints, commonly manifesting as neck, shoulder, and lower back pain. The etiology of MSDs in students is multifactorial, involving prolonged sedentary study, repetitive postures, lack of physical activity, and high psychological stress, as Ikenna *et al*. showed (2). Recent reports indicate that up to 70–80% of medical students experience some form of MSD during their training. These conditions not only impair physical functioning but also exert a negative influence on psychological health, contributing to fatigue, irritability, and decreased concentration. Chronic MSDs have been linked to reduced QoL scores, further reinforcing the need to consider both physical and psychological domains when assessing student well-being (3, 4).

The relationship between QoL, MSDs, and psychological health is complex and bidirectional. Poor QoL may predispose students to musculoskeletal complaints through increased sedentary behaviors and heightened stress, while MSD-related pain and discomfort can exacerbate psychological distress. Moreover, the interplay between these factors can create a self-perpetuating cycle of reduced physical health, impaired academic performance, and deteriorating mental well-being. Understanding these interconnections is vital for designing effective interventions that enhance resilience and promote sustainable health practices among medical students.

To capture the multidimensional nature of QoL and its association with MSDs, validated psychological and clinical tools are widely used in research and practice. Instruments such as the WHOQOL-BREF, SF-36, and the Beck Depression Inventory (BDI) are frequently employed to evaluate global QoL and mental health outcomes, while musculoskeletal-specific questionnaires like the Cornell Musculoskeletal Discomfort Questionnaire (CMDQ), the Oswestry Disability Index (ODI), and the Neck Disability Index (NDI) provide insights into functional limitations and pain severity. In addition, screening tools for stress, anxiety, and depression, including the DASS-21, the Hospital Anxiety and Depression Scale (HADS), and the General Health Questionnaire (GHQ-12), enable a more comprehensive psychological assessment. The integration of these instruments facilitates the evaluation of both general well-being and condition-specific burdens, providing a holistic perspective on student health.

Given the high prevalence of MSDs and the considerable psychological strain faced by medical students, it is imperative to systematically investigate their combined impact on quality of life. Employing standardized assessment tools enables researchers and educators to quantify these effects and identify high-risk groups, while also guiding the development of targeted interventions. Such insights are particularly relevant, as the future healthcare workforce depends not only on academic training but also on the physical and psychological resilience of its members. Accordingly, the

present study aims to explore the interrelationship between QoL, musculoskeletal disorders, and psychological well-being among medical students, drawing on established measurement tools to generate evidence that may inform both institutional policy and individual coping strategies.

MATERIALS AND METHODS

Participants. The research was conducted between January-May 2025 among medical students from different years of study. The respondents were informed about the purpose of the study and the use of the collected data. They were also informed about the confidentiality of the data and that they could stop at any time. No incentive was given to the participants. The research used an online questionnaire, and if they were agreed with the informed consent, they could continue filling in the items. Inclusion criteria: respondents enrolled in medical studies and questionnaires completed before the deadline. Finally, 105 questionnaires were included in the study.

Instruments and Data collection. The questionnaire was constructed using Google Forms application (Alphabet, Mountain View, CA, USA).

a) The first part of the questionnaire gathered socio-demographic and health-related data (such as age, gender, year of study, Body Mass Index - BMI, changes in body composition over time and factors that contributed to this change, chronic disease diagnosis, changes in posture over time, smoker/non-smoker status, drug use, number of hours of sleep per night, engagement in physical activities, and assessment of physical and mental health level).

b) The second part contains questions related to the quality of academic life, such as: number of hours spent per week on academic work, the level of stress or burnout related to academic life, coping mechanisms to deal with stress, various mental health issues, whether studies interfere with the ability to maintain a healthy diet, and university-level changes that could improve students' physical and mental well-being.

c) The third part assesses stress using a classic instrument – the Perceived Stress Scale - Cohen, Kamarch, & Mermelstein, 1983 (5). The instrument comprises 10 items evaluating feelings and participants' thoughts during the last month, and it was designed to measure the degree to which situations in one's life are appraised as stressful. Responses for each item were indicated using a five-point Likert scale (where 0 = Never and 4 = Very Often). There are two subscales in the PSS-10: perceived helplessness, which measures an individual's feelings of a lack of control over their circumstances or their own emotions or reactions, and lack of self-efficacy, which measures an individual's perceived inability to handle problems.

d) The fourth part of the survey assesses exposure to hassles and their influence on health outcomes by way of the Inventory of College Students' Recent Life Experiences *ICSRLE* - Kohn, Lafreniere, & Gurevich, 1990) (6). This scale is comprised of 49 items. Each item is typically rated on a scale (where 0 = Not at all part of my life and 3 = Very much part of my life), and the responses are summed to generate a total score. There are seven factor loadings from the ICSRLE, which are named developmental challenges, time pressure, academic alienation, romantic problems, assorted annoyances, general social mistreatment, and friendship problems.

e) The fifth section evaluates vulnerability to stress by using the Stress Vulnerability Questionnaire - Sharon & Werner Hoeger, 2021 (7).The scale is comprised of

30 items, and it is based on a four-level scoring system from 'strongly agree (1)' to 'strongly disagree (4).'

f) The final part of the survey includes an instrument which measures current or past levels of symptomatology, intensity of symptoms, and number of reported symptoms, respectively – the Brief Symptom Inventory (BSI). It consists of 53 items covering nine symptom dimensions: Somatization, Obsession-Compulsion, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation, and Psychoticism; and three global indices of distress: Global Severity Index, Positive Symptom Distress Index, and Positive Symptom Total - Derogatis, 1995 (8).

Statistical analysis. All analyses for this research were performed by using IBM Statistical Package for Social Sciences (SPSS) Statistics for Windows, version 24 (SPSS Inc., Chicago, IL, USA). Results for descriptive statistics were expressed as means and standard deviations (SD). The normality of data distribution was tested by using the Kolmogorov-Smirnoff test. Given the fact that the data were not normally distributed, bivariate analyses were performed, and non-parametric tests were applied.

To assess comparative results considering gender and diagnosis of a chronic disease, the Mann–Whitney test was performed. Comparative results considering level of physical activity, number of hours of sleep, degree of physical health assumed by the students, degree of mental health assumed by the students, and experiencing burnout due to academic pressure were assessed using the Kruskal–Wallis H test to determine if there are statistically significant differences between more than two groups of an independent variable on a continuous or ordinal dependent variable.

The Spearman correlation was used to test the relationship between variables. A p-value < 0.05 was considered statistically significant.

RESULTS

Socio-demographic and health related data. The participants were aged M = 23.19 ± 14.15 years (with ages ranging between 18 and 37 years old). More than half of the respondents were female (55.2%, N = 58), and more than half are in their 1st (33.3%, N = 35) and 2nd year of study (25.7%, N = 27), respectively.

TABLE I.
Data About Body Composition and Posture

Items	N / %
Changes in body composition	
Increased muscle mass, decreased fat	24 (22.9%)
Increased fat, decreased muscle mass	17 (16.2%)
Lost weight (both fat and muscle)	23 (21.9%)
Gained weight (both fat and muscle)	17 (16.2%)
No significant change	21 (20.0%)
Factors contributed to changes in body composition	
Lifestyle changes (e.g., work, stress, sleep)	36 (34.2%)
Changes in diet, Changes in exercise routine, Lifestyle changes (e.g., work, stress, sleep)	27 (25.7%)
Changes in diet, Changes in exercise routine	7 (6.6%)
Changes in diet, Lifestyle changes (e.g., work, stress, sleep)	7 (6.6%)
Changes in posture over time	
Improved	26 (24.8%)
Worsened	53 (50.5%)
No change	7 (6.6%)

*Frequency and percentages (%)

Results showed that only a third of the participants were of normal weight (30.5%, N = 32), and more than half were underweight (66.7%, N = 70). These changes in body composition experienced by students over the past few years, the main factors that contributed to this change and changes in posture are diverse and are mentioned in first table.

Most participants were non-smokers (74.3%, N = 78) and very few had used illicit drugs (9.5%, N = 10). Approximately one third of the participants engaged in physical activity rarely (35.2%, N = 37), 1-2 times a week (26,7%, N = 28), or 3-4 times a week (26.7%, N = 28). More than half of the participants slept an average of 6-7 hours per night (58.1%, N = 61).

Very few participants suffered from chronic diseases (9.5%, N = 10). Regarding assessment of physical and mental health level, less than half of the participants considered themselves to be in good health, both physically (43.8%, N = 46) and mentally (40.0%, N = 42).

Quality of the academic life

Approximately one third of students spent an average of 10 to 20 hours per week studying (28.6%, N = 30), while the fewest devoted around 40-50 hours (6.7%, N = 7), or even more than 50 hours per week (3.8%, N = 4) to academic work.

More than half of students clearly stated that studies interfere with their ability to maintain a healthy diet (34.3%, N = 36), or only to some extent (36.2%, N = 38).

TABLE II.

Data about Coping Mechanisms and Strategies to Improve Well-Being[1]

Items	N / %
What coping mechanisms do you use to handle stress?	
Hobby (coloring, cooking, listening to music, dancing/partying, reading, walking, traveling, gaming)	26 (24.8%)
Self-encouragement with or without breathing exercises	13 (12.3%)
None/ They don't know how to deal with stress	12 (11.4%)
Sleep	11 (10.4%)
Sport	11 (10.4%)
Meditation/Religion	10 (9.5%)
Talking to someone	8 (7.6%)
Compulsive eating	7 (6.6%)
Smoking	4 (3.8%)
Compulsive TV watching	3 (2.8%)
What changes do you think could improve a students' mental and physical well-being?	
No answer	54 (51.4%)
Having a good community/entourage	8 (7.6%)
More relaxed university schedule	8 (7.6%)
To work mentally with oneself/ To be more relaxed	7 (6.6%)
Extracurricular activities offered by the university	6 (5.7%)
Mental health support/education offered by the university	5 (4.7%)
More support from teachers and the university	5 (4.7%)

[1]Frequency and percentages (%)

Most students experienced study-related stress occasionally (35.2%, N = 37), or frequently (31.4%, N = 33), and more than half experienced burnout due to academic pressure (61.9%, N = 65). However, more than half stated that they do not have access to mental health support (52.4%, N = 55).

When asked *how comfortable they feel when discussing their mental health issues with teachers or colleagues*, on a scale from 1 (very uncomfortable) to 5 (very comfortable), more than a third of students ticked level 3 (31.4%, N = 33).

Coping mechanisms to deal with stress and some of the changes mentioned by students that they believe could improve their physical and mental well-being (tab. II).

Perceived Stress Scale (PSS)

The scale had good internal consistency, as determined by a Cronbach's alpha of 0.86.

The total score for the PSS scale was M = 19.76 ± 7.90, with scores ranging from 0 (0.1%, N = 1) to 37 (0.1%, N = 1). More than half of the students (61.0%, N = 64) were found to have a moderate level of stress. Women scored higher on this instrument (M = 21.93 ± 7.29) compared to men (M = 17.08 ± 7.89).

For PSS subscales, we obtained the following results:

- Perceived helplessness - M = 12.77 ± 7.01,
- Lack of self-efficacy - M = 6.99 ± 3.16.

Inventory of College Students' Recent Life Experiences (ICSRLE)

The Cronbach's alpha score was 0.95 for the total scale. The analysis of Cronbach's alpha revealed the following coefficients: 0.82 for Factor 1 – Developmental Challenge, 0.81 for Factor 2 – Time Pressure, 0.72 for Factor 3 – Academic Alienation, 0.83 for Factor 4 – Romantic Problems, 0.69 for Factor 5 – Assorted Annoyances, 0.82 for Factor 6 – General Social Mistreatment, and 0.83 for Factor 7 – Friendship Problems.

For these ICSRLE subscales, we obtained the following results:

- *Developmental Challenge* - M = 12.40 ± 6.37,
- *Time Pressure* - M = 9.60 ± 4.88,
- *Academic Alienation* - M = 3.61 ± 2.41,
- *Romantic Problems – M* = 2.77 ± 2.72
- *Assorted Annoyances – M* = 3.56 ± 3.02
- *General Social Mistreatment – M* = 5.58 ± 4.25
- *Friendship Problems – M* = 2.97 ± 2.66

The total ICSRLE score was on average M = 50.49 ± 26.45, with scores ranging from 4 (1.0%, N = 1) to 129 (1.0%, N = 1).

Stress Vulnerability Questionnaire (SVQ)

The total score for the SVQ scale was M = 58.16 ± 13.34. More than half of the students scored average or high on this scale, indicating that they were vulnerable to stress (34.3%, N = 36) or even had a high vulnerability to stress (39.0%, N = 31).

The scale had good internal consistency, as determined by a Cronbach's alpha of 0.87.

Brief Symptom Inventory (BSI)

The scale had good internal consistency, as determined by a Cronbach's alpha of 0.98.

The total score for the BSI scale was M

= 63.66 ± 45.35, with scores ranging from 0 (1.9%, N = 2) to 188 (1.0%, N = 1). For the BSI subscales, we obtained the following results:

TABLE III.
BSI Subscales[1]

Subscales	M±SD	Gender differences M±SD		α-Cronbach
		Men	Women	
Somatization	1.10 ± 0.96	0.81 ± 0.90	1.34 ± 0.96	0.88
Obsession-Compulsion	1.51 ± 1.01	1.27 ± 1.02	1.71 ± 0.97	0.89
Interpersonal sensivity	1.26 ± 1.10	1.02 ± 1.01	1.46 ± 1.15	0.84
Depression	1.20 ± 0.96	1.05 ± 0.91	1.33 ± 0.99	0.86
Anxiety	1.30 ± 1.00	1.04 ± 0.98	1.52 ± 0.98	0.87
Hostility	1.13 ± 0.89	1.01 ± 0.95	1.22 ± 0.83	0.78
Phobic anxiety	0.95 ± 1.03	0.83 ± 0.97	1.04 ± 1.08	0.88
Paranoid ideation	1.26 ± 0.94	1.18 ± 0.96	1.29 ± 0.93	0.79
Psychoticism	1.13 ± 0.91	1.00 ± 0.82	1.25 ± 0.98	0.78

[1]Means and standard deviations (M±SD)

Results for the Global Severity Index (GSI), a global index of the BSI , which is the most sensitive indicator of the respondent's distress level and combines information about the number of symptoms and the intensity of distress, are: M = 15.39 ± 11.12, with scores ranging from 0 (1.9%, N = 2) to 44.5 (1%, N = 1).

Comparative and correlational analysis

Results of Mann-Whitney tests showed that there were significant differences depending on gender in PSS scale (z = -3.314, p = 0.001, U = 849.50), ICSRLE scale (z = -2.627, p = 0.009, U = 955.50), and BSI scale (z = -2.237, p = 0.025, U = 997.00). This means that women (Mdn1 = 21.50) are more stressed than men (Mdn2 = 18.00), women (Mdn1 = 55.00) are more affected by minor stressful events than men (Mdn2 = 43.00), and women showed more psychopathological symptoms (Mdn1 = 67.00) than men (Mdn2 = 46.00).

There are significant differences depending on gender in BSI subscales. The results showed that women (Mdn 1 = 1.14) somatized more than men (Mdn2 = 0.57) (z = -3.181, p = 0.001, U = 853.50) and had a higher level of anxiety (Mdn1 = 1.33) compared to men (Mdn2 = 1.00) (z = -2.446, p = 0.014, U = 947.50).

On the Obsession-Compulsion (z = -2.162, p = 0.031, U = 990.00) and Interpersonal Sensitivity subscale (z = -2.039, p = 0.041, U = 1010.00), the results showed that men (Mdn2 = 1.16, Mdn2 = 1.00, respectively) had lower scores than women (Mdn1 = 1.66, Mdn1 = 1.25, respectively).

Significant differences were recorded in terms of diagnosis of a chronic disease (z = -2.323, p = 0.020, U = 262.50), in the sense that students with a positive diagnosis of chronic illness had a higher level of stress (Mdn1 = 28.00), as assessed by the PSS scale, compared to those who were not chronically ill (Mdn2 = 20.00).

Also, comparative analysis for the

ICSRLE scale (z = -3.374, p = 0.001, U = 166.00) showed that those with a diagnosis of chronic disease were more affected by minor stressful events (Mdn1 = 79.00) than those without a diagnosis of chronic disease (Mdn2 = 47.00). At the same time, students with a positive diagnosis of chronic illness had a higher vulnerability to stress (Mdn1 = 68.50), as assessed by the SVQ scale (z = -2.316, p = 0.021, U = 263.00), compared to those who were not chronically ill (Mdn2 = 57.00). Significant differences in this variable were also identified for the BSI scale (z = -2.305, p = 0.021, U = 261.00), in the sense that those with a diagnosis of chronic disease showed more psychopathological symptoms (Mdn1 = 98.50) than those without a diagnosis of chronic disease (Mdn2 = 53.00).

Results of the Kruskal-Wallis test showed that there were significant differences depending on the level of physical activity in the PSS scale ($\chi^2(4) = 11.098$, p = 0.025), in the sense that students who never (Mdn1 = 34.00) or only rarely (Mdn2 = 21.00) practiced sports had a higher level of stress than those who practiced sports daily (Mdn5 = 18.00) or at least 3-4 times a week (Mdn4 = 19.00).

There were significant differences depending on the number of hours of sleep in the SVQ ($\chi^2(3) = 11.185$, p = 0.011) and BSI ($\chi^2(3) = 11.504$, p = 0.009) scales. The results showed that students who slept 4-5 hours a night were more vulnerable to stress (Mdn 2 = 59.00) than students who slept 8 hours a night or more (Mdn4 = 54.00). Additionally, the results showed that students who slept 4-5 hours a night showed more psychopathological symptoms (Mdn2 = 67.00) than students who slept 8 hours a night or more (Mdn4 = 25.50).

Results of Kruskal-Wallis tests showed that there were significant differences depending on the degree of physical health assumed by students in the PSS scale ($\chi^2(4) = 12.276$, p = 0.015) and the SVQ scale ($\chi^2(4) = 12.620$, p = 0.013). This means that those who considered themselves to be in poor physical health had a higher degree of stress (Mdn1 = 29.00) than those who considered themselves to be in excellent physical health (Mdn5 = 15.00) and those who considered themselves to be in poor physical health had a higher vulnerability to stress (Mdn1 = 75.50) than those who considered themselves to be in excellent physical health (Mdn5 = 44.00).

Also, there were significant differences depending on the degree of mental health assumed by students in the ICSRLE scale ($\chi^2(4) = 17.532$, p = 0.002) and the BSI scale ($\chi^2(4) = 23.301$, p = 0.000). This means that those who considered themselves to be in poor mental health were more affected by minor stressful events (Mdn1 = 73.00) than those who considered themselves to be in excellent mental health (Mdn5 = 29.00), and those who considered themselves to be in poor mental health had more psychopathological symptoms (Mdn1 = 66.00) than those who considered themselves to be in excellent mental health (Mdn5 = 21.50).

Results of Kruskal-Wallis test showed that there were significant differences depending on burnout in the PSS scale ($\chi^2(1) = 20.242$, p = 0.000) in the sense that students who felt burnout due to academic pressure were more stressed (Mdn1 = 22.00) than students who did not feel burnout (Mdn2 = 15.00). Also, there were significant differences depending on burnout in the ICSRLE scale ($\chi^2(1) = 6.724$, p = 0.010) indicating that students who felt burnout were more affected by minor stressful events (Mdn1 =

54.00) than students who did not feel burnout (Mdn2 = 36.00).

The Spearman correlational analysis found a positive significant correlation between the level of physical activity and the degree of physical health assumed by the students ($r = 0.42$, $p < 0.001$), indicating that the more students exercised, the healthier they considered themselves physically. The level of physical activity correlated negatively with the PSS scale ($r = -0.27$, $p = 0.004$), meaning that the more sports students did, the lower their stress levels.

The degree of physical health assumed by the students correlated positively with degree of mental health assumed by the students ($r = 0.52$, $p < 0.001$) in the sense that the healthier students considered themselves physically, the healthier they were mentally. Negative correlations were identified between the degree of physical health considered by students and the instruments used in the research: PSS ($r = -0.31$, $p < 0.001$), ICSRLE ($r = -0.21$, $p = 0.031$), SVQ ($r = -0.32$, $p = 0.001$), BSI ($r = -0.21$, $p = 0.026$), which means that the healthier the students were physically, the less stressed they were, the less affected they were by minor stressful events, the less vulnerable they were to stress and the fewer psychopathological symptoms they had.

Also, negative correlations were identified between the degree of mental health considered by students and the PSS scale ($r = -0.51$, $p < 0.001$), the ICSRLE scale ($r = -0.40$, $p < 0.001$), the SVQ scale ($r = -0.28$, $p = 0.003$), and BSI scale ($r = -0.43$, $p < 0.001$), which means that the healthier the students were mentally, the less stressed they were, the less affected they were by minor stressful events, the less vulnerable they were to stress and the fewer psychopathological symptoms they had.

TABLE IV.

Correlation Results

Variable	PSS_ Perceived helplessness	ICSRLE_ Time Pressure	ICSRLE_ Academic Alienation	ICSRLE_ Romantic Problems	BSI_ Somatization	BSI_ Depression	BSI_ Anxiety
PSS_ Perceived helplessness	1	r=0.556** p=0.000	r=0.400** p=0.000	r=0.293** p=0.002	r=0.510** p=0.000	r=0.538** p=0.000	r=0.545** p=0.000
ICSRLE_ Time Pressure	r=0.556** p=0.000	1	r=0.527** p=0.000	r=0.514** p=0.000	r=0.368** p=0.000	r=0.439** p=0.000	r=0.411** p=0.000
ICSRLE_ Academic Alienation	r=0.400** p=0.000	r=0.527** p=0.000	1	r=0.229* p=0.019	r=0.327** p=0.001	r=0.464** p=0.000	r=0.469** p=0.000
ICSRLE_ Romantic Problems	r=0.293** p=0.002	r=0.514** p=0.000	r=0.229* p=0.019	1	r=0.341** p=0.000	r=0.308** p=0.001	r=0.263** p=0.007
BSI_ Somatization	r=0.510** p=0.000	r=0.368** p=0.000	r=0.327** p=0.001	r=0.341** p=0.000	1	r=0.733** p=0.000	r=0.760** p=0.000
BSI_ Depression	r=0.538** p=0.000	r=0.439** p=0.000	r=0.464** p=0.000	r=0.308** p=0.001	r=0.733** p=0.000	1	r=0.784** p=0.000
BSI_ Anxiety	r=0.545** p=0.000	r=0.411** p=0.000	r=0.469** p=0.000	r=0.263** p=0.007	r=0.760** p=0.000	r=0.784** p=0.000	1

A positive correlation was identified between the number of hours of sleep per night and mental health ($r = 0.29$, $p = 0.002$), meaning that the more students slept, the healthier they considered themselves mentally. The number of hours of sleep correlated negatively with the BSI scale ($r = -0.29$, $p = 0.003$), meaning that the more students slept at night, the fewer psychopathological symptoms they had.

Positive correlations were identified between all scales used in the questionnaire. An example of this would be the positive correlations identified between the PSS scale and the ICSRLE ($r = 0.54$, $p < 0.001$), the SVQ ($r = 0.36$, $p < 0.001$), and the BSI ($r = 0.54$, $p < 0.001$) scales, which meant that the more stressed the students were, the more affected they were by minor stressful events, the more vulnerable they were to stress and the more psychopathological symptoms they had.

Strong significant positive correlations were identified between all instruments` subscales (tab. IV)

DISCUSSION

This study highlights several important determinants of stress and psychological well-being among medical students, with particular emphasis on lifestyle, perceived health, and chronic disease status. When compared to previous research, many of our findings align with international patterns.

Students with a diagnosis of chronic disease were significantly more stressed, more vulnerable to stress, and displayed more psychopathological symptoms compared to peers without chronic illness. These results are consistent with earlier findings that medical students with pre-existing conditions such as hypertension, obesity, or diabetes experience greater academic and psychological burden. (9-11) Our findings reinforce the notion that chronic health conditions compound academic stress, magnifying both perceived helplessness and vulnerability.

A strong association was observed between low levels of physical activity and higher stress scores, while regular sports practice correlated with better perceived physical health and lower stress. This supports previous evidence that exercise serves as a protective factor against psychological distress in medical students. Jose J *et al.* (12) and Taylor *et al.* (13) reported similar reductions in anxiety and burnout among physically active students, while Booth *et al.* (14) emphasized the role of inactivity in chronic stress pathways. Collectively, these findings underscore the importance of structured exercise programs in medical curricula to promote resilience.

Students who reported sleeping only 4–5 hours per night were more vulnerable to stress and exhibited more psychopathological symptoms than those who slept 8 hours or more. These results align with evidence from Parto *et al.* (15), Rocha *et al* (16), and Howie *et al.* (17), who identified sleep deprivation as a key contributor to anxiety, depression, and impaired academic functioning in medical students. Our results confirm sleep duration as a central determinant of both perceived mental health and actual psychological symptoms.

Both perceived physical and perceived mental health were strongly associated with stress vulnerability and psychopathological outcomes. Students rating themselves in poor physical health reported higher stress and stress vulnerability, while those rating their mental health as poor were more af-

fected by minor stressful events and exhibited more symptoms. These findings are consistent with Wang *et al.* (18), who showed that life events predicted depression and anxiety particularly among students with higher psychological vulnerability. Similarly, Franzoi *et al.* (19) and Iorga *et al.* (20) found that poorer self-rated health was strongly associated with psychological distress in university students, supporting the link between subjective health perceptions and mental outcomes. Together, these studies reinforce our results by highlighting that both perceived physical and mental health are central predictors of stress sensitivity and psychopathological symptoms in students

A substantial proportion of students reporting burnout also demonstrated higher stress levels and greater sensitivity to stressful events. This finding mirrors reports from Abdel Aziz *et al.* (21), who found high burnout prevalence among UAE medical students, and Maddalena *et al.* (22), who described worsening QoL during high-stress academic periods. Our results confirm burnout as a central mediator of academic distress, suggesting that interventions must address both workload management and psychological support.

Strengths and Limitations of the study: The cross-sectional design limits causal inference, and reliance on self-reported measures may introduce reporting bias. However, the use of multiple validated instruments across a representative student sample strengthens the reliability and generalizability of our conclusions.

Practical implications: The convergence of our results with international evidence suggests that the challenges faced by our medical students are not isolated, but part of a broader global pattern of academic and health-related stress. Interventions such as integrating physical activity programs, promoting healthy sleep habits, ensuring access to mental health services, and addressing burnout through workload reforms could meaningfully improve a student's well-being.

CONCLUSIONS

This study underscores the multifaceted interaction between lifestyle factors, psychological well-being, and perceived QoL among medical students. Our findings demonstrate that inadequate sleep, low levels of physical activity, and the presence of chronic illness are strongly associated with heightened stress vulnerability and increased psychopathological symptoms. Furthermore, burnout and academic pressure emerged as significant contributors to compromised mental health, reinforcing the need for systemic and institutional interventions.

By highlighting the relationships between perceived physical and mental health, stress, and coping mechanisms, this research affirms the value of integrating comprehensive wellness strategies into medical training. Interventions such as promoting adequate sleep hygiene, integrating regular physical activity, and ensuring access to mental health support are not merely desirable but essential in safeguarding the future healthcare workforce. Given that today's medical students are tomorrow's practitioners, prioritizing their well-being should be recognized as a matter of both academic success and public health responsibility.

CONFLICT OF INTEREST AND FUNDING

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ENHANCED ANTI-INFLAMMATORY EFFICACY OF NSAID–ANTIBIOTIC COMBINATIONS IN EXPERIMENTAL SUBACUTE INFLAMMATION

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ENHANCED ANTI-INFLAMMATORY EFFICACY OF NSAID–ANTIBIOTIC COMBINATIONS IN EXPERIMENTAL SUBACUTE INFLAMMATION (Abstract): Subacute inflammation represents a transitional stage between acute and chronic inflammatory processes, characterized by fibroblast proliferation and granulomatous tissue development. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac and ketoprofen are widely used to control these processes. Some antibiotics, including cefuroxime and levofloxacin, have also been reported to exhibit secondary anti-inflammatory or immunomodulatory effects. This study investigated the anti-inflammatory efficacy of these agents, administered individually or in combination, using the cotton pellet-induced granuloma model in rats. **Materials and methods:** Adult Wistar rats were divided into experimental groups receiving intraperitoneal doses of NSAIDs and antibiotics alone or in combination. Granulomatous tissue formation was assessed after subcutaneous implantation of sterile cotton pellets, and the degree of inflammation was evaluated by measuring granuloma development and comparing it across treatment groups. **Results:** Both NSAIDs produced clear reductions in granuloma formation compared with the untreated control group, confirming their significant anti-inflammatory potential. Levofloxacin showed a moderate inhibitory effect, consistent with its reported cytokine-suppressive and immunomodulatory properties, whereas cefuroxime exhibited minimal influence on inflammatory tissue growth. When combined, NSAIDs and antibiotics produced a greater reduction in granuloma formation than either agent alone, with diclofenac-based combinations showing the strongest overall activity. **Conclusions:** The findings indicate that diclofenac and ketoprofen effectively suppress subacute inflammatory responses, and that levofloxacin may contribute complementary immunomodulatory effects. Combined administration enhances the anti-inflammatory outcome, suggesting a potential pharmacological synergy between NSAIDs and certain antibiotics in conditions involving both infection and inflammation. **Keywords**: SUBACUTE INFLAMMATION, DICLOFENAC, KETOPROFEN, LEVOFLOXACIN, CEFUROXIME, GRANULOMA.

INTRODUCTION

Subacute inflammation represents an intermediate phase between acute and chronic inflammatory processes, characterized by persistent cellular infiltration, fibroblast proliferation, and granulomatous tissue

formation (1). Unlike acute inflammation, which manifests rapidly through vascular changes, edema, and exudation, subacute inflammation evolves more slowly and reflects the proliferative phase of tissue repair (2). This stage is critical for understanding the transition from acute exudative responses to chronic tissue remodeling, making it highly relevant in the evaluation of anti-inflammatory and antiproliferative agents (3).

Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their effects primarily through inhibition of cyclooxygenase (COX) enzymes, leading to decreased prostaglandin synthesis (4). During subacute inflammation, characterized by the proliferation of fibroblasts, angiogenesis, and infiltration of mononuclear cells, prostaglandins contribute to the persistence of pain, edema, and tissue swelling (5). By reducing prostaglandin production, NSAIDs diminish vascular permeability, decrease leukocyte migration, and alleviate pain and swelling, thereby promoting the resolution of the inflammatory process (6). Additionally, some NSAIDs may exert secondary effects on inflammatory cytokine release and oxidative stress, further contributing to their anti-inflammatory efficacy during the subacute phase of inflammation (7, 8).

DCF is a potent COX-2 inhibitor, producing strong and sustained anti-inflammatory and analgesic effects (9), while KET demonstrates a more balanced COX-1/COX-2 inhibition, resulting in effective suppression of tissue proliferation and exudate formation, though with slightly lower potency than DCF (10). Prolonged or high-dose use of these NSAIDs can lead to gastrointestinal irritation, ulceration, and bleeding. DCF, in particular, has been associated with an increased risk of cardiovascular events such as myocardial infarction and stroke due to its relative COX-2 selectivity (11, 12). Both drugs may also impair renal function by decreasing renal blood flow and sodium excretion, potentially leading to fluid retention, hypertension, or acute kidney injury (13,14). Therefore, while these drugs are valuable anti-inflammatory agents, they should be used at the lowest effective dose and for the shortest duration necessary, especially in patients with preexisting cardiovascular, renal, or gastrointestinal conditions (15).

Beyond traditional NSAIDs, certain antibiotics, have been reported to influence inflammatory processes indirectly, potentially through modulation of cytokine release, reduction of oxidative stress, or interference with leukocyte activity. Although their primary role remains antimicrobial, these agents may exert adjunctive anti-inflammatory or immunomodulatory actions (16). Cefuroxime (CFX), a second-generation cephalosporin, is a broad-spectrum β-lactam antibiotic effective against both Gram-positive and Gram-negative bacteria, with minimal direct anti-inflammatory activity but potential to indirectly reduce inflammation through infection control (17). Levofloxacin (LFC), a third-generation fluoroquinolone, is a broad-spectrum antimicrobial agent that inhibits bacterial DNA gyrase and topoisomerase IV, exhibiting potent activity against both Gram-positive and Gram-negative organisms and demonstrating additional anti-inflammatory and immunomodulatory effects through suppression of cytokine release and oxidative stress (18, 19).

The present study **aimed** to evaluate the anti-inflammatory efficacy of DCF and KET, administered alone or in combination with CFX or LFC, using the cotton pellet-

induced granuloma model in Wistar rats. The formation and dry weight of granulomatous tissue were used as objective parameters to quantify subacute inflammation. By examining potential pharmacological interactions between NSAIDs and antibiotics, this study sought to elucidate whether such combinations could modify or potentiate the suppression of subacute inflammatory responses, offering insights relevant to both experimental pharmacology and clinical therapeutics.

MATERIALS AND METHODS

Animals. Adult male Wistar albino rats, weighing 200-250 g, were used as the experimental model. The animals were sourced from the National Medical-Military Institute for Research and Development, Băneasa (Bucharest, Romania), and transported under controlled conditions to the Biobase facility of the Grigore T. Popa University of Medicine and Pharmacy Iasi.

Upon arrival, rats underwent a mandatory 14-day quarantine period. They were accommodated in individually ventilated cages within the experimental animal facility of the CEMEX. The environment was maintained at a temperature of 20 ± 4°C, relative humidity of 50±5%, and a 12:12-hour light/dark cycle to simulate natural circadian rhythms. The housing environment was carefully regulated, ensuring stable thermal conditions (approximately 20±4°C), balanced humidity levels (around 50±5%), and a 12:12-hour light/dark cycle to simulate natural circadian rhythms. Throughout the study, the animals had continuous access to drinking water and a nutritionally balanced laboratory diet. Prior to experimental manipulation, animals underwent a 24-hour acclimatization period to reduce stress and allow adaptation to the new environment.

To control for circadian variation in physiological parameters, all experimental procedures were performed within a fixed time frame, between 7:00 a.m. and 19:00 p.m. each day. Throughout the study, rats had unrestricted access to a standardized commercial rodent diet (pelleted form). Individual food intake was monitored daily to ensure nutritional consistency. Fresh drinking water was supplied ad libitum through automated systems designed to maintain cleanliness and prevent spillage.

On the day of the experimental procedures, animals were subjected to a fasting period during which both food and water were withheld to avoid potential interference with biochemical and physiological measurements.

The cotton pellet-induced granuloma model. The experiments were conducted under strictly controlled laboratory conditions at the Prof. Ostin C. Mungiu Advanced Research and Development Center for Experimental Medicine (CEMEX), within the Grigore T. Popa University of Medicine and Pharmacy Iași, Romania. All procedures adhered to standardized environmental parameters and animal care protocols to ensure experimental consistency.

For the experiment, nine groups of five animals each were used, receiving intraperitoneal administrations every 12 hours for three days at the same volume as follows: Group C: saline solution - positive control; Group KET: ketoprofen 15 mg/kg; Group DCF: diclofenac 15 mg/kg; Group CFX: cefuroxime 20 mg/kg; Group LFC: levofloxacin 5 mg/kg; Group KET+CFX: ketoprofen 15 mg/kg + cefuroxime 20 mg/kg; Group KET+LFC: ketoprofen 15 mg/kg + levofloxacin 5 mg/kg; Group DCF+CFX: diclofenac 15 mg/kg + cefuroxime 20 mg/kg; Group DCF+LFC:

diclofenac 15 mg/kg + levofloxacin 5 mg/kg. All injectable solutions were freshly prepared immediately before administration and pre-warmed to 36.7°C. Sterile, single-use syringes were employed for all injections.

Each animal was weighed individually using a high-precision automated balance (Sartorius AG, UK; accuracy ±0.1 g). Dosages were adjusted throughout the study to account for any variations in body weight.

At the beginning of the experiment, rats were anesthetized via inhalatory anesthesia with Isoflurane – Isoflutek, KRKA. Following anesthesia, the dorsal area was shaved, and a small incision was made at each implantation site. Sterile cotton pellets, each weighing 62 mg, were inserted subcutaneously, one the right flank. The implanted pellets acted as foreign bodies, eliciting a localized inflammatory response characteristic of subacute granulomatous inflammation (20). The development of granulomatous tissue was monitored visually throughout the observation period.

On day eight, the animals were weighed again and then anesthetized before being euthanized through isoflurane overdose. The granulomatous tissue encapsulating each cotton pellet was meticulously excised and separated from the surrounding tissue. The wet pellets were first weighed to record their wet weight, then dried in an incubator at 60 °C for 18 hours until a constant (completely dry) weight was reached. After drying, the pellets were weighed again. The amount of exudate (in mg) was determined by subtracting the dry weight from the wet weight. Granulation tissue formation (dry granuloma weight) was calculated by subtracting the weight of the cotton pellet (62 mg) from the constant dry weight.

The inhibition of granuloma weight gain (%) was calculated according to the following formula (21): [(Dry weight of granuloma in treated animals - Dry weight of granuloma in C) / Dry weight of granuloma in C] x 100.

Statistical analysis. Data analysis was performed using SPSS software, version 24 (IBM Corp., Armonk, NY, USA). Group comparisons were primarily conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons when significant differences were identified. Results were expressed as mean ± standard deviation (SD), with variance and interquartile range also computed. For comparisons between two independent groups, Student's t-test was applied; when normality or homogeneity of variance assumptions were not met, the nonparametric Kruskal-Wallis test was used instead. Correlation analyses employed Pearson's coefficient for parametric data and Spearman's rank correlation for nonparametric data. Simple and multiple linear regression models were applied to evaluate the influence of one or more independent variables on the dependent variable. Statistical significance was established at $p<0.05$.

Ethical considerations. All experimental activities were performed at CEMEX in full compliance with the ethical principles governing animal experimentation, as outlined in the European Directive 2010/63/EU (22) and the Romanian Law No. 43/2014 regarding the protection of animals used for scientific purposes (23). The study protocol was thoroughly evaluated and authorized by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy of Iasi (Approval No. 553/06.03.2025), as well as by the Romanian National Sanitary Veterinary and Food Safety Authority (Authorization No. 79/15.04.2025).

RESULTS

Body weight variation in experimental animals serves as an indirect but valuable indicator of general health status, metabolic balance, and potential systemic effects of the tested compounds. In subacute inflammation models such as the cotton pellet-induced granuloma test, weight gain over the observation period is expected due to normal growth and recovery processes. Conversely, a reduced increase or weight loss may indicate systemic stress, altered metabolism, or potential drug-induced adverse effects. The C group exhibited a mean body weight increase of +7.5 g after seven days, reflecting normal physiological growth under experimental conditions. Treatment with anti-inflammatory agents led to a noticeable attenuation of weight gain, with the magnitude of this effect varying across groups (tab. I).

Both KET and DCF groups showed a statistically significant reduction in body-weight gain compared to controls, with mean increases of +5.4 g ($p<0.05$) and +3.8 g ($p<0.01$), respectively. This pattern suggests that NSAID administration may slightly suppress appetite and nutrient absorption or influence metabolic rate through prostaglandin inhibition, mechanisms known to contribute to reduced weight gain during prolonged treatment. The more pronounced effect observed in the DCF group aligns with its stronger COX-2 inhibitory potency and systemic anti-prostaglandin action. CFX and LFC, demonstrated modest, nonsignificant or mildly significant decreases in weight gain (+6.7 g, and +5.9 g, respectively), indicating that these agents, when administered alone, exert limited systemic impact on growth and metabolism (tab. I).

When combined with NSAIDs, all dual-treatment groups exhibited a further reduction in weight gain compared with the control. The DCF-based combinations produced the most marked effects (+3.6 g, and +3.3 g, both $p<0.01$), suggesting possible additive systemic influences or metabolic interactions between DCF and the co-administered antibiotics. KET-based combinations also resulted in significant, though slightly less pronounced, reductions (+5.1 g, and +4.9 g, respectively) (tab. I).

TABLE I.

Variation in body weight of rats seven days after pellet implantation

Groups	Mean changes in animal`s body weight 7 days after subcutaneous pellet implantation (g).
C	+7.5±0.3
KET	+5.4±0.01*
DCF	+3.8±0.05**
CFX	+6.7±0.1
LFC	+5.9±0.3*
KET+CFX	+5.1±0.5*
KET+LFC	+4.9±0.3**
DCF+CFX	+3.6±0.05**
DFC+LFC	+3.3±0.03**

Data are expressed as the mean ± SD for five animals per group.
*p<0.05, **p<0.01 compared with the C group.

All the experimental groups showed an increase in granuloma mass after seven days compared to M0, confirming the successful induction of subacute inflammation through cotton pellet implantation. However, the extent of granulomatous tissue formation varied significantly between treatment groups, reflecting the anti-inflammatory effects of the tested substances. The C group exhibited the highest granuloma mass after seven days (112.2±6.33 mg), consistent with the ex-

pected progression of fibroblast proliferation, collagen deposition, and inflammatory cell infiltration in untreated subacute inflammation (fig. 1).

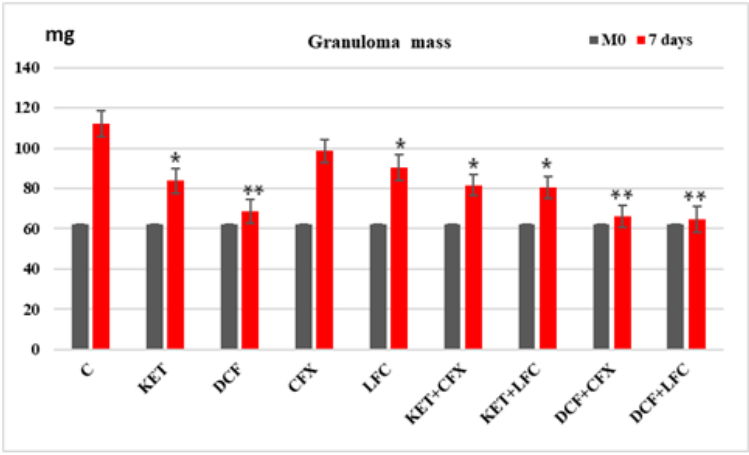

Fig. 1. Changes in granuloma weight 7 days after subcutaneous pellet implantation. Data are expressed as the mean ± SD, based on 5 animals per group. *p<0.05; **p<0.01 *vs.* Control-p group.

Administration of NSAIDs markedly reduced granuloma formation. Both KET and DCF significantly inhibited granulomatous tissue proliferation compared to the control group (p<0.05, and p<0.01, respectively). The stronger inhibitory effect observed with DCF suggests greater suppression of the proliferative phase of inflammation, likely due to its higher COX-2 inhibitory potency and consequent reduction in prostaglandin-mediated fibroblast activation and collagen synthesis. Among antibiotic monotherapies, LFC induced a mild but statistically significant decrease (p<0.05). This finding aligns with reports that fluoroquinolones may exert ancillary anti-inflammatory or immunomodulatory effects through inhibition of pro-inflammatory cytokines and oxidative mediators, while β-lactams such as CFX have minimal impact on inflammatory pathways (fig. 1). Combination treatments generally enhanced the anti-inflammatory outcome compared to individual drug administration. The KET+CFX, KET+LFC, and DCF+CFX groups all exhibited significant granuloma inhibition (p<0.05, or p<0.01), suggesting additive or synergistic effects between NSAIDs and antibiotics. The most pronounced reductions were observed in the DCF+CFX and DCF+LFC groups (p<0.01), indicating that DCF-based combinations were particularly effective in limiting fibroproliferative tissue formation (fig. 1).

The effects of the tested treatments on granuloma formation were evaluated by determining the percentage inhibition of granuloma weight gain in the pellet-induced granuloma model. All treatment groups produced varying degrees of inhibition compared with the control group, DCF produced the highest inhibitory effect among the single-drug treatments, with a mean inhibition of 38.9±2.33%, followed by KET at 25.3±1.82%, LFC at 19.4±2.21%, CFX at 12.1±2.67% (tab. II).

TABLE II.
The inhibition of granuloma weight gain

The inhibition of granuloma weight gain	
Groups	**%**
KET	25.3±1.82*
DCF	38.9±2.33**
CFX	12.1±2.67
LFC	19.4±2.21*
KET+CFX	27.3±1,67*
KET+LFC	28.3±2.37*
DCF+CFX	41.0±1.55**
DCF+LFC	42.5±1.45**

Data are expressed as the mean ± SD for five animals per group.
*p<0.05, **p<0.01 compared with the C group.

Combination therapy generally enhanced the inhibitory response. The combination of DCF with LFC produced the greatest inhibition (42.5± 1.45%), slightly exceeding that of DCF + CFX (41.0±1.55%). The KET-based combinations also showed improved inhibition compared with KET alone, with 27.3± 1.67% for KET+CFX and 28.3±2.37% for KET+LFC (tab. II).

DISCUSSIONS

The cotton pellet-induced granuloma model in rodents is a classical and widely accepted approach for studying the subacute phase of inflammation. When sterile cotton pellets are implanted subcutaneously, they provoke a localized and sustained inflammatory reaction marked by fibroblast proliferation, collagen synthesis, macrophage and lymphocyte infiltration, and the development of new blood vessels. Over time, these cellular and molecular events culminate in the formation of granulomatous tissue around the pellet (20). Measuring the dry weight of this tissue provides a reliable quantitative index of fibroplasia and tissue repair. Consequently, a reduction in granuloma mass reflects the anti-proliferative or anti-inflammatory potential of tested compounds, offering valuable insight into mechanisms involved in the resolution of subacute inflammation (24).

In this study, the control group exhibited the greatest increase in body weight, reflecting normal physiological growth. Treatment with NSAIDs led to a moderate reduction in weight gain, most evident in DCF-treated animals. This effect is likely related to COX inhibition, which can influence gastrointestinal integrity, appetite, and metabolism. Animals receiving antibiotics, alone or in combination with NSAIDs, showed only mild weight suppression, suggesting minor metabolic or gastrointestinal stress without signs of overt toxicity or behavioral disturbance.

Analysis of granuloma mass provided complementary evidence of systemic drug effects. All pharmacological interventions reduced granuloma formation relative to controls, indicating effective modulation of subacute inflammatory responses. Among the NSAIDs, DCF produced the strongest inhibition of granuloma tissue development, followed by KET. The pronounced efficacy of DCF aligns with the suppression of prostaglandin synthesis, fibroblast proliferation, and collagen deposition (25). KET, also significantly reduced granuloma mass, though to a lesser degree. These outcomes support existing evidence that COX inhibition attenuates fibroproliferative inflammation and may modestly influence body-weight dynamics during prolonged inflammatory states.

The antibiotics displayed distinct profiles. LFC significantly decreased granuloma weight, whereas CFX produced only a minor, non-significant effect. The superi-

or activity of LFC may stem from its documented ancillary anti-inflammatory actions, particularly the inhibition of cytokine release (e.g., TNF-α, IL-6, IL-1β) (19) and suppression of NF-κB signaling, resulting in reduced leukocyte infiltration and fibroblast activation (26). Further research has reported that LFC effectively reduces pulmonary inflammation through the suppression of pro-inflammatory cytokines and chemokines, including TNF-α, IL-6, and MCP-1 (19,27,28). It also demonstrates significant anti-inflammatory effects *in vitro* by reducing proinflammatory cytokine secretion in human bronchial epithelial cells culture, indicating potential immunomodulatory benefits in addition to its antibacterial activity in chronic pulmonary infections (28-30). Complementary findings have also indicated that LFC exerts inhibitory activity on the lipoxygenase, which plays a role in lipid peroxidation and oxidative inflammatory pathways (31, 32). Collectively, these studies support the view that LFC exhibits measurable anti-inflammatory actions beyond its antimicrobial role, likely mediated through modulation of cytokine signaling and oxidative stress responses.

CFX, by contrast, exhibits limited interaction with inflammatory mediators and may even trigger mild pro-inflammatory responses under certain conditions. CFX, is primarily recognized for its broad-spectrum antibacterial efficacy, with scarce data indicating anti-inflammatory or immunomodulatory effects on human bronchial epithelial cells (29, 30). Therefore, while LFC appears to exert a modest yet consistent anti-inflammatory effect, CFX shows minimal evidence of such activity when administered alone.

Combination treatments revealed notable interactions. Co-administration of NSAIDs with either antibiotic enhanced the inhibition of granuloma formation, with the DCF-LFC pairing demonstrating the greatest effect. This potentiation likely arises from complementary mechanisms, NSAID-mediated suppression of prostaglandin pathways, alongside antibiotic modulation of cytokine activity and oxidative stress. The DCF combinations consistently outperformed those involving KET, underscoring the stronger anti-proliferative impact of selective COX-2 inhibition. The findings confirm that DCF exerts the most potent anti-inflammatory action in this subacute granuloma model, while LFC contributes additional immunomodulatory benefits, particularly when used in combination. Although CFX shows limited direct anti-inflammatory properties, it may enhance NSAID efficacy through pharmacodynamic interaction. These results highlight the potential therapeutic value of combining NSAIDs with specific antibiotics, especially in clinical contexts characterized by concurrent infection and inflammation. Nevertheless, further investigations are required to elucidate the precise mechanisms underlying these interactions, evaluate long-term safety, and determine whether the observed experimental synergy translates into tangible clinical advantages.

CONCLUSIONS

The present study demonstrated that both DCF and KET exerted significant anti-inflammatory effects in the cotton pellet-induced granuloma model, confirming their efficacy in modulating the proliferative phase of subacute inflammation. Among the antibiotics tested, LFC produced a moderate but statistically significant reduction in granuloma formation,

consistent with its reported immunomodulatory and cytokine-suppressive properties, while CFX showed minimal direct anti-inflammatory activity. Combination therapy enhanced the inhibition of granulomatous tissue formation compared with single-drug treatments, particularly in DCF-based regimens, suggesting additive or possibly synergistic effects between NSAID-mediated prostaglandin suppression and antibiotic-mediated cytokine modulation. These interactions may hold clinical relevance in conditions characterized by coexisting infection and inflammation, where combined anti-infective and anti-inflammatory strategies could improve therapeutic outcomes. Further studies involving larger cohorts, extended treatment durations, and molecular assays are required to confirm the observed pharmacodynamic interactions and to elucidate the mechanisms underlying the immunomodulatory potential of antibiotic-NSAID combinations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SAFETY OF CONTRAST ADMINISTRATION IN COMPUTED TOMOGRAPHY ANGIOGRAPHY IN PEDIATRIC CONGENITAL HEART DISEASE: A 9-YEAR SINGLE CENTER COHORT OF 697 EXAMS

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SAFETY OF CONTRAST ADMINISTRATION IN COMPUTED TOMOGRAPHY ANGIOGRAPHY IN PEDIATRIC CONGENITAL HEART DISEASE: A 9-YEAR SINGLE CENTER COHORT OF 697 EXAMS (Abstract): Pediatric cardiovascular congenital disease (CHD) diagnosis benefits greatly from thoracic computed tomography angiography (CTA), with an increasing number of referrals. **The aim** of this study was to evaluate the safety of CTA examinations in pediatric patients with congenital cardiovascular malformations. **Materials and methods**: A 9-year retrospective analysis of pediatric CTA reports was performed. 697 consecutive examinations were included, with recorded data consisting of demographic data of the patient and procedure-related information (cannulation type, site of venous access, type and volume of contrast agents, injection flow rate, dose and adverse events). **Results**: The median patient age was 199 days (interquartile range IQR [15,1714.5]), with a male: female ratio of 1.29. The most frequent peripheral cannulation site was the left antecubital fossa 265 cases (38.02%), and the most frequently used cannula size was 24-gauge (331 cases); a central line was used in 101 cases (14.49%). The flow rates for peripheral and central venous catheters were 0.3 to 5 mL/s, median 1.00, [0.8, 2.0] and 0.5 to 4 mL/s, median 0.9 [0.8, 1.0] respectively. Nine cases of contrast media extravasation (1.29%), four minor allergic reactions (0.6%), two cases of placement of the central venous catheter in the artery (0.29%), one case of air embolus (0.14 %) and one anaphylactic shock (0.14%) were recorded. **Conclusions**: In a single-center cohort of 697 pediatric CTAs for congenital heart disease over 9 years, complication rates related to contrast and venous access were low (extravasation ~1.3%; allergic reactions ~0.6%; anaphylaxis ~0.14%), all resolved favorably. Central line incidents were rare. The results support the safety of contrast administration in pediatric CTA of congenital heart disease, with the recommendation to verify the prescan and standardize protocols**.** **Keywords***:* CONGENITAL HEART DISEASE, CT ANGIOGRAPHY, CONTRAST MEDIA, PEDIATRIC.

INTRODUCTION

CT Angiography (CTA) has become a vital non-invasive diagnostic tool with extremely high clinical value for cardiovascular pathology due to recent advancements in multidetector CT scanner technology that have enabled faster anatomical coverage and high submillimeter spatial resolution for larger body scan volumes(1, 2).

Formerly considered in the context of

pediatric use due to associated exposure to ionizing radiation, CTA has recently become a robust imaging modality, providing excellent input for pre and post-therapeutic management of congenital heart disease (CHD)(3-6).

Including a broad spectrum of indications, in the context of CHD, CTA mainly focuses on the anatomical mapping of normal and variant cardiac chambers and the great vessel arrangements, proximal and distal pulmonary arteries, the imaging of normal and abnormal venous anatomy, the detailed analysis of coronary vessels (especially important for surgical planning to treat Tetralogy of Fallot (TOF)), congenital or acquired arterial stenosis, and assessment of aortopulmonary collateral arteries in patients with pulmonary atresia(4, 7-10).

Pediatric cardiovascular CT imaging is performed after intravenous contrast medium injection, with multiple techniques available for contrast agent administration(11). The main challenge in CTA for pediatric CHD is the use of small-bore angiocatheters, which sometimes must be placed in unusual and minute access sites to deliver small volumes of contrast medium while obtaining the appropriate vascular enhancement(12-14).

Due to the possibility of contrast extravasation, volume, maximum flow rate, contrast type, and osmolarity (15-17), must be considered. Pediatric patients, especially those with preexisting cardiac dysfunction, have lower tolerance to intravascular fluid changes, which can be triggered if a hyperosmotic contrast medium is provided, so non-ionic, low- or iso-osmotic iodinated contrast material is preferred(18-20) even if it could provide less enhancement(21).

For children undergoing CTA, contrast material is delivered by a power injection through small peripheral angiocatheters (22-26 gauge) placed in antecubital veins (preferably the right arm, to avoid hardening artifacts in the innominate vein) or in feet, as well as central venous catheters (jugular or femoral). A high viscosity contrast agent increases the pressure associated with intravascular injection, ultimately limiting the maximum injection rate. This can cause failure to obtain the desired flow rate or even catheter failure and extravasation of contrast material with possible vessel injury. The use of power injectors has become the most common method for delivering contrast media for CTA(12, 22-25).

The aim of this study was to evaluate the safety of CTA exams in pediatric patients with congenital cardiovascular malformations. Our hypothesis was that CTA examinations performed in a pediatric congenital cardiovascular population represent a rather safe procedure, with a rate of incidents similar to that in the general population.

MATERIALS AND METHODS

A retrospective review of data collected from computed tomography angiography examinations reports of a single center- a tertiary cardiovascular teaching hospital was performed, following Ethics Committee retrospective approval (5718/08.07.2024), with exemption from informed consent for the file analysis. For minor patients, consent for the medical procedure/imaging was obtained from the legal guardian, in accordance with institutional policy.

Patient and procedural data were collected from consecutively performed CTA. All patients under 18 years of age underwent computed tomography angiography to evaluate cardiovascular anatomy or function between February 2015 and July 2024.

Examinations were considered and included in the database if they were ordered for a patient with congenital cardiovascular disease to evaluate anatomy or function.

Exclusion criteria included CTA examinations performed for evaluation of non-cardiovascular indication, incomplete demographic or reporting records, and reports mentioning non-diagnostic scans. Acquisition protocols have been developed according to multi-society recommendations, considering the available infrastructure.

Relevant information was collected from radiological records, including patient demographics (age, gender, weight, height, indication), procedure-related data (cannulation type, site of venous access, type and volume of contrast agents used, injection flow rate), and adverse events (erythema, nausea, vomiting, anaphylactic shock) or technical issues (extravasation, line misplacement). Examinations in which any of the above-mentioned data were missing were excluded from the study.

In pediatric cardiac CT imaging using the Mallinckrodt OptiVantage system, it is essential to balance image quality and radiation exposure. For children, 80-100 kVp is typically used to minimize radiation, with mAs adjusted according to the patient's weight: 1-30 mAs for infants and 30-60 mAs for older children. The CARE dose system optimizes the mAs automatically based on body size and anatomical area. Collimation is set tightly around the heart to reduce unnecessary radiation, and the pitch is typically 1.0-1.5, ensuring optimal resolution while maintaining scan speed. ECG gating is used to synchronize the scan with the cardiac cycle, usually capturing the 50%-75% phase to reduce motion artifacts. Contrast is administered at 1.5-2.5 mL/kg of body weight, with concentrations of 300-370 mg/mL, and followed by 0.5-30 mL saline to flush the contrast from the system. The contrast temperature is maintained at 37°C to prevent reactions and ensure smooth injection. These settings are carefully adjusted to provide high-quality cardiac imaging while minimizing risks, such as radiation exposure and contrast-related issues, especially in pediatric patients.

The flow rate used in each case was established according to the local protocol, depending on the cannula size, ranging from 0.3 mL/s in the case of central lines and 26-gauge cannula up to 5 mL/s in case of large-bore 16-gauge cannula.

The amount of contrast medium used varied depending on the patient's weight and body height ranging from 3 to 100 mL. In all cases, a bolus tracking technique was used, with a threshold of 150 Hounsfield units and a region of interest determined by clinical indication.

Until approximately 2019, none of the patients underwent pre-medication for contrast agent reaction prophylaxis. Starting in that year, all patients capable of receiving oral medication were treated with Medrol 32 mg (in general between 0.5-1 mg/kg) 12 hours before examination and Medrol 32mg + Loratadine 5/10 mg one hour before. If the patients were unable to take oral medication, we replaced each corticoid with 4-8 mg/kg of hydrocortisone IV. Premedication was introduced not only for patients with a history of contrast reactions but also to minimize the risk of adverse reactions, even in the absence of prior tests, given the underlying cardiac conditions of the patients. For those already under treatment, their medication remained unchanged prior to CTA.

Adverse events (AE) were defined as

contrast-related and technical- related.

AE related to contrast included bitter taste, erythema, nausea, vomiting, and anaphylactic shock. To reduce bias, warming sensation and bitter taste were considered physiological and were not included. They were evaluated after contrast administration and up to 24 hours following the examination since all patients were represented by hospitalized patients.

The technical related events were assessed immediately after contrast agent administration. The presence of extravasation of the contrast agent, misplacement of the catheter and air embolus were recorded.

Data normality was evaluated using a Kolmogorov-Smirnov test.

Continuous variables are expressed as mean +/- standard deviation (SD) if data has normal distribution or as median and interquartile range (IQR) if data is skewed.

Comparison of medians was made using Mann-Whitney tests, and correlation strength analysis was assessed using Spearman test.

All statistical calculations were performed using IBM SPSS Statistics 26, with a p-value <0.05 considered statistically significant.

RESULTS

Data from 697 examinations performed at one institution were retrospectively included in the study. The median patient age at the time of the scan was 199.00 days (range: 0 days to 17 years; IQR [15,1714.5]). Females accounted for 44.5% of the study population (n=310), while males were 55.5%, resulting in a male-to-female ratio of 1.29. The median male age was 245 days, IQR [15,2102]; was statistically significantly different ($P = 0.045$) from the median female age, which was 153 days, IQR [15,1097].

The median weight was 6.0 kilograms, IQR [3.4,15], while the median height was 65.00 centimeters, IQR [53,105].

The most common primary indications for the studies were vascular abnormalities - including aortic coarctation, ascending aortic ectasia, and aortic arch hypoplasia — as well as complex congenital heart malformations such as TOF (including double outlet right ventricle), transposition of the great arteries, and total anomalous pulmonary venous return.

Before surgical or interventional treatment, aortic coarctation, complex congenital heart malformations, and TOF accounted for 55.73% of all cases, representing 306 examinations of a total of 549. Following surgical correction, the most frequent indications remained similar, with aortic coarctation, Tetralogy of Fallot, and transposition of the great arteries comprising 60.81% of all cases (90 examinations out of 148).

When using peripheral access, the most frequent cannulation site was the left upper limb antecubital fossa, 265 cases (38.02%) followed by the right upper limb, 242 cases (34.72%). Scalp veins and inferior limb peripheral cannulas were used less frequently, representing less than 12.19% of the total sites (fig. 1).

A central line was used in 101 cases (14.49%), placed in the superior vena cava (72 cases), inferior cava vein tributaries (25 cases) or umbilical veins (4 cases).

Figure 1 illustrates the distribution of venous access sites and the size (gauge) of the devices used in patients. The x-axis indicates the location of the venous access. The y-axis shows the number of patients who received access to that site. The colors within the bars represent the size (gauge) and the type of catheter / cannula used.

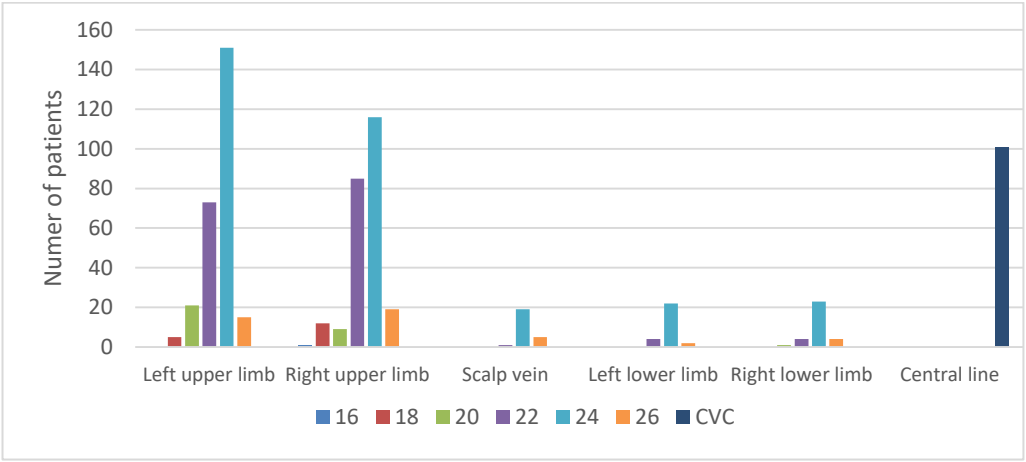

Fig. 1. Venous access sites and size (gauge)

As the gauge number increases (moving from a 16 gauge up to a 26 gauge and then a central line), the median flow rate consistently and significantly decreases, confirming that larger cannulas (lower gauge numbers) permit much higher flow rates. For example, the 18-gauge cannula shows the highest median flow-rate 2.5 and the largest range, IQR [0.9, 3.5], while the 24- and 26-gauge cannulas have the lowest median flow rate 0.8 and the least variability, IQR [0.8,1.0], demonstrating the critical impact of catheter diameter on intravenous fluid delivery speed. The central line is comparable with 24- and 26-gauge cannulas (fig. 2).

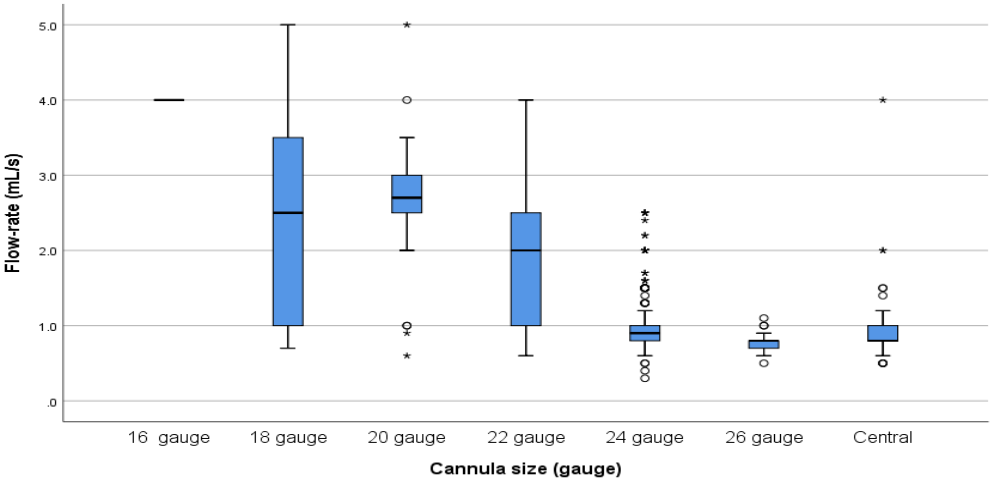

Fig. 2. Relation between flow-rate (mL/s) and cannula size (gauge)

In the second figure the asterisks indicate far out values, the central box represents the values from the lower to upper quartile (25 to 75 percentile) and the mid-

dle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outside and far-out values, which are displayed as separate points (fig. 2).

Four allergic reactions and an anaphylactic shock with Iodixanol 320 were recorded. All cases are resolved favorably after medical treatment.

With peripheral cannulation, nine cases of subcutaneous extravasation of contrast media occurred (1.29%) with a 95% confidence interval ranging from 0.68%-2.44%. This narrow confidence interval suggests a low and stable rate of extravasation in the study population, indicating that the procedure was performed safely and consistently. They occurred while using 22 to 26 gauge, eight being placed in the upper limb and one in the lower limb, and without connection to the injection flow rate (between 0.6 mL/s and 2.5 mL/s) or the administered media (Iopromide 370, Iomeron 350 and Iodixanol 320) (tab. I).

TABLE I.

Extravasation cases, including injection site, cannula gauge, flow rate, and type of contrast agent used.

Extravasation	Place	Gauge	Flow mL/s	Agent
Extravasation 1	Left upper limb	26 gauge	0.6	Iopromide 370
Extravasation 2	Right lower limb	24 gauge	0.8	Iomeprol 350
Extravasation 3	Left upper limb	22 gauge	0.8	Iomeprol 350
Extravasation 4	Left upper limb	24 gauge	0.8	Iomeprol 350
Extravasation 5	Left upper limb	24 gauge	0.8	Iomeprol 350
Extravasation 6	Left upper limb	24 gauge	1	Iomeprol 350
Extravasation 7	Left upper limb	22 gauge	1	Iomeprol 350
Extravasation 8	Left upper limb	22 gauge	2.3	Iodixanol 320
Extravasation 9	Right upper limb	22 gauge	2.5	Iodixanol 320

The use of central line was not associated with incidents due to contrast medium injection, however, we report two cases of central venous catheter misplacement into the artery, which were detected immediately prior to the exam (2/101= 1.98% with a Wilson CI of 0.55%- 6.98%) and an injection incident, one air embolus (1/101= 1.01% with a Wilson CI of 0.19%-5.43%).

The Mann-Whitney U test showed no significant differences in injection flow rate between examinations with and without extravasation (1.18 mL/s vs 1.31 mL/s, $U = 2648.00$, $p = 0.447$), indicating no association between flow rate and extravasation occurrence.

Reactions were evaluated using the ACR-ESUR classification. Three cases of erythema and one case of nausea were observed, both classified as Grade 1 (minor reactions), as they were mild and self-limiting. A case of anaphylactic shock occurred, classified as Grade 3 (severe reaction), requiring immediate medical intervention (tab. II).

TABLE II.

Types of injected contrast agents and adverse reactions

Type of contrast agent	Erythema	Nausea/ Vomiting	Anaphylactic shock	Grand Total Cases
Iomeprol 350				387 (55.5%)
Ioversol 350	1			112 (16%)
Iopromide 370	1			122 (17.5%)
Iodixanol 320	1	1	1	76 (10.9%)
Total Number of Reactions	3 (0.45%)	1 (0.14%)	1 (0.14%)	697

Following the implementation of the universal premedication protocol in 2019, a distinct cessation of adverse reactions was observed. While the period between 2015 and 2019 showed an incidence of approximately 1.2 reactions per year, no contrast-related adverse events were recorded in the study population from 2020 onward. Technical events were in total 12 (12/697=1.72% with a 95% CI 0.75%-2.69%) distributed approximately 1.2/year (fig. 3).

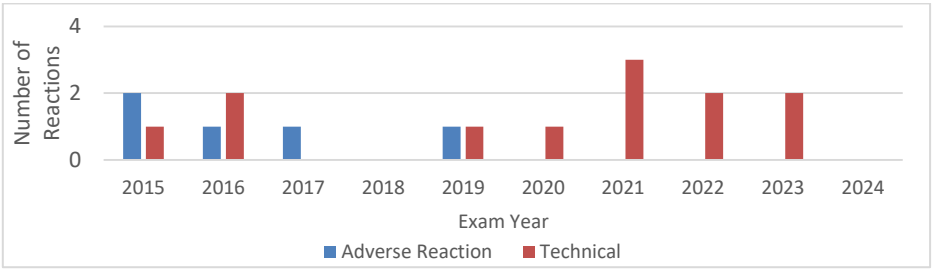

Fig. 3. Total number of incidents per year

Figure 3 presents annual distribution of contrast-related adverse events and technical complications (2015-2024). Technical events occurred at a rate of approximately 1.2 per year. No contrast-related adverse reactions were recorded from 2020 onward, coinciding with the introduction of the universal premedication protocol.

DISCUSSION

Using power injectors with small cannulas, especially via non-implanted central venous catheters, is challenging. Catheter type, injection rate, pre-warming, and pressure affect outcomes, and patient factors must guide decisions.

When the contrast agent was used, tried and adjusted to the size and quality of the vein, neither the extravasation rates nor the contrast reaction frequencies were significantly different from those reported for CT angiography. The extravasation rate was 1.29% (95% CI: 0.68%-2.44%) in our group, 0.36% (95% CI: 0.10%-1.31%) in Amaral et al. and 0.74% (95% CI: 0.47%-1.17%) in Barrera et al. The overlapping 95% CIs (0.68%-2.44%, 0.10%-1.31, 0.47%-1.17) suggest no statistically significant difference between studies. Amaral et al. reported that the risk of extravasation correlated with high

injection rate and pressure. Although, in our examination, the Mann-Whitney U test showed no significant differences in injection flow rate between examinations with and without extravasation (1.18 mL/s *vs.* 1.31 mL/s, U = 2648.00, p = 0.447), indicating no association between flow rate and extravasation occurrence (22, 26-30).

Our center's treatment yielded a few minor adverse reactions related to contrast agent (0.6% with a Wilson CI of 0.24%-1.50%) and one anaphylactic shock (0.14% with a Wilson CI of 0.02%-0.80%), when using low-osmolar iodinated contrast media, all which resolved favorably following conservative treatment; in this regard our experience stays in accordance to previously reported data

When speaking about the limitations of the study, one was the relatively small sample size, and all data was collected from a single center. Many examinations were performed under sedation, so some contrast-related incidents may have gone unnoticed. Children may also have difficulty reporting reactions. Due to the retrospective design, only adverse events documented in radiology reports were collected and late complications such as kidney failure or central line infections could not be assessed. Moreover, over the years, the protocol has undergone several modifications; therefore, its heterogeneity may have influenced the results.

CONCLUSIONS

In this nine-year cohort, pediatric congenital heart disease CTA demonstrated low rates of technical and contrast-related complications. Peripheral extravasation was infrequent and self-limited. Central venous access was associated with rare line placement incidents, underscoring the importance of pre-scan verification. Further stratified analyses suggest that universal premedication may contribute to low rates of hypersensitivity. Overall, the procedure is safe for these pediatric CHD patients, as also observed in similar studies (28, 31, 32).

CONFLICT OF INTEREST AND FUNDING

All authors declare no conflict of interest and no funding received.

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SEGMENTAL VASCULAR BURDEN AND OUTCOMES IN DIABETIC *VS.* NON-DIABETIC CHRONIC LIMB-THREATENING ISCHEMIA AFTER ENDOVASCULAR REVASCULARIZATION

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SEGMENTAL VASCULAR BURDEN AND OUTCOMES IN DIABETIC *VS.* NON-DIABETIC CHRONIC LIMB-THREATENING ISCHEMIA AFTER ENDOVASCULAR REVASCULARIZATION (Abstract): Peripheral artery disease (PAD) progressing to chronic limb-threatening ischemia (CLTI) is associated with high morbidity and limb loss. Diabetes mellitus (DM) significantly influences disease distribution and clinical outcomes. The Bollinger score, GLASS classification, and WIfI staging are complementary tools for assessing anatomical burden and limb threat. This study aimed to analyze the relationships between these scoring systems in diabetic CLTI, with particular focus on infra-popliteal versus global anatomical evaluation. **Materials and methods**: This single-center retrospective study included 136 patients with CLTI undergoing endovascular revascularization between May 2021 and May 2023. Baseline characteristics, angiographic severity, WIfI stage, Bollinger scores (whole limb, above- and below-knee), GLASS staging (global, femoropopliteal and infra-popliteal), and outcomes (technical success, bypass conversion, major amputation, and one-year mortality) were analyzed. **Results**: Patients were divided into diabetic (DM, n=85) and non-diabetic (NDM, n=51) groups. Diabetic patients more frequently presented with infra-inguinal stenoses >50% (55.3% *vs.* 35.3%, p=0.024) but fewer complete occlusions (83.5% *vs.* 96.1%, p=0.028). WIfI stage 1 was more common in the DM group (54.1% *vs.* 33.3%, p≈0.08). Bollinger scores were lower in diabetics for the whole limb (30.07±16.34 *vs.* 36.78±16.09, p=0.018) and below-knee segment (20.53±15.88 *vs.* 26.86±13.93, p=0.015). **Conclusions**: Diabetic CLTI is characterized by a distal angiographic pattern with predominant infra-popliteal stenoses and lower additive Bollinger scores. Segmental infra-popliteal assessment showed stronger clinical relevance than global evaluation, with below-knee Bollinger and GLASS infra-popliteal stages most closely associated with WIfI severity. These findings support a segment-focused approach in endovascular planning for diabetic patients.

Keywords: CHRONIC LIMB-THREATENING ISCHEMIA, PERIPHERAL ARTERIAL DISEASE, DIABETES MELLITUS, ENDOVASCULAR REVASCULARIZATION, BOLLINGER SCORE, GLASS CLASSIFICATION, WIFI STAGING.

INTRODUCTION

Peripheral artery disease (PAD) progressing to chronic limb - threatening ischemia (CLTI) is associated with high

morbidity, limb loss, and mortality, particularly in patients with diabetes mellitus (DM). Over the past two decades, endovascular therapy has become the first-line strategy for most infra-inguinal lesions, supported by contemporary guidelines and randomized evidence demonstrating lower peri-procedural risk compared with open bypass in appropriately selected patients (1-4). Percutaneous transluminal angioplasty remains the cornerstone of treatment, with adjunctive stenting used in selected cases of residual stenosis, flow-limiting dissection, or complex lesions.

Diabetes mellitus significantly alters the anatomical and biological characteristics of PAD. Diabetic arteriopathy is typically distal, multi-segmental, and frequently associated with calcification, impaired collateralization, and microvascular dysfunction. This phenotype results in more severe infra-popliteal involvement and a predominance of stenotic rather than extensive occlusive lesions. Mechanistically, medial arterial calcification, microangiopathy, and endothelial dysfunction have been identified as key contributors, influencing both procedural feasibility and long-term durability of endovascular interventions (5-7).

To standardize evaluation and guide clinical decision-making, complementary scoring systems are widely used. The Society for Vascular Surgery WIfI classification assesses wound extent, ischemia, and foot infection to estimate amputation risk and the potential benefit of revascularization (8, 9). The Bollinger score provides a semi-quantitative assessment of segmental atherosclerotic burden across the lower limb arterial tree (10-12). The Global Limb Anatomic Staging System (GLASS) integrates femoropopliteal and infra-popliteal disease complexity to predict technical success and durability of revascularization strategies (1, 13, 14). Together, these tools offer a comprehensive framework combining clinical severity, anatomical burden, and procedural complexity.

Clinical outcomes in CLTI are influenced by multiple lesion- and patient-related factors, including lesion type (stenosis versus occlusion), lesion length, multilevel disease, runoff status, and comorbidities such as DM and chronic kidney disease. These factors directly affect technical success, patency, and limb salvage, highlighting the importance of standardized assessment and reporting (1, 4, 15). However, comparative data evaluating diabetic versus non-diabetic CLTI using integrated scoring systems (WIfI, Bollinger, and GLASS) remain limited in Eastern European populations.

Therefore, this study aimed to analyze the characteristics of diabetic patients with CLTI undergoing endovascular revascularization and to evaluate the relationships between WIfI stage, Bollinger score (whole limb; above- and below-knee), and GLASS classification (global, femoropopliteal, and infra-popliteal), with particular emphasis on segmental infra-popliteal assessment compared with global limb evaluation.

MATERIALS AND METHODS

Study design. This was a single-center, retrospective observational study conducted at the Vascular Surgery Clinic of Sf. Spiridon County Clinical Emergency Hospital Iași, Romania. The study included consecutive patients diagnosed with CLTI who underwent endovascular revascularization between May 2021 and May 2023. The study protocol complied with institutional and ethical standards, and all patients pro-

vided informed consent for the procedures performed.

Patients. A total of 136 patients with CLTI were included. Patients were stratified into two groups based on the presence of diabetes mellitus: diabetic (DM, n=85) and non-diabetic (NDM, n=51). CLTI was defined as ischemic rest pain lasting more than two weeks, with or without tissue loss. Diabetes mellitus was defined based on documented medical history, ongoing antidiabetic treatment, or HbA1c ≥6.5% at admission or during hospitalization. Patients with acute limb ischemia, isolated aorto-iliac disease, recent surgical bypass, or without indication for revascularization were excluded.

Data collection. Baseline demographic characteristics, cardiovascular risk factors, and comorbidities were recorded. Angiographic evaluation was performed using digital subtraction angiography. The Bollinger score was calculated as a segmental additive measure of atherosclerotic burden, based on lesion severity and distribution (10-12). Scores were obtained for the whole limb, as well as for above-knee and below-knee segments. The GLASS classification was used to assess femoropopliteal and infra-popliteal lesion complexity and to determine overall limb staging (1, 13, 14). Clinical severity was evaluated using the Society for Vascular Surgery WIfI classification, according to established criteria (8, 9). WIfI staging was reconstructed retrospectively based on available clinical and hemodynamic data.

Endpoints. The primary endpoint was technical success, defined as restoration of inline flow to at least one pedal artery with less than 30% residual stenosis on completion angiography. Secondary endpoints included early (30-day) limb-related outcomes - major amputation, conversion to surgical bypass, and repeat revascularization - as well as late outcomes, defined as one-year all-cause mortality.

Statistical analysis was performed using *SPSS version 29*. Continuous variables were expressed as mean ± standard deviation (SD) and compared using t-Student's test or the Mann-Whitney U test, depending on data distribution. Categorical variables were expressed as absolute numbers and percentages and compared using the χ^2 test or Fisher's exact test, as appropriate. Correlations between scoring systems were assessed using Spearman's rank correlation coefficient. Agreement between scoring classifications was evaluated using Cohen's kappa coefficient. Logistic regression analysis was performed to explore predictors of technical failure; detailed model outputs are not presented, as they fall beyond the primary scope of the present study. Variables with potential clinical relevance or statistical significance in univariate analysis were included in the multivariate model. Model performance was evaluated using the Hosmer-Lemeshow goodness-of-fit test and Nagelkerke R^2. Statistical significance was defined at $p < 0.05$.

RESULTS

Baseline characteristics. A total of 136 patients with CLTI were included in the study, of whom 62.5% (n=85) had diabetes mellitus. Baseline clinical characteristics are presented in first table.

The two groups were comparable in terms of age and sex distribution, with a mean age of approximately 70 years in both groups (p=0.737). Urban residence was significantly more frequent among diabetic patients (72.9% *vs.* 49.0%, p=0.005). The prevalence of hypertension (85.9% *vs.*

74.5%), chronic kidney disease (18.8% *vs.* 13.7%), and prior stroke (11.9% *vs.* 3.9%) was higher in the diabetic group; however, these differences did not reach statistical significance.

Angiographic findings and WIfI stages. Angiographic characteristics and WIfI stages are summarized in table II.

TABLE I.
Baseline characteristics of patients

Parameter	NDM Group (n=51)	DM Group (n=85)	p-value
Female, n(%)	12 (23.5)	27 (31.8)	0.304
Urban residence, n (%)	25 (49.0)	62 (72.9)	0.005**
Age, years (mean ± SD)	69.78±11.38	69.89±8.57	0.737
Hypertension, n (%)	38 (74.5)	73 (85.9)	0.097
Obesity, n (%)	4 (7.8)	13 (15.3)	0.203
Heart failure, n (%)	17 (33.3)	24 (28.2)	0.531
Atrial fibrillation, n (%)	15 (29.4)	16 (18.8)	0.154
Prior mi, n (%)	2 (3.9)	7 (8.2)	0.483
Chronic coronary syndrome, n (%)	8 (15.7)	17 (20.0)	0.530
Cardiomyopathy, n (%)	1 (2.0)	5 (5.9)	0.410
Dilated cardiomyopathy, n (%)	4 (7.8)	4 (4.7)	0.473
Chronic kidney disease, n (%)	7 (13.7)	16 (18.8)	0.443
Prior stroke, n (%)	2 (3.9)	10 (11.9)	0.210

TABLE II.
Angiographic severity according with WIfI stages in the diabetes mellitus (DM) and non-diabetes (NDM) groups.

Variable	NDM Group (n=51)	DM Group (n=85)	p-value
>50% stenosis, n (%)	18 (35.3)	47 (55.3)	0.024*
Any occlusion, n (%)	49 (96.1)	71 (83.5)	0.028*
WIfI stage 1, n (%)	17 (33.3)	46 (54.1)	0.083+
Stents present, n (%)	11 (21.6)	20 (23.5)	NS

*NS non-significant

Patients in the diabetic group more frequently presented with stenoses greater than 50% compared with non-diabetic patients (55.3% *vs.* 35.3%, p=0.024). In contrast, complete occlusions were less frequent among diabetics (83.5% *vs.* 96.1%, p=0.028). WIfI stage 1 was more common in the diabetic group (54.1% *vs.* 33.3%),

although this difference did not reach statistical significance (p≈0.08). The presence of stents was similar between groups.

Bollinger and GLASS scores

Bollinger and GLASS scores are presented in table III.

TABLE III.

The Bollinger score in the DM group *vs.* the NDM group

Parameter	NDM Group	DM Group	p-value
Bollinger whole leg, mean ± SD	36.78±16.09	30.07±16.34	0.018*
Bollinger above knee, mean ± SD	9.92±8.01	9.54±6.96	0.941
Bollinger below knee, mean ± SD	26.86±13.93	20.53±15.88	0.015*
Glass global: stage 1, n (%)	23 (45.1)	28 (32.9)	NS
Glass global: stage 2, n (%)	18 (35.3)	35 (41.2)	NS
Glass global: stage 3, n (%)	10 (19.6)	22 (25.9)	NS

Diabetic patients exhibited lower Bollinger scores for the whole limb (30.07±16.34 *vs.* 36.78±16.09, p=0.018) and for the below-knee segment (20.53± 15.88 *vs.* 26.86±13.93, p=0.015). No significant differences were observed for above-knee scores. Global GLASS staging showed a similar distribution between the two groups, with no statistically significant differences across stages.

Outcomes of revascularization. Early limb-related outcomes and one-year mortality are presented in table IV.

TABLE IV.

Success rate in the DM group *vs.* NDM group

Outcome	NDM Group (n=51)	DM Group (n=85)	p-value
Technical success, n (%)	37 (72.5)	64 (75.3)	0.71
Bypass, n (%)	6 (11.8)	10 (11.8)	1.00
Major amputation, n (%)	2 (3.9)	8 (9.4)	0.32
Death, n (%)	1 (2.0)	3 (3.5)	1.00

Technical success was achieved in 74.3% of cases, with no significant difference between diabetic and non-diabetic patients (75.3% *vs.* 72.5%, p=0.71). Bypass conversion rates were identical in both groups (11.8%). Major amputation occurred more frequently in the diabetic group (9.4% *vs.* 3.9%), without statistical significance (p=0.32).

In-hospital mortality was low and comparable between groups (3.5% *vs.* 2.0%, p=1.00).

Correlations between scoring systems. Correlations between WIfI stage, Bollinger score, and GLASS classification are presented in table V.

TABLE V.

Correlations between WIfI stage, Bollinger score and GLASS classification.

Correlation	ρ	p-value
WIfI ↔ Bollinger (whole leg)	0.290	<0.001
WIfI ↔ GLASS (global)	0.173	0.044
Bollinger (whole leg) ↔ GLASS (global)	0.643	<0.001
Above - knee: Bollinger ↔ GLASS-FP	0.762	<0.001
Below - knee: WIfI ↔ Bollinger-IP	0.266	0.002
Below - knee: Bollinger-IP ↔ GLASS-IP	0.713	<0.001

A strong correlation was observed between Bollinger whole-limb score and global GLASS stage (ρ=0.643, p<0.001). Moderate correlations were identified between WIfI stage and Bollinger score (ρ=0.290, p<0.001), and a weaker correlation between WIfI stage and GLASS classification (ρ=0.173, p=0.044). At the segmental level, the strongest association was observed between infra-popliteal Bollinger score and GLASS infrapopliteal stage (ρ=0.713, p<0.001).

Correlations heatmap illustrating these relationships are shown in the first figure.

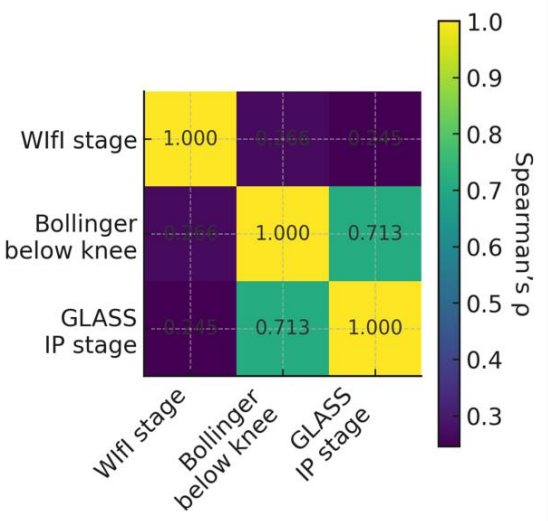

Fig. 1. Spearman's correlation heatmap (ρ) between WIfI stage, infra-popliteal Bollinger score (below-knee burden), and GLASS infra-popliteal (IP) stage.

DISCUSSION

This study provides a comparative evaluation of diabetic and non-diabetic patients with CLTI undergoing endovascular revascularization, integrating 3 complementary scoring systems: WIfI, Bollinger, and GLASS. The combined use of these tools allowed a structured assessment of clinical

severity, anatomical burden, and procedural complexity. Our findings confirm that diabetes significantly alters the angiographic pattern of peripheral arterial disease. Patients in the DM group more frequently presented with infra-inguinal stenoses >50% and fewer complete occlusions, accompanied by lower Bollinger scores for both the whole limb and the below-knee segment. This pattern reflects the known pathophysiology of diabetic arteriopathy, characterized by diffuse, calcific, predominantly distal disease with relative preservation of luminal continuity (15, 20-22).

At first glance, the lower Bollinger scores observed in diabetic patients may appear contradictory to the established concept of diffuse and severe disease in diabetes. However, this discrepancy likely reflects a limitation of occlusion-weighted scoring systems, which may underestimate disease severity in the presence of heavily calcified but patent distal vessels (23-25). Thus, in diabetic CLTI, anatomical burden is not fully captured by additive occlusion-based metrics alone. In contrast, patients in the non-DM group more frequently exhibited proximal and occlusive lesions, consistent with a classical macroatherosclerotic pattern. These differences underline the importance of considering disease phenotype when interpreting angiographic scores and planning revascularization. The observed trend toward a higher prevalence of WIfI stage 1 in diabetic patients suggests a potential mismatch between clinical presentation and anatomical severity. In this population, neuropathy and early-stage tissue loss may lead to lower wound grades despite advanced an infrapopliteal disease. This highlights the limitation of relying solely on clinical staging and supports the need for detailed distal anatomical assessment. A key finding of our study is the strong correlation between infra-popliteal Bollinger and GLASS scores ($\rho = 0.713$), which was higher than correlations observed at the global or femoropopliteal level. This reinforces the central role of the infra-popliteal compartment in determining both anatomical complexity and clinical severity in CLTI, particularly in diabetic patients. Similar observations have been reported in contemporary studies emphasizing the predominance of distal disease in diabetes (15, 20-22). The agreement analysis further supports this concept, showing moderate-to-substantial concordance between Bollinger and GLASS overall, with stronger agreement in the DM group. In contrast, the weak association between femoropopliteal GLASS stages and WIfI underscores that proximal anatomy alone does not adequately reflect limb threat. The modest agreement between infra-popliteal GLASS and WIfI in diabetic patients suggests that distal disease has a more direct clinical impact. These findings are consistent with the pathophysiological model proposed by Ferraresi *et al*., in which small artery disease (SAD) and below-the-ankle (BAD) involvement play a critical role in tissue perfusion, even after technically successful revascularization (27). Histopathological studies further support the presence of diffuse distal obstruction and microvascular dysfunction in CLTI, particularly in diabetic limbs (28). In this context, angiographic scoring systems may act as indirect markers of perfusion impairment rather than direct measures of microcirculatory status.

From a procedural standpoint, technical success rates were comparable between groups, confirming that diabetes does not preclude effective endovascular revascular-

ization. However, clinical outcomes tended to be less favorable in the DM group, with higher rates of major amputation and slightly increased mortality. Although not statistically significant, these trends are consistent with previous reports highlighting the influence of systemic factors such as infection, neuropathy, and impaired wound healing on outcomes in diabetic CLTI (17, 29). The relationship between lesion characteristics and procedural success also differed between groups. While stent implantation was associated with higher technical success in the non-DM group, this effect was less evident in diabetic patients, possibly due to smaller vessel caliber, diffuse disease, and increased calcification. These findings support a more selective use of stenting in infra-popliteal disease, in line with contemporary endovascular strategies.

Overall, the integration of WIfI, Bollinger, and GLASS provides a comprehensive framework for CLTI evaluation. WIfI reflects clinical severity, Bollinger quantifies anatomical burden, and GLASS estimates procedural complexity. Their combined use allows a more accurate characterization of disease and may improve decision-making in endovascular planning. The strong segmental correlations observed below the knee support the routine inclusion of infra-popliteal scoring, particularly in diabetic patients. Our results are consistent with prior studies, including BASIL and subsequent analyses, which demonstrated the prognostic value of anatomical scoring systems in CLTI (12).

At the same time, our findings highlight potential limitations of global scoring approaches and support a shift toward segment-focused evaluation, especially in populations with a high prevalence of diabetes.

From a clinical perspective, recognizing the infra-popliteal predominance of diabetic PAD may facilitate earlier intervention, optimize revascularization strategies, and improve multidisciplinary management of CLTI.

A final aspect to consider is the retrospective, single-center design, which may limit generalizability. In addition, WIfI staging was reconstructed retrospectively and long-term outcomes were not systematically assessed. Despite these limitations, the study provides relevant insights into the segmental distribution of disease and the complementary role of integrated scoring systems in diabetic CLTI.

CONCLUSIONS

In diabetic patients with CLTI, anatomical evaluation may benefit from moving beyond global limb scoring toward segmental infra-popliteal assessment. When planning endovascular revascularization, below-knee Bollinger burden and GLASS infra-popliteal staging, integrated alongside WIfI, better reflect distal disease severity and procedural complexity. These findings support a segment-focused approach to endovascular management in diabetic CLTI.

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CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding regarding this scientific research.

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ASYMPTOMATIC COMPLEX TESTICULAR NEOPLASIA ASSOCIATED WITH ORCHIEPIDIDYMITIS. CASE REPORT

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ASYMPTOMATIC COMPLEX TESTICULAR NEOPLASIA ASSOCIATED WITH ORCHIEPIDIDYMITIS-CASE REPORT (Abstract): Testicular germ cells tumors (TGCTs) are rare (2-3 new cases per 100,000 men per year) accounting for 1-2% of male malignancies and ranking fourth among genitourinary cancers. Testicular cancer is the most common cancer in young men 15 to 35-year-old. With approximately 50% of the TGCTs being pure seminomas and 50% non-seminomas, "radical orchiectomy" provides the histological diagnosis and should be performed before any further treatment, unless the clinical situation requires immediate chemotherapy in patients with a clear germ cell malignancy based on elevated tumor markers. Any testicular mass of uncertain ranking must be explored by the inguinal approach to verify or exclude malignancy. We present the case of a 30-year-old patient admitted to the Urology unit of the "Dr. C.I. Parhon" Clinical Hospital for pain in the right hemiscrotum for two weeks. Physical examination revealed the increase in size and local inflammatory changes of the right hemiscrotum. Later during hospital stay, the patient mentioned that the hemiscrotum had been progressively increasing in size over the past 6 months. An ultrasound exam was performed, and serum tumor markers were determined. Based on the results, the patient underwent radical orchiectomy by inguinal approach. **Keywords**: ORCHIEPIDIDYMITIS, GERM CELL TUMOR, TREATMENT.

Testicular germ cells tumors (TGCTs) are divided into two categories: seminomas and nonseminomas. The last category can subsequently be divided into choriocarcinoma, teratoma, embryonal cell carcinoma and yolk-sac tumor. Risk factors for TGCT are: family history, cryptorchidism, testicular dysgenesis, Klinefelter syndrome, Down syndrome, feminized testicle syndromes, persistent Müllerian duct syndrome and hermaphroditism (1).

The serum tumor markers alpha-fetoprotein (AFP) (≤5.80 IU/mL or <10 ng/mL), human chorionic gonadotropin (hCG) (<2.6 mIU/mL) and lactate dehydrogenase (LDH) (135-225 U/L) are essential in determining the presence of tumor and its prognosis, and for assessing the postoperative outcome (1). The recommended treatment for TGCTs is radical orchiectomy (2).

CASE REPORT

We present the case of R.C., a 31-year-old male patient admitted to the Urology Department of the "Dr. C.I. Parhon" Clinical Hospital from Iasi for fever and shivers which started a few days before his admission and pain in and increase in size of the right hemiscrotum over the last two weeks.

His personal medical history included: hepatic steatosis, ulcerative pancolitis in the remission phase (treated with Infliximab) and stage I-II pulmonary sarcoidosis (on corticotherapy). Physical examination revealed class II obesity, painful right hemiscrotum both spontaneously and on palpation, warm and with overlying congested tissue. Laboratory tests showed no biochemical or blood count changes suggestive for the febrile syndrome. Based on the collected data, our first diagnostic supposition was "suppurative right orchiepididymitis" and the patient was given Cefuroxime 0.5g, the fever diminishing within the first 24 hours.

Later during his admission to the Urology Clinic, the patient mentioned that his right hemiscrotum had been slowly increasing in size over the past 6 months. The scrotal ultrasound revealed an inhomogeneous right testicle (fig. 1), increased in size, with imprecise limits and negative Doppler signal.

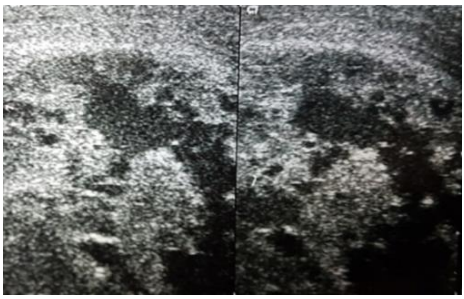

Fig. 1. Ultrasonography of the right hemiscrotum.

This result was suggestive for a highly disorganized testicular parenchyma either due to suppuration or neoplastic process. Tumor markers AFP, *hCG* and *LDH* were determined as part of the preliminary diagnosis. The blood results came back positive: AFP – 689.8 IU/mL, hCG - 10.6 mIU/mL, LDH - 288 U/L. As a result, the surgical intervention for the drainage of the supposed suppuration was canceled to prevent the tumor from metastasizing.

The patient was discharged on Cefuroxime 0.5g for the treatment of orchiepididymitis with the recommendation to return after two weeks to undergo radical orchiectomy. *Abdominal and pelvic CT scan* for the detection of lymphadenopathy was not performed due to the lack of certainty in differentiating inflammatory from metastatic lymphadenopathies. In this case, waiting for the disappearance of inflammatory lymphadenopathies might have taken long enough for the testicular tumor to disseminate.

After two weeks the patient was readmitted for surgical intervention. With the patient in dorsal decubitus, under general anesthesia and orotracheal intubation, a 10-cm oblique incision just 2 cm superiorly and parallel with the inguinal ligament was performed. After the section of the muscular and aponeurotic layers and dissection of the fascia of the external oblique muscle, the right spermatic cord (or funiculus spermaticus) was exposed. The spermatic cord was not isolated with ease, due to the adherences resulted from the previous inflammation.

The spermatic cord was clamped (fig. 2); the testicle was ascended through the inguinal canal and brought to surface through the surgical wound.

The funiculus spermaticus was sectioned

and sutured with 2 double suture wires. Surgical hemostasis was performed, the anatomical defects were repaired, the surgery ending with the placement of sterile bandage.

Fig. 2. The clamped spermatic cord

Gross examination described an enlarged testicle due to a low-consistency and heterogeneous mass, which was occupying almost the entire testicular parenchyma and was invading the tunica albuginea (fig. 3).

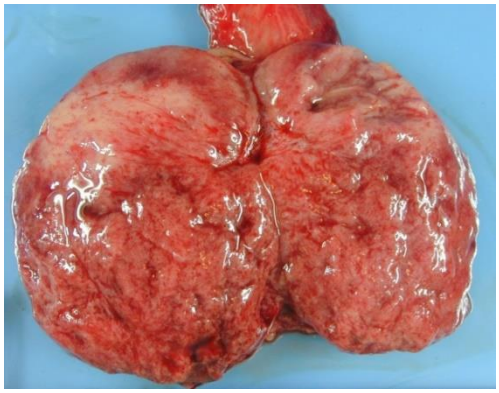

Fig. 3. The macroscopic appearance of the tumor

Microscopic examination highlighted germ cell tumor proliferation, with necrosis and hemorrhagic areas, which was invading the testicle and tunica albuginea. The microscopic appearance had a complex pattern: embryonal carcinoma with areas of mature and immature teratoma, associated with areas of seminoma (fig. 4-5).

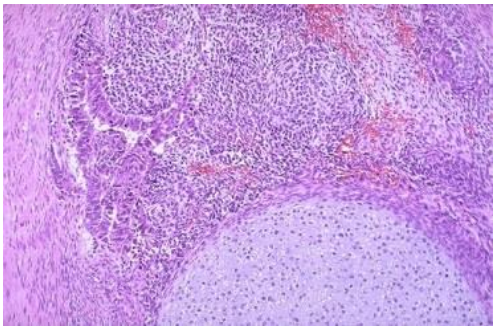

Fig. 4. Microscopy - embryonal carcinoma and teratoma

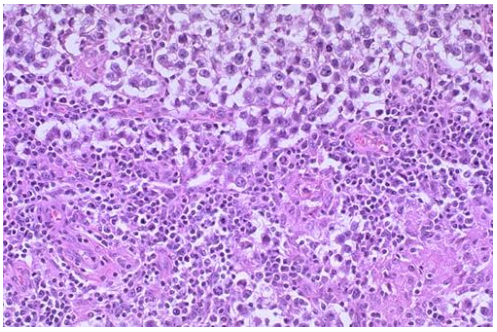

Fig. 5. Microscopy - embryonal carcinoma and seminoma

The diagnosis was positive for pT2NxMx "Mixed germ cell tumor".

The patient's postoperative outcome was favorable and was discharged after three days and instructed to have the tumor marker levels determined after three and six postoperative weeks and an *abdominal and pelvic CT scan* six weeks postoperatively. After three weeks there was a decrease in tumor marker levels: AFP 34.60 IU/mL, hCG 4.6 mIU/mL, LDH 184 U/L.

After six weeks postoperatively, although the AFP reached normal blood levels (3.13 IU/mL) and LDH was still within normal range, the hCG levels were increasing (8.6 mIU/mL). The CT exam at five months postoperatively revealed multiple mediastinum and abdominal lymphadenopathies (epiphrenic, epigastric, interportocaval, later aortic bilateral, peripancreatic), bilateral common iliac lymphadenopathies (left side = 12/27 mm, right side = 18/14 mm) and an adenopathic conglomerate located in the paraaortic lymph nodes, 52/32/60mm in size. At five months postoperatively, the tumor markers continued to rise (AFP=41.48 ng/mL, hCG=22.1 mIU/mL, LDH=217 U/L). Following these results, the patient was admitted to the Oncology Unit, where he received, up until seven months postoperatively, 4 cycles of the recommended chemotherapeutic "BEP Protocol" (Bleomycin 30 Units x 3 cycles, Etoposide 240 mg x 4 cycles, Cisplatin 80 mg x 4 cycles) (fig. 6), during which he developed stage IV neutropenia.

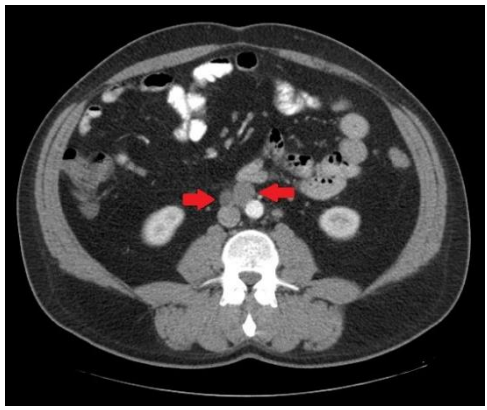

Fig. 6. Interaortocaval adenopathies

A second CT scan at 8 months postoperatively revealed a slight decrease in size of the abdominal and mediastinal adenopathies, suggestive for a partial response to the chemotherapy treatment. The patient is now regularly followed-up at the Oncology and Urology Units.

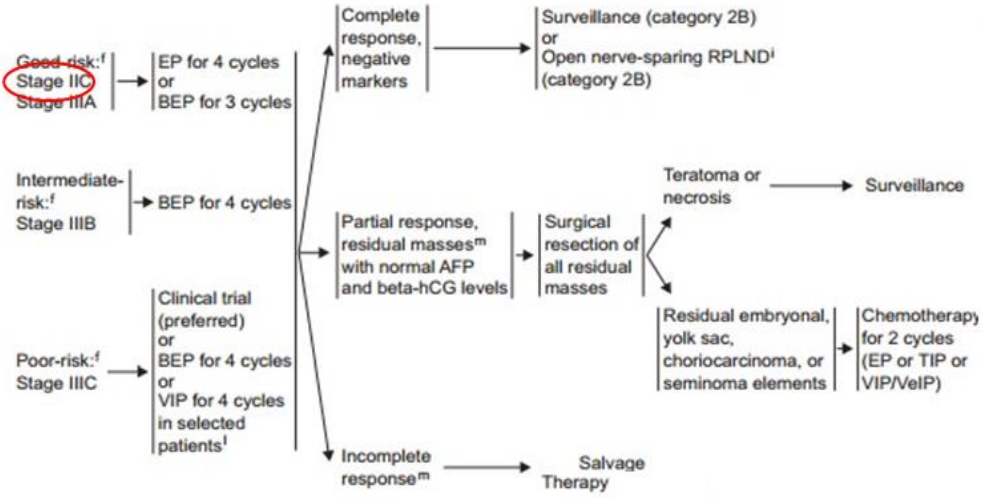

Fig. 6. Stage IIC postoperative recommended treatment

TABLE I

TNM Classification of Seminoma Testicular Cancer

T	Description	N	Description	M	Description
x	Primary tumor cannot be assessed	x	Regional lymph nodes cannot be assessed	0	No distant metastasis
0	No evidence of primary tumor	0	No regional lymph node metastasis	1	Distant metastasis
is	Intratubular germ cell neoplasia	1	Metastases to single or multiple lymph nodes, each < 2 cm in size	1a	Nonregional nodal or pulmonary metastasis
1	Tumor limited to the testis and epididymis or tumor invasion into the tunica albuginea only	2	Metastases to single or multiple lymph nodes, >2 cm but < 5 cm in size	1b	Distant metastasis other than to nonregional lymph nodes and lungs
2	Tumor extending through the tunica albuginea with involvement of the tunica vaginalis	3	Metastases to lymph node, >5 cm in greatest dimension		
3	Tumor invades the spermatic cord				
4	Tumor invades the scrotum				

DISCUSSION

Considering all the previous information, the patient was diagnosed with cT2N3M0 (tab. I), S1 (tab. II), stage IIC (tab. III) (4) testicular cancer. The particularity of the case is how a painful orchiepididymitis revealed a hidden neoplasia by forcing the patient to see a urology specialist, just like in a similar case reported by A. Baletti et al. (6), and how the associated pathology changed the patient management. Although the standard management in neoplasia is a preoperative CT/IRM scan to identify any lymphatic or extra lymphatic lesion suspected of being a metastasis, in our patient's case the preoperative CT scan was abandoned due to the existing orchiepididymitis, which caused inflammatory lymphadenopathies.

Combining surgical and chemotherapy treatment, stage IIC seminomas are considered as having a good prognosis (1) and after the chemotherapy treatment is completed, the serum tumor markers are measured, and a thoraco-abdominopelvic CT scan is performed.

If the residual tumor mass is absent and the tumor markers are within the normal range, the patients require only follow-up, but if the tumor mass persists, like in our patient, a PET scan is recommended (5). A positive PET scan requires a biopsy to be performed, followed by surgical excision or radiotherapy. On the other hand, a negative PET scan requires no further procedure.

TABLE II

Serum levels of tumor markers

	LDH (U/L)	hCG (mUI/mL)	AFP (ng/mL)
SX	-	-	-
S0	Normal	Normal	Normal
S1	<1.5x normal	<5000	<1000
S2	1,5-10x normal	5000-50000	1000-10000
S3	>10x normal	>50000	>10000

TABLE III

Testicular seminoma staging

Stage	T	N	M	S
0	Tis	N0	M0	S0
I	T1-T4	N0	M0	SX
IA	T1	N0	M0	S0
IB	T2-T4	N0	M0	S0
IS	Any T	N0	M0	S1-S3
II	Any T	N1-N3	M0	SX
IIA	Any T	N1	M0	S0-S1
IIB	Any T	N2	M0	S0-S1
IIC	Any T	N3	M0	S0-S1
III	Any T	Any N	M1	SX
IIIA	Any T	Any N	M1a	S0-S1
IIIB	Any T	N1-N3	M0	S2
	Any T	Any N	M1a	S2
IIIC	Any T	N1-N3	M0	S3
	Any T	Any N	M1a	S3
	Any T	Any N	M1b	Any S

CONCLUSIONS

Our paper presented the case of a young patient with an asymptomatic testicular germ cell tumor identified and later diagnosed during his hospitalization in the Urology department for another disease: acute orchiepididymitis on a tumoral testicle. If this inflammatory disease had not occurred, the tumor would have developed for a long period of time without any type of symptom, in which case it might have been identified only after systemic metastases might have occurred. Our paper also presents the investigations, as well as the surgical and medical treatments our patient underwent since the diagnosis.

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CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS. CASE REPORT

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CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS. CASE REPORT (Abstract): We present the case of an obese patient with diabetes, hypertension, and dyslipidemia diagnosed 12 years ago with rheumatoid polyarthritis, non-responding to disease-modifying antirheumatic drugs (DMARDs) and anti-TNF antibodies who developed symptomatic episodes of tachyarrhythmia with important prognostic consequences. Cardiovascular risk assessment and the adoption of cardiovascular disease prevention strategy are integral part of the management of the patient with rheumatoid polyarthritis. **Keywords**: RHEUMATOID POLYARTHRITIS, DIABETES MELLITUS, CARDIO-VASCULAR RISK, BIOLOGICAL THERAPY, ARRHYTHMIA.

The diseases of the connective tissue are characterized by a complex multifactorial etiology and pathogenesis. Mortality and cardiovascular morbidity are higher in individuals with rheumatoid polyarthritis (RP) compared to the general population. Rheumatoid polyarthritis is an independent risk factor for cardiovascular disease. The degree of disease activity in RP is directly correlated with the cardiovascular risk (1). The early and aggressive treatment of RP reduces the rate of cardiovascular events. The association of therapy with statins and angiotensin converting enzyme inhibitors reduces the cardiovascular risk in patients with RP (2).

CASE REPORT

We present the case of a 57-year-old rural patient diagnosed with stage 4 seropositive rheumatoid polyarthritis and Steinbrocker class III functional capacity at the age of 45; the patient was initially treated with DMARDs (methotrexate and leflunomide) and corticosteroids, discontinued due to the diagnosis of pulmonary fibrosis; he then received biological therapy with infliximab, discontinued following the detection of some axillary lymphadenopathies (complex workup confirming they were of inflammatory type in the context of the collagen disease), later switched

to rituximab, with therapeutic response and a decrease in disease activity scores. The patient presented to the hospital for dyspnea on light-moderate exertion and palpitations with onset approximately 24 hours earlier, with irregular, fast heartbeats following prolonged high-intensity physical activities. He associated the traditional risk factors for coronary disease – diabetes mellitus, dyslipidemia, obesity, arterial hypertension.

Physical examination showed: good general health, absence of fever, conscious, third-degree obesity (BMI=41.20 kg/m^2), gynecomastia (fig. 1), lipodermatosclerosis, SpO_2=97% on room air, BP=160/100 mmHg, ventricular rate = 65 beats/minute, rhythmical heart noises, aortic atheromatosis, abdominal panniculus adiposus mobile with breathing, sensitive to palpation in the right-side hypochondrium, without signs of peritoneal irritation, hepatomegaly located 2 cm below the costal margin. Examination of the musculoskeletal system revealed: peripheral algofunctional syndrome (irreducible flexum of the left elbow at 10 degrees), „rheumatoid hand“ appearance with bilateral tumefaction of radiocubitocarpal joint and metacarpophalangeal joints II-III, bilateral swan neck deformity of the middle finger, bilateral Z-deformity of the thumb“, radial deviation of the carpal bones and ulnar deviation of fingers, atrophy of the dorsal interosseous muscles, bilateral tumefaction of the knee with liparthritis, without local warmth, cracking upon movement, discrete bilateral tumefaction of the tibiotarsal joint, bilateral deforming arthrosis of the metatarsophalangeal joint of the big toe, „rheumatoid foot“ appearance.

Electrocardiogram findings: pauci symptomatic episodes of bradycardia-tachycardia – polymorphic extrasystolic ventricular arrhythmia expressed through doublets, ventricular tachycardia phases/beats (fig. 2). Bio humoral tests revealed hyperglycemia, hypercholesterolemia, hepatic cytolysis syndrome, absence of the inflammatory syndrome and myocardial cytolysis. Immunological assessment revealed the presence of the rheumatoid factor and elevated level of anti-cyclic citrullinated peptide antibody (250 IU). Urine test revealed proteinuria and chest X-ray the absence of progressive pleuropulmonary lesions, rectilinear left mid aortic arch, aortic knob calcification, right-hand side basal accentuated pulmonary sketch (fig. 3). The abdominal and pelvic ultrasound imaging confirmed the hepatic steatosis and pancreatic lipomatosis and did not describe other changes. The echocardiogram revealed ischemic heart disease with delayed relaxation type diastolic dysfunction and incipient degenerative aortic valve changes, ejection fraction = 52%, progressing on the background of a chronic immune-inflammatory status, to which a complicated diabetes mellitus is added.

Fig. 1. Third degree obesity

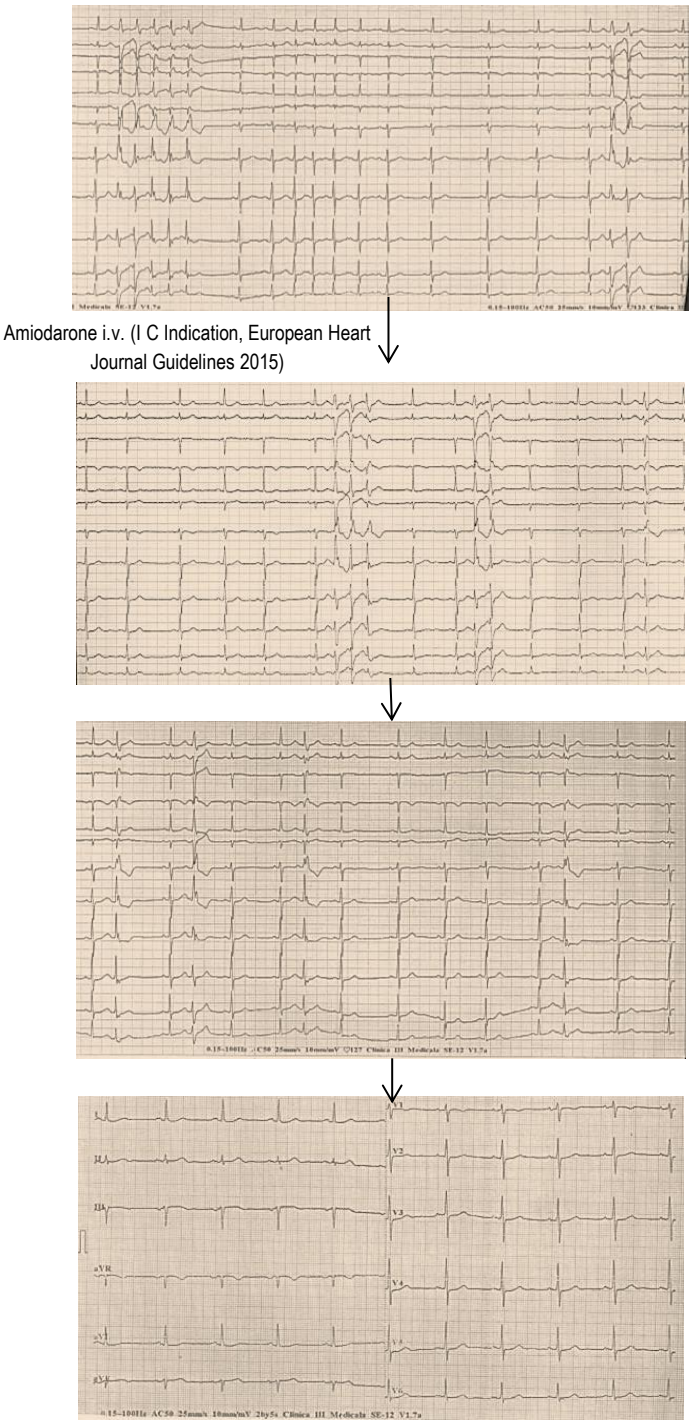

Fig. 2. Electrocardiogram evolution

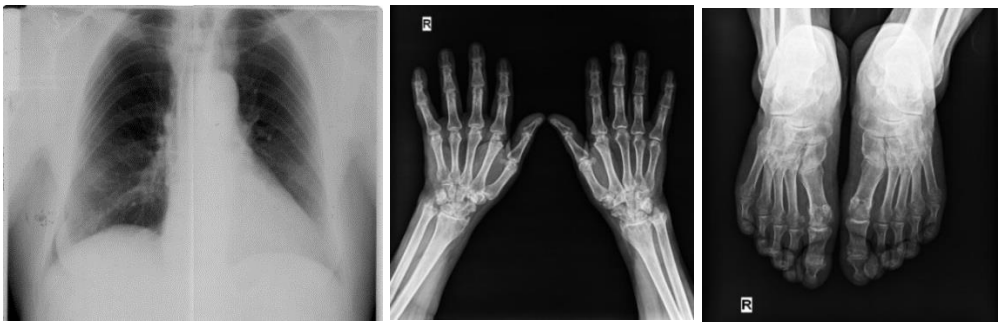

Fig. 3. X-ray aspects

According to the SCORE (Systematic COronary Risk Evaluation) charts which are used to assess the *total death risk* due to cardiovascular disease (coronary and non-coronary) in the last 10 years, the patient was at intermediate risk (3%) (3,4). Following the analysis of Framingham score parameters, which estimate the *coronary cardiovascular accident risk* in the following 10 years, the patient also showed an intermediate risk (14.5%) (3, 4).

The disease activity scores in collagen disease [DAS28 (Disease Activity Score28) = 3.68; SDAI (Simplified Disease Activity Index) = 14.9; CDAI (Clinical Disease Activity Index) = 14] indicated a moderately active disease with an average functional impact (HAQ (Health Assessment Questionnaire) = 1.75) and EULAR (European League Against Rheumatism) response = 0.51 (5).

The electrocardiographic evolution was slowly favorable under specific treatment, arrhythmia being interpreted within the context of silent myocardial ischemia of rheumatoid polyarthritis and diabetic neuropathy.

The prognosis is poor, not only due to the immediate complications of the associated pathology, but also to the distant complications which progress to locomotor disability, development of opportunistic infections or neoplasia because of the immunocompromised state.

DISCUSSION

We reported the case of an obese patient with diabetes, hypertension and dyslipidemia who associated an autoimmune disease – rheumatoid polyarthritis – (*with higher incidence in women!*), showed no response to DMARDs and anti-TNF (Tumor Necrosis Factor) antibodies, with cardiovascular risk score multiplied (x1.5) by the presence of the systemic disease, and who developed symptomatic episodes of tachycardia with important prognostic consequences.

The cardiovascular and rheumatic diseases can coexist (6, 7). The risk of cardiovascular events in rheumatoid polyarthritis is independent, yet amplified by the presence of traditional risk factors (8, 9). Endothelial dysfunction represents the initial link in the development of vascular complications and can be induced by the oxidative stress generated by hyperglycemia and/or insulin resistance (10, 11). The correct and early risk stratification is essential for guiding the treatment. The therapeutic scheme is guided by patient's comorbidities (12).

In these patients, a careful post-discharge monitoring is necessary. Long-term monitoring must include lifestyle change, correction of risk factors and secondary prevention (13). The early recognition and aggressive therapeutic approach of rheumatic disease will reduce the risk of

cardiovascular disease and improve survival and quality of life (14, 15).

CONCLUSIONS

This case describes a patient with rheumatoid inflammatory disease and high cardiovascular risk. Rheumatoid arthritis and cardiovascular diseases share common pathophysiologic mechanisms, which have as consequence the acceleration of process of the atero-forming process. Cardiovascular risk assessment and adoption of cardiovascular disease prevention strategy are integral part of the management of patients with rheumatoid arthritis. Patients with chronic immunoinflammatory RA require regular cardiac screening (clinical examination, electrocardiogram and, in certain conditions, echocardiography). The cardiovascular affection in connective tissue disease requires a dual treatment: of the underlying disease and of the cardiac affection itself according to the current specific guidelines.

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MULTIPLE MYELOMA COMPLICATED WITH TAKOTSUBO CARDIOMYOPATHY: CASE REPORT

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MULTIPLE MYELOMA COMPLICATED WITH TAKOTSUBO CARDIOMIOPATHY: CASE REPORT (Abstract): Multiple myeloma (MM) is characterized by clonal proliferation caused by the expansion of monoclonal plasma cells, in which, due to immunoglobulin deposition (M protein/amyloid), renal dysfunction or another organ involvement like cardiac amyloidosis can be periodically encountered. We present the clinical case of a 61-year-old man admitted to our clinic with acute low back pain in the lumbar region. One month earlier, the patient was diagnosed with Takotsubo cardiomyopathy. The lumbosacral spine x-ray showed lumbar vertebral compression fractures and skull x-ray revealed various well-circumscribed lytic bone lesions. The typical M-peak (M band of IgG-kappa monoclonal gammopathy was found) was indicated by serum immunofixation. **Keywords:** TAKOTSUBO SYNDROME, MYOCARDIAL INFARCTION, MULTIPLE MYELOMA, CARDIAC AMYLOIDOSIS, CHEMOTHERAPY.

Multiple myeloma (MM), also known as plasma cell myeloma, is a cancer of plasma cells which was historically defined by end-organ damage, especially hypercalcemia, renal failure, anemia, and bone lesions (CRAB features) that can be associated with the neoplastic process (1). The following three new criteria are used in the diagnosis of MM: greater than 60% clonal plasma cells on bone marrow biopsy, serum free light chain (sFLC) ratio of > 1 (the involved sFLC must be > 100 mg/l), and more than one unequivocal focal lesion on cutting-edge imaging (low dose whole body computed tomography, magnetic resonance imaging, or 18-F-fluorodeoxyglucose-positron emission tomography) (2). Takotsubo cardiomyopathy, also known as "broken heart syndrome", is a transient cardiac syndrome that involves left apical akinesis without obstructive atherosclerotic coronary artery disease. It is believed that this syndrome is triggered by an episode of emotional stress; it frequently occurs in female patients, 12% of patients with an uncertain diagnosis of acute coronary syndromes undergoing cardiac catheterization. Currently, few data in the literature advocate that multiple myeloma can be complicated with Takotsubo cardiomyopathy before the

initiation of chemotherapy (3-11).

CASE REPORT

A 61-year-old man was admitted to University Hospital in Amman, Jordan, with low back pain which lasted for about four weeks. One month earlier, the patient had been admitted to another hospital where he was diagnosed with Broken Heart Disease (Takotsubo cardiomyopathy) and hyperchromic macrocytic anemia. He had been smoking for 40 years-one packet a day-but he did not consume alcohol. He had positive family history of cardiovascular diseases (CVD) but no personal history of other CVD or cardiometabolic syndrome.

At admission, the physical examination showed normal weight (BMI of 24 kg/m²), resting blood pressure (BP) of 120/70 mmHg and heart rate of 85 beats per minute; his respiratory rate was 18 breaths/min, and he was afebrile.

Blood test results showed hyperchromic macrocytic anemia (RBCs = $2.73*10^6/mm^3$, Hb = 11.2 gm/dL, MCV = 122.3 fl, MCH = 40.8 pg). Erythrocyte sedimentation rate, a non-specific indicator of inflammation (normal range for men < 50 years: 0-15 mm/hour) was highly increased (136 mm/h). As to the lipid profile, an important cardiovascular risk factor, the levels of all components: Triglycerides, Total Cholesterol, LDL-Cholesterol, and HDL-Cholesterol were within normal range. However, total protein level was too high = 9.5 g/dL, globulin = 6.4 g/dL and albumin level was too low = 3.1 g/dL. The fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) presented a satisfying short-term and long-term control of blood sugar levels. The renal function, which is evaluated by creatinine and urea levels, was normal, and so were the liver function and the hydro-electrolytic balance. At admission the biological markers of myocardial necrosis-CPK, CK-MB and Troponin T were within normal range. Serum immunofixation showed the typical M-peak (M band of IgG-Kappa monoclonal gammopathy was detected), FLC Kappa = 1500 mg/dL, FLC Lambda = 12.2 mg/dL, and Kappa/Lambda ratio = 122.9. The calcium level was normal. The liver enzyme levels and kidney function were normal, too.

The patient underwent resting electrocardiography (ECG) which showed ST elevation in anterior precordial leads (V1-V5). Fully developed Pardee's waves were seen in precordial leads V2-V3-V4-V5. Sinus tachycardia was about 90 beats per minute; however, the heart rhythm was regular. The normal electrical heart axis (QRS axis) was preserved. Consequently, the patient displayed only electrical signs of lesion and necrosis, specific for supra acute myocardial infarction; however, there were no disturbances of electrical function (fig. 1).

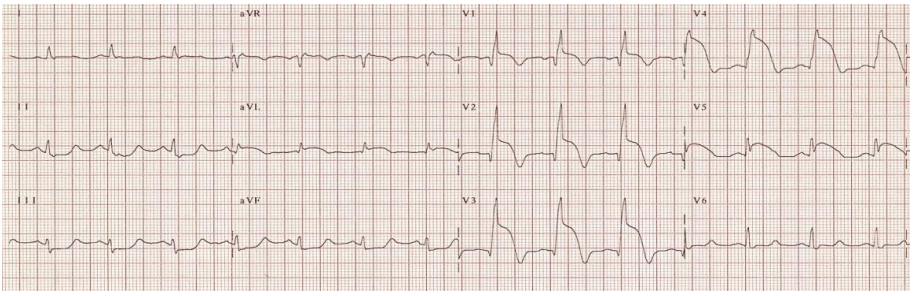

Fig. 1. Electrocardiogram at rest revealed STEMI

The patient underwent chest radiography which showed cardiomegaly with a cardiothoracic ratio of 69.3% and bilateral pulmonary congestion (fig. 2).

The 2D transthoracic Doppler echocardiography demonstrated apical akinesis of the anterior wall. Coronarography showed normal left main artery (LM), left circumflex coronary artery (LCX) and anteroapical akinesis of the left ventricle wall (fig. 3).

Disseminated multiple myeloma has two common radiological appearances, although it should be noted that initially radiographs may be normal despite the presence of symptoms. The two main diffuse patterns are: numerous, well-circumscribed lytic bone lesions (more common), and punched out lucencies, raindrop skull, endosteal scalloping (fig. 4).

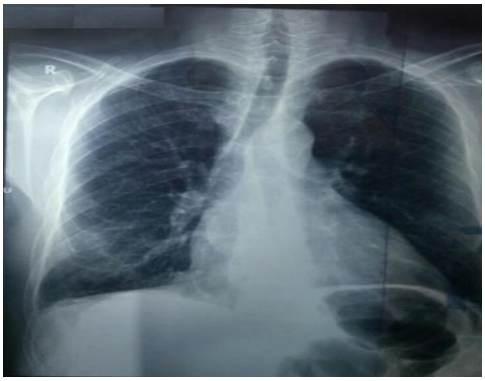

Fig. 2. Chest X-Ray- cardiomegaly with a cardiothoracic ratio of 69.3% and bilateral pulmonary congestion

Fig. 3. Coronarography with normal LM, LCX, LAD and anteroapical akinesis of LV

Fig. 4. Skull x-ray with numerous, well-circumscribed, lytic bone lesions and punched out lucencies

DISCUSSION

Multiple myeloma (MM), also known as myelomatosis, is a rare type of hematological malignancy which accounts for approximately 10% of all hematological malignancies. Osteolytic bone destruction, anemia, recurrent infections and renal impairment are caused by the neoplastic proliferation which is defined as an uncontrolled reproduction of plasma cells in the bone marrow (12).

The diagnostic criteria for MM were updated in 2014 by the International Myeloma Working Group (IMWG) to add specific biomarkers which can be used to make the diagnosis of the disease in patients who did not present CRAB features. Moreover, MM bone disease can be diagnosed by using cutting-edge imaging methods such as computed tomography (CT) and positron emission tomography-CT (PET-CT) Bone pain and bone fractures, anemia, increased susceptibility to infections, hypercalcemia, neurological manifestations, hyper viscosity syndrome, renal failure and progressive osteolytic vertebral fractures are among the most frequently encountered characteristics of MM (13-14).

Approximately 15-20% of the patients suffering from MM secrete monoclonal light chains only, without the expression of the normal immunoglobulin heavy chain, and this constitutes light-chain multiple myeloma. “CRAB” is the abbreviation for high calcium, abnormal kidney function, anemia, and bone lesions. All initial treatments are started when myeloma is symptomatic. MGUS and smoldering myeloma should be carefully followed-up. Bence Jones proteinuria is a disorder characterized by the excretion of monoclonal light-chain protein. MM, which is usually confined to the bone marrow, sometimes infiltrates into other tissues; this phenomenon is known as extramedullary plasmacytoma (EMP). MM is defined as the monoclonal proliferation of bone marrow plasma cells producing multiple bone lesions and overproduction of a monoclonal protein (M-protein) that could amass in tissues (amyloidosis) (15).

In our case the serum immunofixation showed the typical M-peak (M band of IgG-Kappa monoclonal gammopathy was detected) that is specific for the diagnosis of MM.

Takotsubo cardiomyopathy (TCM) is a transient cardiac syndrome that involves the left ventricular (LV) apex akinesis and mimics acute coronary syndrome; this condition is triggered by emotional or physical stress and usually resolves completely (16).

Even though TCM is usually reversible, it can be associated with significant morbidity, such as cardiac arrhythmia. The repolarization abnormalities characteristic to TCM can be associated with characteristic T-wave anomalies and QT prolongation that place patients at increased risk for ventricular arrhythmia, as well as for torsade de pointes. Life threatening arrhythmias such as VT and SCA are prevailing among men, while TTC has been agreed upon to be a disease mainly of females.

TTC may lead to severe heart failure and hemodynamic instability, even if most patients with Takotsubo cardiomyopathy (TTC) have benign clinical course and prognosis. TTC mimics the clinical characteristics of acute anterior wall myocardial infarction (AMI). Generally, clinicians often have difficulties in making the diagnosis whenever cardiac catheterization and angiography are contraindicated or causes adverse effects. In addition, differential diagnosis is also crucial since electrocardiogram findings in patients with Takotsubo

cardiomyopathy and acute coronary syndrome are similar, consisting among others in ST-segment elevation, T-wave inversion, QT-prolongation. Normally, apical involvement with hypercontraction of basal left ventricle (apical type) is prevalent; however, abnormal types affecting basal, mid-ventricular, and right ventricular myocardium have been depicted as well. Patients suffering from acute coronary syndrome die while hospitalized and this is usually caused by hidden conditions (17).

CONCLUSIONS

This case underlines the importance of suspecting a TTC in all patients with myocardial infarction and normal coronarography, mainly if they do not have any cardiovascular disease or symptom at the onset of a major cardiovascular event.

This disease should be suspected more frequently in all patients even though it is rarer in male patients especially if they develop myocardial infarction in the absence of any cardiovascular disease.

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POLYCYSTIC LUNG DISEASE: CASE REPORT

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POLYCYSTIC LUNG DISEASE: CASE REPORT **(**Abstract): Polycystic lung disease is a particular condition characterized by the presence of multiple air spaces, distributed in both lungs, expression of a syndrome covering rare and multisystem diseases. With the exception of centrilobular emphysema, diseases like lymphangio-leiomyomatosis, Langerhans cell histiocytosis, or lymphoid interstitial pneumonia are rare condition. Clinical, tomographic and histopathologic criteria could be used for the differential diagnosis. We report the case of polycystic lung disease in a 64-year-old male non-smoking firefighter who presented an associated chronic hepatitis C virus infection. Using high-resolution computed tomography, we describe the distribution, size and shape of pulmonary cysts, compatible with centrilobular emphysema. The associated lesions consisted in cystic bronchiectasis and non-significant interstitial linear fibrosis. Based on a literature review, we outline clinical and computed tomography appearances of other causes of polycystic lung disease. **Keywords**: POLYCYSTIC LUNG DISEASE, CENTRILOBULAR EMPHYSEMA, LUNG CYST, COMPUTED TOMOGRAPHY.

Polycystic lung disease (PLD) has an estimated incidence ranging from 3,4 to 7,8 per 1,000,000 population (1, 2). The condition is characterized by the presence of multiple cystic air spaces, usually distributed in both lungs. The cystic lesions should first be differentiated from pulmonary cavities depending on the aspect of the cyst wall, which usually is regular and thinner than 2-3 mm (1-3). The mechanisms leading to cyst formation could be different, depending of the main pathological process at the origin of the syndrome. Except for some types of centrilobular emphysema (CLE), PLD includes rare diseases such as lymphangio-leiomyomatosis, Langerhans cell histiocytosis, lymphoid interstitial pneumonia, pulmonary metastases, Birt Hogg Dubé syndrome and other diseases (1-4). Occasionally, the cysts could contain a small amount of fluid.

High-resolution computed tomography was used to distinguish the characteristics of the pulmonary lesions present in PLD. Per this method, CLE, one of the most frequent types of pulmonary emphysema, was describe as a form of PLD and an important component of the chronic obstructive pulmonary disease (COPD). Less frequent types are represented by pan lobular

and paraseptal emphysema (5). Generally, pulmonary emphysema was associated with an abnormal inflammatory response induced in lung tissue, related to chronic exposure to cigarette smoke and other noxious particles and gases (5).

This paper describes and discusses a case of PLD diagnosed in a 64-year-old nonsmoking firefighter. Clinical and functional data were integrated with high-resolution computed tomography, which describes the distribution, size and shape of pulmonary cysts, compatible with CLE. The associated lesions consisted in cystic bronchiectasis and non-significant interstitial linear fibrosis.

CASE REPORT

A 64-year-old man presented to the hospital because a 2-year history of intermittent episodes of cough with sputum production associated with NYHA class II exertional dyspnea. The family history revealed that the patient had one sister with essential hypertension and type 2 diabetes mellitus and one brother with gastric ulcer. Patient significant medical history consisted of one episode of pneumonia at the age of 30 and other subsequent lung lesions (PLD at the age of 47 years and non-cystic fibrosis bronchiectasis at the age of 60). Bronchiectasis was confirmed by bronchoscopy, and sputum analysis at that time revealed overgrowth of Enterobacter Cloacae and Pseudomonas Aeruginosa. A diagnosis of chronic C hepatitis was documented when the patient was 57-year-old and the immediately initiated antiviral treatment was suspended after 7 months due to the absence of a sustained virological response. The patient received conventional hepatoprotective treatment. He was a nonsmoker but used to be a firefighter and he related chronic respiratory exposure to a polluted environment.

On physical examination, he was found to be overweight (body mass index 28.3 kg/m^2, abdominal circumference 97 cm), afebrile, with acral cyanosis and hand clubbing. The patient had an increased anterior posterior diameter of the chest, prolonged expiratory time, inspiratory crackles and ronchi at the lung bases. Heart auscultation revealed an accentuated pulmonic component of the second heart sound, suggesting pulmonary hypertension. Otherwise, the cardiovascular examination was less significant, without signs of right heart failure. On admission, heart rate was 72 bpm, regular rhythm, and blood pressure was 130/80 mmHg.

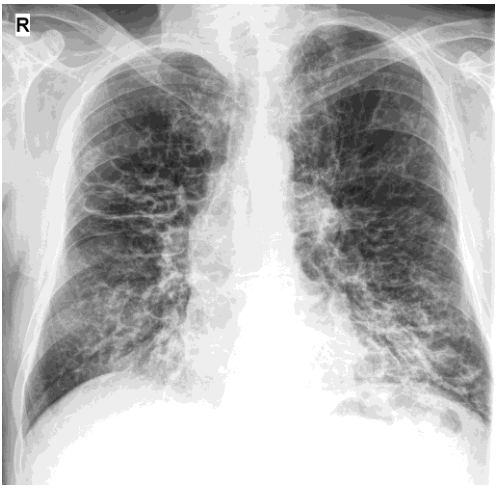

Fig. 1. Poster anterior chest X-ray showing multiple cysts associated with fibrosis and bronchiectasis in both lungs.

Laboratory findings were within normal ranges, excepting for a decrease in HDL-cholesterol (37 mg/dl). Chest X-ray showed bilateral basal emphysema associated with appearance of fibrosis (linear densities) and bronchiectasis. Both upper pulmonary lobes presented multiple rounded and confluent

transparencies, giving the appearance of PLD (fig. 1). Further assessment consisted in a thoracic computed tomography which showed a type of change compatible with PLD (fig. 2, 3); multiple rounded transparencies were present in both lungs, most commonly found it in right upper and left inferior lobe, all with very thin wall and of various sizes up to 38/30 mm, some with minimal fluid content. Other dilations, far smaller, present in the same lung field, were the expression of the associated cystic bronchiectasis. Some inflammatory, small size lymph node enlargements were present in both hilar regions of the lungs. Pulmonary function tests demonstrated a combined defect, with the predominance of airflow obstruction (flow expiratory volume – FEV_1 was 1.23 L, 38,8% of predicted, forced vital capacity – FVC 1.91 L, 46,9% of predicted and FEV_1/FVC% ratio was 85,3%). Pulse oximetry value was normal. Electrocardiogram showed sinus rhythm at 72 beats/min and reduced amplitude of the QRS waves in the extremity leads. Using echocardiography, we noticed the enlargement of the right chambers cavities, compared with the left ones and the right ventricle systolic pressure was estimated at 40 mmHg. The patient received long-term tiotropium inhaler 18 μg/day in combination with mucolytic agents and antibiotics during acute episodes.

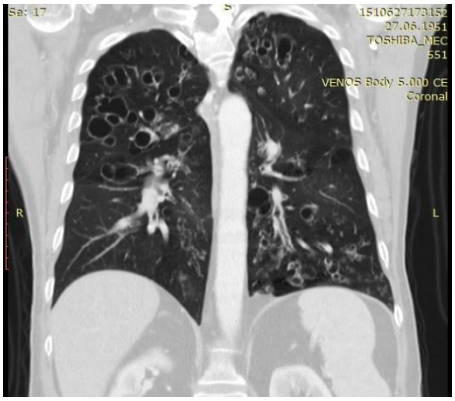

Fig. 2. CT of the lungs (coronal view) – multiple thin wall cysts, more numerous in right upper and left inferior lobes.

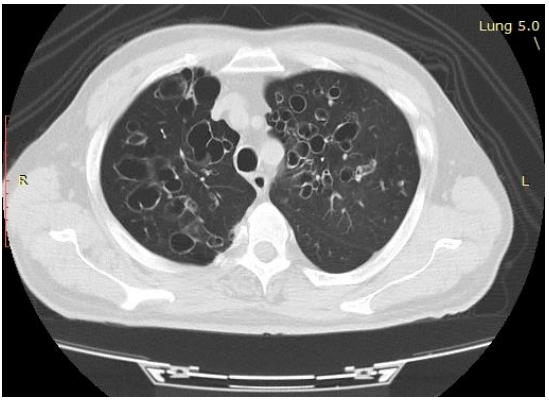

Fig. 3. CT of the lungs (axial view) – multiple thin wall cysts, larger in the right lung.

DISCUSSION

PLD can be encountered in the adult population clinically asymptomatic or accompanied by dyspnea, often suspected after chest radiological examination and confirmed by high-resolution computed tomography (3,4). The patient develops a clinical and radiological syndrome, which is due to a multisystem, but rare disease. The exception is CLE, more frequently encountered in smokers and patients exposed to other noxious particles (3, 4). In our case, chest X-ray showed multiple rounded and confluent transparencies and computed tomography confirmed the presence of disseminated pulmonary cysts. Based on computed-tomography studies, a lung cyst is defined as a round parenchymal area of low attenuation, with a well-defined wall thinner than 2-3 mm, contain-

ing air, but rarely a solid material or a small amount of liquid. In contrast, a cavitary lesion will develop mainly in an area of pulmonary consolidation, mass or nodule and the cavity wall will be thicker than 4 mm (3,6,7). For the final diagnosis, the clinical characteristics should be correlated with the shape and distribution of the cysts on the computed tomography and, in many cases, with histopathology features. The main diseases that may cause pulmonary cysts are presented in table I (3, 7).

TABLE I

The principal causes of PLD (3, 7)

Pathologic condition	Clinical findings / Appearance on computed tomography
Centrilobular emphysema	Clinical history of smoke and dust exposure/ Upper lobe predominance, non-perceptible or thin cyst wall, presence of a centrilobular artery
Langerhans cell histiocytosis	Young patient, cigarette smoker/ Combination of nodules, cavitating nodules and cysts
Lymphangio-leiomyomatosis	Young women with dyspnea, recurrent pneumothoraxes, hemoptysis/ Thin wall rounded cysts, may involve the juxtaphrenic recesses
Lymphoid interstitial pneumonia	Women in 5th decade, associated with collagen vascular disorder or HIV infection/Cysts mixed with ground-glass opacities and nodules
Hypersensitivity pneumonitis	Diffuse granulomatous inflammatory disease caused by exposure to organic and anorganic particles/ Few cysts in 10% of cases
Desquamative interstitial pneumonia	Smoking history/ Ground-glass aspect and small cysts
Metastases (sarcoma, squamous cell cancer, carcinoma of the bladder, melanoma)	Tumoral syndrome/ Different sizes and lower predominance
Neurofibromatosis type I	Café-au-lait spots, neurofibromas, optic nerve glioma, Lisch nodules, skeletal lesions/ Cysts in both upper lobes
Pneumatoceles	Caused by pneumonia (*Pneumococcus* species, *Escherichia coli*, *Klebsiella* species, *Staphylococcus* species, *Pneumocystis jiroveci,* trauma and inhalation of hydrocarbon fluid/Thin wall cysts

Other causes far less commonly mentioned in literature are cystic fibro histiocytic tumor of the lung, tracheal papillomatosis, cystic mesenchymomas, light-chain disease or Birt Hogg Dubé syndrome (pneumothoraxes, renal cell carcinomas, skin fibro-folliculomas) (3).

Our patient had a history of pneumonia at the age of 30 and chronic exposure to noxious gases, he never smoked and there are no arguments supporting the clinical features of a multisystem disease. The

pneumonic episode could have contributed to the progression to polycystic CLE. Computed tomography revealed the characteristics of the cysts and the association with bronchiectasis and mild fibrosis.

Generally, particular cases will require correlation with histopathology findings or a highly specific biomarker, such as an increased serum VEGF- D level in lymphangio-eiomyomatosis (2).

Another particularity of this reported clinical case is represented by the chronic infection with hepatitis C virus, but probably unrelated to the pulmonary findings. The pulmonary disease was relatively stable on chronic treatment and during the 7 months of antiviral treatment the clinical and radiological course was unchanged. Cases of chronic hepatitis C virus infection associated with or complicated by pulmonary diseases (sarcoidosis, pulmonary fibrosis, asthma) (8-10), occasionally due to antiviral therapy, have been reported in the literature (11). However, a study published in 2014 demonstrated that hepatitis C virus infection is not an independent risk factor for COPD (12).

CONCLUSIONS

Our paper presented a particular case of PLD in a male nonsmoking firefighter who associated a chronic infection with hepatitis C virus. The clinical course, stable over years, and the computed tomography findings are highly suggestive of a CLE, one of the main causes of PLD.

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THE IMPACT OF SOCIOECONOMIC STATUS AND NUTRITIONAL BIOCHEMICAL MARKERS ON QUALITY OF LIFE OF CHRONIC DIALYSIS PATIENTS

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THE IMPACT OF SOCIOECONOMIC STATUS AND NUTRITIONAL BIOCHEMICAL MARKERS ON QUALITY OF LIFE OF CHRONIC DIALYSIS PATIENTS (Abstract) **Aim**. This cross-sectional study aimed to determine the relationship between socio-economic status and laboratory values with mental and physical components of QoL (quality of life) in chronic dialysis patients. **Material and methods:** 254 patients were included in the study and divided into two groups: 243 hemodialysis (HD) patients and 11 peritoneal dialysis (PD) patients. QoL was measured by the Short Form 36(SF-36). Scores for all dimensions are expressed on a 0-100 scale. Higher score indicates better health status. Two more questions related to monthly food expenditure and the presence/or lack of family support were added. Laboratory investigations included the following nutritional parameters: serum albumin and creatinine. **Results:** Our study results showed that compared to PD group in HD patients QoL was compromised in all SF-36 subscale except physical functioning, role limitations due to physical functioning and role limitations due to emotional functioning. Also, the presence of supportive family members/ caregivers showed a significant improvement of all QoL scores. HD patients who spend at least 90 euros monthly on groceries/food had higher values of SF-36 items. Better QoL is associated with high pre-dialysis serum creatinine and serum albumin levels. **Conclusions:** Future work should incorporate larger and more balanced sample sizes and patient recruitment from multiple dialysis centers to truly capture the variability in patient characteristics. **Keywords:** HEMODIALYSIS, QUALITY OF LIFE, SF-36, SOCIO-ECONOMIC STATUS.

Chronic illnesses and their treatments, including *End-Stage Renal Disease* (ESRD) and dialysis, have great impact on the functioning and well-being of patients. Dialysis is both life-saving and life-altering, changes a person's lifestyle completely and has great impact on physical, mental, and social well-being (1). The Kidney Disease Quality of Life (KDQoL) instrument was developed for assessing the

quality of life of individuals with kidney disease and on RRT (renal replacement therapy). Short-Form 36 Health Survey (SF-36) consists of eight dimensions, generating a profile of health-related quality of life (HRQoL) (2). In patients with ESRD QoL is influenced by the type of RRT. It is unclear whether hemodialysis (HD) or peritoneal dialysis (PD) is associated with a higher HRQoL (3).

In Romania, data on QoL among chronic dialysis patients is limited. Most studies in dialysis population showed that higher income is positively correlated with role emotional and mental health (4). Low socio-economic status is associated with poor health outcomes (5). In addition to the inherent discomfort caused by the disease and social problems the patient experiences difficulties in fulfilling social, professional, family obligations, up to temporary or permanent work incapacity (6).

None of the currently favored biochemical nutritional markers has been demonstrated to accurately reflect nutritional status in CKD (7). Serum albumin is still being used for research purposes and, in the clinical setting, as a biomarker of nutritional status. Malnutrition is highly prevalent in ESRD patients and is a major contributor to poor quality of life (8).

The objectives of our study were: 1. to evaluate QoL among patients undergoing chronic dialysis therapy; 2. to determine if there is any correlation between socio-economic status and nutritional laboratory data with QoL.

MATERIAL AND METHODS

This cross-sectional study, performed at the B. Braun Hemodialysis Unit, Botosani Romania, included 254 patients meeting the following criteria: 1) on regular dialysis therapy for more than three months; 2) no apparent mental diseases to avoid potential misinterpretation of the questions; 3) 18 years and above, 4) stable condition, meaning no hospital admission at least one month prior data collection. Written informed consent was obtained from all patients before enrollment in the study. 243 HD patients and 11 PD patients completed the SF-36 questionnaire.

Quality of life questionnaire was measured using a RAND Short Form 36-Items Health Survey (version 1.0) which taps eight health concept: 1) Physical Functioning (PF); 2) Role Limitations due to Physical Functioning (RPF); 3) Bodily Pain (BP); 4) General Health Perceptions (GH); 5) Vitality (VT); 6) Social Functioning (SF); 7) Role Limitations due to Emotional Functioning (REF); and 8) Mental Health (MH) (9). Raw scores on each dimension are transformed into a score ranging from 0-100. Higher scores indicate better health. To evaluate socio-economic status, we added to this questionnaire two more questions: 1. Does the patient have any caregivers at home to cook for him/her regularly-following the nutritional recommendations received at the Dialysis Center? 2. Does the patient spend at least 90 Euros per month on food/groceries?

Medical history and laboratory data (serum albumin and creatinine) were obtained from patients medical and dialysis record.

The statistical study was conducted in SPSS 16.0 for Windows. We determined the frequency distributions and we determined the elements of descriptive statistics for the numerical parameters: mean value, mean standard error (MSE), standard deviation (SD), minimum and maximum values. We used the *t* test and the Mann-Whitney nonparametric test (U) for inde-

pendent samples of the comparative study between the groups for numerical variables, having previously checked the hypothesis that variables abide the normal law using the Kolmogorov-Smirnov fitting test. The level of significance $p<0.05$ was considered statistically significant.

RESULTS

Assessing quality of life among the chronic dialysis patients by SF-36 questionnaire revealed significant differences between the two patient groups, except for PF, RPH şi REP; overall global QoL score was significant lower in patients undergoing HD (p=0.013). In PD group SF had the highest score (77.27 ± 12.27) and RPF the lowest score (50.00 ± 27.38). In HD patients, the lowest score was on VT (30.41± 21.71) and GH (35.14 ± 22.79). It is noteworthy that PD patients scored significantly better on VT, MH and SF than HD patients (tab. I). Regarding bodily pain, HD patients scored significantly lower (more pain) than PD patients (p=0.022) (tab. I).

TABLE I

Global and group coefficients of all eight dimensions and relevant laboratory values. For each scale, SF-36 the score ranges from 0-100, with a higher score indicating better QoL

	All patients n=254 mean ±SD	HD group n=243 mean ±SD	DP group n=11 mean ±SD	P
PF	53.07±37.11	52.43±37.73	67.27±12.72	0.520
RPF	43.33±45.83	43.03±46.51	50.00±27.38	0.209
REF	57.91±43.34	57.51±44.06	66.68±21.09	0.924
VT	31.78±22.26	30.41±21.71	62.27±7.53	0.000
MH	44.11±15.41	42.74±13.92	74.18±16.91	0.000
SF	47.74±27.03	46.41±26.76	77.27±12.27	0.000
BP	56.15±26.74	55.37±26.70	73.40±22.08	0.022
GH	36.23±23.12	35.14±22.79	60.45±16.50	0.001
QoL global score	46.15±25.45	45.24±25.62	66.44±5.47	0.013
Serum				
Albumin	3.88±0.33	3.90±0.31	3.30±0.36	0.000
Creatinine	9.83±2.73	9.83±2.67	9.68±3.963	0.858

SF-36 subscale abbreviations: PF-Physical functioning; RPF- Role limitation due to physical functioning; REF- Role limitation due to emotional functioning; VT-Vitality; MH-Mental health, SF-Social functioning; BP-Bodily pain; GH, General health perception; HD-hemodialysis; PD- peritoneal dialysis; p<0.05 –significant statistic.

169 (69.54 %) from HD patients declared that at least one family member help them in kitchen and cook for them following dietitian recommendations. In HD patients, the presence of supportive family members/ caregivers was associated with a significant improvement on all scores rating the QoL except MH score. In PD group, there were no relatives to help the respondents (tab. II).

TABLE II

Comparative data in both groups regarding socio-economic factors and laboratory values

MEAN ±SD		PF	PF	RPH	RPH	REP	REP	VT	VT	MH	MH	SF	SF	BP	BP	GH	GH	Global QoL	Globa QoL
		HD	PD	HD	PD	HD	PD	HD	PD	HD	PD	HD	PD	HD	PD	HD	PD	HD	PD
Caregivers/ Family	Yes	56.6 ±36.5	-	48.8 ±47.0	-	49.1 ± 25.5	-	57.8 ±27.1	-	37.6 ±22.3	-	48.5 ± 24.6	-	62.9 ±42.4	-	32.8 ±21.0	-	42.9 ±14.9	-
	No	38.3 ±36.9	-	24.6 ±39.6	-	37.5 ± 28.2	-	47 ± 24.4	-	26 ± 21.0	-	34.5 ± 24.7	-	41.5 ±44.8	-	23 ±21.7	-	41.8 ± 1.7	-
	P value	p=0.001	-	p=0.000	-	p=0.004	-	p=0.005	-	p=0.000	-	p=0.000	-	p=0.002	-	p=0.003	-	p=0.338	-
Monthly food budget	>90 euros	69.4 ±33.0	60.00	62.5 ±4.7	100.0	57.2 ± 20.9	50.0	64.5 ±24.9	77.5	45 ±22.6	20.0	57.4 ±22.1	63.3	71.6 ±36.0	100.0	38.9 ±17.7	55.0	45.4 ±15.0	44.0
	<90 euros	34.5 ±33.4	68.3 ±3.9	22.7 ±39.0	44.4 ±24.2	35.2 ± 27.3	80.5 ±9.0	45. ±25.2	72.5 ±24.5	24.5 ±7.3	63.3 ±10.0	32.4 ±22.2	66.2 ±5.8	43.0 ±46.6	62.9 ±20.0	21.6 ±21.6	62.2 ±7.5	40 ±12.6	76.0 ±14.6
	P value	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-
Serum albumin (g/dl)	<4	31.2 ±32.2	68.0 ±13.1	17.8 ±34.5	45.0 ±22.9	32 ±25.7	80.0 ±8.7	43.3 ±24.3	73.0 ±23.2	22.7 ±15.8	64.5 ±10.1	29.5 ±20.8	66.7 ±5.6	37.9 ±45.5	63.3 ±18.9	19.0 ±20.9	63.0 ±7.5	38.5 ±12.9	77.2 ±14.3
	>4	76.2 ±28.0	60.000	71.3 ±41.7	100.0	62.0 ±17.7	50.0	68.9 ±22.4	77.5	49 ±21.4	20.0	62.8 ±17.8	63.3	79.4 ±29.8	100.0	43.1 ±14.2	55.0	47.5 ±13.4	44.0
	P value	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-	p=0.000	-
Serum creatinine mg/dl	≥	72.3 ±30.5	62.5 ±3.5	65.9 ±44.3	75.0 ±35.3	58.7 ±22.8	68.7 ±26.5	67.9 ±19.1	77.5 ±0.0	42.4 ±21.2	40.0 ±28.2	58.4 ±21.1	66.9 ±5.1	72.3 ±38.8	83.3 ±23.5	38.4 ±19.1	62.5 ±10.6	45.7 ±14.2	66.0 ±31.1
	≤	47.6 ±37.8	68.3 ±13.9	37.5 ±45.4	44.4 ±24.2	43.4 ±26.8	79.1 ±8.8	52.3 ±27.4	72.5 ±4.5	33.4 ±22.8	65 ±10.6	42.0 ±25.6	66.3 ±5.8	53.9 ±44.5	62.9 ±20.0	28.5 ±21.8	62.2 ±7.5	42.0 ±13.7	76.0 ±14.6
	P value	p=0.000	-	p=0.000	-	p=0.001	-	p=0.000	-	p=0.009	-	p=0.000	-	p=0.009	-	p=0.011	-	p=0.139	-

SF-36 subscale abbreviations: PF-Physical functioning; RPF- Role limitation due to physical functioning; REF- Role limitation due to emotional functioning; VT-Vitality; MH-Mental health, SF-Social functioning; BP-Bodily pain; GH-General health perception; HD-hemodialysis; PD- peritoneal dialysis; Caregivers/ family yes- presence of the members' family, no- lack of family; p <0.05 –significant statistic.

114 (46.91%) from HD patients who were spending at least 90 euros monthly on groceries/food had higher values of SF-36 items than the others. Only one patient in PD group used to spend more than 90 euros per month but he showed only sporadic improvement in the SF-36 items (tab. II).

Half of the HD sample had serum albumin levels below the normal range (tab. II). In the presence of low serum albumin levels the most affected scores were RPF and VT (17.82±3.04 and 19.07±1.84, respectively). The lowest decline was found in MH score. Serum creatinine correlates well with all SF-36 scores, meaning that low levels correspond to low QoL. In HD group a significant correlation was identified between low serum creatinine and RPF, PF and REF scores (p=0.000, p=0.000 and p=0.009, respectively). The lowest correlation was found with MH score (p= 0.139).

DISCUSSION

Dialysis care has increased in importance throughout the world because of the growing prevalence of patients receiving RRT, its related morbidity and mortality and high social and financial costs (10). This study aimed at describing HRQoL (Health Related Quality of Life) within the context of dialysis care. Global QoL score was almost 40 % higher in PD patients compared to HD group, perhaps due to the slightly advanced age in HD group, although PD patients had lower food expenditure and lacked support from family members. The small number of PD patients could be considered a limitation of our study. Liem et al. (11) conducted a systematic review on HRQoL of HD an DP patients but did not find statistically significant differences between dialysis modalities. Vacaroiu et al. (12) found that PD was a better option for dialysis in elderly people. However, considering that dialysis treatment is chronic, maybe these statistically significant results need more careful observation regarding changes that occur as the disease progresses.

Among our cohort of patients, we found that economic factors like monthly food expenditure and family support varied in an expected direction showing poverty levels and material disadvantage for this population. Almost all our study patients have low monthly retirement income and healthcare allowance, characterizing a population with low socio-economic status. Also, nearly half of HD patients spent monthly on food at least 90 Euros compared to 10% of the PD group. So, in HD group, the patients who met this criterion presented a statistically significant improvement in all QoL scores. In PD group, only one patient had a monthly food budget of more than 90 euros which was sporadically reflected in improved QoL scores. One theme remains questionable: subjectivity of monthly food spending reported by the patients. Many of them did the shopping together with their family so such an assessment may also be over- or underestimated. Poverty, low income, lack of knowledge and access to information contribute to low QoL (13).

Mingardi et al. (14) in a multi-center study aimed at better understanding the relationship between albumin level and SF-36 scores, classified the patients into four groups from low to high albumin level. The results showed that with increasing serum albumin levels better self-reported HRQoL is registered. It is said that serum creatinine level indicates skeletal muscle mass, although an elevated level before dialysis means having suboptimal dialysis or long time without dialysis. The present study showed that high serum creatinine and albumin levels are associated with better QoL. Low PF and RPF scores, 2 items that indicates physical activities were associated with the lowest serum creatinine levels which can be linked with a possible low muscle mass.

CONCLUSIONS

This study highlights the decline of quality of life in patients with low socio-economic status, especially in the vulnerable subgroups of single and financially deprived patients. In our study the alterations of nutritional biomarkers was associated with low QoL. Prevention of protein-energy malnutrition can improve and maintain a high QoL in chronic dialysis patients. The limitations of the research were: single-center cross-sectional study; because of the small number of patients in PD group statistical estimation could not be obtained.

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DIETARY VITAMIN D, CALCIUM, AND MAGNESIUM INTAKE IN TYPE 2 DIABETES PATIENTS

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DIETARY VITAMIN D, CALCIUM, AND MAGNESIUM INTAKE IN TYPE 2 DIABETES PATIENTS (Abstract): **Aim**: To estimate specific nutrient intake in patients with type 2 diabetes. **Material and Methods**: The study was conducted on a group of subjects with type 2 diabetes. The collected data included: anthropometry, lifestyle, blood measurements, and the mean daily nutrient intake assessed by the EPIC food frequency questionnaire. **Results**: The study group included 101 subjects with type 2 diabetes mellitus (DM) with a mean age of 60.6±10 years, of which 45 men (59±10.6 years) and 56 women (62±9.6 years). The average energy intake was 1714.4±713.9 Kcal/day, with a statistically significant gender difference (1877.7±850.9 kcal/day for men, 1583.1±554.9 kcal/day for women, p = 0.039), and the average daily micronutrient intake was 2.44±1.44 μg/day for vitamin D, 724.5±263.2 mg/day for calcium (Ca), 266.7±98 mg/day for magnesium (Mg), which was low compared to dietary recommendations. Calcium intake was correlated with intake of carbohydrates, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), fiber and energy. Vitamin D intake was correlated only with protein intake (R=0.695), while magnesium intake correlated with intake of fibers, energy, carbohydrates and SFA. **Conclusions**: Our study in patients with type 2 diabetes showed a reduced average intake of calcium, magnesium and vitamin D compared to standard recommendations, requiring an individualized approach. **Keywords**: NUTRIENTS, FOOD FREQUENCY QUESTIONNAIRE, TYPE 2 DIABETES MELLITUS.

The assessment of nutritional intake through food survey consists in collecting information on the amount and type of foods ingested and on calculating nutrient intake based on their composition. Even tough dietary research methods (dietary patterns research) are imperfect and the methodological aspects of analysis have some limitations, they are widely used for nutritional recommendations and food policies (1,2). Food frequency questionnaires (FFQ) are the most commonly used dietary assessment method in epidemiological studies. FFQ are used to characterize dietary exposures and to explore the relationship between diet, risk of illness and/or disease progression. Diabetes is an extremely important medical condition, given its prevalence, negative impact on life expectancy and quality and the extremely high costs involved (3). Medical nutrition therapy has an important role in the prevention and management of type 2 diabetes.

The aim of this study was to assess the

specific nutrient intake in a group of patients with type 2 DM and to compare these with the standard dietary recommendations.

MATERIAL AND METHODS

The study group included 101 adult subjects with type 2 DM who did not take inulin and were scheduled for the regular medical checkup at the diabetes ambulatory unit; patients' mean age was 60.6±10 years, 45 were men (mean age 59±10.6 years) and 56 women (mean age 62±9.6 years). Participants were interviewed face-to-face about lifestyle, area of residence, education, occupation, physical activity, medical history. To assess dietary intake, we used the EPIC FFQ which was validated earlier on a Romanian population (4). The questionnaire consists of 130-items (foods and drinks) divided into 10 major food groups, and subjects were asked to specify the frequency of their consumption during the last year. Portion sizes were evaluated by discussion and demonstration drawings. Data collected via questionnaire were analyzed by FETA-FFQ EPIC Tool for Analysis (5), which converted data on food consumption into nutrient intake. In addition, information regarding diabetes duration and chronic complications were obtained. Weight, height, and waist circumference (WC) were measured and body mass index (BMI) was calculated. Also measured were systolic (SBP) and diastolic blood pressure (DBP). Blood was collected for biological parameter measurement (blood glucose, glycated hemoglobin - HbA1c). Statistical analysis was performed using SPSS v17. The significance of differences between the mean values of the studied variables was checked using the Mann-Whitney test, ANOVA and chi-square test for independent samples, considering statistically significant those differences with a level of significance of $p<0.05$.

RESULTS

Of the analyzed parameters, compared by gender, a statistically significant difference was found for weight, height and BMI. Regarding the presence of specific chronic complications of diabetes, 5.1% of the study subjects had retinopathy, 22.2% - neuropathy, 1% - peripheral arterial disease (PAD), and 76% hypertension, with gender differences, but lacking statistical significance (tab. I).

The average energy intake was 1714.4±713.9 Kcal/day, with a statistically significant difference between genders (1877.7±850.9 kcal/day for men, 1583.1±554.9 kcal/day for women, $p=0.039$). Otherwise, there were no statistically significant differences between men and women in macro- and micronutrients intake (tab. II). The percentage of caloric macronutrients was balanced according to guidelines, as follows: carbohydrates 49.02% (48.8% for men, 49.2% for women), proteins 19.2% (19.1% for men and 19.3% for women) and lipids 31.88% (32.1% for men, 31.5% for women). Total energy intake correlated with the intake of carbohydrates, proteins and lipids. These percentages are consistent with dietary recommendations (6).

Only 13.9% of subjects ingested the recommended daily dose of calcium (1200 mg/day): 13.3% of men and 14.3% of women ($p>0.05$). As to magnesium, 25.7% of subjects ingested the recommended daily dose (22.2% of men and 28.6% of women, $p>0.05$) and 5% (4.4% of men and 5.4% of women, $p> 0.05$) ingested the recommended daily dose of vitamin D. No statistically significant correlation was found between the intake of vitamin D, calcium, and magnesium and age, BMI, duration of diabetes

and HbA1c. However, there was a statistically significant inverse correlation between vitamin D intake and fasting blood glucose (R = -0.272, p = 0.017).

TABLE I

General characteristics of the study group

Parameters	Mean±SD; 95% CI			
	Total (n=101)	Men (n=45)	Women (n=56)	Sig. *
Age (years)	60.6±10.1;58.6-62.6	59±10.6;55.8-62.2	62±9.6;59.4-64.5	>0.05
Weight (kg)	88±16.5;84.8-91.3	91.6±16.3;86.7-96.5	85.1±16.2;80.8-89.5	0.048
Height (cm)	166.3±9;164.6-168.1	173.2±6;171.4-175	160.8±6.9;159-162.6	<0.001
BMI (kg/m^2)	31.8±5.4;30.7-32.8	30.5±5.1;28.9-32	32.8±5.4;31.4-34.3	0.026
WC (cm)	105.3±12.1;102.9-107.7	105.1±11.2;101.7-108.5	105.5±12.9;101.9-109	>0.05
Duration of DM	5.5±5.1;4.4-6.6	5.8±6;3.9-7.8	5.3±4.5;4-6.5	>0.05
Glycaemia (mg/dl)	143.3±35.2;135.4-151.3	141.7±34.1;130.2-153.3	144.8±36.5;133.2-156.3	>0.05
HbA1c (%)	6.9±1.2;6.7-7.2	7±1.3;6.6-7.4	6.9±1.1;6.6-7.2	>0.05
	N, %			
Retinopathy	5.1	6.8	3.6	>0.05
Neuropathy	22.2	13.6	29.1	>0.05
Nephropathy	0	0	0	
PAD	1	0	1.8	>0.05
Stroke	4	6.7	1.8	>0.05
Myocardial infarction	1	2.2	0	>0.05
Angina	8	8.9	7.3	>0.05
Hypertension	76	68.9	81.8	>0.05

*between Men and Women

TABLE II

Daily intake of energy and nutrients

	Mean±SD; 95% CI			
Parameters	Total (N=101)	Men (N=45)	Women (N=56)	Sig.
Energy (Kcal/day)	1714.4±713.9; 1573.4-1855.3	1877.7±850.9; 1622.1-2133.4	1583.1±554.9; 1434.5-1731.7	0.039
Carbohydrates (g/day)	210.2±93.6; 191.7-228.7	229.4±113.9; 195.2-263.6	194.8±70.8; 175.8-213.7	>0.05
Fibers (g/day)	18.8±9.2;17-20.6	19.3±11.2;15.9-22.6	18.5±7.3;16.5-20.4	>0.05
Proteins (g/day)	82.4±39;74.7-90.1	90±49.4;75.1-104.8	76.3±27;69.1-83.5	>0.05
Lipids (g/day)	61.6±29.7;55.7-67.4	65.1±36.5;54.1-76.1	58.8±22.8;52.7-64.9	>0.05
MUFA (g/day)*	22±11.7;19.7-24.3	23.3±14.2;19.1-27.6	20.9±9.1;18.5-23.4	>0.05
PUFA (g/day)*	12.6±6.6;11.3-13.9	13.1±8;10.7-15.5	12.1±5.2;10.7-13.5	>0.05
SFA (g/day)*	21±9.7;19.1-23	22.1±11.6;18.6-25.6	20.2±8;18-22.3	>0.05
Calcium (mg/day)	724.5±263.2; 672.6-776.5	736.9±278.9; 653.1-820.7	714.6±251.9; 647.1-782	>0.05
Vitamin D (μg/day)	2.44±1.44;2.16-2.73	2.73±1.57;2.26-3.2	2.21±1.29;1.87-2.56	>0.05
Magnesium (mg/day)	266.7±98.1; 247.3-286	268.4±98.1; 238.9-297.9	265.3±98.9; 238.8-291.8	>0.05

* MUFA=monounsaturated fatty acids, PUFA= polyunsaturated fatty acids, SFA=saturated fatty acids

The intake of vitamin D, calcium, and magnesium was positively statistically significantly correlated with all macronutrients analyzed (energy, carbohydrates, fibers, proteins, and lipids). Thus, we conducted a multivariate linear regression to find out which of the macronutrients and energy significantly influence the intake of micronutrients. Regarding calcium intake, it depended on carbohydrate, saturated fat, monounsaturated fat, fiber and energy intake, in that order, explaining 83.5% of the total calcium intake. Vitamin D intake remained significantly influenced only by protein intake (R = 0.695). Magnesium intake was influenced by the intake of fiber, energy, carbohydrates and saturated fat in a proportion of 90%. Subsequently, ROC curve analysis highlighted that for the diabetics in our study an intake of 259.03 g carbohydrates would suffice to provide the recommended daily dose of calcium. This would mean that this population should increase carbohydrate intake to 60% of daily energy intake, or increase caloric intake, in order to reach the recommended calcium intake.

DISCUSSION

A norm caloric balanced diet usually provides all the nutrients, and supplements are required only in at risk sub-populations or when deficiencies are demonstrated (6). Some authors (7) have shown that a high-carb low-fat diet has been associated with a higher risk of developing type 2 diabetes. Foods with a high glycemic index together with an increased proportion of lipids, were also found to increase the risk of diabetes (8). Schulze *et al*. (9) concluded in the EPIC-Potsdam study that replacing carbohydrates with protein and PUFA was associated with a reduced risk of type 2 diabetes. A systematic review (10) based on prospective randomized controlled trials showed that nutrients, other than carbohydrates, affected glycemic control and explained the mechanisms by which these nutrients influenced insulin secretion and action. Magnesium supplements decrease the risk of complications in people with diabetes, improving insulin secretion and insulin signaling (11). The results of a large prospective study suggested the beneficial role of both vitamin D and calcium intake in reducing the risk of type 2 diabetes (12). Food supplementation is preferred both in preventing and treating diabetes (13).

When analyzing the questionnaire-derived data we found that most of our subjects, both male and female, had a lower average daily intake of micronutrients (vitamin D, calcium, and magnesium) compared to standard recommendations (1200 mg Ca, 5 μg vitamin D and 320 mg Mg for women and 420 mg men), but without statistically significant gender differences. These nutritional deficiencies are however consistent with the statistical data on the Romanian population, namely a low intake of fruits and vegetables, fish and dairy products (14). It is also suggested that low vitamin D levels are an independent risk factor in diseases prognosis (15). Interaction between vitamin D and calcium is known and treatment of these deficiencies is done together. The consumption of foods rich in calcium is safe in terms of cardiovascular risk, use of calcium supplements should be reserved for patients who truly need supplementation (16). Magnesium depletion is a common problem in patients with DM and it affects glycemic status and progress of complications (17). Magnesium is a cofactor for vitamin D biosynthesis, transport, and activation. The consequences of suboptimal magnesium status in diabetic populations eating

modern diets are important because of the interactions of magnesium with the nutrients calcium and vitamin D. All three must be supplemented simultaneously (18).

Given that the proportion of macronutrients is according to guidelines, it means that increasing the food amount of vitamin D, calcium and magnesium, could be achieved only by increasing the calories. Therefore, one emerging conclusion is that each case should be addressed individually. Correcting these deficiencies would improve glycemic control and reduce the risk of complications.

CONCLUSIONS

In our group of diabetics, calcium intake was insufficient and depended on the intake of carbohydrates, saturated fat, monounsaturated fat, fiber and energy. Vitamin D intake was low and depended only on protein intake. Magnesium intake was low and was also influenced by the intake of fiber, energy, carbohydrates and saturated fat. Evaluating the sources of vitamin D, calcium and magnesium for each patient could lead to correct recommendations of either dietary adjustments or use of supplements.

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NEWS

CORRELATION OF MONOCYTE/HDL CHOLESTEROL RATIO WITH SYNTAX SCORES IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE

Monocytes play a pivotal role in the inflammatory cascade, being the first inflammatory cell type that infiltrates early atherosclerotic plaques. The study authors investigated the role of monocyte/HDL cholesterol ratio in predicting the severity and complexity of coronary lesions quantified by the SYNTAX score in patients with stable coronary artery disease undergoing coronary angiography. The study was conducted over a 3-year period, 428 patients underwent coronary angiography being investigated. All patients were clinically stable. They were excluded from the study patients with an acute coronary syndrome, malignancy, acute or chronic inflammatory diseases of the liver, kidney or other organ and patients who had previously undergone percutaneous coronary intervention and coronary artery bypass grafting. Transthoracic echocardiography was performed in all patients, and left ventricular ejection fraction (LVEF) was calculated using Simpson's method. Severe coronary artery disease was defined as having a SYNTAX score ≥ 23. The patients were divided into two groups: those with low SYNTAX score (< 23) and those with high SYNTAX score (≥23). Of the 428 patients included in the study, 161 patients had a SYNTAX score ≥ 23. The treatment with ACE inhibitors or angiotensin receptor blockers, beta blockers, statins and acetyl salicylic acid were similar in low and high SYNTAX score groups. Also, age, gender, hypertension, smoking status, instance of diabetes mellitus, hyperlipidemia, and positive family history were similar between groups. In the patients with SYNTAX score ≥ 23, multivessel disease showed a higher incidence. Monocyte/HDL cholesterol ratio and C-reactive protein (CRP) were significantly higher in patients with high SYNTAX score ($p<0,001$). As well, WBC count, total bilirubin, LVEF and HDL level were significantly correlated with SYNTAX score in stable coronary artery disease. Left ventricular ejection fraction was lower in the group with high monocyte/HDL cholesterol ratio and high SYNTAX scores. The cutoff value of monocyte/HDL cholesterol ratio that predicted a high SYNTAX score was 24, with a sensitivity of 66% and a specificity of 65,1%. In conclusion, monocyte/HDL cholesterol ratio is significantly associated with SYNTAX score. Monocyte/HDL cholesterol ratio can be used as a prognostic marker in patients with stable coronary artery disease, because it is an quickly accessible and inexpensive test (H. Kundi, E. Kiziltunc, M. Cetin, H. Cicekcioglu, Z. G. Cetin, G. Cicek, E. Ornek. Association of monocyte/HDL-C ratio with SYNTAX scores in patients with stable coronary artery disease. *Herz* 2016; 41(6): 523–529).

Nicoleta Dima

EXTRADIGESTIVE MANIFESTATIONS OF GASTROESOPHAGEAL REFLUX DISEASE: DEMOGRAPHIC, CLINICAL, BIOLOGICAL AND ENDOSCOPIC FEATURES

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EXTRADIGESTIVE MANIFESTATIONS OF GASTROESOPHAGEAL REFLUX DISEASE: DEMOGRAPHIC, CLINICAL, BIOLOGICAL AND ENDOSCOPIC FEATURES (Abstract): Gastroesophageal reflux disease (GERD) with extradigestive manifestations is a disorder increasingly recognized both by gastroenterologists, pneumologists, otolaryngologists and cardiologists. **Aim:** To evaluate the demographical, clinical, biological and endoscopic features of the patients with gastroesophageal reflux disease and extradigestive manifestations (chronic laryngitis, asthma, pseudoangina). **Material and methods:** Prospective case-control study, including 137 patients selected from patients referred to the Iasi Institute of Gastroenterology and Hepatology between July 2014 - September 2015. In the presence of typical GERD symptoms (heartburn or regurgitation), the patients were assessed by upper digestive endoscopy for the detection or exclusion of esophagitis. Despite the absence of esophageal lesions, the patients were further assessed by impedance-pHmetry. **Results:** Depending on the dominant extradigestive manifestation, the patients were assigned into 3 groups: 94 chronic laryngitis patients, 24 asthma patients and 19 pseudoangina patients. Females were more frequent among pseudoangina patients (68.4%). Mean age of the male patients with dysphonia or asthma was lower (p=0.002), the majority (78.1%) living in urban areas. Obesity was predominant in pseudoangina group (52.6%), as compared to dysphonia group (16%) the differences being statistically significant (p=0.002). A share of 57.9% of pseudoangina patients were dyslipidemic, in contrast to dysphonia (24.5%) or asthma group (37.5%) (p=0.013). Esophagitis was also more frequent at pseudoangina group (84.2%), but with no significant statistical difference between the study groups (79.8% and 75%, respectively) (p=0.115). It seems that *Helicobacter pylori* infection tends to be protective in patients with GERD and pseudoangina (RR=0.61), but it can not be extrapolated to the general population (p=0.459). **Conclusion:** GERD with extradigestive manifestations is a prevalent and heterogeneous disease. There are demographic, clinical, biological and endoscopic differences between patiens with extradigestive GERD. **Keywords:** GASTROESOPHAGEAL REFLUX DISEASE, EXTRADIGESTIVE MANIFESTATIONS.

Gastroesophageal reflux disease (GERD) with extradigestive manifestations is a topic debated in literature in the last decades. It became an important diagnostic issue for both gastroenterologists and specialists in other medical fields, like otolar-

yngology, cardiology, pneumology and dentistry, as demonstrated by the increasing number of patients suspected of GERD which are referred to gastroenterologists from these services. Apparently a banal disease, GERD has a significant morbidity, consuming human and material resources and burdening health system budget. So far, in Romanian literature there are limited data on extradigestive GERD.

The aim of this study was to evaluate extradigestive GERD patients in terms of demographic, clinical, biological and endoscopical features.

MATERIAL AND METHODS

A prospective case-control study including 137 patients referred to the Iasi Institute of Gastroenterology and Hepatology Iasi from otolaryngology, cardiology and pneumology services in the interval July 2014-September 2015 was performed. Included in the study were adult patients previously diagnosed with asthma, non-cardiac chest pain or chronic laryngitis in whom GERD was suspected. Written informed consent for the participation in the study was obtained. Excluded from the study were the patients with prior gastric or esophageal surgery, malignant tumors, esophageal motility disorders (achalasia, scleroderma, myopathies), alarm signs (hematemesis, jaundice, unexplained weight loss), psychiatric disorders, pregnant women, peptic ulcer, active tuberculosis or other acute lower respiratory tract infections, unstable arrhythmias or myocardial infarction in the last 6 months.

Demographic data, personal history of diseases and smoking and anamnesis were recorded in individual files. All patients were tested for *Helicobacter pylori* antibodies by ELISA. As it is known that dyslipidemia and obesity are risk factors for GERD, the cholesterol and triglycerides serum levels were analyzed and body mass index (BMI) was calculated in all patients. Obesity was considered if BMI was greater than 30 kg/m^2.

Depending on the presence/absence of typical symptomatology (heartburn or regurgitation), the patients were differently investigated in order to diagnose or exclude GERD. The patients presenting typical manifestations underwent a therapeutic test with double-dose PPI (Pantoprazole 40 mg BID), while the patients not presenting the classical symptoms were assessed by upper digestive endoscopy (UDE) for the detection of the lesions of esophagitis or hiatus hernia. In some cases, biopsies were taken and submitted to anatomo-pathological exam for diagnosing or excluding a Barrett's esophagus. Patients without esophageal lesions were further assessed by impedance-pH metry in order to make a certain diagnosis of GERD.

The data were statistically analyzed using *SPSS 18.0.* The used parametric tests were: student's t- test for comparing the means, χ^2-test for comparing frequency distribution and Pearson's test correlation coefficient. A p value < 0.05 was regarded as statistically significant.

RESULTS

According to the predominant extradigestive manifestation, the patients were assigned into 3 groups: group I included 94 patients with chronic laryngitis, group II-24 patients with asthma and group III-19 patients with non-cardiac chest pain.

Demographic features. Both non-cardiac chest pain group and chronic laryngitis group showed a slightly tendency towards females (68.4% and 52.1%, respectively), while asthma patients were more likely to be men (58.3%), the majority (78.1%) living in urban areas. The study patients were homogenous in terms of gender (p=0.218): 47.4% were males and 52.6% were females (fig. 1).

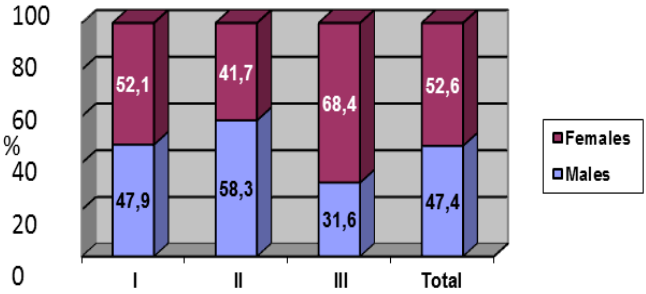

Fig. 1. Gender distribution

The age of reflux laryngitis patients ranged from 20 to 83 years, with a mean of 50.33±15.10 years and a median of 50 years. In the asthma group the mean age was slightly lower than in group I (50.25 ±14.14 years) and the median was also 50 years. The age of non-cardiac chest pain patients ranged between 44 and 74 years, with a median of 59 years and a mean age of 58.84±8.33 years, which is significant higher compared to the other two groups (p=0.05) (tab. I). The age > 50 years induced an estimated risk of GERD four times higher in the pseudoangina group (p=0.012).

Mean age was lower in males presenting asthma and laryngitis (p=0.002).

Of the 137 patients enrolled, 1 was under 20 (0.7%) and 3 (2.2%) were over 80 years of age. In group I the pick frequency was between 40-49 years (26.6%), in group II-between 50-59 years (33.3%) and in group III-between 60-69 years (47.4%) (p=0.026) (fig. 2).

TABLE I.

Descriptive statistical indicators of age (years) by study groups

Study group (Lots)	N	Mean	Standard deviation	Std. error	Confidence interval		Min	Max	p F_{ANOVA} test
					- 95%CI	+95%CI			
I	94	50.33	15.10	1.56	47.24	53.42	20	83	0.050
II	24	50.25	14.14	2.89	44.28	56.22	18	77	
III	19	58.84	8.33	1.91	54.83	62.86	44	74	
Total	137	51.50	14.41	1.23	49.06	53.93	18	83	

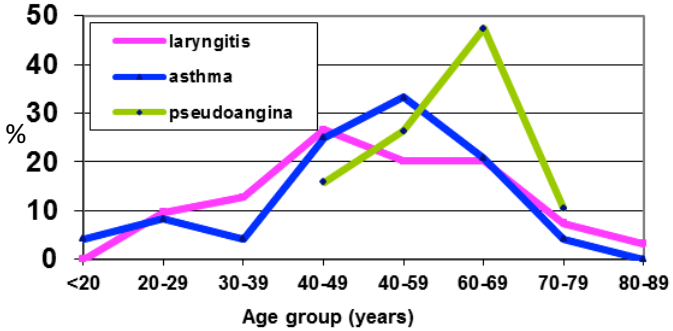

Fig. 2. Percent distribution of events by age

Clinical features. Typical reflux symptoms were more frequent in asthma subjects (54.2%), but there were no significant statistical differences between this group and laryngitis (39.4%) and non-cardiac chest pain patients (36.8%) (p=0.384) (fig. 3).

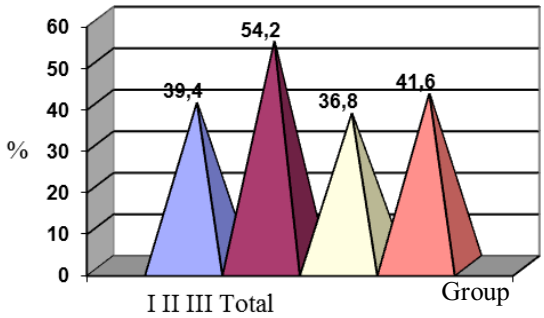

Fig. 3. Distribution of typical symptoms by study groups

The highest frequency of obese patients was found in the pseudoangina group (52.6%) and the lower in the laryngitis group (16%), with significant statistical differences (p=0.002); the overall percentage of obese people was 23.4%.

According to smoking status, the patients' distribution by study groups was homogenous. Although the group with pseudoangina recorded the lowest rate of smoking patients (5.3%), there were no statistical differences between reflux laryngitis (18.1%) or GERD-related asthma patients (25%) (p=0.234).

Biological features. Dyslipidemia was found in 57.9% of patients presenting non-cardiac chest pain, which is a significantly higher frequency in comparison with the other two groups (24.5% for laryngitis and 37.5% for asthma) (p=0.013).

Helicobacter pylori infection was identified in 52.6% of all GERD patients, without any significant statistically differences between study groups; the fewest cases of infection with *Helicobacter pylori* were identified in the pseudoangina group (p=0.619).

Endoscopic features. The percentage of patients presenting esophagitis on UDE examination was found to be higher in the group with non-cardiac chest pain (84.2%) compared to the other two groups (79.8%, 75% respectively) (p=0.115). Statistical analysis showed Barrett's esophagus in only 6.6% of GERD patients, but with no significant differences between the study groups (p=0.457). Hiatus hernia occured in 63.2% of pseudoangina patients and in only 36.2% and 41.7% of patients with laryngitis and asthma, respectively (p=0.094).

DISCUSSION

Our study is one of the few studies on extradigestive GERD conducted in our country. It is known that GERD with or without extradigestive manifestations is found in approximately equal proportions in both genders, with a slight tendency towards males (1, 2). In our study there was a gender homogeneity of the study groups, with a slightly higher frequency of women in the group with chest pain of non-cardiac cause.

GERD can occur at any age, but it is most commonly diagnosed after 40 years

(3, 4). The age of our patients showed wide variations in the three study groups. The mean age was 50 years both in patients with laryngitis and asthma, but in the pseudoangina group the mean age was significant higher (58 years), suggesting that age is a risk factor for this category of GERD patients. The highest frequency of young patients was recorded in the group with laryngitis, while older patients were most numerous in the group with pseudoangina.

In the literature (5, 6, 7) it is postulated that smokers, obese and dyslipidemic patients are more likely to have GERD. In our study, a quarter of asthma patients were smokers, with a 1.57 times higher relative risk of developing GERD. It appears that non-smoking status may be a protective factor in patients with pseudoangina, but this result cannot be extrapolated to general population. We found obesity in less than a quarter of extra digestive GERD patients, of which half were in group III. The highest percentage of patients with dyslipidemia was found in the group with non-cardiac chest pain. The relative risk to develop GERD of these patients is 3 times higher, which reinforces the idea that dyslipidemia is a factor contributing factor to the development of GERD.

It is well known that typical symptoms of reflux (heartburn/regurgitation) may be absent in patients with extradigestive GERD in a percentage that reaches 50% (8, 9). We obtained similar results, an increased proportion of patients experiencing both typical and extradigestive manifestations being identified in the asthma group.

As to the endoscopic features, lesions of esophagitis were observed in a high percentage of our patients (62.8%), especially in those with pseudoangina, result that is not consistent with studies in the literature (10, 11). Barrett's esophagus has been identified in a minority of cases regardless of dominant extradigestive manifestation. Hiatus hernia, known as a contributing factor in GERD (12), was present in comparable percentages in the laryngitis and asthma group, exceeding 50% of cases only in the pseudoangina group.

The literature data referring to the association of *Helicobacter pylori* infection and GERD are contradictory. Some studies (13, 14, 15) support the protective role of this bacteria in patients with GERD, while other studies (16, 17) postulate that *Helicobacter pylori* infection is a favorable factor in GERD. In our patients, *Helicobacter pylori* infection was identified in just over half of the cases, the lowest frequency being in the group with pseudoangina. This result would suggest that in this category of individuals *Helicobacter* infection may play a protective role in the development of GERD (RR = 0.61), but the result cannot be extrapolated to the general population.

CONCLUSIONS

Our study supports the idea that extra digestive manifestations of GERD are frequent and heterogeneous. There are differences between patients with GERD and chronic laryngitis, asthma and non-cardiac chest pain in terms of demographic, clinical, biological and endoscopic features. We demonstrated that extra digestive manifestations correlate with smoking, obesity and dyslipidemia, as contributing factors. The typical symptoms are absent in about half of extradigestive GERD cases, making the diagnosis being sometimes difficult. It is obvious that *Helicobacter pylori* infection influences the course of GERD, but is still not clear if it is in a positive or a negative way.

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CERVICAL SPINE LESIONS IN RHEUMATOID ARTHRITIS PATIENTS

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CERVICAL SPINE LESIONS IN RHEUMATOID ARTHRITIS PATIENTS (Abstract): **Aim of the study**: to gather clinical and laboratory data on rheumatoid arthritis patients with cervical spine damage (incidence and prevalence, correlation between duration of disease and the time of lesion onset, to assess signs and symptoms and the role of laboratory investigations). The spine is an axial organ with an important role in support and resistance. It is a pillar with a very complex morphological and functional structure. The vertebral column is crossed by many kinematic chains. The main problem of the cervical spine caused by rheumatoid arthritis is cervical instability which describes all cervical lesions that can lead to neurovascular damage or major disturbance of pain generating statics at movement. The evolving disease shows chronic inflammation of the synovium, which is a self-maintained process and an immunologically induced phenomenon. The chronic inflammation of the synovium forms granulation tissue that invades peripheral joints towards the center and causes ligament cartilage and bone damage. **Material and methods:** The present paper investigated cervical spine lesions in 107 rheumatoid arthritis patients who were admitted to the 1st Rheumatology Clinic of Iasi Rehabilitation Hospital between January 2013 and December 2014. Our study focused on assessing signs and symptoms seen in spine affected by rheumatic disease. **Results and Discussions**: the disease causes destructive lesions due to granulomatous infiltration of rachidian structures and medullary sheaths. These lesions lead to damaged discs and instability that produces subluxations and dislocations. The suboccipital region is most affected; in other regions of the spine, high lesions of C4-C5 prevail, where osteolysis damage of spinal apophyses are found. In atlas and axis joints, rheumatoid arthritis causes the inflammation of bursa, synovium and joint capsule and leads to synovial pannus formation. This causes the destruction of cartilage and subchondral bone. Atlantoaxial dislocation is caused by erosive synovitis of atlanto-epistrophic joint, atlanto-odontoid joint and serous bursitis separating the odontoid process from the transverse ligament. **Conclusions:** The dominant symptom of cervical spine damage was pain associated with stiffness and limited joint mobility, muscle stiffness, poor posture. **Keywords:** CERVICAL INSTABILITY, DISLOCATION, DESTRUCTIVE EROSIONS, RADIOLOGICAL CRITERIA.

Cervical spine lesions in rheumatoid arthritis (RA) patients lead to increased physical pain, with local radicular bone marrow involvement that aggravates the functional deficit, worsening the vital prognosis. The impairment of the cervical spine can be explained only partly by its morphological and dynamic characteristics (1).

During the evolutive process, the chronic inflammation of the synovial membrane is a self-maintained, immunologically induced process that leads to the formation of granulation tissue invading peripheral joints towards the center and causing cartilage and bone ligament lesions (2).

Atlanto-axial dislocation occurs at the C1-C2 segment (anterior more than 3 mm between the arch of C1 and the C2 odontoid, with synovial proliferation in the C2 odontoid joint and the anterior arch of C1 that can lead to rupture of the transverse ligament and alar ligaments) (3).

Other lesions of the cervical segment caused by rheumatoid arthritis include: vertical axial subluxation = collapse of the lateral joints between C1 and C2; lateral atlantoaxial subluxation with pain in the frontal and suboccipital region and anterior inclination of the head posture; posterior atlanto-axial subluxation; C2 and C3 are the most commonly affected, while 10 to 20 percent of C3 and C4 are affected. The therapeutic intervention depends on the moment of survey or on the presence or absence of neurological signs (4).

Destructive lesions found in the rest of the cervical spine affect mainly the C3-C4 and C4-C5 segments, the lower cervical spine being less affected (5).

The present study focused on monitoring clinical and laboratory data of cervical spine lesions in rheumatoid arthritis patients.

Aims of the study: The evaluation of the incidence and prevalence of cervical spine damage in rheumatoid arthritis cases hospitalized in the 1st Rheumatology Clinic, Iasi Rehabilitation Hospital, the correlation between the duration of the disease and the onset of cervical spine lesions in rheumatoid arthritis patients, the evaluation of signs and symptoms that occur in the rheumatoid damage of the cervical spine and the the role of laboratory investigations in the diagnosis of vertebral instability (standard radiography, CT examination, MRI examination).

MATERIAL AND METHODS

The study included a group of 107 RA patients who were admitted to the 1st Rheumatology Clinic, Iasi Rehabilitation Hospital between January 2013 and December 2014.

The inclusion criteria were the 1987 ACR classification criteria for RA and the results of the radiological examination (2).

Exclusion criteria included RA patients who did not show clinical or radiological damage to the cervical spine and RA patients with only degenerative changes. The demographic characteristics in the group of patients were initially evaluated through descriptive statistical methods; statistical correlations between clinical and laboratory parameters (e.g. Pearson correlation) were subsequently performed.

T-student and chi-square tests were used for the statistical evaluation of data. They were clinically significant when p is less than or equal to 0.05. The database was systemized with Excel (Microsoft Office), and the statistical analysis used the statistical programs MedCalc and Epi Info 2000.

RESULTS

RA incidence and prevalence are reported differently according to the diagnostic criteria used in population studies (table I).

Out of the total number of 20 (18.69%) RA patients with cervical spine involvement admitted during the study period, 13

(12.14%) were women and 7 (6.54%) were men. A predominant involvement of female patients was noted, showing a regular sex distribution of RA patients and cases with cervical lesions in the sense that female cases are about three times more frequent than male cases. Female gender is a factor of poor prognosis of the disease.

TABLE I
Incidence and prevalence in the study group

Year	2013	2014	Total
Total number of RA patients	46	61	107
RA with cervical damage - new cases	6	9	15
RA with cervical damage - reappraisal	2	3	5
Incidence of cervical damage in RA patients	13.33%	20.00%	33.33%
Prevalence of cervical damage in RA patients	17.39%	14.75%	32.14%

54.4% of the study group patients were from urban areas, possibly explained by the higher medical visit rate of the urban group. The values related to the duration (in years) of RA were dispersed, ranging from 0 to 35 years of disease, most patients (64.5%) being in the group with a duration of less than 5 years (table II).

TABLE II
Distribution of the study group according to the duration of the disease

Years	< 5	5-10	10-15	15-20	>20
Patients in the study group (%)	61.31	14.50	5.60	3.40	4.40

The study group patients were aged over 45 years and cervical lesions were present in patients with a duration of the disease of at least 10 years in cases with onset before the age of 65 and occurred early in patients with senile RA. Damage to the cervical spine was higher in elderly patients with a longer duration of the disease and especially in the more severe types of disease.

Specific symptoms occurred gradually in most patients from a period of several months to several years. The dominant symptom was joint pain exacerbated at night associated with prolonged morning stiffness and swelling of various joint areas that have led to functional impairment, the higher, the more regions were affected by arthritis. Several clinical onset forms were observed, i.e. monoarticular, oligoarticular, polyarticular. Other onset forms were extraarticular manifestations such as muscle pain, tendonitis or ductal syndromes (especially carpal tunnel syndrome). The polyarticular type of disease onset with rapid development of deformities and joint ankyloses was mostly seen in patients with cervical damage.

TABLE III
Distribution of general signs in RA patients

General signs	%
Asthenia, fatigue	97
Loss of appetite	45
Transient arthralgia	91
Raynaud's syndrome, acrocyanosis	49
Weight loss	12
Irritability	83
Depression, insomnia	85

The main clinical manifestations were pain, swelling, morning stiffness and functional discomfort (table III).

Inflammatory cervicalgia and cervical stiffness was present in all study group patients studied, but the elderly with senile RA, aged 65 years, cervicalgia showed also degenerative changes. Signs suggesting the localization of the rheumatoid process at the cervical level in the study group were found in varying proportions (tab. IV).

The dominant element was cervical and cervical-cephalic pain associated with stiffness of the extensor, trapezius or sternocleidomastoid muscle. Almost half of the patients showed a poor posture of the cephalic extremity with slight anteflexion.

16% of patients had severe neurological damage caused by cervical changes, while the altered sensitivity seen in the dermatomes of the cervical plexus roots was frequent.

TABLE IV

Symptoms of cervical damage in RA patients

Clinical signs of cervical damage in rheumatoid arthritis	%
Scalp tenderness	66%
Tenderness of the spinous apophyses, cervical chain	95%
Altered sensitivity of the cervical region and upper limbs	45%
Muscle tenderness	74%
Muscle weakness in regional muscles and upper limb end	54%
Fasciculations	1%
Transient hearing loss	3%
Sensory ataxia	1%
Sensitivity of the carotid sinus	9%

The study of global and segmental mobility of the cervical spine showed a severe limitation of movements in the upper region in 37% of the cases and an overall mobility limitation in 41% of the patients.

TABLE V

Extraarticular manifestations in RA study group patients

Extraarticular manifestation	%
Subcutaneous nodules	39.7
Tenosynovitis	31.0
Myopathy	36.3
Cardiomyopathy	6.1
Rheumatoid lung	24.3
Pleurisy	0.93
Pulmonary fibrosis	7.5

In patients with severe cervical damage, extraarticular manifestations included changes in the skin, subcutaneous tissue and juxtaarticular tissues, respiratory and cardiovascular systems (table V).

Skin manifestations – 39.7% subcutaneous nodules were seen in patients with cervical lesions. This was correlated with a relatively long duration of the disease and with severe forms of the disease. They were associated with seropositive forms of the disease and with other systemic manifestations such as rheumatoid lung.

Nonspecific cutaneous manifestations (pale skin, hypopilosity, dull brittle nails, hyperpigmentations) were also seen. Some of them express the inflammatory anemia caused by the long evolution of the disease in the selected patients.

Skeletal muscles and tendons - the study group patients often showed myalgia (in the shoulder girdle) and muscle weakness,

clinically expressed as muscle atrophy caused by inactivity, secondary to the joint pain of the disease. The anatomo-pathological basis was represented by degenerative lesions of muscle fibers.

Respiratory manifestations - multinodular rheumatoid lung, radiographically shown, clinically manifested as dry cough, moderate dyspnea and intermittent hemoptysis were present in 14 (58.3%) patients. Pleurisy was observed in 1 (0.93%) patient; as the pleurisy was quantitatively reduced, it was reabsorbed following appropriate antibiotic and anti-inflammatory therapy.

Cardiovascular manifestations - difficult to assess, given that patients in the study group were over 45 years old and physiological changes of involution are closely intricated with pathological changes that cannot be properly classified. Chest pain, dyspnoea on exertion or tachycardia are difficult to interpret. Arterial hypertension was present in 63% of patients.

Digestive manifestations - clinically, 14 (35%) patients had hepatomegaly at baseline. Following administration of NSAIDs, 31% of patients had epigastralgia and heartburn. Gastric or gastroduodenal ulcer, radiographically shown, was present in 3 (15.00%) patients.

Neurologic manifestations - compressive neuropathies, such as carpal tunnel syndrome, were found in 9 (45%) patients and resulted from existing periarticular changes. The manifestations were more pronounced in the dominant limb. Damaged brachial plexus roots gave rise to radicular syndromes characterized by pain, paresthesia, hypoesthesia in the corresponding dermatome and the decrease of osteotendon reflexes. A significant percentage of patients showed this manifestation. These clinical changes were present mainly in patients with specific cervical radiological changes, such as subaxial dislocation. Cervical-brachial neuralgia was seen in 7 (6.54%) patients.

Facial paralysis was observed in 2 (10%) patients, probably caused by the damage of the spinal nucleus of the trigeminal nerve, as patients had atlantoaxial dislocation. Several patients showed symptoms of dysphagia and dysphonia, which can be attributed to altered hypoglossal nerve (XII), vagus nerve (X) and glossopharyngeal nerve (IX). By using the Redlung-Johnell method, radiological changes such as vertical atlanto-axial dislocation were seen in these patients.

Renal manifestations. The research showed that 2 (10%) patients had a proteinuria of 1.5 g / 24 hours, one patient having a proteinuria of 1g / 24 hours. A high frequency of urinary tract infections was seen in women (38.6%) due to associated risk factors found in RA patients with decreased immune defense, hormonal changes, frequent hospitalizations and consumption of NSAIDs.

Hematologic changes - anemia was present in 17 (8.00%) patients (out of the total number of 20 patients). The most common cases showed normocytic and hypochromic anemia, as well as and hyposideremia; serum Fe values were low in some patients. The anemia seen in these patients can be attributed to the long evolution of the inflammatory disease and it is associated with iron absorption disorders, moderate decrease of erythropoiesis, hemolysis and blood loss.

3% of the study group patients had clinical manifestations of diplopia that might be caused by the damage of the abducens nerve (VI).

The following laboratory parameters and their correlations were followed in patients with cervical lesions caused by RA: biological inflammatory syndrome,

immunological changes - rheumatoid factors, standard radiological examination - flexion / extension; CT and MRI were used in patients with neurological symptoms.

Inflammatory syndrome - the assessment of ESR and CRP values, the blood count were useful for both diagnosis and the survey of the progression of the disease by determining their dynamics.

Anterior atlanto-axial dislocation was the most common form of cervical instability (65% of study group patients). Combined dislocations such as anterior atlanto-axial dislocation associated with the subaxial dislocation were far less numerous, 7 (35%) patients, as compared with 13 (65%) patients with anterior atlanto-axial dislocation. Other forms of dislocations were much less common.

The radiological examination has shown fractures of the odontoid in 1 patient; erosions of the interapophyseal joint in 4.2%; odontoid secondary osteosclerosis in 2.5%; the appearance of diffuse osteoporosis was associated with about 47% of the total number of patients. CT was performed in 4 patients and MRI in 3 patients.

DISCUSSIONS

Anterior atlanto-axial subluxation is the most frequent classical involvement of cervical spine in RA. The profile radiography of the cervical spine, C1-C2 - centered, shows a distance of more than 3 mm between the posterior edge of the anterior arch of the atlas and the anterior edge of the odontoid apophysis. The anterior subluxation of the atlas to the axis is possible due to the rupture or distension of the transverse and cruciform ligaments, following the chronic inflammation of the atlanto-axial joint (6, 2).

Subluxation causes the narrowing of the retro-odontoid part of the spinal canal and the compromise of the neuraxis at the bulbo-medullary junction.

Clinically, the alteration of this joint can be completely asymptomatic, with a latent evolution, or may lead to severe cervical pain, high and posterior with inflammatory rhythm. Neurological complications are quite rare and include Lhermitte's sign or neurological signs of the upper limb: sensory disorders, movement disorders (3,4).

The literature reports also cases of sudden death (5), mostly following road accidents or during intubation for general anesthesia. Therefore, the arthritic condition must be systematically investigated before any surgical intervention (4,8).

Our findings showed also that the positive diagnosis is based on radiological examination (profile radiography of the cervical spine, C1-C2-centered, dynamic, flexion and extension) and on CT examination (a posterior detachment of the odontoid apophysis related to the anterior arch of the atlas is observed on the cross sections of the spine) similar to the reports of other authors (11,12).

The cervical spine damage in RA might involve also the middle and lower spine under the form of posterior interapophyseal arthritis. This could cause joint slipping or subluxations between vertebral bodies. These lesions are easily noticed on dynamic flexion / extension radiographs. These areas of the cervical spine are affected in 15% of the cases. The results concur with literature data already mentioned (13,14).

CONCLUSIONS

Our study showed that cervical spine lesions lead to increased physical pain, with local radicular bone marrow involvement that aggravates the functional deficit, worsening the vital prognosis.

The main disorder of the cervical spine

damaged by rheumatoid arthritis is cervical instability. The damage of the cervical segment caused by RA occurs late in the evolution of the disease, when destructive cartilage and bone lesions, axial deformities, extensive muscular atrophy and, in some cases, even fibrous or bony ankylosis are present.

The dominant symptom of cervical spine damage was pain associated with limited joint mobility, muscle stiffness and poor posture.

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SELENIUM STATUS IN AUTOIMMUNE THYROIDITIS

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SELENIUM STATUS IN AUTOIMMUNE THYROIDITIS (Abstract): Selenium (Se) is an important element that exerts its effects through selenoproteins. The thyroid gland has the highest Se concentration and specific selenoprotein enzymes families are crucial in the thyroid hormone metabolism. There is little evidence on the link between Se and thyroid autoimmune disease, therefore future studies are required to elucidate the nature of this association. **Aim**: To evaluate the Se status in euthyroid subjects with autoimmune thyroiditis**. Material and methods**:FromJanuary 2014 to January 2015 we recruited 100 consecutive euthyroid subjects with autoimmune thyroiditis, living in the same region and with normal iodine intake. Serum concentrations of Se, thyroid antibodies (antithyroperoxidase –TPOAb – and antithyroglobulin – TgAb), thyroid-stimulating hormone (TSH), and thyroid ultrasound were performed in all patients. **Results:** Mean age of the study group was 48.87±12.83 years, range: 18-82 years. Since thyroid pathology is more frequent in the 5th – 6th decades of life we selected the age of 50 for the comparative analysis of the results (51% of patients were under 50). No statistical age-group differences in antibody levels were found: mean TPOAb = 420.95 IU/ml, p=0.840; mean TgAb = 327.98 IU/ml, p=0.977. TSH mean was 2.14 µIU/ml, with no significant age-group differences (p=0.176). Se levels ranged between 8.05 - 998.50µg/ with a mean value of 294.96µg/L and no significant differences between age groups (p=0.158). Thyroid ultrasound showed inhomogeneity in 89%, nodules in 35% of patients, and a mean thyroid volume of 11.72ml, with no significant age-group differences (p=0.366). The low TSH levels were associated with low Se levels in 11.6% of cases, but the direct correlation was statistically insignificant (r = 0.116; R^2 = 0.0161; p = 0.371). Depending on TSH percentiles, mean Selevels showed no significant differences, however pointing out the highest mean value at the 25th percentile (F = 0.441, df = 61, p = 0.646). A negative correlation trend was found between Se and TPOAb (r=-0.2276) or TgAb (r=-0.2190) but lacking statistical significance (p=0.099). **Conclusion:** Our results showed a weak negative correlation between Se and antithyroid antibodies, suggesting that selenium supplementation may improve the course of thyroid autoimmunity. **Keywords**: SELENIUM, AUTOIMMUNE THYROIDITIS.

Selenium (Se) was first isolated in 1817 by Jacob Berzelius (1), and its name derives from Selene – goddess of the moon. Se is a metalloid present in the environment, water, soil and air, generally in very low concentrations (< 1µg/g) (1) and is an essential element for human health.

Se has a concentration-dependent effect in the body, being an antioxidant at nano- or micromolar levels while in higher concentrations it has a prooxidant effect.

The normal plasma Se concentrations of range between 60 and 120µg/l or 0.8 ± 0.36µmol/l being related to its intake through diet and the daily dose should not exceed 400 µg/day, dose at which toxic effects were observed (2).

Se deficiency can affect human health in different ways. The severe endemic deficiency is associated with Keshan disease (congestive cardiomyopathy) and Kaschin-Beck disease (chronic, endemic osteochondropathy), and the mild one with various limited expression of Se-dependent enzymes. Causes of deficiency are dietary low intake and poor intestinal absorption. Selenosis is the most common disease as a result of over limit intake of Se from food (3).

The majority of Se in tissues is found in proteins (seleno-proteins, selenotrisulphides and other acide-labile Se compounds) (4). In humans, the thyroid gland has the highest Se concentration as a constitutive part of Dio 1 and 2 thatconvert thyroxine (T4) to triiodothyronine (T3); Gpx 1 and 4, which protect thyreocytes against H_2O_2 released during thyroglobulin iodination; Gpx3, regulating H_2O_2 concentration in the lumen of thyroid follicle; selenoprotein P and Txnrd 1, having an antioxidant effect (5).

In normal nutritional conditions, the liver is the primary source of plasma T3, due to liver deiodination of T4 by Dio 1 (which actually contributes to the release of T3 by the thyroid). In Se deficiency, the primary source of T3 is the thyroid by increasing thyroid activity of Dio1 which allows maintaining normal concentrations of plasma T3 despite reduced activity of hepatic Dio (6).

Selenium deficiency leads to a decrease in GPx activity that results in the accumulation of H_2O_2 in the thyroid and thus enhancing apoptosis. Since H_2O_2 is essential in the synthesis of T3 and T4, its accumulation could be the activator of increased thyroid production of T3 and T4. Probably Dio 1 is required to maintain an optimum T4/T3 balance in these conditions (6).

Selenium was shown to have an important role in lymphocytic chronic thyroiditis. Most authors consider that Se affects the autoimmune system by controlling the production of ROS (Reactive Oxygen Species) and Se supplementation determines a stimulating activity on glutathione peroxidases and thioreductases. Another path in which Se is involved in autoimmune thyroiditis may be by inhibiting the expression of HLA-DR molecules (7, 8).

Currently, there are no ways to stop the autoimmune process in chronic thyroiditis; levothyroxine treatment only addresses to the consequences of autoimmune aggression (9).

Given current knowledge, selenium therapy to reduce aggression and to prevent autoimmune thyroid dysfunction, remains the only therapeutic alternative, whose effectiveness needs to be supported by more evidence.

MATERIAL AND METHODS

We conducted a one-year prospective study (January 2014 - January 2015) in the

Department of Endocrinology of the "St.Spiridon" Hospital Iasi. One hundred consecutive subjects with euthyroid autoimmune thyroiditis and normal iodine intake were selected. Serum concentrations of TPOAb, TgAb, thyroid-stimulating hormone (TSH), Se and thyroid ultrasound were performed in all patients. In a second phase, Se was randomly administrated to half of these patients and re-assessed them after 3 months.

Inclusion criteria: female patients aged 18 to 2 years with normal thyroid function (TSH =0.4-4μIU/ml) and autoimmune thyroiditis (TPOAb>35UI/ml or/and TgAb> 40UI/ml) able to sign informed consent. All patients included in our study signed the informed consent. *Exclusion criteria*: major co-morbidities making it difficult to participatein the study, thyroid treatment (LT4 or anti-thyroid drugs) previous or at the time of enrollment; pregnancy or breast-feeding; malabsorption or consumptive syndromes, other autoimmune diseases, and intake of selenium supplementation above 70 μg/day; inability to read or understand Romanian; or lack of informed consent.*Variables studied*: age, sex, titersof TPOAb and TGAb, TSH, volume (V) and echostructure (E) at thyroid ultrasound, and plasma Se levels.

TSH and thyroid Abs were measured by chemiluminescence at the hospital laboratory.

Se measurements were performed by atomic absorption spectrometry using the GF with platform HR-CS-AAS contra 600 AnalytikJena (10). The method we used involves several steps: fresh blood samples were preserved by freezing at -25° C; they were defrosted and homogenized before metal digestion; metal digestion for Se analysis also had several steps. A mixture of 1ml total blood with 3ml of nitric acid 65% and 1ml hydrogen peroxide 30% was allowed 15 minutes to react. To realize the digestion process we kept the samples at 145°C for 5 minutes, at 190° for 10 minutes and finally at 100°C for 10 minutes. After the digestion process the samples were transferred into 25 ml decontaminated Duran volumetric flasks. The flasks containing the samples were filled with ultrapure water up to a volume of 25ml,shaked few times and then the content was transferred in 15ml bottles for the final metal analysis.

The collected data were processed and interpreted by using the SPSS 18.0 software and statistical and mathematical tests.

RESULTS

Mean age of the study group was 48.87±12.83 years, range: 18-82 years. The study group was divided into two groups according to their age, with age 50 as the demarcationpoit, justified by the fact that thyroid pathology is more frequent after the 5th–6th decade of life. 51% patients were aged under 50 years. No statistical age-group differences in antibody levels were found: mean TPOAb = 420.95 IU/ml, p = 0.840; mean TgAb = 327.98 IU/ml, p = 0.977. For TSH the mean value was 2.14 μIU/ml with no significant age-group differences (p=0.176). Se levels ranged between 8.05-998.50 μg/L with a mean value of 294.96 μg/L (p=0.158). Thyroid ultrasound showed inhomogeneity in 89%of patients, nodules in 35%, and a mean thyroid volume of 11.72 ml (p=0.366) (tab. I).

Depending on TSH percentiles, mean Se levels showed no significant differences, however pointing out the highest mean value at the 25th percentile (F = 0.441, df = 61, p = 0.646). A negative correlation trend was found between Se and

TPOAb (r=-0.2276) (Fig.1) or TgAb (r=-0.2190), but lacking statistical significance (p=0.099) (fig. 2).

The low TSH levels were associated with low Se levels in 11.6% of cases, but the direct correlation was statistically insignificant (r = 0.116; R^2 = 0.0161; p = 0.371) (fig. 1).

The same result (negative Se-ATPO Ab correlation) was obtained when we divided our study group into 3 subgroups depending on Se levels (tab. II).

TABLE I

Statistical indicators for Se, TPO and Tg Abs, TSH, VT (Mean SD , min – max)

	Se (µg/L)	**TSH** (µIU/ml)	**TPOAb**(IU/ml)	**TgAb** (IU/ml)	**VT(ml)**
<50y	256.76 246.70 (8.05-998.50)	1.860 0.769 (0.569-3.67)	328.97 348.75 (10-1000)	337.83 595.29 (10-2372)	11.00 7.98 (0.83-47.7)
>50y	344.48 229.58 (25.08-771.25)	1.777 0.949 (0.4-3.93)	326.95 359.96 (10-1000)	478.23 569.93 (10-1946)	12.47 8.20 (3.91-44.41)
Total	294.96 241.48 (8.05-998,50)	1,830 0.864 (0.4-3.93)	327.98 352.49 (10-1000)	440.08 609.23 (10-2372)	11.72 8.08 (0.83-47.07)

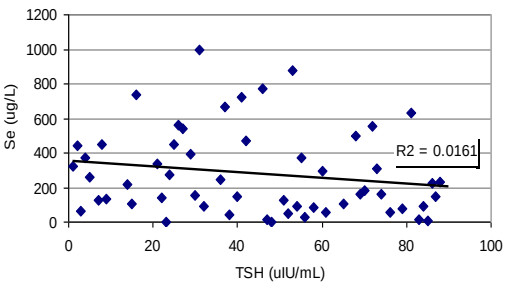

Fig. 1. Correlation between Se and TSH

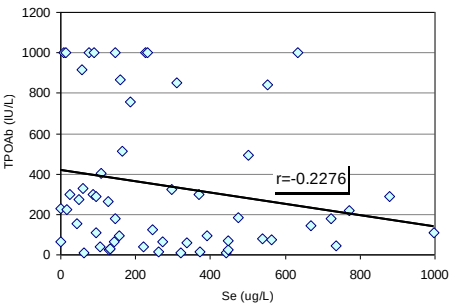

Fig. 2. Correlation between Se and TPOAb

TABLE II

Statistical indicators for Se- TSH and Se- ATPO

t test	TSH	ATPO		corel	Se vs TSH	Se vs ATPO
mVSn	0.839941	0.885068		m	0.19813	-0.43639
mvsM	0.86379	0.276061		n	-0.00761	-0.12693
nvsM	0.430623	0.420775		M	0.130626	-0.14692

m : Se <60 µg/L, n: Se = 60-120 µg/L , M: Se >120 µg/L

DISCUSSION

Se is an essential micronutrient in many aspects of human health, which plays a major part in optimal endocrine response, immunomodulation and inflammatory process. The most frequent disease involving the thyroid gland is chronic autoimmune thyroiditis, an inflammatory process which progressively destroys the gland. Several studies have examined the potential benefits of Se in this disease to assess if its supplementation may be effective in in-

creasing the antioxidant defenses (11).

In the present study we measured Se levels in the blood of euthyroid patients with autoimmune thyroiditis. This is the second study which provides information on Se status in Romanian patients with autoimmune thyroiditis. The fist data on Se level in the Romanian patients were provided by a multicenter study published in 2014 (12) which recruited patients from four European countries (Greece, Romania, Austria, Italy) (tab. III).

TABLE III
Selenium status in different European countries

Author	Country	Selenium levels(plasma µg/l)	
		Patients with TAI	Patients without TAI
Krassas, Pontikides, Tziomalos*et al*. Se status in patients with autoimmune et non-autoimmune thyroid disease from four European countries	Italy (2014)	106.0 ± 41.9 (n=13)	116.8 ± 34.2 (n = 56)
	Greece (2014)	81.3 ± 50.7 (n=81)	82.3 ± 27.8 (n = 56)
	Austria (2014)	83.1 ± 31.2 (n=36)	93.4 ± 31.2 (n = 25)
	Romania (Timisoara 2014)	86.6 ± 23.3 (n=30)	80.4 ± 31.0 (n = 22)
Present study, The effect of Se supplementation on antioxidant status, hormonal, autoimmune and ultrasonographic profile in euthyroid patients with chronic autoimmune thyroiditis	Romania (Iasi 2015)	294.96 ± 241 (n=100) blood	ND

When comparing the results of these two studies we found that the Se levels reported in 2015 were higher than those from the multicenter study. This difference can be explained by the fact that we used total blood which contains more Se than plasma. In our study Se was determined by atomic absorption spectrometry (see methods). The method we used is an original one but there are various methods for Se determination: nuclear activation analysis (NAA), elecrothermal atomic absorption spectrometry (ET AAS), hydride generation atomic absorption spectrometry (HG AAS), fluorimetry, X-ray fluorescence analysis (XFA), and gas-liquid chromatography (GCL) (Bem EM). Chromogenic reagents used for Sedetermination by various spectrophotometric methods are: dithiozone, chromo tropicacid, J-acid, Variamine Blue (12).

Usually, these methods are used to determine Se in plasma, serum, whole blood and other tissues. Se concentration in the serum and plasma have similar values (plasma contains 81% of the Se of whole

blood and 94% of serum) and correlates with Se ingestion. Se in whole blood varies less and correlates better with GPx(3). The obtained Se levels, even if the same tissue is used, can vary depending on method. Another variable is the number of patients (the 2015 Iasi study had a greater number of patients) and the heterogeneity of the study group. The iodine intake is also very important. Anamnestically, our patients had a normal iodine intake. Previous studies reveled that Moldavia is aniodine deficient region, except Iasi, where the iodine intake is normal (13).

Levels of Se also vary by geographic region(type of soil) which influences the dietary Se intake. The dietary Se intake is in the range 7-4990 mg/day, with different mean values for each geographical area (40µg/day in Europe, 93µg/day in women and 134µg/day in men in the USA). Also, the dietary Se concentrations are dependent on the soil Se content, form and distribution of Se in foods (Se from plant source foods is a more bioavailable form than animal source foods) (13).

China, India, Middle–East and some European countries have a very low content of Se in soil resulting in Se deficiency in the local population (3). Soilsof the driest regions concentrate high quantities of Se and alkaline soils release more Se than the acid ones (3).

Selenium content of foodstuffs varies in a broad range between countries, depending mainly on selenium content in soils and its availability (bioavailability) for plants. The bioavailability for plants depends mainly on the chemical forms of selenium in the soil, but is also influenced by some physicochemical parameters of the soil. The absorption of Se^{+6} is generally higher than that of Se^{+4}. The selenium content in animal products depends on its content in the diet consumed by the animals (14).

In most countries, legumes,cereals and derivatives are the main source of selenium intake, due to their predominance in the diet. In few cases, meat, poultry and seafood are the main contributing food categories to selenium intake (14).

Agricultural activities can influence Se levels in foodstuffs. In some areas of the world Se has been added to fertilizers in order to increase Se levels in cultivated plants and indirectly improve selenium status in humans (3).

As to our study it is difficult to integrate the results of Se determinations because we donot have the normal Se levels for our population and we donot know the content of Se in our soil. So we compared our results with those reported by other European countries (Table III). The different results are accounted for by the differences in dietary intake and soil content.

It is difficult to obtain unbiased estimates of dietary trace-element intake, no matter of the epidemiological method used. Due to high variability in Se content of different foodstuffs and interindividual diet variations, the procedure for estimating Se dietary intake is questionable (14). The recommended adult daily Seintake varies considerably between authors, countries and organizations. Schrauzer and White (cited by Dharmasena, 2014) estimated a daily Se intake per person in the range 90-168 µg. In Asia, Africa and many European countries the daily Seintake is below the recommended dose. Se deficiency has negative consequences mostly in seniors, pregnant and lactating women, growing and developing children (3). In order to provide a safe total Se intake the reference dose (RfD) from all nutritional sources for a 70 kg adult has

been set at 350µg/day, corresponding to 5 µg Se/kg body weight/day (11).

We also found that Se status correlated positively with age, which is in line with the findings from most similar studies (11).

Regarding TPO Abs, the available data show that in 11 Se supplementation trials in patients with autoimmune thyroiditis, 7 have shown benefit and positive effects on serum TPO-Ab concentrations. Another fact is that high Se levels in the blood are associated with low TPO levels (15).

Our study revealed a negative correlation between Se and TPO – Ab but lacking statistical significance (p=0.099). Our study group was divided into 3 groups according to Selevel (and compared them with the normal Se levels in Europe - plasma Se =60g/L -120g/L). We found no significant differences between TSH and ATPO values depending on Se level. Instead, for low Selevels we obtained a negative correlation with ATPO-Ab, which suggests the possible benefits of selenium treatment in autoimmune thyroiditis (tab. II).

New studies are required to evaluate the evolution of antithyroid antibodies during Se supplementation and the development of reliable methods for direct determination of Se level in the body and especially the amount necessary to ensure homeostasis and prevention of thyroid specific autoimmune pathology. Also, little is known about the effect of mild selenium deficiency in the context of normal iodine intake (situation specific for our country).

CONCLUSION

In our study the low Selevels were associated with high TSH levels, suggesting apossible involvement ofselenium inthyroid function.

Our results showed a weak negative correlation between selenium and antithyroid antibodies, so selenium supplementation may improve the evolution of thyroid autoimmunity.

Meaningful clinical outcomes should be demonstrated before Se supplementation can be routinely suggested in patients with thyroid autoimmunity.

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NEWS

CARDIAC REHABILITATION AFTER SURGICAL CORRECTION OF CONGENITAL HEART DISEASE

Comprehensive cardiac rehabilitation is an important element of the comprehensive management of grown-up congenital heart disease patients after surgical correction of congenital heart disease but access to this treatment is still limited. To assess the effect of a comprehensive cardiac rehabilitation program on physical capacity, exercise tolerance, quality of life, and severity of depressive symptoms in grown-up congenital heart disease patients long-term after surgical correction of congenital heart disease. Implementation of a comprehensive cardiac rehabilitation rogram improves physical capacity, exercise tolerance, and quality of life and reduces depressive symptoms in patients late after surgical correction of congenital heart disease. Introduction of such programs seems reasonable as a supplement to the holistic care for grown-up congenital heart disease patients (Gierat-Haponiuk K, Haponiuk I, Szalewska D, Chojnicki M, Jaworski R, Niedoszytko P, Leszczyńska K, Bakuła S. Effect of complex cardiac rehabilitation on physical activity and quality of life during long-term follow-up after surgical correction of congenital heart disease. *Kardiol Pol.* 2015;73(4):267-73).

Teodor Vasilcu

TUBEROUS SCLEROSIS –PRENATAL DIAGNOSIS OF CARDIAC RHABDOMYOMA – CASE REPORT

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TUBEROUS SCLEROSIS – PRENATAL DIAGNOSIS OF CARDIAC PHABDOMYOMA (CASE REPORT) (Abstract). Primary cardiac tumors are rare in medical practice, with a prevalence of 0.0017-0.28% in autopsy examinations. In intrauterine life, the incidence of cardiac tumors has been reported to be of approximately 0.14%. Rhamdomyoma is the most frequent tumor formation during fetal period and childhood, accounting for 70% of all primary tumors in the fetus, neonate and infant. Diagnosing heart rhabdomyomas occurs most often by accident during fetal morphology at 22 weeks. We will present a case of a newborn diagnosed with fetal echocardiography in the 23 week of gestation having multiple cardiac tumors. **Keywords**: TUBEROUS SCLEROSIS, FETAL ECOGRAPHY, HYPO-MELANOTIC MACULES, CARDIAC RHABDOMYOMA.

Advances in medicine and technology made it possible to diagnose a growing number of diseases, many of them in the fetal period.

Cardiac tumors can be benign or malignant having as a starting point muscle layers or pericardium. These can be primary or secondary, of the metastatic type.

Primary cardiac tumors are rare in medical practice, with a prevalence of 0.0017-0.28% at autopsy examinations. In intrauterine life, the incidence of cardiac tumors has been reported to be approximately of 0.14%. Most of them have a benign nature, only 10% of them being malignant (1, 2, 3, 4).

Rhabdomyoma is the most frequent tumor formation during fetal period and in childhood, accounting for 70% of all primary tumors in the fetus, newborn and infant (5,6). It is most often located in the ventricles but it may also originate in the atria (7, 8).

Diagnosing heart rhabdomyomas occurs most often by accident during fetal morphology at 22 weeks. While the association between tuberous sclerosis and heart rhabdomyomas has been recognized, association with only one tumor formation cannot lead to the diagnosis of tuberous sclerosis.

Tuberous sclerosis is a monogenic, multisystemic disease with dominant autosomal transmission, high penetration and variable expressivity, characterized by skin, cerebral and characteristic visceral manifestations.

CASE REPORT

We are presenting the case of a female newborn, born by caesarean section, cranial presentation, with a birth weight of 4000 g

and one minute Apgar 8 score, admitted to the Cardiology Department of ”Sf. Maria” Emergency Children's Hospital in Iaşi for seizures accompanied by perioronasal cyanosis.

From the hereditary familial information, we could observe that both parents are apparently healthy and the patient’s brother was postnatally diagnosed with pyloric stenosis.

Regarding pregnancy history, we found that the mother was diagnosed with chickenpox at 23 weeks of gestation.

The obstetrical control performed at 26 weeks of amenorrhea, the mother did a fetal ultrasound and morphology, based on which the doctor identified echogenic images in the brain and in the heart.

In the brain, there was a bilateral presence of non-homogenepus hyperechogenic images, imprecisely demarcated: at the right hemisphere in temporal lobe measuring 11/6 mm and in frontal lobe 4/4 mm and in the left hemisphere, in frontal lobe measuring 10/7 mm and 5/4 mm.

In the heart, the obstetrician noted the presence of a heterogeneous hyper echogenic images, vaguely defined, with dimensions of 6/4 mm in the thickness of the right ventricular wall. He also noticed an image with the same specifications having 13/7mm (fig. 1, 2) in the thickness of the papillary muscle of the left ventricle.

Therefore, based on fetal morphology, the obstetrician established the diagnosis of cardiac tumor (rhabdomyoma).

Postnatally, an echocardioecography was performed that revealed multiple formations, most in the left ventricle, measuring 18/16/12 mm, with polylobate contours, with implantation to the side wall of the ventricle. In the interventricular septum, in the apex and in the thickness of the myocardium, three other formations of infracentimetric size and in the right ventricle, another oval image of 6/7 mm under septal cusp of the tricuspid.

Fig. 1. Fetal echocardiography, hyperechoic formation, non-homogenous in the right ventricle.

Fig. 2. Fetal echocardiography –presence of the two imprecisely defined hyperechogenic on the right and left ventricle.

Clinical examination during last hospitalization showed that the patient had good general condition, pale skin, perioronasal cyanosis, multiple hypopigmented macules, spread on the entire body surface, the largest measuring 2/6 cm, located on the left flank. No murmur was detected by listening. The abdomen was supple, depressive, mobile with respiratory movements. In

terms of neuromotor activity, the child was developed normally.

Biologically, we could note the presence of a mild thrombocytosis and hyposideremia.

Performed electroencephalogram showed a spontaneous route with motion artifacts, with releases of ample waves in all derivations.

Electrocardiography revealed a sinus rhythm with a 160-170 beats/minute, electrical axis 60 degrees, PQ =0.12s, with normal morphology, QT=0.26s (upper limit).

Transfrontal ultrasound was normal, no modifications in the brain substance were found.

As the child has numerous seizures, treated at that moment with Tonotil, we recommended a neurological examination that showed depigmented patches of different sizes on the trunk and limbs, epileptic spasms in extension and generalized motor seizures. Therefore, the pediatrician made the diagnosis of Bourneville tuberous sclerosis and West syndrome with lesions and recommended a CT brain scan.

Since tuberous sclerosis is a multisystemic disease with dominant autosomal transmission, we also recommended that should be performed that raised again the suspicion of Bourneville tuberous sclerosis. Samples for DNA tests were collected that were transferred for confirmation to a specialized laboratory.

Also, it was recommended to parents that an echocardiography should be made to see if any of them showed changes typical to tuberous sclerosis. Cardiological investigations of family members showed no evidence of cardiac tumors in none of them.

The ophthalmologic examination that we recommended, including fundus examination, revealed no occurrence of tumors in the retina.

Ultrasounds performed during hospitalization showed the presence of left ventricular of tumors measuring 17.5/11.1 mm and 25.2/16.7mm. On the side wall of the right ventricle, we were able to observe two growths of 4.2/1.8 mm and 3.6/1.8mm. Inside the right ventricle, a tumor of 8.1/6.7 mm was found, and at the tip of the left ventricle two tumor formations of 10.7/4.3mm and 12.9/3.1-5.3mm, respectively. The patient also showed left ventricular dysfunction and permeable oval foramen (fig. 3, 4, 5).

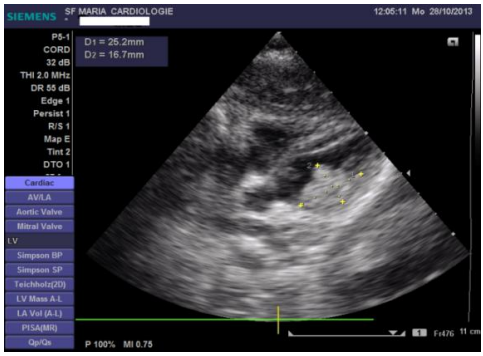

Fig. 3. Echocardiography - subcostal section. Shows numerous hyperchogenic formations in the right and left ventricle

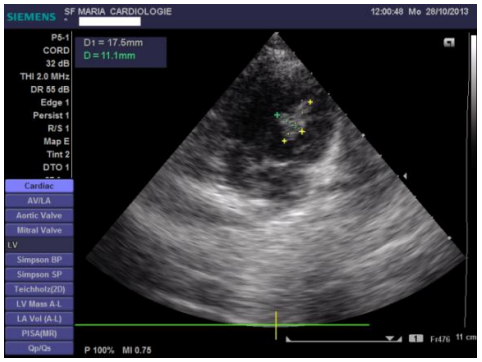

Fig. 4. *Echocardiography apical 4 chamber view – shows 12 tumoral formations*

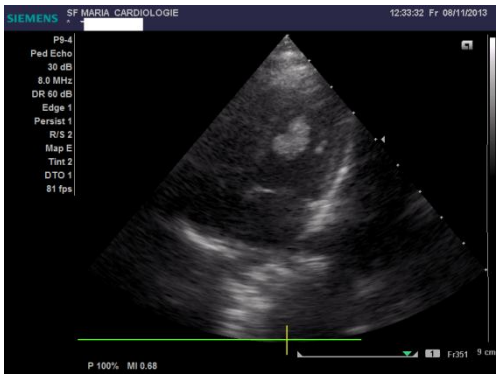

Fig. 5. Echocardiography, apical 4 chamber view. Tumor formations at the top of left ventricle

The performed thoracoabdominal CT scan showed an expansive formation, solid density, iodophile, natively and post-contrast applied intravenously homogenous, well defined, with regular outline, which occupies the upper floor and the floor above middle mediastinum, with upper sizes 24/65/45 mm having no effect on the mass and with anterior relation to anterior chest wall; posterior relation to trachea, the internal carotid artery, vascular pedicle and the front of the heart; lateral to pulmonary pleura and superior to a plan section passing through the inferior edge of the clavicle (fig. 6, 7).

Fig. 6. Formations in upper floor and middle floor of anterior mediastinum

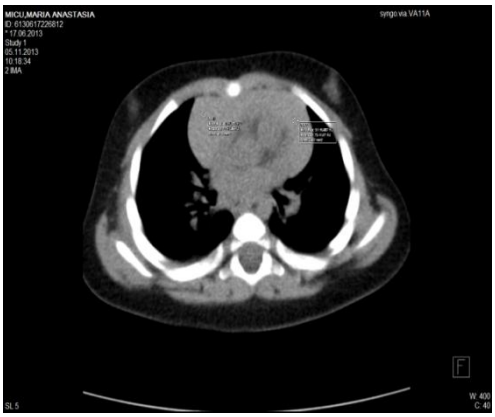

Fig.7. Thoracoabdominal CT scan – showing mediastinal formations

CT brain examination showed hyper dense areas after intravenous contrast, disseminated to the brain substance, with varying sizes, the largest of 9/7mm, in the left frontal lobe, without mass effect on adjacent structures (fig. 8).

Fig. 8. Brain CT showing hypersense areas after CIV in brain substance

Final diagnosis was: Bournevile tuberous sclerosis that was attributed to changes in the skin, presence of tumors in the brain and heart, permeable oval foramen, left ventricular dysfunction and West syndrome with lesions.

DISCUSSION

Rhabdomyomas are the most common

cardiac tumors found in newborn and children at a rate of 51-96% associated with tuberous sclerosis (9).

While the association between tuberous sclerosis and heart rhabdomyomas has been recognized, the association with only one tumor formation cannot diagnose tuberous sclerosis.

TABLE I
Positive diagnostic criteria for tuberous sclerosis

Major criteria	Minor criteria
1.Hypomelanotic macules (≥3, at least 5-mm diameter) 2. Angiofibromas (≥3) or fibrous cephalic plaque 3. Ungual fibromas (≥2) 4. Shagreen patch 5. Multiple retinal hamartomas 6. Cortical dysplasias 7. Subependymal nodules 8. Subependymal giant cell astrocytoma 9. Cardiac rhabdomyoma 10. Lymphangioleiomyomatosis (LAM) 11. Angiomyolipomas (≥2)	1. "Confetti" skin lesions 2. Dental enamel pits (≥3) 3. Intraoral fibromas (≥2) 4. Retinal achromic patch 5. Multiple renal cysts 6. Nonrenal hamartomas

Detecting multiple growths in the heart during fetal period diagnoses the Bourneville syndrome before the appearance of skin signs or seizures in the postnatal period (10, 11).

Tuberous sclerosis is a monogenic, multisystemic disease with dominant autosomal transmission, high penetration and variable expressivity.

This occurs in response to mutations of two tumor suppressing genes, TSC1 coding hamartin and TSC2 coding tuberin. TSC2 gene is located near the gene involved in the production of polycystic kidney disease, which in case of larger deletions (seen in 2% of cases) makes patients show both pathologies.

To confirm the clinical diagnosis, the criteria of "The 2012 International Tuberous Sclerosis Complex Consensus Group" are used. The presence of two major or one major criteria makes the diagnosis to be certain (15).

In the presented case, the clinical diagnosis clinical diagnosis was made on the presence since intrauterine life of heart rhabdomyomas, confirmed later postnatally by both echocardiography and by the CT examination, the presence of growths in the brain and hypomelanotic macules (>3) (13,14).

The following complications may occur in the future: arrhythmias, most commonly ventricular, loss of myocardial function due to the increase of the tumor mass located in the side wall of the left ventricle, in the papillary muscles and in the interventricular septum that may be conducive to decreased cardiac output and congestive heart failure. It has been observed that the Wolf-Parkinson-White syndrome has a higher incidence in patients with tuberous sclerosis, it is about 2% (1.12). Depending on the size of the tumor and the appeared complications, death may occur in some cases.

CONCLUSIONS

The evolution of techniques detecting congenital defects or diseases in the early stages allowed the pre-natal diagnosis of

numerous pathologies. Today, the suspicion of tuberous sclerosis may be raised since the pre-natal period, if the presence of cardiac rhabdomyomas is identified in the fetus during fetal morphology.

As specific features of the case, we could observe the pre-natal diagnosis of tumor formation during fetal morphology and even though tuberous sclerosis was with dominant autosomal transmission, no family members had skin changes or heart tumors, raising suspicion of a de novo gene mutation. However, to support this aspect genetic testing is recommended.

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CAUSES OF DEATH IN PATIENTS WITH STAGE 0-II BREAST CANCER

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CAUSES OF DEATH IN PATIENTS WITH STAGE 0-II BREAST CANCER (Abstract): **Aim**: To analyze the main causes of death in patients with stage 0-II breast cancer who undergo breast conserving surgery or radical mastectomy, and to establish the role of imaging surveillance protocols following breast cancer treatment. **Material and methods:** We conducted a retrospective medical record review between January 2005 and December 2012, when breast cancer was the primary cause of death for 113 inpatients. All patients were admitted to the Oncology Clinic of the Iasi Regional Cancer Institute (IRCI), Romania. Patients were stratified by clinical stage 0, I and II, of which 33 (29.2%) patients were managed by breast conservation therapy and 80 (70.8%) underwent radical mastectomy. From the patient medical records all diagnostic imaging studies performed (ultrasound, radiography and computed tomography) were identified and analyzed according to a standard protocol for imaging the postoperative breast. **Results:** Bone, liver, lung, lymph nodes and local-regional recurrence were the most common sites for metastasis, while the most frequent cause of death were metastases to the liver, pleura, lung and brain. The time interval between recurrence and death ranged from 0-24 years among patients with one type of metastasis, and decreased to 0-3 years since the last recurrence for patients with multiple metastases. **Conclusions:** The current imaging protocol for monitoring the postoperative breast could be optimized to improve the prognosis and quality of life in patients with stage 0-II breast cancer. **Keywords**: BREAST CANCER, METASTASES, IMAGING PROTOCOL.

The main cause of death for women in Europe is breast cancer (1, 2). Metastases to the liver, pleura, lung and brain, diagnosed by clinical and imaging protocols, are most often responsible for patient death (3). Diagnoses of local recurrence or distant metastases in infra clinic stages allow immediate therapeutic decision making in order to improve the quality of life. In this context, the current imaging surveillance protocols following breast cancer treatment are aimed to be improved based on internationally agreed guidelines proposed by the European Society for Medical Oncology (ESMO) (4), St. Gallen Consensus Conference, Advanced Breast Cancer Consensus Conference (ABC), the German Gynecological Oncology Working Group (AGO), and National Comprehensive Cancer Network (NCCN).

The objectives of this study were: to assess the risk of local recurrence and death after breast cancer surgery; to investigate the benefits and limitations of imaging

studies in detecting local recurrence and distant metastases, and to improve the current imaging surveillance protocol following breast cancer treatment.

MATERIAL AND METHODS

We conducted a retrospective medical record review between January 2005 and December 2012, when breast cancer was the primary cause of death for 113 in patients admitted to the IRCI Oncology Clinic.

Breast cancer patients were stratified according to clinical staging at the time of diagnosis into stage 0, I and II. All patients had undergone breast surgery between January 1983 and December 2011, of which 33 (29.2%) patients were managed by breast conservation therapy and 80 (70.8%) underwent radical mastectomy.

Data drawn from patient medical records were compared using multiple regression analysis, regression coefficient, frequency distribution, chi-square test and Pearson's product-moment r ($-1 < r < 1$, the more closely the two variables are related, $r = 0$, there is no relationship between the variables). A P value of less than 0.05 was considered statistically significant. All statistical tests were performed with the Statistical Package for the Social Sciences (SPSS for Windows, Version 16.0. Chicago, IL, USA).

RESULTS

Of the 113 patients included in the study, 49 (43.4%) patients died of breast cancer-related causes and 64 (56.6%) patients had other causes of death (cardiac pathology, stroke, colon, stomach, pancreas and ovary neoplasms). Deaths resulting from breast cancer were due to local recurrence or distant metastases. The most common types of recurrence were bone metastases - 40 (35.4%) patients, liver metastases - 24 (21.2%) patients, lung metastases - 22 (19.5%) patients, local recurrence - 20 (17.7%) patients, and lymph node metastases - 18 (15.9%) patients. Most of the recurrences occurred within 3 years following the surgery. The mean survival time from recurrence to death ranged from 0.95 years (in patients with brain metastases) to 5.73 years (patients with local recurrence) (tab. I).

TABLE I

Case distribution by type of recurrence, minimum, maximum and mean survival time from recurrence to death

Type	Patients (%)	Minimum survival time (years) from recurrence to death	Maximum survival time (years) from recurrence to death	Mean survival time (years) from recurrence to death
Local recurrence	20 (17.7)	<1	24	6
Bone metastases	40 (35.4)	<1	10	3
Liver metastases	24 (21.2)	<1	8	2
Lung metastases	22 (19.5)	<1	5	1
Lymph node metastases	18 (15.9)	<1	11	3
Pleural metastases	12 (10.6)	<1	3	1
Brain metastases	11 (9.7)	<1	5	1
Skin metastases	9 (8)	<1	8	2
Peritoneal metastases	2 (1.7)	1	2	2

Of the 33 patients treated with breast conserving surgery, 11 (33.3%) patients had no recurrence and 22 (66.6%) patients had local recurrence or single/multiple organ metastases. Of the 22 patients with local recurrence or distant metastases in 17 (77.2%) the cause of death was breast cancer.

Of the 80 patients treated with radical mastectomy, 22 (27.5%) patients had no recurrence, and 58 (72.5%) patients had local recurrence or single/multiple organ metastases. Of the 58 patients with local recurrence or single/multiple organ metastases, 34 (58.6%) patients had breast cancer as the cause of death.

Comparative evaluation of local recurrence and distant metastases in breast cancer patients treated with breast conserving surgery or radical mastectomy showed that bone, liver, pleural and skin metastases were diagnosed in similar frequencies. Local recurrence, lymph node and brain metastases were more frequently observed in patients treated with breast conserving surgery. Metastatic spread to the lungs was significantly more frequent in patients treated with radical mastectomy.

Regardless of breast cancer surgery type, lung, pleural, liver and brain metastases were causes of death in breast cancer patients ($p<0.0001$). Pearson's product-moment correlation coefficient showed a significant negative correlation between mean survival time from recurrence to death and the presence of lung (r= -0,562), pleural (r= -0,394), liver (r= -0,506) and brain (r= -0,375) metastases.

Imaging methods used to diagnose recurrence /distant metastases were:

- chest CT ± chest radiograph, for the assessment of lung and pleural metastases;
- abdominal ultrasound ± CT scan for the detection of liver metastases;
- CT brain ± MRI for the detection of brain metastases.

DISCUSSION

In this study group we found that the most common sites of secondary relapse include the bone, liver, lung, and local lymph nodes. Leading causes of breast cancer death were liver, pleural, lung and brain metastases (5-9). While breast cancer patients with bone metastases and local recurrence had the longest survival interval (10 years for bone metastases and 24 years for local recurrence), the most aggressive lesions were brain metastases that caused death within the first 2 years after diagnosis (fig. 1).

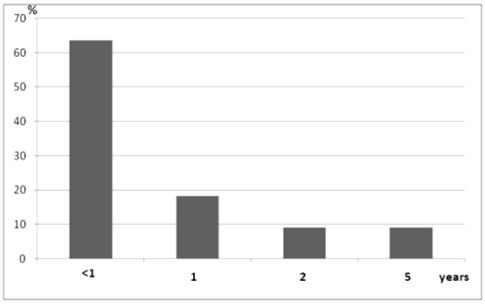

Fig. 1. Time interval from the diagnosis of brain metastases diagnosis to death in breast cancer patients

Patient survival was significantly influenced by relapses. We found that in patients with one metastatic lesion death occurred within 24 years from the time of their diagnosis, while in patients with two or more metastatic lesions death occurred within 3 years after the last recurrence.

All patients included in this study were monitored with different imaging tests at different times. For the diagnosis of lung and pleural metastases chest X-rays were

performed each year. When a clinical suspicion of lung or pleural metastasis was raised, thoracic CT was performed. It is noteworthy that metastatic lesions less than 5 mm in diameter are often not visible on standard chest X-rays (10). Hence, the necessity to include thoracic CT in the imaging surveillance protocols. For the diagnosis of liver metastases abdominal ultrasound at various time intervals (every 6 months or annually) was performed, and in doubtful cases an abdominal CT scan. The study showed that liver metastases occurred in the first 3 years after surgery, so it is appropriate to perform an abdominal ultrasound every 6 months during the first three years after surgery, then yearly. It was also noted that homogeneous ultrasound appearance of the liver does not exclude the presence of secondary lesions (11). The introduction of abdominal CT scans in imaging surveillance protocols for breast cancer patients could diagnose the liver metastases at infra clinic stage. For the diagnosis of brain metastases when clinical suspicion was raised, a cranial CT scan was indicated. In 3 of the 11 cases with brain metastases, brain MRI was indicated to establish the uniqueness of the lesion. Surgical treatment of brain metastases was indicated only if the lesion was unique and had an easily approachable location (12, 13). MRI imaging remains decisive in patients with brain metastases, which can be treated surgically.

CONCLUSIONS

In our study, the most common causes of death in breast cancer patients treated with breast conserving surgery or radical mastectomy were metastases to the liver, pleura, lung and brain. Optimizing current imaging surveillance protocols in patients surgically treated for breast cancer by using ultrasound, computed tomography and MRI can improve the quality of remaining life.

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NEWS

EMULSIFYING AND ANTIOXIDANT PROPERTIES OF APPLE AND GRAPE TANNINS

Preparation of an emulsion requires amphiphilic compounds that can stabilize it. The emulsions are unstable due to the surface tension between water and oil which prevents the increase of the interfacial area. Moreover, the stabilizing agents are needed to control the two competing (attracting and repulsive) forces that occur in the preparation process. Another problem would be the oxidation of lipids which are added in an emulsified system. Therefore, compounds with both stabilizing and antioxidant properties are needed. Tannins are a class of polyphenols that are widely spread in nature. Figueroa-Espinoza *et al*. studied the properties of catechin, apple and grape seed tannins, as well as their stabilizing and antioxidant capacity. They used catechin and tannin fractions isolated from grape seeds (*Vitis vinifera*, var. Shiraz) and apples (*Malus sylvestris*, var. Kermerrien). The degree of polymerization of tannin fractions was studied by HPLC after depolymerization. The antioxidant properties were evaluated using the conjugated autoxidizable triene method. The oil in water emulsions stabilized with tannins were compared with the emulsions obtained using polyvinylalcohol (PVA) and polyoxyethylene hydrogenated castor oil. The results showed that, in certain conditions, oxidized tannins provide an emulsion stability comparable to that of PVA. Furthermore, grape tannins showed higher antioxidant effects in comparison with the apple tannins. Therefore, oxidized tannins could be used as an alternative source of antioxidant emulsifiers (Figueroa-Espinoza MC, Zafimahova A, Maldonado Alvarado PG, Dubreucq E, Poncet-Legrand C. Grape seed and apple tannins: Emulsifying and antioxidant properties. *Food Chem* 2015; 178: 38-44).

Constantin Ciobanu

SMOKING CESSATION, PULMONARY REHABILITATION AND QUALITY OF LIFE AT SMOKERS WITH COPD

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SMOKING CESSATION, PULMONARY REHABILITATION AND QUALITY OF LIFE AT SMOKERS WITH COPD (Abstract): **Aim** was to compare the quality of life (QoL) at the smokers with COPD who quit smoking and have completed a pulmonary rehabilitation program with those who received usual treatment. **Material and Methods**: 437 smokers with COPD stages II and III were counseled and treated to smoking cessation and completed COPD Assessment Test (CAT) at the beginning and at the end of 12 weeks of treatment. 113 patients were enrolled in a 12 weeks supervised pulmonary rehabilitation program (PR group) and 324 smokers received usual treatment for COPD and were monitored for 12 weeks (non-PR group). The initial HbCO > 2%, and every patient completed an initial CAT. **Results:** Initially, CAT scores varied between 26 and 38. At the end of the period, CAT scores in the PR group were between 7 and 16 and in the non-PR group CAT scores were between 20 and 26, and additionally the smoking cessation failure at an important number of non-PR members (45.98%) (in PR group, 16.81%). There were reported as well higher rates of long-term abstinence in the adherent patients to PR than the other group. **Conclusions**: COPD smokers who completed the PR presented important QoL improvements, better CAT score than COPD smokers usually treated. A better PR adherence seems to be related with higher rates of sustained long term abstinence. **Keywords:** COPD, SMOKING CESSATION, PULMONARY REHABILITATION, CAT.

Smokers included in pulmonary rehabilitation (PR) is an often debate, considering PR a real aid to smoking cessation and improving the quality of smokers life.

PR is an integral part of the clinical management and health maintenance of those patients with chronic respiratory disease who remain symptomatic or continue to have decreased function despite standard medical treatment. PR aims to reduce symptoms, decrease disability, increase participation in physical and social activities, and improve the overall quality of life (QoL) for patients with chronic respiratory disease (1, 2, 3, 4, 5, 6).

The COPD Assessment Test (CAT) is a new questionnaire for people with Chronic Obstructive Pulmonary Disease (COPD). It's designed to measure the impact of the condition on a person's life, and how this

changes over time. The CAT is very simple to administer, and aims to help doctors and nurses better manage a patient's COPD. The CAT is a short, simple and validated assessment, which measures the impact of COPD on a patient's life in an objective manner (7, 8).

The objective of this study was to compare the improvement of quality of life, evaluated through CAT, between smokers with COPD who completed a PR program complementary of the smoking cessation treatment and those who received usual treatment.

MATERIAL AND METHODS

Data were obtained from the database of Smoking Cessation Center, Rehabilitation Clinical Hospital, Iasi, Romania.

Inclusion criteria were:

1) evidence of COPD according to GOLD standards (2010) (9);

2) an initial HbCO > 2%, that suggests active smoking status (10);

3) an initial COPD Assessment Test (CAT) completed by every patient.

From 437 smokers who presented at the Smoking Cessation Center between January 1 and September 1, 2011, were diagnosed with COPD stages II and III (GOLD 2010), only 113 patients (25.8%) were enrolled in a 12 weeks supervised PR program, complementary the smoking cessation treatment (PR group), and 324 smokers

74.1% received usual treatment for COPD in addition the treatment for stop smoking, monitored as well for 12 weeks (non-PR group). All patients completed the CAT questionnaire at the beginning and at the end of the determined period.

The CAT has eight items. Patients read the two statements for each item, which describe the best and worst scenario, and decide where on the scale (0-5) they fit. This system was chosen because it is reliable and simple to use. Scores for each of the 8 items are summed to give single, final score (minimum 0, maximum 40) – this is a measure of the overall impact of a patient's condition on their life.

Scores for the individual items within the questionnaire will provide insight into the relative influence that the different components of COPD have on its overall impact on a patient's life – thus, they will highlight problematic areas, which can be explored further during consultation and ultimately addressed through intervention.

Current management guidelines – such as those produced by the National Institute of Health and Clinical Excellence (NICE) (11) and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) – emphasize the need for regular and effective patient monitoring and assessment. The CAT has been developed in line with the goals of these guidelines, with the aim of making these tasks easier and less time-consuming.

The data collected were analyzed in a specific program for statistical analysis (Statistical Package for Social Sciences ™-*SPSS 17.0*).

RESULTS AND DISCUSSION

PR group COPD subjects mean aged 59.02 +/- 11.424 (lim. 40-86 years old) were classified in stage II (n=50) and COPD stage III (n=63). Non-PR group COPD subjects, mean aged 67.19 +/- 10.45 (lim. 42-88) were classified in stage II (n=177) and III (n=147).

Initially, CAT mean scores of smokers with COPD were 26.84 in stage II and 30.98 in stage III and finally decreased to 10.32 and 13.95. At the end of the 12

weeks period, the CAT scores variations in the PR group were between 6-16 in COPD II patients and 7-16 in COPD III ones, comparing to the non-PR group, where CAT scores shown little improvements since the beginning of the treatment, with values between 20-26 (fig. 1), additionally to smoking cessation failure at an important number of non-PR members (45.98%) than the PR group (16.81%). There were reported as well higher rates of short and long-term abstinence at the adherent patients to PR than the other group.

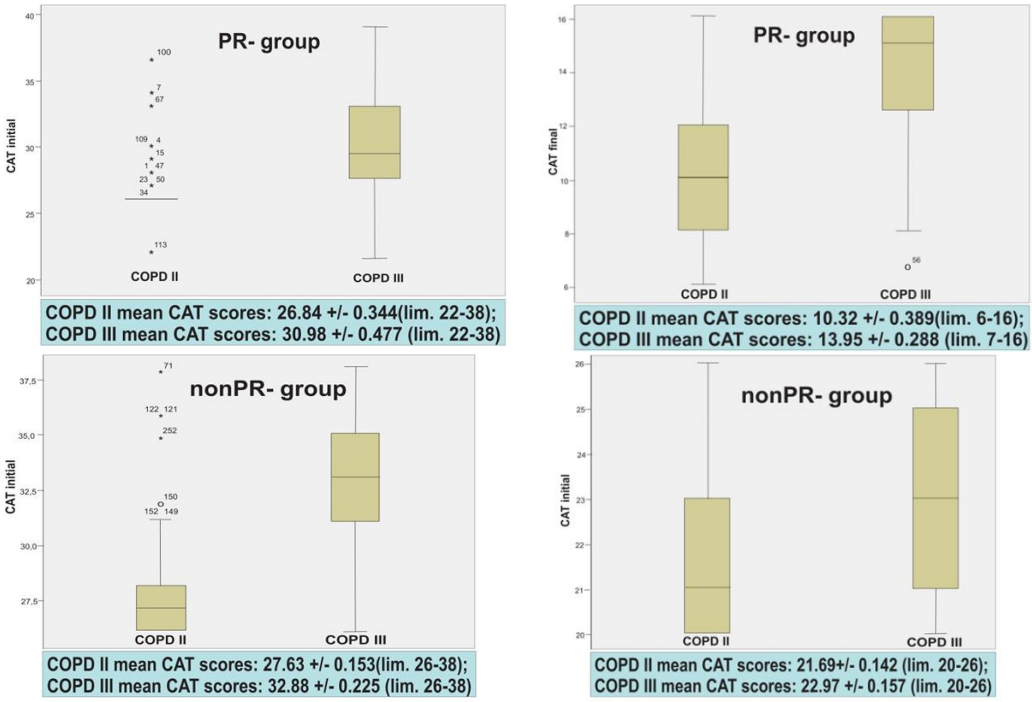

Fig. 1. CAT scores distribution by COPD stages in PR group and non-PR group

CONCLUSIONS

Although current smokers are often expected to have a small PR adherence even they intend to stop smoking, COPD smokers who completed the PR presented important QoL improvements, better CAT score than COPD smokers usually treated. Moreover, a better PR adherence seems to be related with higher rates of sustained long term abstinence.

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NEWS

POLIO PUZZLE AT THE BEGINNING OF 2015

Polio eradication is a very expensive campaign since 1988. In January, Ministers of Health worldwide convened at WHO's Executive Board meeting, to set global public health policies. Among other topics, representatives reviewed the current polio epidemiology and global preparedness plans for the phased removal of oral polio vaccines. The first wild poliovirus type 1 (WPV1) case of 2015 is reported this week, from Pakistan. The case had onset of paralysis on 3 January, from Khyber Pakhtunkhwa. A total number of 6 cases of polio with wild virus were confirmed in Pakistan until 4 February 2015. Afghanistan: no new WPV1 cases have been reported in the past weeks and the most recent case had onset of paralysis on 4 December 2014. Nigeria's total WPV1 case count for 2014 remains 6, compared to 53 in 2013. In Nigeria, the Expert Review Committee on Polio Eradication and Routine Immunization met last month to discuss aggressive strategies to urgently eradicate both wild- and vaccine-derived polioviruses in the country as rapidly as possible. National Immunization Days NIDs are planned in Cameroon in January using trivalent oral polio vaccine (OPV) and in Cameroon, Chad, and Gabon in February using bivalent OPV. Subnational Immunization Days SNIDs are planned in the Central African Republic in February and in Angola, Cameroon, the Central African Republic and the Democratic Republic of the Congo in March. The total number of cases that were reported in the Horn of Africa in 2014 was 6: 1 WPV1 in Ethiopia and 5 WPV1s in Somalia. Three cases of WPV1 were reported in the Middle East in 2014 2 in Iraq and 1 in Syria. Even as polio programme staff across West Africa support efforts to control the Ebola outbreak affecting the region, efforts are being made in those countries not affected by Ebola to vaccinate children against polio to create a buffer zone surrounding the Ebola affected countries. The Ebola crisis in western Africa continues to have an impact on the implementation of polio eradication activities in Liberia, Guinea and Sierra Leone. The Polio Eradication and Endgame Strategic Plan 2013–2018 is a comprehensive, long-term strategy that addresses what is needed to deliver a polio-free world by 2018. (WHO. Global polio eradication initiative, 2015).

Alina Manole

RARE PEDIATRIC CUTANEOUS T-CELL LYMPHOMA

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RARE PEDIATRIC CUTANEOUS T-CELL LYMPHOMA (Abstract): We describe a rare case of an adolescent with T-cell cutaneous lymphoma, having a history of intense pruriginous, erythematous and hyperpigmented skin lesions. Several considerations regarding diagnostic work-up and treatment must be emphasized. Erythematous, pruritic patches, unresponsive to common topical/general treatment should raise the suspicion of more serious immuno-allergic or neoplastic conditions, among which, cutaneous lymphoma could be a rare, but a realistic possibility in pediatric field. Long term follow-up by a pediatric haemato-oncologist and dermatologist team is mandatory for these patients. **Keywords:** CUTANEOUS LYMPHOMA, CHILD, PARAPSORIASIS.

In pediatric oncology Non-Hodgkin's Lymphoma is the third most common solid tumor, and constitute 5-7% of all malignancies, most often located in abdominal and thoracic regions. However, Non-Hodgkin lymphomas may have atypical primary presentations like otorhinolaryngology (1), central nervous system (CNS) or skin. Cutaneous T-cell lymphoma (CTCL) is a non-Hodgkin lymphoma that commonly occurs in elderly individuals (2,3,4,5). It is characterized by skin infiltration of malignant T lymphocytes. Mycosis fungoides (MF) is a subtype of CTCL while Sézary syndrome is its leukemic variant. The incidence varies among different age groups, with only 0.5 - 5% of cases occurring before 20 years old (3). The wide variety of clinical features and its similarities with other dermatoses may delay the diagnosis in early stages of MF. An indolent course is noticed in children and adolescents diagnosed with MF restricted to skin involvement (5). These patients require a multidisciplinary approach-pediatric haemato-oncologists, pathologists and dermatologists to improve their outcome.

CASE REPORT

We report the case of an adolescent girl who presented with a 5-months history of generalized body patches. The lesions were highly pruriginous, with mixed, erythematous and hyperpigmented regions of different sizes and shapes, with an initial chest and abdomen concentration, and a subsequent diffusion towards the upper and low-

er limbs. A cutaneous tumor of 5 cm below the right costal edge noticed. (fig. 1). Associated inguinal lymphadenopathy was observed. The patient denied drug abuse and no history of exposure to chemicals or radiation could be found.

No hepatosplenomegaly, intraoral lesions or arthralgia were found.

Fig. 1. Erythematous patches on the trunk and abdomen, erythematous plaque on the abdomen

Laboratory tests showed peripheral blood eosinophilia, with no other abnormalities. Antibodies against Cytomegalovirus, Epstein Barr virus, Toxoplasma were negative. Screening for B, C hepatitis B and C viruses, HIV, EBV, and CMV were performed for differential diagnosis of skin lesions and confirmation of chronic hepatitis and hepatic neoplasms (4).

Biopsy samples from skin lesions, lymph nodes and cutaneous tumor and subsequent histopathological and immunohistochemical examination were performed. Both skin and tumor biopsy revealed the presence of atypical epidermotropic lymphoid infiltrate with cerebriform nuclei extend into the dermis, suggestive for Mycosis fungoides (fig. 2). Also, small plaque parapsoriasis was present in the skin lesions. Lymph node biopsy revealed reactive lymphadenitis. The biopsy samples were sent to Timisoara County Emergency Clinical Hospital for further investigations. The immunohistochemistry analysis revealed positive CD2, CD3, CD4, CD5, CD7, CD8 markers and negative CD20, CD30 markers in the atypical lymphocytes from skin and tumor samples, confirming the diagnosis of MF (fig. 3).

Computed tomography of the neck, chest, abdomen and pelvis showed multiple enlarged lymph nodes in the cervical chain, axillary region (measuring up to 2.62 cm), mesentery (measuring up to 1.05 cm) and in the inguinal region (measuring up to 1.82 cm).

The bone marrow aspiration showed normal cellularity at different stages of maturation. *Clinical, laboratory and histopathological* findings established the diagnosis of Mycosis Fungoides, T3N0M0B0 - tumor stage, IIB, according to International Society for Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer (ISCL/EORTC) revision to the staging of MF and Sezary Syndrome (8).

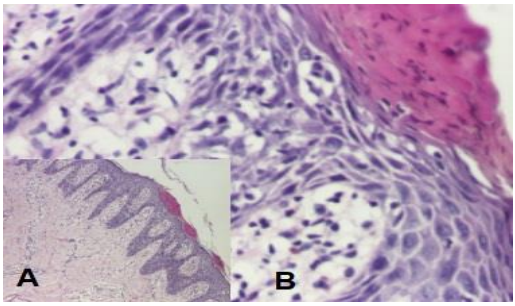

Fig. 2. A) Infiltrate into the epidermis and dermis (^{x}40 HE stains);
B) Infiltrate with abnormal cells with cerebriform nuclei, epidermotropic infiltrations of malignant cells (^{x}200 HE stains).

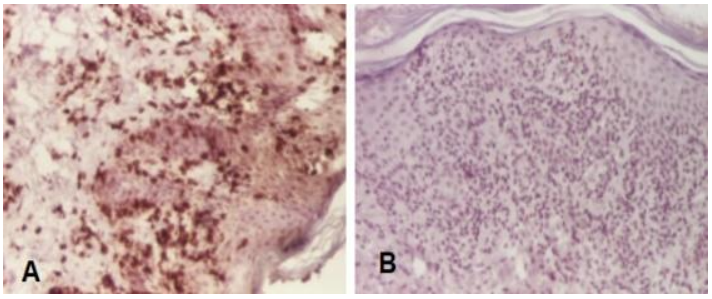

Fig. 3. A) Immunostaining - Abnormal cells positive for CD3(^{x}100);
B) Immunostaining - Abnormal cells negative for CD20 (^{x}100)

An initial regimen treatment with moderate potency topical steroids concomitant with narrowband ultraviolet B (NB-UVB) were used, followed-up by chemotherapy - cyclophosphamide, doxorubicin, vincristine and prednisone (regimen CHOP)-12 cycles, given in 21 days interval, and narrowband ultraviolet B-10 phototherapy sessions. CHOP chemotherapy sequence consisted in: Prednisone 100 mg PO daily Days 1 to 5, Doxorubicin 50 mg /m² IV Day 1, Vincristine 1.4 mg /m² IV (max 2 mg) Day 1, Cyclophosphamide 750 mg /m² IV Day 1.

The computed tomography and PET-CT were performed at the end of the treatment and showed no abnormalities. We noted a partial clinical response with the disappearance of pruritus and improvement of the skin lesions.

DISCUSSION

According to our previous experience (9, 10). pediatric neoplastic diseases may include some uncommon type of malignancies, requiring different confirmation procedures within the diagnosis process.

CTCL are quite rare among children with MF being a T-cell lymphoproliferative disorder and the major subtype of CTCL (2, 3). Data on clinical features, management, treatment response, disease progression in children are limited. MF is difficult to be distinguished from other entities in early stages due to its clinical polymorphism, mimicking benign skin disorders, that frequently occurs in pediatric age, such as eczema, atopic dermatitis, pityriasis, psoriasis. Therefore, consequently multiple biopsies may be necessary for

an accurate diagnosis (11, 12, 13).

In our case, the diagnosis of MF was suggested by histological and immunostaining parameters. Also, the association with small plaque parapsoriasis was observed in our patient.

Parapsoriasis manifests as large plaque and small plaque parapsoriasis (SPP). Both are caused by T-cell infiltration in the skin. If large plaque parapsoriasis is the earliest stage of CTCL (14,15), SPP is a benign form (16), but there were reported cases of progression to MF in adult population (17).

Our patient was diagnosed in an advanced stage-tumor stage IIB according to International Society for Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer revision to the staging of MF and Sezary Syndrome (8).

Topical and systemic corticosteroids are used for treating advanced stages of MF. Systemic chemotherapy along with phototherapy is the most useful treatment schemes for advanced stages (4,18,19). In our case, no hypertension occurred, although it is known that this treatment regimen is prone to such complication (20, 21, 22). One should consider early diagnosis, fast and effective communication with stakeholders, as well as reflection upon the moral values when life is at stake, to a good prognosis (23, 24, 25, 26). Despite the several therapies reported, none of them is well-defined for children and adolescents. Our patient completed twelve cycles of systemic chemotherapy and narrowband ultraviolet B. Currently she is treated only with topical corticosteroids. Follow-up period for our patient is 2 years. She didn't develop so far, any complication or disease progression, but still has an inferiority complex about her aesthetic look.

CONCLUSIONS

We reported a rare case of juvenile-onset of MF. Long follow-up period is needed for our patient. Further studies are required for a better understanding of the outcome and prognostic implications of MF in pediatric age.

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THE CORRELATION BETWEEN THE ANKLE-BRACHIAL INDEX AND THE METABOLIC SYNDROME

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THE CORRELATION BETWEEN THE ANKLE-BRACHIAL INDEX AND THE METABOLIC SYNDROME (Abstract): On a global scale, the cardiovascular diseases constitute the prime factor of death and invalidity. The premature mortality percentage caused by these varies from 4% in high developed countries to 40% in underdeveloped countries. Atherosclerosis is the most important etiological factor. The presence of various degrees of atherosclerosis in a certain vascular area (in our case, the lower limb arteries), increases the probability of affecting other areas as well (coronary, cerebral, renal, mesenteric arteries). **Aim:** The evaluation and description of the correlations between the ankle-brachial index levels and the cardiovascular risk factors, taken individually or as part of the metabolic syndrome. **Material and method**: The values of the ankle-brachial index were divided in normal and abnormal. The evaluated cardiovascular risk factors were: age, sex, arterial hypertension, obesity, smoking, high levels of cholesterol and basal glucose, low levels of HDL-cholesterol. **Results**: There were significant statistical differences between the normal ankle-brachial index lot and the one with abnormal values, specifically in patients with diabetes mellitus and metabolic syndrome. More so, the study demonstrates that the ankle-brachial index is considerably smaller in patients with metabolic syndrome. **Conclusions:** The simple measurement of the afore mentioned index, as a atherosclerosis marker for the lower limb arteries, represents an independent prediction over the metabolic syndrome and the conventional risk factors, in the development of the cardiovascular diseases. The routine measurement of this parameter in medical practice might imply the early diagnosis of high risk manifested cardiovascular disease patients. **Keywords**: ANKLE-BRACHIAL INDEX, METABOLIC SYNDROME, CARDIOVASCULAR RISK FACTORS

The ankle-brachial index (ABI) is defined as the ratio between the systolic blood pressure, measured on ankle level, and the brachial level one, lying down, 5 minutes after resting.

The ratio is derived as follows: the blood pressure is measured on both arms, and the highest value is considered; then it is measured in the pedis and tibial bilateral posterior arteries (using a sphygmomanometer and a Doppler instrument), selecting the highest value as well. From the resulted two values of the ratio, the lowest value is further considered (1). This technique is maneuverable, non-invasive and reliable. It is applied on a large scale in medical practice to confirm the diagnosis and severity of the peripheral arterial disease (2). More

so, ABI is an indicator of generalized atherosclerosis, as the literature associates low values with an increased rate of coronary and cerebrovascular damage, as well as with the presence of numerous cardiovascular risk factors, that suggest the independent nature of it predicting cardiovascular events (3).

The metabolic syndrome, heterogeneous entity considering the gender differentiation, is currently defined, as the association of at least 3 out of 5 criteria, each with its own established reference values (4).

MATERIAL AND METHODS

A retrospective observational study was performed on 189 patients hospitalized in the Cardiovascular Rehabilitation Clinic of the Clinical Rehabilitation Hospital Iasi, between September 2011 and April 2012 (7 months).

The patients were divided in two batches, based on the values of ABI: first batch, with abnormal values (ABI $\leq$ 0.9) and the latter, with normal values (ABI between 0.9 and 1.4) (5).

Subjects with ABI values higher than 1.4 were excluded from the study, due to an increased level of errors determined by the arterial stiffness (6). Other exclusion criteria were: secondary arterial hypertension, severe chronic renal disease (dialyzed patients), major cardiovascular events (myocardial infarction, stroke, and chronic heart failure, myocardial or peripheral revascularization) (7).

RESULTS AND DISCUSSION

The gender repartition varied: 74.1% (140) male subjects, and 25.9% (49) female. Patients' age ranged from 37 up to 83 years. In addition, 59.8% (113) came from urban areas, while the remaining 40.2% (76) resided in rural areas. No statistic age difference was observed amongst the study group, between the two genders (p=0.606) (fig. 1).

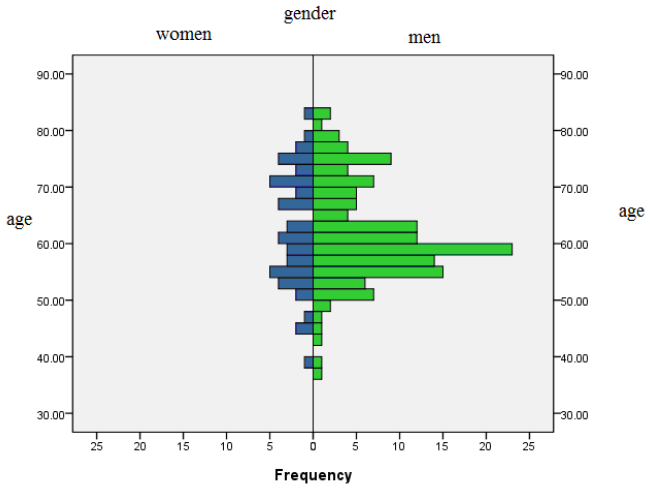

Fig. 1. Age over sex distribution

The maximum systolic blood pressure level, depending on the ABI values, registered the following results:

- ABI $\leq$ 0.9 batch, values between 120-290 mmHg, with an average value of 180 mmHg;

- ABI > 0.9 batch, values between 135-260 mmHg, with an average value of 190 mmHg.

There were no significant differences between the two batches (8) (fig. 2).

The statistical analysis of the cholesterol (fig. 3) and HDL-cholesterol levels (fig. 4) depending on the ABI values revealed the following:

- normal distribution of cholesterol and HDL-cholesterol levels on ABI ≤ 0.9 subjects (107-345 mg/dl, with an average of 187 mg/dl for cholesterol and 19-82 mg/dl, with an average of 40 mg/dl for HDL-cholesterol);

- non normal distribution on ABI > 0.9 patients (110-351 mg/dl, with an average of 193 mg/dl, and 27-80 mg/dl, with an average of 41 mg/dl respectively).

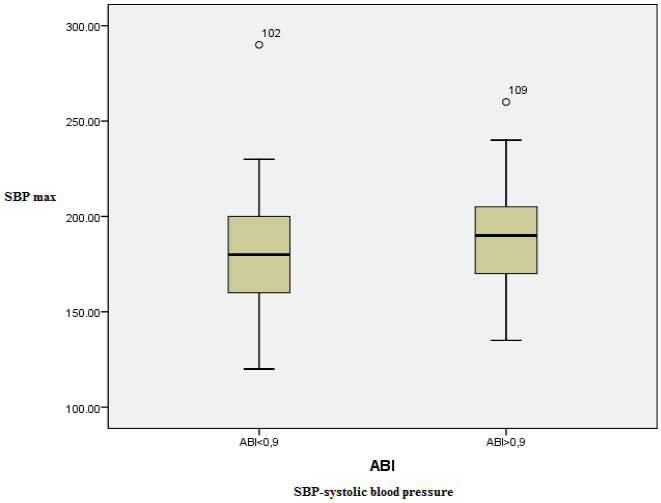

Fig. 2. The correlation between ABI and SBPmax

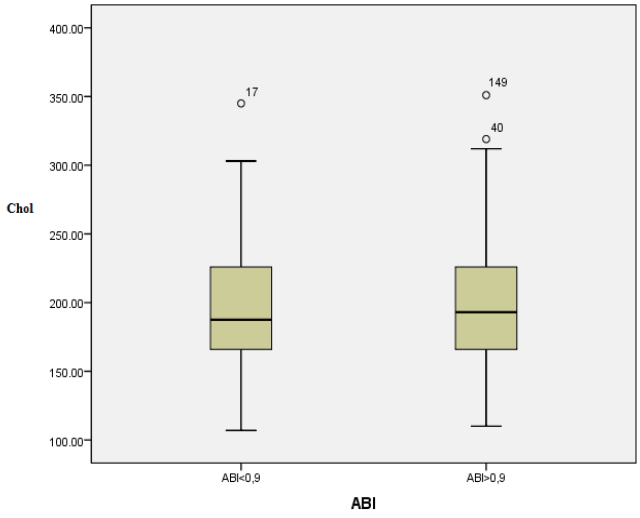

Fig. 3. ABI and cholesterol levels

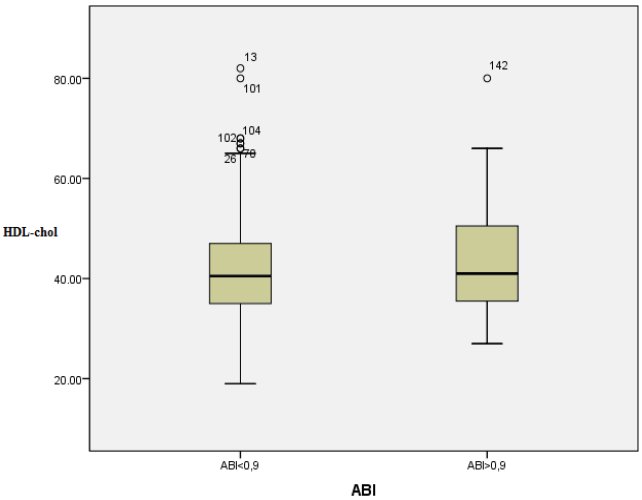

Fig. 4. ABI and HDL-cholesterol levels

Again no significant differences were observed (9).

In ABI ≤ 0.9 batch, there were 26 patients with diabetes mellitus, compared to 43 from the second batch, with a significant statistical difference between the two (p=0.049) (10).

The analysis of the correlation between smoking and ABI values showed no differences (11) (fig. 5).

The study batches' average body mass index (BMI) was 29 kg/m^2, with a variation between 18 and 49 kg/m^2, without a significant difference (p=0.227) (12, 13) (fig. 6).

In the ABI ≤ 0.9 batch, there were 16 patients with left ventricular hypertrophy (LVH), compared with 20 patients from the second batch, with no significant statistical difference between the two (p=0.714).

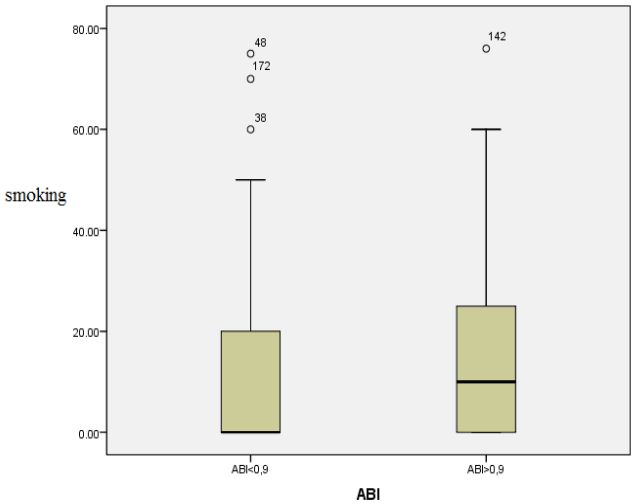

Fig. 5. The correlation between

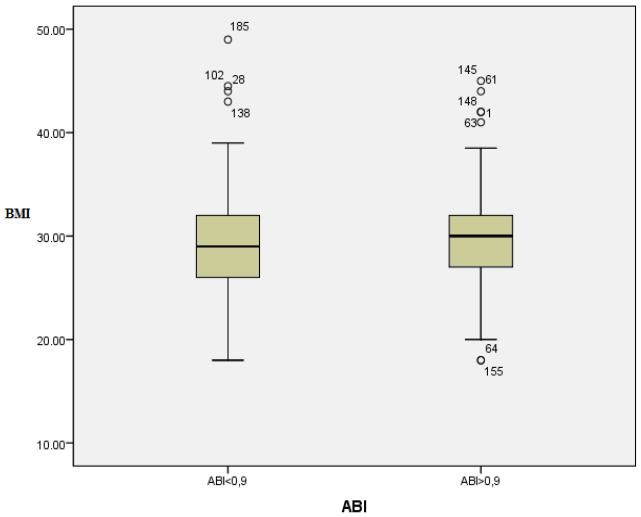

Fig. 6. ABI and BMI smoking and ABI

Regarding the main objective of the present study, establishing a correlation between the ABI values and the presence of the metabolic syndrome, it was demonstrated that the distribution of the latter was non-normal for both batches; the metabolic syndrome was present on 49 patients with ABI ≤ 0.9 and 32 patients with ABI > 0.9, with a significant statistical difference (p=0.003); moreover the ABI values were considerably lower on patients with metabolic syndrome (14) (fig. 7).

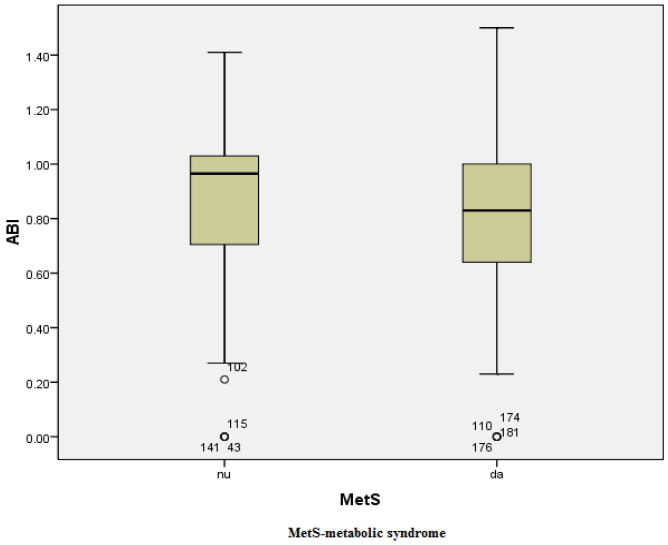

Fig. 7. ABI and the metabolic syndrome

CONCLUSIONS

This study strengthens the literature results and demonstrates that the low ABI values (as a marker for atherosclerosis in lower limbs arteries) represent an independent predictor in relation with the metabolic syndrome and conventional risk factors, in the development of cardiovascular disease. Introducing the routine measurement of this parameter in medical practice might imply the early diagnosis of high risk manifested cardiovascular disease patients.

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SNEDDON SYNDROME: RARE DISEASE OR UNDER DIAGNOSED CLINICAL ENTITY? REVIEW OF THE LITERATURE RELATED TO A CLINICAL CASE

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SNEDDON SYNDROME: RARE DISEASE OR UNDER DIAGNOSED CLINICAL ENTITY? REVIEW OF THE LITERATURE RELATED TO A CLINICAL CASE (Abstract). Sneddon syndrome is defined by the association of livedo racemosa and recurrent cerebrovascular ischemic lesions. The annual incidence is 4/1,000,000. This syndrome particularly affects young women, some reports suggesting a family predisposition. It is a chronic, progressive, arterio-occlusive disease of unknown etiology that involves small and medium-sized arteries. It is usually associated with antiphospholipid antibodies. We report the case of a female patient with Sneddon syndrome with significant family history, personal history of stroke, epilepsy, migraine, cardiovascular involvement, three miscarriages, cognitive decline, noncompliant to therapy, in the absence of antiphospholipid antibodies. This paper aims to analyze the main characteristic features and management of Sneddon syndrome by conducting a literature review related to a clinical case. **Keywords:** SNEDDON SINDROME, CEREBROVASCULAR DISORDER, LIVEDO RACEMOSA, ANTI-PHOSPHOLIPID ANTIBODIES.

Sneddon syndrome is a condition characterized by the association of livedo racemosa and ischemic strokes (especially in the middle cerebral artery territory); it was named after the British dermatologist Sneddon, the first to draw attention to this association in 1965. Sneddon syndrome affects mainly women (80%) aged 20-40 years; livedo racemosa precedes neurologic impairment and the first cerebral ischemic event usually occurs before the age of 45. The annual incidence is estimated at 4/1,000,000 but data suggest that Sneddon syndrome is under diagnosed; it has been estimated that Sneddon syndrome affects 1:2000 stroke patients and the prognosis for these patients is poor (1).

It is a chronic, progressive, non-inflammatory, arterial occlusive disease of unknown cause that affects small and medium sized arteries in the brain and skin, although there are studies that state its system-

ic nature. Cardiovascular system is the most commonly involved (hypertension, ischemic heart disease, valvular disease), but the visual system, gastro-intestinal system and kidneys may also be affected. The association with antiphospho-lipid antibodies is controversial (0-85%).

Some studies describe a significant family history in Sneddon syndrome patients, and autosomal dominant with incomplete penetrance or autosomal recessive arrays were suggested (2, 3, 4).

Sneddon syndrome is not currently regarded only as a clinical diagnosis but rather as a common clinical manifestation of different diseases. Various authors have classified the disorder in primary Sneddon syndrome (without evidence of etiological factors) and secondary (autoimmune, thromboembolic, associated with systemic lupus erythematosus, associated with antiphospholipid syndrome) (5). No treatment has proven effective for this condition.

Pathophysiology. Recent data suggest that Sneddon syndrome begins as an inflammatory, possibly immunologically-mediated disorder which develops into the migration and proliferation of smooth muscle cells in small and medium-sized arteries that result in vessel stenosis. The disease progresses in four stages: early stage (I), characterized by attachment of lymphohistiocytic cells and detachment of endothelial cells followed in stage II by total or partial occlusion of the lumen with a plug of fibrin and lymphohistiocytic cells; the plug is then replaced in stage III by proliferating subendothelial cells and stage IV is characterized by fibrosis and stenosis of the vessel (1,6).

Clinical diagnosis. Sneddon syndrome is a clinical diagnosis as there is still no specific test for it. Livedo racemosa is usually located on the limbs (100%), trunk (89%), buttocks (74%), face (15%), hands and feet (59%). Neurological manifestations occur after an ischemic stroke, usually in the middle cerebral artery territory and usually consist of hemiparesis, sensory deficits, aphasia and dysarthria, but visual field deficits and drop attacks may also occur. TIAs are common and spinal infarcts, intracerebral, subarachnoid and intraventricular hemorrhages were also reported (1).

Other neurological manifestations associated with Sneddon syndrome are headache, vertigo (the most common), chorea, epilepsy, myelopathy, migraine, hemicrania, acute encephalopathy, amnesia, internuclear ophthalmoplegia, nystag-mus, ophthalmic artery occlusion, myasthenia gravis, polyneuropathy, Raynaud syndrome (1). In almost all cases the disease advances to cognitive dysfunction, personality changes, depression and late dementia.

Impairment of other systems has been reported: cardiovascular (hypertension, valvular, aortic or mitral regurgitation usually, ischemic heart disease), ocular, gastrointestinal, renal, gynecological (miscarriages, sterility), suggesting a systemic nature of the disease (1, 7, 8).

Of the clinical manifestations, migraine and headache appear to be most strongly associated with the risk of a new stroke (9).

Laboratory exams. Recent studies support the importance of skin biopsy and cranial imaging in the diagnosis of Sneddon syndrome. Wohlrab *et al.* used a skin biopsy method that required taking a deep punch biopsy (4 mm) from the white areas, the sensitivity of the method increasing with the number of biopsies: 27% one biopsy, 53% two biopsies, and 80% with three biopsies (10). Ultrastructural and immunohistochemical investigations have shown that the migrating cells from the media contain smooth muscle actin (smooth muscle cells or myofi-

broblastic cells). Hilton and Footitt reported the main findings in the brain tissue biopsy to be small cortical infarcts associated with the occlusion of the small and medium arteries and prominent focal hyperplasia of the media in the small arteries, similar to the skin findings (1).

MRI can detect cortical and/or subcortical arterial infarcts, usually multiple, and periventricular white matter abnormalities. Diffuse cortical-subcortical atrophy has been reported in advanced stages. The results of cranial angiography are inconsistent, some studies describing various abnormalities (irregular vessel calibre, occlusion or stenosis of major cerebral vessels, medium and small vessel stenosis, large networks of fine collateral vessels and granulomatous leptomeningeal infiltration, transdural anastomoses, lobar intracerebral hemorrhage with "pseudo-angiomatosis" pattern), others did not report any abnormalities (1,11).

Stockhammer suggested using MRI and duplex sonography in detecting large vessel occlusions, while Canepari *et al*. have demonstrated a strong relation between SPECT analysis of cerebral perfusion, clinical findings, and disease progression; functional imaging associated with MRI seem to have a good sensitivity in the follow-up of these patients (11, 12, 13).

The combination of Sneddon syndrome and antiphospholipid antibodies (APAs) has been reported in quite variable percentage, ranging from 0 to 85%, most authors suggesting values of 40-50% (1). Some authors have concluded that thrombocytopenia, proteinuria, epilepsy, and mitral regurgitation occur more often in the presence of APAs associated with Sneddon syndrome, whereas livedo network appears to be broader in Sneddon syndrome without APAs. Others have found that the presence of APAs indicates a lower risk of thrombosis and a much less severe course of the disease (1, 14, 15).

Numerous other prothrombotic abnormalities have been reported: heterozygous factor V Leiden mutation, deficiency of protein Z, resistance to activated protein C, increased platelet aggregation, and increased ß-tromboglobulin, modified plasminogen activator/inhibitor ratio, familial antithrombin III deficiency, increased factor VII activity (1, 16, 17, 18, 19). Our patient tested negative for APAs, proteins C and S were normal, although bleeding and clotting parameters were modified.

Differential diagnosis. Differential diagnoses include other conditions that associate livedo racemosa and cerebrovascular events such as: APA, systemic lupus erythematosus (SLE)- it can present with livedo racemosa (11.7%), especially associated with anti-Ro antibodies, and neurological symptoms (amnesia, cognitive impairment, depression), Divry van Bogaert syndrome, thrombangiitis obliterans - reported as a rare complication of Buerger disease, is clinically similar to Sneddon syndrome.

Other differential diagnoses to be considered are polyarteritis nodosa, cholesterol emboli syndrome, livedoid vasculopathy. Rarely, livedo racemosa may be associated with: hematological abnormalities (essential thrombocythemia, polycythemia vera, pernicious anemia, DIC, cryofibrino-genemia), hyperthyroidism, rheumatoid arthritis, Sjogren's syndrome, derma-tomyositis, scleroderma, temporal arteritis, calciphylaxis in chronic dialysis patients, hyperoxaluria, atrial myxoma, post-streptococcal syndrome, borreliosis (1).

Course. The natural course of Sneddon syndrome is marked by recurrent ischemic strokes accompanied by progressive cerebral atrophy and dementia, leading to disa-

bility and a mortality of 9.5% over an observation period of 6.2 years (11).

Treatment. Due to lack of controlled trials, the management of Sneddon syndrome patients remains controversial. Corticosteroids and immunosuppressive therapy appear to be ineffective. It has been found that the presence of APAs in Sneddon syndrome may influence the management and patients with positive APA respond well to anticoagulant treatment with warfarin in high doses (20). Wohlrab *et al.* proposed a long-term triple prophylactic therapy with pro-rheological agents (prostaglandin E1), against myocyte proliferation (Captopril) and antiplatelet that led to halting disease progression over 3-5 years (1). Hannon *et al.* reported improved memory and emotional status after treatment with cyclophosphamide (21), while other authors state that intravenous thrombolysis may be effective and safe in patients with Sneddon syndrome (22); also, these patients should avoid smoking and oral estrogen contraceptives (1).

Prognosis. It seems that patients who associate APAs have a better prognosis than those without APAs, wand a better response to anticoagulant treatment. Systemic involvement, especially cardiovascular, leads to a poorer prognosis and the presence of migraine in patients with Sneddon syndrome is associated with an increased risk of stroke (9, 10). The best follow-up methods for preventing cerebrovascular ischemic lesions in patients with Sneddon syndrome seem to be the brain imaging techniques (MRI and SPECT) (13).

Although Sneddon syndrome is a clinical diagnosis, it requires multiple investigations, necessary for initial assessment of systemic involvement, prognosis and treatment plan.

CASE PRESENTATION

To illustrate the complexity of approaching such a patient we present the case of a 42-year-old woman admitted to the Department of Internal Medicine of the Iasi "Dr. C.I. Parhon" Hospital who upon admission showed uncontrolled high blood pressure values, frontal occipital headache of moderate intensity (5 p/10 on the visual analog scale), faintness, vertigo accompanied by nausea and vomiting, visual disturbances, muscle weakness and chest pain.

Fig. 1. Livedo racemosa of the left arm

Fig. 2. Livedo racemosa of the legs

Physical exam revealed skin changes characteristic for "livedo racemosa" (fig. 1, 2), visible on the thighs, arms and trunk, with onset at the age of 22 years and

trophic disorders of the legs.

Neurological exam revealed left spastic hemiparesis, left facial paresis, central motor deficit of the left limbs.

Our patient had a significant family history, a grandmother who died of stroke, a brother who suffered from stroke, the mother who had livedo reticularis and a personal history of three miscarriages, grade 3 arterial hypertension, epilepsy and migraine.

Known with neglected arterial hypertension, nonsmoker, denied use of estrogen products, our patient presented an episode of left faciobrachial motor deficit resolved within 3-4 days at the age of 34. At the age of 39 she suddenly experienced a left limb motor deficit following strenuous exercise; glucocorticoids and antiplatelet agents were administrated and the course was favorable.

The suspicion diagnoses were Sneddon syndrome, antiphospholipid antibody syndrome, and systemic lupus erythematosus; for confirming the diagnosis we performed full blood and biochemical tests, autoimmune markers, coagulation tests, heart examination (ECG, Holter, transthoracic echocardiography), CT, MRI and skin biopsy and the results were:

- Brain MRI: multiple ischemic brain lesions located in the right parietal and left occipital lobes (fig. 3) while cranial CT showed an insular frontal ischemic sequels and cerebral atrophy;
- Laboratory tests: the absence of antiphospholipid antibodies, p-ANCA and c-ANCA, ANA and anti-dsDNA antibodies were negative, protein C and S were normal, while bleeding and coagulation time were decreased (BT = 1'00 ", CT = 5'48");
- Skin biopsy: discreet perivascular lymphocytic infiltrate and bulging of vascular endothelium;
- Holter: systolic values ranged between 140-160 mm/Hg and echocardiography revealed normal heart cavities, with a LVEF = 65%.

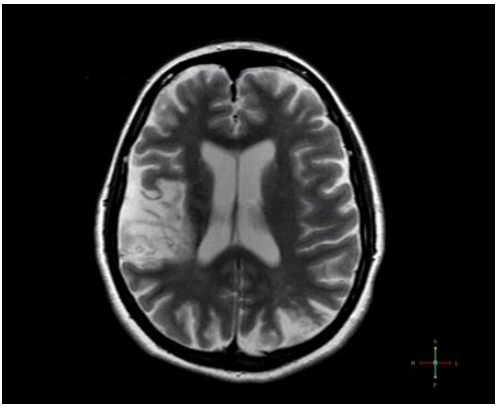

Fig. 3. MRI imaging: Ischemic lessions located in the right parietal and left occipital lobe

In our patient, a positive diagnosis of Sneddon syndrome was based on the presence of clinical symptoms (livedo racemosa, neurological and cognitive impairment), history of stroke, and brain imaging suggestive of ischemic changes accompanied by cerebral atrophy.

The most eficient and used drugs for arterial hipertension asociated with angina pectoris seems to be ß blockers and angiotensin receptor blockers II /sartans (23) and our pacient was treated with ß blocker, vasodilator, antiplatelet, oral anticoagulant and neurological treatment, resulting in halting the progression of the disease, with good blood pressure control and absence of a new cerebral ischemic event or degradation of cognitive status, but unchanged neurologic manifestations.

As our patient had a suggestive family history, personal history of two strokes, cognitive dysfunction (suggestive of an

advanced stage of disease), migraine, cardiovascular abnormalities (hypertension, angina pectoris), pro-thrombotic status (suggested by the 3 miscarriages), tested negative for APAs (usually associated with poor anticoagulant response and a more severe course of the disease) and was non-compliant, the prognosis was poor.

CONCLUSIONS

Although considered a rare disease, new studies show that Sneddon syndrome is actually an under diagnosed clinical entity and recognizing this disease is important in the management and subsequently in the outcome of these patients. Therapeutic conduct in these cases is difficult in the absence of a well-established protocol. Management of patients with Sneddon's syndrome should be multidisciplinary because morbidity is significant (recurrent stroke, cardiac ischemia, hypertension, miscarriages, cognitive impairment). The evolution is progressive; therefore the follow-up for these patients should be regularly done, both by clinical examination and by analyzing MRI, SPECT and echocardiography. In the absence of effective treatment, management of these patients involves removing known modifiable risk factors (smoking and eliminating estrogen products, cardiac ischemia, hypertension, hypercoagulability, epilepsy).

Our patient had multiple risk factors and was diagnosed with Sneddon syndrome 20 years after the first symptoms appeared. A more prompt diagnosis would have led to a more rapid initiation of appropriate treatment and as such to an improved prognosis.

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DETERMINANTS OF INADEQUATE WEIGHT GAIN IN PREGNANCY

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DETERMINANTS OF INADEQUATE WEIGHT GAIN IN PREGNANCY. **Aim.** The aim of the study was to explore the anthropometric maternal characteristics and prenatal care as determinants of pregnancy weight gain. **Material and methods.** We conducted a cross-sectional study on a total of 400 pregnant women admitted to Cuza-Vodă Obstetrics and Gynecology Clinical Hospital, Iaşi. Information on demographic characteristics, number of prenatal visits, and education on nutrition and food changes occurring during pregnancy were recorded in a structured questionnaire. Anthropometric parameters analyzed were pregestational BMI (body mass index) and weight gain during pregnancy. **Results.** Weight gain was associated with pregestational BMI category. An increase in weight more than recommended occured more frequently in overweight (53.1%) and obese women (66.7%) ($p<0.001$). Weight gain during pregnancy was related to area of residence, age, APCU (adequate prenatal care utilization) index. The multivariate analysis identified the following variables as significant determinants of pregnancy weight gain: inadequate prenatal care, BMI and changes in diet. **Conclusions.** Pregestational BMI and changes in diet during pregnancy identified as determinants of weight gain suggests that overweight and underweight women must carefully be counseled regarding recommendations for weight gain in pregnancy. Tracking diet changes is important to ensure that a weight gain lies within the guidelines recommendations. **Keywords:** PREGESTATIONAL BMI, WEIGHT GAIN DURING PREGNANCY, PRENATAL VISITS, NUTRITIONAL EDUCATION

The main purpose of recommendations for weight gain during pregnancy is represented by a normal weight of newborns. Numerous factors can influence weight gain in pregnant women, such as: previous nutritional status, age, parity, race and dietary habits (1). Overweight is associated with an increased risk for preeclampsia, gestational diabetes and caesarean section (2, 3). The pre- and postnatal nutritional environment is important in shaping the future phenotype of the organism, influencing the risk of insulin resistance, type 2 diabetes and cardiovascular disease in adult life. The aim of the study was to explore the determinants of inadequate weight gain during pregnancy.

MATERIAL AND METHODS

We conducted a cross-sectional study on

a sample of 400 women, admitted to Cuza-Vodă Obstetrics and Gynecology Clinical Hospital, Iasi, in August-September 2010. Exclusion criteria were multiple pregnancy, patient refusal and obstetric pathology.

A standardized questionnaire provided information on age, pregestational weight, area of residence, marital status, years of formal education, parity and prenatal care utilization (date of registration, the number of medical visits to the family doctor and obstetrician). Weight and height were measured in duplicate and recorded according to the standard protocol. Prepregnancy nutritional status was classified based on BMI (body mass index), according to the World Health Organization (WHO) criteria. Weight gain during pregnancy was divided into three categories (less than 9 kg, 9-15 kg and above 15 kg) according to general practitioner guidelines (4).

Adequacy of prenatal care utilization was assessed by determining the Adequacy of Prenatal Care Utilization Index which takes into consideration the timing of prenatal care initiation and the number of prenatal visits. The index is based on ACOG (American College of Obstetricians and Gynecologists) recommendations for low risk pregnancies and has 4 categories. Inadequate prenatal care includes women who were registered after the fourth month or had less than 50% of the number of recommended visits in pregnancy. Women registered after the fourth month with a total of 50-79% of visits are included in the intermediate care category. Initiation of prenatal consultation in the first 4 months of pregnancy and a total of 80-109% of visits correspond to adequate care category. Adequate plus category refers to the initiation of prenatal consultation in the first 4 months and total medical checks of more than 110% compared to the number recommended (5).

The statistical package *SPSS 13.0* for Windows (Chicago, IL, USA) was used for data analysis. For the prediction of inadequate weight gain, multinomial logistic regression was used. Significance values of Goodness-of-Fit test were higher than 0.05, so the model was adequate. Access to data observation sheet, the mother interview and the measurements were made with the agreement of management of Cuza-Vodă Obstetrics and Gynecology Hospital, and with the approval of Science and Ethics Committee of the University of Medicine and Pharmacy ”Grigore T. Popa”- Iaşi.

RESULTS

Almost half of the participants (45.8%) were from rural areas. The mean age was 27.53 years, and 63.8% of all women were between 19 and 30 years old. Pregnancy occurred in married couples in 80.75% of cases. Primiparous women were predominant (49.5%) and also those with a second child (32.3%) (tab. I).

A proportion of 80% of women presented to the family doctor during the first trimester of pregnancy. The mean total number of medical visits during pregnancy was 9.58±4.88.Almost half of women (53.5%) had an adequate plus prenatal care level, evaluated by APCU (adequate prenatal care utilization) index, while 23% of them had inadequate care during pregnancy. A proportion of 42.3% of women received advice about nutrition in pregnancy. Only 33% declared they had no change in diet during pregnancy. Most women were advised to breastfeed their children (83.5%).

The frequency of underweight women was 11.8%, 66.6% were normal weight,

17% overweight and 4.6% were obese. Only 52.6% of women achieved a weight gain during pregnancy as recommended by the guide for general practitioners' (9-15 kg). Pregnancy weight gain was influenced by area of residence and age. The number of women with weight gain less than 9 kg was higher among those from rural areas. Pregnant women younger than 20 years old had more frequently a lower weight gain than recommended, but rarely an increase of more than 15 kg (tab. II).

TABLE I
Sociodemographic characteristics of the studied sample

		No.	**%**
Area of residence	urban	217	54,3
	rural	183	45,8
Age (years)	< 20	356	89,0
	≥ 20	44	11,0
Formal education (years of schooling)	1-4	16	4,0
	5-8	86	21,5
	9-12	162	40,5
	>12	136	34,0
Marital status	married	323	80,8
	unmarried	77	19,2
Planned pregnancy	yes	359	89,8
	no	40	10,0

TABLE II
Sociodemographic characteristics related to weight gain during pregnancy

Socio-demographic characteristics		**Weight gain categories, %**			**P**
		< 9 kg	**9-15 kg**	**> 15 kg**	
Area of residence	Urban	9,3	48,6	42,1	**,001**
	Rural	17,5	57,8	24,7	
Planned pregnancy	Yes	13,5	51,1	35,3	,197
	No	6,1	66,7	27,3	
Civil status	Married	32,7	48,1	19,2	,173
	Unmarried	12,7	50,6	36,6	
Age (years)	≥20	12,0	51,3	36,7	**,021**
	< 20	20,5	64,1	15,4	
Formal education (schooling years)	< 9	11,6	51,0	37,3	,095
	≥ 9	16,7	57,8	25,6	
Parity	≤ 2	11,9	51,6	36,6	,128
	> 2	17,7	58,1	24,2	

Most of underweight women had an adequate weight gain during pregnancy. However, 39.5% had a weight gain of more than 15 kg. Women with obesity had more frequently a reduced weight gain compared to other categories, but the proportion of those with large increases in weight remained high (p=0.001) (tab. III).

Pregnant women with adequate prenatal care had a higher mean weight gain compared to those with inadequate or intermediate APCU index (p<0.001). Significant differences were seen between women with inadequate and adequate care (12.53 kg *vs.* 14.42 kg., p = 0.041) and among those with inadequate and adequate + APCU index (12.53 kg *vs.* 15.05 kg, p<0.001). Weight gain of pregnant women with adequate + APCU index was also significantly higher than the one seen in those with intermediate and adequate prenatal care (15.05 kg *vs.* 12.53 kg, p <0.001, respectively 15.05 kg *vs.* 14.42 kg, p=0.007). (fig. 1).

A weight gain under the recommendations was noticed in 20.4% of women who received advice on diet during pregnancy, compared to 25.5% of women who did not receive such information. An increase in weight greater than recommended occurred in 35.3% of women who received advice about diet, compared to 33.8% of them who have not received such advice. Weight gain within the recommendations was more common among women who received advice on nutrition during pregnancy, even if there was no statistical significance (44.3% *vs.* 40.7%, p = 0.496).

TABLE III

Weight gain during pregnancy according to pregestational BMI

Weight gain, kg	Categories of BMI, %			
	Under weight	Normal weight	Over weight	Obese
<9	4,7	5,5	12,7	33,3
9-15	55,8	60,5	54	27,8
>15	39,5	34	33,3	38,9

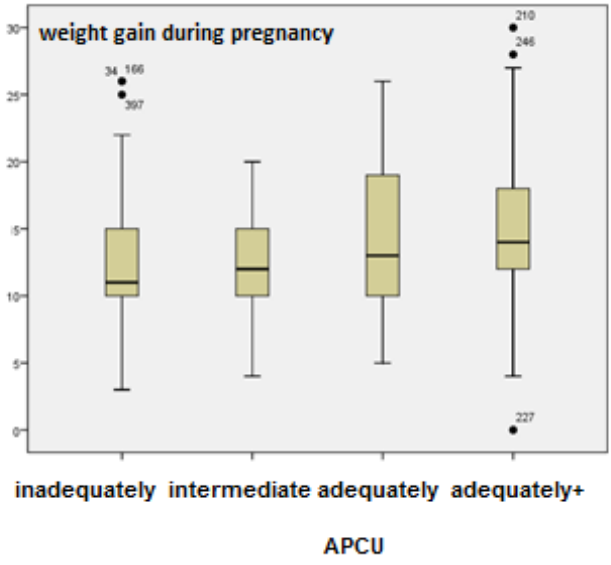

Fig. 1. Association between weight gain and APCU index

Multinomial logistic regression was performed to determine which of the following factors determinants of inadequate pregnancy weight gain are: area of residence, age, education, marital status, parity, planned pregnancy, APCU index, nutritional advice, pregestational BMI, changes in diet during pregnancy (tab IV).

TABLE IV
Determinants of weight gain during pregnancy

Weight gain during pregnancy[a]			***P***	**OR**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Less than recommended	APCU index	**Inadequate**	**,009**	**3,064**	**1,318**	**7,123**
		Intermediary	,657	1,301	,407	4,156
		Adequate	,374	1,565	,583	4,201
		Adequate +				
	Age	<20 years	,535	1,358	,517	3,568
		>20 years				
	Dietary changes	Eat less	,063	2,390	,953	5,993
		Eat more	,643	,818	,349	1,914
		Avoid salty foods	,903	,923	,256	3,325
		Eat more salty	,520	,462	,044	4,864
		Avoid certain foods	,773	1,279	,241	6,772
		No dietary changes				
	Pregestational BMI (kg/m^2)	**<18,5**	**,002**	**,117**	**,031**	**,444**
		18,5-24,9	**,000**	**,041**	**,007**	**,243**
		<25-29.9	,110	,317	,078	1,296
		>30	.	.[b]	.	.
More than recommended	APCU index	Inadequate	,136	,600	,307	1,174
		Intermediary	**,023**	**,292**	**,101**	**,846**
		Adequate	,368	1,364	,694	2,681
		Adequate +	.	.[b]	.	.
	Age	**<20 years**	**,036**	**,346**	**,128**	**,935**
		>20 years				
	Dietary changes	**Eat less**	**,009**	**,065**	**,008**	**,501**
		Eat more	**,007**	**2,117**	**1,229**	**3,649**
		Avoid salty foods	,738	1,146	,517	2,536
		Eat more salty	,874	1,135	,237	5,434
		Avoid certain foods	,532	1,416	,476	4,2207
		No dietary changes				
	Pregestational BMI (kg/m^2)	<18,5	,172	,416	,118	1,463
		18,5-24,9	,375	,538	,137	2,117
		<25-29.9	,350	,525	,136	2,029
		>30	.	.[b]	.	.

b-reference category

Variables that significantly contributed to distinguish between the three categories of weight gain during pregnancy were: APCU index; pregestational BMI; changes

in diet and age. A weight gain lower than the recommended was associated with inadequate prenatal care, pregestational BMI < 18.5 kg/m^2 and normal pregestational weight. Weight gain higher than recommendations was associated with changes in diet, age less than 20 years and an intermediate value of APCU index.

DISCUSSION

Prenatal care is essential for screening and prevention of complications associated with pregnancy (6). WHO recommends initiation of prenatal care during the first 4 months of pregnancy (7). Continuity (8) and frequency of prenatal care (9) have positive effects on mother and child health. APCU index combines in a single variable two quantitative factors: gestational age, in months, at beginning of prenatal care and the number of prenatal visits during pregnancy. Because prenatal care needs to be appropriate, registration should be done during the first trimester and the number of prenatal visits should be at least nine. Although the name includes the term appropriate, the index does not refer to the content of prenatal consultation (5).

Our data show an improved addressability to general practicionner during the first trimester of pregnancy compared to previous studies. In the study conducted by IOMC "Nutritional status of pregnant women" almost 6% of women received no prenatal visit during their last pregnancy, and 68.8% of mothers were presented at the first prenatal visit during the first trimester of pregnancy (79.2% in urban and 58.5% rural) (10). The Reproductive Health Survey 2004, most women (94%) received prenatal care, and 74% were considered in the first trimester. Only 17% were considered in the second and 2% in the third trimester. In 1999 it was found that 89% of women received prenatal care (10). The self-reported weight was used in the present study. For accurately assessing this parameter it is recommended to use the self-reported weight in the first two months of pregnancy (11). However, there are studies showing that mothers declared a value that correlates with real weight (12).

Pregnancy is considered an appropriate time for promoting healthy changes in dietary habits (13). Characteristics of intervention methods, content and frequency of educational measures and socio-economic peculiarities led to discordant results of interventional studies. The impact of recommendations on weight gain during pregnancy has been shown in numerous studies, indicating that messages during prenatal consultation may influence attitudes and perceptions of pregnant women on optimal growth. The impact of recommendations on appropriate weight gain may be influenced by family and friends attitude on prenatal education (14).

In this sample, it has been observed that only 57.6% of women had an optimal weight gain. Studies in the U.S. have shown that 30-40% of pregnant women have a weight gain outside the limits recommended by the Institute of Medicine (15). Our results show that the number of prenatal visits influenced the proportion of women with optimal weight gain. Pregnant women with inadequate prenatal care had more frequently a weight gain below the recommended limits. Weight gain within the recommendations was more common among women who received advice on nutrition during pregnancy. Other studies concluded that belonging to certain ethnic groups (Hispanic), educa-

tion level, decreased pregestational BMI and the lack of nutritional advice were determinants of lower than recommended weight gain (14). Overweight, young age and multiparity had a higher weight gain than considered appropriate by the IOM (14). Weight gain above the recommended level was associated with overweight and obesity in a study conducted in Brazil (16).

CONCLUSIONS

Pregestational BMI, APCU index and changes in diet during pregnancy identified as determinants of weight gain suggests that overweight and underweight women must carefully be counciled regarding recommendations for weight gain in pregnancy. Tracking diet changes is important to ensure that a weight gain lies within the guidelines recommendations.

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INFECTIOUS ENDOCARDITIS WITH FIVE LOCALISATIONS - CASE REPORTS

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INFECTIOUS ENDOCARDITIS WITH FIVE LOCATIONS - CASE REPORTS (Abstract) Infectious endocarditis is defined as an infection of the endocardial surface area of the heart which can include one or more cardiac valves, mural endocardium or a septal defect. We present the case of a patient, aged 8, hospitalized in Hemato-Oncology Department with pontine glioma, who was diagnosticated with infectious endocarditis with five localisations.
K**eywords**: ENDOCARDITIS, CARDIAC VALVES, SEPTAL DEFECT.

Infectious endocarditis is defined as an infection of the endocardial surface area of the heart which can include one or more cardiac valves, mural endocardium or a septal defect (1, 11). Its intracardiac effects include severe valve insufficiency which can lead to congestive cardiac insufficiency that is hard to treat and to myocardial abscesses. If it is left untreated, the infectious endocarditis is, in general, fatal (2, 6).

Endocarditis can be classified into:

• native valve endocarditis, acute and subacute;

• early and late prosthetic valve endocarditis;

• secondary endocarditis in intravenous drug users (4, 7).

Rheumatic diseases represent now less than 20% of the cases and 6% of the patients with cardiac rheumatic disease finally develop infectious endocarditis. Congenital cardiac diseases represent 15 % of the cases, with bicuspid aortic valve being the best known example (10).

Acute endocarditis is caused most frequently by *Staphilococcus aureus*, it appears on a normal valve, and is rapidly destructive, produces metastatic foci and if not treated is fatal in less than 6 weeks (5, 8). Subacute endocarditis is caused usually by viridans streptococci, it appears on valves with pre-existent injuries, it does not determine metastatic foci and, if not treated, it needs more than 6 weeks or even a year to become fatal (8,9).

Other congenital anomalies which lead to the appearance of endocarditis include ventricular septal defect, persistence arterial channel and tetralogy of Fallot. Atrial septal defect is rarely associated with infectious endocarditis. Mitral valve prolapse is the most common predisposing factor, more frequently found in young patients and is the predisposing condition in 30% of the cases of native valve endocarditis in this age group. Infectious endocarditis complicates 5% of the cases of asymmetric septal hypertrophy, which usually involves

the mitral valve (2, 3).

CASE REPORT

A 8 years and 2 months old male patient is transferred to the IVth Clinic of Hematology Oncology with signs and symptoms of acute cardio-respiratory insufficiency: generalized edema, dyspnea with orthopnea, hepatomegaly of under 4 cm below the rim, tachycardia, low blood pressure, rales on bilateral, the reason for which he is transferred to Intensive Therapy Section.

The patient at the debut of the disease at the beginning of October 2008 with headache resistant to symptomatic treatment. After a week, convergent squint of the left eye appears. The ophthalmological and neurological examination recommends correction of the squint by wearing glasses. In December 2008, mother notices balance disorders, nasal voice and discrete swallowing disorders.

CT and IRM examinations showed a cerebral edema in December 2008, and a suspicion of brainstem glioma in January 2009. Progressive deterioration of the general state, persistence of balance disorders, progressive squint and swallowing disorders imposed treatment with chemotherapy and radiotherapy.

During the fourth course of chemotherapy, the patient's state aggravated quickly, with signs and symptoms of acute cardiorespiratory failure.

The symptoms described earlier were resolved under treatment with Cefort Tienam, Dopamine, Tramadol, Furantril, ACC, HHC, tapping, physiotherapy, secretion aspiration, Brofimen, the patient being transferred to Oncology.

Later evolution of the disease was aggravated by the appearance of some complications: acute enter colitis reflecting the immunosuppressed state, hepatocytolisis syndrome, iatrogenic Cushing syndrome, thrombocytopenia secondary to CHT (chemotherapy).

Under an objective clinical examination, it was observed that the general state of the patient was extremely poor, with preserved consciousness. The child presented Cushingoid facies, ringed, with pale skin, dry, with multiple bruises and stretch marks post-venous, with peripheral cooling trend. The patient had aphthous stomatitis and dry lips, muscular system hypotonic and hypokinetic weakness. Stetacustic lung could detect the presence of a coarse vesicular murmur with disseminated bronchial rales on both lung areas.

Heart sounds were rhythmic, cardiac frequency of 84b/min and blood pressure of 97/40mm Hg. In the digestive system, slowed intestinal transit was present, lack of appetite, hepatomegaly 3-4 cm below the costal margin, of soft consistency, with rounded front edge.

Patient reacts only to painful stimuli, balance disorders and psychomotor retardation specific to age.

Laboratory examinations could reveal the presence of microcytic hypochromic anemia, thrombocytopenia and absent inflammatory markers, hepatic cytolysis syndrome, hyperglycemia, remaining examinations were within normal limits.

Electrocardiogram showed sinus tachycardia 140b/min, QRS axis +30 degrees, PQ = 0.12 and rounded T waves in all leads (fig. 1, 2).

Chest radiograph (postero-anterior incidence) showed lung drawing accentuated hiliobazal, bilateral and heart displayed on the diaphragm (fig. 3).

Given the patient's neurological status,

computed tomography performed on 15.12.2008 was reconsidered, an examination which confirmed the diagnosis of glioma Pontin (fig. 4, 5, 6, 7),

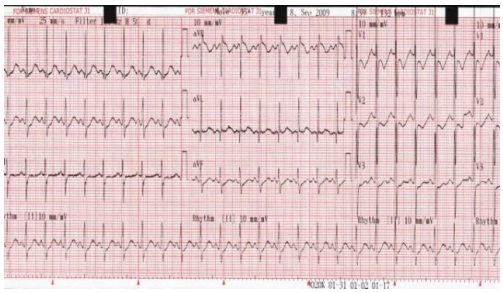

Fig.1. EKG – sinus tachycardia

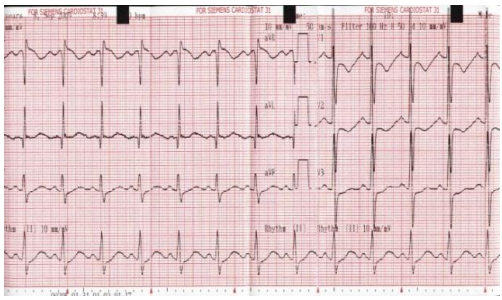

Fig. 2. EKG – where T rounded in all leads

Fig. 3. Chest radiography – accentuated lung drawing hiliobazal, bilateral, heart displayed on the diaphragm

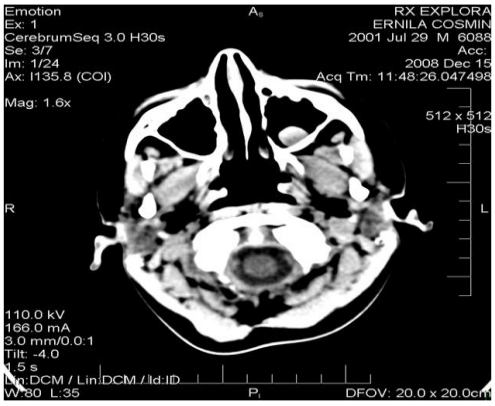

Fig. 4. Cranio-cerebral computer tomography - glioma Pontin

Fig. 5. Aspects of computed tomography

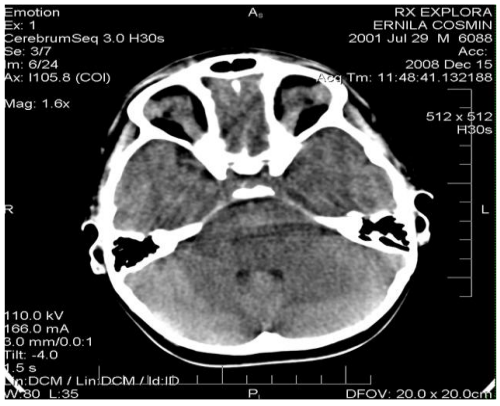

Fig. 6. Aspects of computer tomography

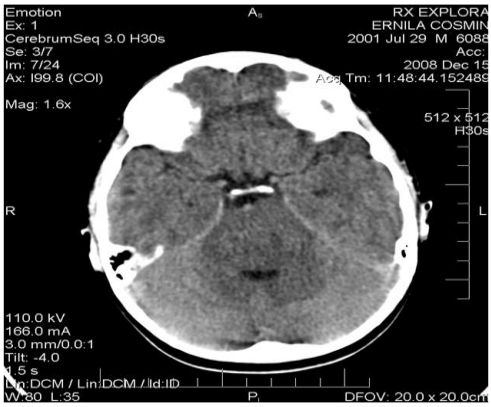

Fig. 7. Aspects of computer tomography

Neurosurgical examination revealed that the patient was conscious, with poor cooperation and left hemiparesis.

Doppler echocardiography performed on 08/06/2009 showed an intraventricular vegetation 1/1cm, located at the apical level that does not cause hemodynamic disturbances (fig. 8). Route ECG performed immediately revealed paroxysmal supraventricular tachycardia, AV = 170b/min, PQ = 0.12 and indefinite electrical axis with biventricular overload. Cardiothoracic radiography reveals a pronounced lung and heart drawing displayed on the diaphragm.

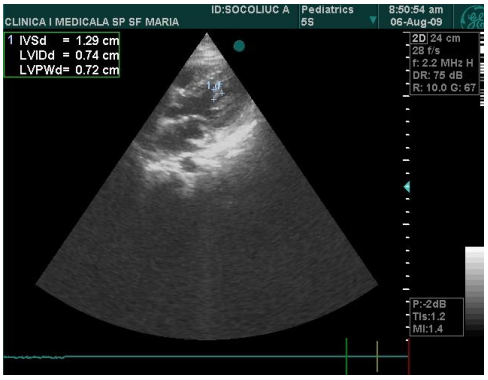

Fig. 8. Aspects of echocardiography Vegetation on apical level of the left ventricular

Echocardiography performed on 11.09.2009 highlights hiperechogene formation with dimensions of 1.52 mm / 0.57 mm VMA at the top left ventricular formation of 1/0, 4cm, VMA perforation, mitral insufficiency degree III, severe tricuspid degree III, pulmonary insufficiency degree III, raising the suspicion of pulmonary valve endocarditis (fig. 9).

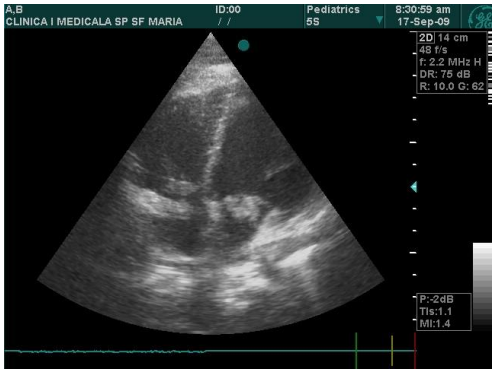

Fig. 9. Aspects on echocardiography Vegetations on mitral valve and tricuspid valve

The echocardiography performed on 17.09.2009 the endocarditis is diagnosed with 5 locations: mitral valve, aortic valve, pulmonary valve, tricuspid valve and ventricular tip.

During admissions, the patient was given antibiotics in dual therapy, initially with Ciprofloxacin Targocid and subsequently with Tienam and Cefort, for hemodynamic support Dopamine was administered, and Spironolacton for cardiac function, Furantril, under which the evolution of symptoms was weakly positive, finally, the patient died.

Final diagnoses were: 1. brainstem glioma; 2. syndrome pontomezen-cephalic; 3. VI nerve paresis left; 4. convergent strabismus left eye; 5. postchemotherapy ane-

mia; 6. secondary thrombocytopenia, aphthous stomatitis; 7. endocarditis with five locations: mitral valve, aortic valve, pulmonary valve, tricuspid valve, top right ventricular; 8. perforation of anterior mitral valve, 9. sinus tachycardia; 10. hepatic cytolysis postchemotherapy.

CONCLUSIONS

The peculiarity of the case lies in the presence of five outbreaks of endocarditis. The first ultrasound localization was described on 06.08.2009 at the top of right ventriclem, size of 1/1 cm after the patient received broad spectrum antibiotics and antifungal agents, and one month later it was described in other four sites of endocarditis.

This is explained by the patient's immunosuppressed status (by chemotherapy with Temodal). Also, chemotherapy is responsible for anemia, thrombocytopenia, hepatic cytolysis syndrome and nitrogen retention syndrome.

During the 9 hospitalizations, the patient had an episode of paroxysmal supraventricular tachycardia, for which he received treatment with metoprolol, this was the only episode.

Another feature of the case is the age of cerebral glioma–being outside the two peaks of incidence of the disease by age.

Patient prognosis was reserved, finally the patient died despite of all the efforts.

The prognosis of infectious endocarditis was also reserved as the etiologic agent could not be identified; the mortality reported in literature is of 20-25%.

Glioma Pontin has the worst prognosis, with average survival of 9-12 months, despite treatment, the patient survived nine months after being diagnosed.

Favorable prognosis factors in glioma Pontin are represented by neurofibromatosis, symptoms of less than 12 months before the diagnosis, exofitic localization, favorable histology, extra cervico medullary or focal tectal tumors, and calcification on computed tomography. Factors of unfavorable prognosis are under 2 years, with cranial nerve palsy and unfavorable histology.

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NEWS

IMPORTED HUMAN RABIES CASES WORLDWIDE

Authors from the Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France, carried out a study about 60 cases of human rabies in international travelers, reviewed from 1990-2012. A significant proportion of the cases were observed in migrants or their descendants when emigrating from their country of origin or after a trip to visit friends and relatives or for other reasons (43.3%). The cases were not necessarily associated with long-term travel or expatriation to endemic countries; moreover, cases were observed in travelers after short trips of two weeks or less. A predominance of male patients was observed (75.0%). The proportion of children was low (11.7%). Cases from India and Philippines were frequent (16 cases/60). In a significant proportion of cases (51.1%), diagnosis was challenging, with multiple missed diagnoses and transfers from ward to ward before the final diagnosis of rabies. Among the 28 patients whose confirmed diagnosis was obtained ante-mortem, the mean time between hospitalization and diagnosis was 7.7 days (median time: 6.0 days, range 2-30) including 4 cases with a diagnosis delayed by 15 or more days. In 5 cases, a patient traveled through one or more countries before ultimately being hospitalized. Factors that played a role in delaying the diagnosis of rabies in a number of cases were a low index of suspicion for rabies in countries where the disease has been eradicated for a long time or is now rare, a negative history of animal bites or exposure to rabies, and atypical clinical presentation of the disease. Clinical symptomatology of rabies was complex and commonly confuses physicians. Furthermore, failure in diagnosing imported cases in more developed countries is most likely related to the lack of medical familiarity with even the typical clinical features of the disease. (Carrara P, Parola P, Brouqui P, Gautret P. Imported human rabies cases worldwide, 1990-2012. *PLoS Negl Trop Dis.* 2013; 2; 7(5): e2209)

Alina Manole

MENINGITIS SEPSIS AFTER IUD INSERTION, A CASE PRESENTATION

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MENINGITIS SEPSIS AFTER IUD INSERTION, A CASE PRESENTATION (Abstract): *Neisseria meningitidis* is a normal commensal of human mucous membranes that is no longer considered to be restricted to the nasopharynx. Due to the practice of oral sex, the mucous membranes of the cervix, urethra or anus have become a potential infection site for this bacterium. Inserting an intrauterine device (IUD), can alter the protective barrier of the endocervical mucosa, allowing for bacterial infection and systemic spread. We present a case report of a 40-year old woman who presented with abdominal pain, spotting and fever after inserting an IUD and developed a fulminant septical shock. Blood cultures and cultures from ascites showed the presence of *Neisseria meningitidis* group Y. From our knowledge, there are a few cases presented in the literature of toxic shock syndrome after IUD insertion, caused by *Staphylococcus aureus* or *Streptococcus* group A, but this is the first case of meningococcal sepsis after IUD insertion described. So, even though IUDs rarely cause significant infection, physicians should consider this device as a possible source in reproductive-aged women with the clinical features of sepsis. **Keywords**: MENINGOCOCCUS, IUD, SEPSIS

Neisseria meningitidis, an encapsulated, gram-negative diplococcus, is a normal commensal of human mucous membranes. It is no longer considered to be restricted to the nasopharynx, although this is the most frequent site identified as its reservoir (1). Meningococci are spread through respiratory droplets, normally requiring close and prolonged exposure or direct contact with saliva and respiratory secretions. Spread can occur from an asymptomatic carrier or from a patient who is ill. Occasionally, shortly after the onset of colonization, *N. meningitidis* strains might penetrate the mucosal membrane and gain access to the bloodstream. Bacteria that are able to survive and multiply in the circulation may then cross the meninges and gain access to the cerebrospinal fluid. Of the various forms of meningococcal disease, the most common ones are meningitis and severe sepsis with often a rapid fatal outcome (2).

Due to the practice of oral sex, the mucous membranes of the cervix, urethra or anus have become a potential infection site for this bacterium, and by inserting an IUD, the protective barrier of the endocervical mucosa can be altered, allowing for bacte-

rial infection and systemic spread.

Genital infection caused by *Neisseria meningitidis* (*N. meningitidis*) is not a common infection met in a gynecological practice, even if over the years there have been described cases when *N. meningitidis* was isolated from the urogenital tract, mostly as associated with subclinical infection (3-5) but also with cervicitis and even with salpingitis and peritonitis (1, 6). From our knowledge no case of sepsis related to a gynecological meningitis infection after IUD insertion was described until now.

Intrauterine devices (IUD) are a common used contraceptive method and even if it is known their association with a slightly increased risk for pelvic inflammatory diseases(cervicitis,endometritis, salpingitis, peritonitis), it is considered that the benefits of IUD insertion generally outweigh the risks (7).

In this case report we present a young woman that suffered a fulminant meningococcal sepsis presumably after an IUD insertion.

CASE REPORT

A 40-year-old woman, in a stable relation for one and a half years, zero-gravid, presented in November 2012 to the Obstetrics and Gynecological hospital, Malmö, Sweden, with lower abdominal pain and spotting after inserting an IUD. Anamnestic, she was suffering from Sjögren's syndrome and in 1997 had been operated for thyroid cancer, now on substitution medication.

For two weeks back, inserted a Copper-IUD and have had since then low-moderate abdominal pain and vaginal spotting. She presents to the gynecological emergency department with abdominal pain and fever. Her triage vital signs were blood pressure (BP) 112/63 mm Hg, pulse 97 beats/min, and temperature 39.3°C.

Initial examination showed tenderness of the lower abdomen without muscular defense. There was a brownish discharge from the cervix at speculum examination and the patient reported severe pain on dislocation of the cervix and uterus. No adnexal masses were palpated. A wet smear of the vaginal discharge showed leukocytosis and clue-cells. Cervical specimens were taken for culture of pathogenic *Neisseria gonorrhea* and *Chlamydia trachomatis*. Vaginal ultrasound showed a normal uterus with the IUD lying in the endometrial cavity and normal ovaries; no fluid in the Douglas pouch was noted at that time. Venous blood proves showed a normal level reactive protein C at 1.7 mg/ml, WBC count 3.6/mm^3 and normal renal, hepatic and electrolytes values.

The diagnosis of endometritis is set and the patient is sent home with oral treatment with Doxycillin, Metronidazol and antiinflammatory. Seven hours later, she wakes up with abdominal pain, diarrhea, vomiting and rash and comes by ambulance to the emergency hospital with the initial suspicion of an allergic reaction to medication. The examination shows intense abdominal pain, an increased liver, peripheral cyanosis, hypotonia, petechiae, but no neck stiffness and normal lungs auscultation. Afebrile at incoming, in the next hour the temperature rises to 40°C, triage vital signs are now BP 90/60 mmHg, pulse 120beats/min. Laboratory results show WBC count 0.8/mm^3, platelets 11/mm^3, creatinine 165mg/dl, reactive protein C 67mg/dl, base excess - 10mmol/l and lactic acid 8mmol/l. Deteriorates fast, needing intubation. Cultures are taken from blood, urine, throat,

nose and an initial dose of Meropenem and Tobramicyn is administered. A CT thorax and abdomen without contrast, shows a lot of fluid in the abdomen, an increased liver with edema around the intrahepatic veins and bilateral infiltrates at the basis of the lungs with pleural fluid. With a fulminant progress of sepsis, the patient becomes anuric, with distended abdomen and marmorated extremities and coagulopathy. The IUD is removed and diagnostic laparoscopy is performed next morning but only via the camera port because of the patient's increased abdominal pressure. The image (of poor quality) shows normal appearance of the bowels that flow in serous fluid. The genital organs are not seen, but there are no signs of peritonitis or abscess. Culture from the ascites is taken.

In a matter of hours she develops dry gangrene in fingers and toes, probably after septic embolism and aggressive vasopressor treatment.

At this time the culture results become available and they are positive for *Neisseria meningitidis* group Y, in blood and the peritoneal fluid.

Under broad spectrum antibiotic treatment, respiratory support and continuous renal replacement therapy, the patient starts to recover, but ten days after admission, her evolution becomes unstable again with intense abdominal pain and hypotension, and requires laparotomy because of suspicion of internal bleeding. Under operation spleen rupture is found, presumably after spleen infarct as secondary complication to sepsis and a splenectomy is performed, the internal genitalia are also inspected but except some secondary fibrin deposits on the uterus, no other pathology is seen.

After another complication in form of candida sepsis with retinitis, she finally slowly recovers and after nine weeks of hospitalization, is discharged with planned follow ups at orthopedic, hand surgery, endocrinology and ophthalmology clinics.

DISCUSSION

Sepsis after insertion of an IUD is a rare condition. The few cases found described in literature referred to toxic shock syndrome after IUD insertion caused by Streptococcus group A (8, 9) and *Staphylococcus aureus* (10). We haven't found any case reporting meningococcal sepsis after an IUD insertion. In this case presentation, there was no available culture from cervix or from the removed IUD, but beside the positive blood culture, the only other positive culture for *N. meninigococcus* was in the peritoneal fluid. Since the patients symptoms started together with the IUD insertion, and her only complain was gynecological, this was considered the primary focus.

There are at least 13 serogroups of *N. meningitidis*, with the most important being serogroups A, B, C, Y and W-135 (11). Meningococcemia is defined as dissemination of meningococci into the bloodstream. The fundamental pathologic change in meningococcemia is widespread vascular injury characterized by endothelial necrosis, intraluminal thrombosis, and perivascular hemorrhage. Endotoxin, cytokines, and free radicals damage the vascular endothelium, producing platelet deposition and vasculitis. The deleterious effects of cytokines play a major role in the pathogenesis of meningococcemia by causing severe hypotension, reduced cardiac output, and increased endothelial permeability (12). Management should focus on the treatment of the urgent clinical issues in addition to the administration of specific antibiotic

therapy. Empirical antibiotic therapy for suspected meningococcal disease consists of a third-generation cephalosporin to cover the various other (potentially penicillin-resistant) bacteria that may produce an indistinguishable clinical syndrome (13).

As isolation of *Neisseria gonorrhea* has been reported from pharynx (14), findings of *N. meninigitidis* in urogenital sites has been also described over the years, most probably due to changes in sexual behavior and habits during the last few decades, where the majority of cases of infection occur via the urogenital contact, with the nasopharynx acting as the major reservoir in healthy carriers (3).

There have been found healthy carriers of meningococci in urethra, cervix and rectum in both males and females, where meningococci were isolated from pharynx of partners (15,16). There are also cases described of urethritis, anitis cervicitis, salpingitis (1, 3, 6, 16) associated with the presence of *N. meninigitidis.*

CONCLUSIONS

Even though IUDs rarely cause significant infection, emergency physicians should consider this device as a possible source in reproductive-aged women with the clinical features of sepsis. And since the isolation of *Neisseria meningitidis* has been reported from urogenital sites, it is recommended in cases of suspected sepsis related to an IUD insertion to take also in consideration *Neisseria meningitidis* as a potential pathogen, along *Streptococcus group A* and *Staphylococcus aureus.*

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NEWS

MESOTHELIOMA NOT ASSOCIATED WITH ASBESTOS EXPOSURE

Despite asbestos being identified as the single most important cause of malignant mesothelioma, the tumor is known to occur in only 10% to 20% of heavily exposed individuals. In addition, about 20% of the patients have no history of asbestos exposure even after detailed assessment. It should be realized that although there is a strong link between the development of malignant mesothelioma (MM) and asbestos exposure, not all cases are etiologically related to asbestos exposure. The proportion of cases attributable to asbestos exposure varies between the sexes and country, according to occupation and use of amphibole asbestos. A number of other agents have been implicated in the causation of MM but the best evidence appears to relate to another mineral fiber erionite and to irradiation. Erionite has emerged as the most important example of non asbestos-mediated cause of mesothelioma in regions such as Turkey where exposure to this type of fiber is highly prevalent. Ionizing radiation is a recognized human carcinogen and an established risk factor for several different types of cancers including hematologic malignancies and solid tumors. Cases of MM of the pleura, peritoneum, and pericardium have been reported in humans after therapeutic irradiation and thorium dioxide administration. Also, there is a strong experimental, epidemiologic and molecular evidence to suggest a possible carcinogenic or cocarcinogenic role of viruses such as SV40, a monkey polyoma virus, in the induction of MM. Although the polyoma virus SV40 has been unexpectedly discovered as an effective cocarcinogen of asbestos in the causation of animal mesothelioma, its potential role in human mesothelioma remains unproven. (Bharat Jasani, Allen Gibbs. Mesothelioma Not Associated With Asbestos Exposure. Arch Pathol Lab Med. 2012;136:262–267).

Doina Butcovan

A CASE OF TURNER SYNDROME ASSOCIATED WITH SEVERE COARCTATION OF THE AORTA

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A CASE OF TURNER SYNDROME ASSOCIATED WITH SEVERE COARCTATION OF THE AORTA (Abstract): Turner Syndrome (TS) is a chromosomal abnormality due to the complete or partial lack of chromosome X, with clinical polymorphic elements: small size, hypogonadism, various visceral abnormalities. About half of the cases may suffer from congenital heart abnormalities. We describe hereunder a case of TS diagnosed with coarctation of the aorta due to high blood pressure detected by accident. **Key words:** TURNER SYNDROME, COARCTATION OF THE AORTA

Turner Syndrome (TS) is a chromosomal abnormality due to the complete or partial lack of chromosome X. TS incidence is about 1 case/ 2500 new-born female babies. TS pregnancy frequency is much higher, and many foetuses are lost by miscarriages (10% of miscarriages have TS). The TS syndrome is a generic one, the most common chromosomal abnormality being homogeneous monosomy 45,X (55% of the cases), followed by structural abnormalities of chromosome X (25% of the cases) and one normal cell line mosaic in 20% of the cases.

The clinical symptoms vary, whereas the subjects' psychological and motor development is within normal limits. At birth, this condition characterized by the subjects' small size, upper and lower limb lymphedema, webbed neck; 5% of the cases have severe cardiac malformations (severe coarctation of the aorta) requiring urgent surgery. The characteristics that are constantly present during childhood, adolescence and adulthood are the subjects' small size and, with age, delayed puberty, ovarian failure and infertility (1, 2).

These are accompanied with less common conditions such as: autoimmune diseases (thyroiditis, diabetes mellitus, celiac disease). 44.5% of the cases have congenital heart abnormalities (less severe coarctation of the aorta, bicuspid aortic valve, pulmonary and coronary artery abnormalities), whereas 40% of the patients suffer from early hypertension since their adolescence (3). The diameter of the aorta must be monitored the subjects' whole life, as there is a risk of aortic dilatation. Hypoacusis due to frequent otitis and horseshoe kidney are also included in this category (4, 1).

Medical management consists of growth hormone therapy during childhood, then

estrogen-progesterone supplementing during puberty. Trans-thoracic echography is recommended even when no clinical symptoms are present and is designed to detect possible aortic valve abnormalities or aortic dilatation (5).

The subjects' life quality, self-esteem and social integration are much better when puberty is not induced too late. The prognosis depends on the associated abnormalities: congenital heart abnormalities, hypertension, diabetes mellitus, obesity, thyroiditis, osteoporosis (2).

CASE REPORT

Patient DRM, aged 14 years and 3 months, came for primary amenorrhea, repeated hypertension (highest value 150/80 mmHg) and systolic murmur.

Family medical history. The patient is the second child of an unrelated apparently healthy couple, the mother 31 years of age and the father 37 years of age (at the time of conception). No other congenital anomalies, genetic diseases or reproduction disorders run in the family.

Personal physiological history. The pregnancy had a normal evolution. The birth was a term birth, the delivery was natural, head first, APGAR score 7 (1), weight 2000g, upper limb lymphedema. No data on the size and head circumference at birth were available.

Personal pathological history. The patient's first medical examination occurred when she was 13 years and 11 months old, in the endocrinology ward, where she went for primary amenorrhea. High blood pressure and systolic murmur are detected on this occasion, for which reason she was referred to the pediatric cardiology clinic.

Status upon hospitalization (14 years 3 months). The clinical examination reveals: small size (G = +1.16 DS, T = -2.33 DS, Pc = +0.88 DS), normally colored teguments and mucous membranes, lymphedema on the dorsal surface of the hands and feet, webbed neck, hard and soft palate dysmorphism, great inter-nipple distance, low hair level on the nape, many nevi. Slightly dysmorphic facies, earns positioned low and slightly flaring nose. Athletic constitution with broad shoulders and narrow hips. The patient also had cubitus valgus, clubfoot, shortening of the 4-5 metacarpal and metatarsal bones, hypoplasic nails and kyphotic-scoliotic posture. As concerns the cardiac system, we noticed the presence of collateral circulation in the thoracic cage, a III/6 degree systolic murmur in the left II-III intercostal space with interscapular-vertebral irradiation and in the epigastric region, clear peripheral pulse in the radial arteries, weaker pulse in the lower limbs (dorsalis pedis artery and posterior tibial artery, which is possibly also due to the lymphedema). The blood pressure was 160/80mm Hg in the upper limbs, and 140/70mm Hg in the lower limbs. The patient also complained of vertigo, fronto-occipital cephalalgia and bilateral hypoacusis.

During her hospitalization we detected high blood pressure (150/80 mmHg); the results of the complete blood count, hepatic and renal tests, glycemia and urine tests were normal; the fist X-ray reveals that the bone age is in agreement with the chronological age, fertile growth cartilages; the pelvic echography reveals that both kidneys have normal morphology, uterus 25/9.9/26 mm, left gonad 15.5 mm, no right gonad is detected; the hormonal tests reveals subclinical hypothyroidism (TSH = 7.47 µUI/ml, fT4 = 17.95 pmol/l, AAT-PO = 8.82 UI/ml); thyroid echography – hypoechogeneous thyroid.

Echocardiography revealed a severe aortic coarctation.

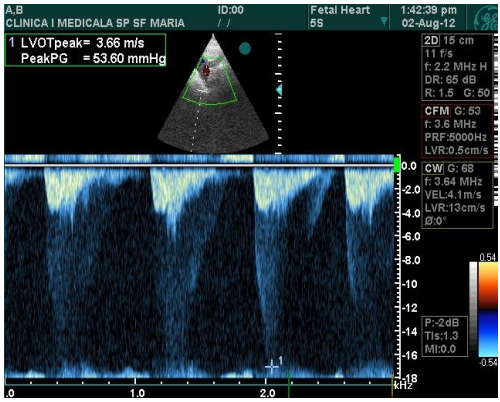

Fig. 1. Patient D.R.M.: echocardiography (suprasternal view) - severe coarctation of the descending aorta

The genetic examination sets a certain diagnosis, namely Turner Syndrome, homogeneous monosomy X, karyotype 45,X.

As a coarctation of the aorta was suspected based on clinical grounds, a thoracic angio CT scan was performed, which revealed a significantly narrowed area (0.73 cm diameter) on the descending thoracic aorta 0.8 cm below the origin of the left subclavicular artery; the caliber of the ascending aorta was 3.27 cm (2.14 cm in the prestenotic area and 2.06 cm in the poststenotic area); considerable collateral circulation visible in the cervico-dorsal area, dilatation of the left subclavicular artery 1.42 cm, of the right brachiocephalic arterial trunk 2.16 cm, of the internal thoracic arteries and of the collateral branches of the descending thoracic aorta; partially aberrant pulmonary venous drainage with possible aterio-venous fistula, pulmonary perfusion disorders. The spine was also affected (spine compression at the level of the D3 and D12 vertebrae, D5-L1 multiple intrabody disk herniations and slight kyphosis.

The final diagnosis was Turner Syndrome by homogeneous monosomy X, tight coarctation of the descending thoracic aorta, subclinical hypothyroidism, lower limb lymphedema, kyphosis.

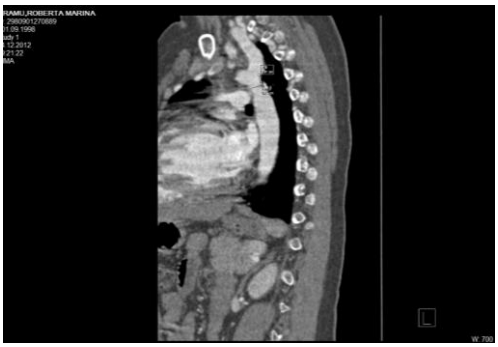

Fig. 2. Patient D.R.M – Angio-CT: area narrowing descending thoracic aorta, the vertebral body subsidence cuneiform D3, D12, D5-L1 multiple intrabody disk herniations and slight kyphosis

Fig. 3. Patient D.R.M – descending aorta coarctation

Therapy

We recommended endocarditis prophylaxis, specific hypertension therapy and avoidance of physical exertion. Measures should be taken to prevent the complications of congenital abnormalities, either by dilating the stenosis area by means of a balloon and stent, or by specific surgical procedures (6). The proband will also be given Euthyrox 100 μg/day for her subclinical hypothyroidism.

Evolution and Prognosis

The asymptomatic forms of coarctation of aorta are detected due to high blood pressure values accompanied by cephalalgia, repeated nosebleed or after a stroke. The prognosis depends on the success of the cardiac abnormality treatment and on hypertension persistence after surgery.

Given the patient's bone age, ~ 14 years, and her heart abnormality, GH therapy is tardy and will be avoided. The management of female patients suffering from Turner Syndrome proposed by Kavoussi et all. includes cardiologic supervision in outpatient clinics even when there are no clinical signs, blood pressure measuring on each visit to the doctor, renal echography and aggressive urinary infection therapy, TSH, glycemia and cholesterol dosing, annual hepatic tests, early scoliosis detection, hypoacusis due to repeated otitis, growth hormone therapy until the bone age of 15 years, then estrogen-progesterone therapy, integration in a support group and psychological counselling designed to prevent any psychosocial integration difficulties (7, 8).

As literature reports a frequent association between TS and autoimmune diseases (Hashimoto thyroiditis, type 1 DM, celiac disease, juvenile rheumatoid arthritis, psoriasis, veal skin) and since the patient already has subclinical hypothyroidism, careful pediatric follow-up is required for the early detection of such complications (9).

The specificity of the case consists of the association between a coarctation of the descending thoracic aorta, a severe form diagnosed at the age of 14 years, and the Turner Syndrome.

CONCLUSIONS

The clinical manifestations, extremely suggestive of Turner Syndrome and of cardiac abnormality, require early genetic and cardiologic exploration for a certain diagnosis setting, adequate therapy and prevention of serious possible complications of the coarctation of the aorta.

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IS THERE A RISK FOR LYMPHOMA OR EXTRACOLONIC CANCER IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE?

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IS THERE A RISK FOR LYMPHOMA OR EXTRACOLONIC CANCER IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE? (Abstract). An important issue in the management of inflammatory bowel disease (IBD) is the risk for lymphatic or extracolonic malignancies reported in patients receiving prolonged immunosuppressive therapy and/or therapies with biological agents. Azathioprine (AZA), 6-mercaptopurine (6-MP), methotrexate, and anti-TNF (infliximab, adalimumab) are reference drugs for IBD forms unresponsive to conventional therapies. The administration of these drugs is a high responsibility because IBD itself is associated with an increased risk for cancer, namely colon cancer. The possibility of a drug-induced additional risk remains controversial, the relative risk for lymphoma being estimated at 1.2%. For extracolonic malignancies, there are variations in standardized incidence per reference population in terms of location (skin, liver and biliary tract, uterine cervix, prostate, etc.), and also IBD phenotype (ulcerative colitis or Crohn's disease). The uncertainty regarding the occurrence of neoplasia in IBD patients for many years on immunosuppressive therapy and/or biological agents is a strong argument both for treatment discontinuation and it's monitoring by inclusion in screening programs. In this paper we aimed to approach the conceptual model risk-benefit in the therapy with imunosuppressive and biological agents given the controversies in the literature generated by the drug-induced risk for malignant lymphatic and extracolonic tumor in patients with IBD. **Key-words:** INFLAMMATORY BOWEL DISEASE, EXTRACOLONIC CANCER, LYMPHOMA.

Inflammatory bowel disease (IBD) is a condition of chronic intestinal inflammation of unknown cause that includes two entities with distinct clinical and morphological features: ulcerative colitis (UC) and Crohn's disease (CD). In UC inflammation is limited to mucosa and is present only in the rectum and colon, and in CD the inflammatory process is transparietal and may affect any segment of the digestive tract, from mouth to near the anus (perianal disease). Descriptive epidemiology data show that IBD is a common disease worldwide, with large region-to-region variations in incidence. In the United States about 2 million people are affected and the worldwide incidence of UC is 0.5 to $24.5/10^5$ inhabitants (1). Europe is characterized by an uneven distribution of the disease, while South-Eastern Europe is less exposed to the risk of disease occurrence, fact supported by the low incidence in this area. In Romania the incidence of IBD is low, with an epidemiological profile dominated by the frequency UC, the incidence of which is approximated to $1/10^5$ inhabit-

ants. Regional data from a 2006 study in North-Eastern Romania show an IBD incidence of $1.75/10^5$ inhabitants, with an upward trend for CD and severe forms, mainly of UC (2).

IBD AND COLORECTAL CANCER

An IBD feature is the occurrence during disease progression of colorectal cancer (CRC), first recognized as a complication of UC in 1925 by Crohn and Rosenberg (3). Since then, several epidemiological studies have confirmed this increased risk, but its magnitude remains controversial due to variations in diagnostic methods. CRC occurs in 2-4% of patients with UC and accounts for 1% of all colorectal cancers in the general population (4). A number of factors that define the clinical features and progression of this disease, alone or in combination, may increase or decrease the risk for CRC. Factors associated with this increased risk are: disease duration, extension of colitis, sclerosing cholangitis (SC), family history of CRC, early onset of disease, and severity of inflammation (5). Main factors correlated with the risk for CRC are disease duration and extension. Early and prolonged exposure of intestinal mucosa to chronic inflammation is responsible for the occurrence of metaplasia - dysplasia - neoplasia sequence (6).

This context requires the use in this population group of endoscopic surveillance methods (colonoscopy) for cancer detection. A prerequisite in lowering this risk is mucosal healing with restoration of normal structure and function. This goal requires the administration of drug groups with immune mediation actions which are responsible for inducing long-term immunosuppression, thus increasing the risk for the occurrence of lymphoma and extracolonic cancer. The risk of extracolonic cancer in IBD patients is increased both by chronic intestinal inflammation and immunosuppressive treatment. This is a concern both for doctors and patients when treatment with purines and/or anti-TNF agents is considered.

One example is azathioprine (AZA), which by being incriminated in the increased risk for malignancy in patients with rheumatoid arthritis or kidney transplant, drew attention to the likelihood of increased risk in IBD patients on long-term immunomodulatory therapy. However, the risk of long-term immunomodulatory therapy-induced cancer remains controversial in both theory and practice, because IBD itself is associated with an increased risk for cancer, and most published studies found no risk for lymphoma or cancer with 5-year treatment; this needs to be also demonstrated for much longer treatments (7). It clearly comes out that the responsibility of gastroenterologists who use these drugs is high, and that he has to be aware of both its benefits and risks in order to provide optimal care to IBD patients.

We now present the drug classes used in IBD and the results of studies on the risk of extracolonic cancer and lymphoma.

THERAPEUTIC PRINCIPLES IN IBD

IBD is a group of chronic diseases, continuous or recurring in flares interrupted by remission, which requires long term, sometimes lifetime, treatment. The therapeutic goals are focused on inducing remission and maintaining it, with the ultimate goal of mucosal healing, which decreases the risk of cancer. Induction treatment aims at controlling clinical signs, reducing acute complications rate, and improving the qual-

ity of life.

Remission induction therapy uses the following drugs classes over a limited period of time:

- 5-aminosalicylate acid derivatives (5-ASA, mesalazine), used only in mild and moderate UC; historically, sulfasalazine and mesalazine, were used for remission induction in mild and moderate forms of CD, but cumulative evidence from different studies indicate that these drugs are less or even ineffective in treating patients with CD (8);
- corticosteroids, represent the most effective therapeutic group in inducing clinical remission used in the treatment of flares in the moderate and severe forms of IBD, or in cases with insufficient response to aminosalicylate treatment; corticosteroids include agents with oral, parenteral and topical administration. The use of corticosteroids in the treatment of IBD is based on its multiple immunosuppressive and anti-inflammatory actions intervening as a modulator or suppressor (high dose) in the production of inflammatory and immune response mediators; their effectiveness is proven if used properly, but no more than three months to avoid corticodependence (inflammatory recurrence when drug discontinuation or dose reduction are attempted) or corticoresistance (persistence of clinical manifestations on treatment) reported in approximately 50% of patients who required hormone treatment (9);
- biological therapy, represented by anti TNF alpha antibodies, is useful both in moderate- severe UC and CD (10);
- cyclosporine, is the drug of choice in toxic megacolon from UC; side effects limit its long term use and dosage (11).

Long-term maintenance of remission is sometimes a life time treatment. If some therapeutic classes, such as 5-ASA derivatives, prevent the risk for CRC, other drug groups (immunomodulators and biological agents) are incriminated in the increased risk for lymphoma and extracolonic cancer.

The drug classes used for maintenance of remission are (12):

- 5-ASA (mesalazine), used only in UC;
- purines, represented by azathioprine, 6-mercaptopurine, and methotrexate, used in CD and in patients with UC who developed corticodependence;
- anti TNF alpha antibodies.

The groups of drugs with risk of extracolonic cancer are purines and biological agents. If data on long term purine therapy are numerous, for biological agents data are available since 1998, when Infliximab was approved for the treatment of moderate and severe CD. In 2007, Adalimumab (humanized monoclonal antibody) and in 2008 Certolizumab (Fab fragment of humanized anti-TNF) (13) were introduced in the treatment of these diseases. The latest class of biological agents is represented by Natalizumab, an anti-integrin α monoclonal antibody.

IBD AND HEMATOLOGIC CANCERS

Studies on large series of patients with IBD demonstrate an association between CD and non Hodgkin's lymphoma (NHL) and between UC and leukemia. CD-myelodisplasic syndrome relationship has not been proven, and in terms of increased risk of lymphoma in patients exposed to purines the available results are controversial. In IBD patients followed up for long periods of time (18 years, between 1983 and 2001) 9 cases of lymphoma were found, with the mention that no patient had received purines prior to the diagnosis (14).

Conversely, other studies show an increased risk of lymphoma in patients exposed to purines. AZA and 6-MP are involved in lymphoma occurrence in patients treated for rheumatoid arthritis and prevention of graft rejection. A therapeutically-induced additional risk remains controversial, the relative risk for lymphoma being estimated at 1.2% with an estimated incidence in the multiple IBD patient series of 0.2-0.4%.

In terms of quality of life, the benefits of a 4-year immunosuppressive therapy outweigh the risk of developing cancer, and with a 5-year treatment no risk of lymphoma or cancer was found. The uncertainty regarding the occurrence of neoplasia in UC patients for many years on immunosuppressive therapy is strong reason for treatment discontinuation (15).

With biological therapy, IBD patients undergo prolonged immunosuppression with increased risk for hematological cancer in cases previously treated with purines. But given that most patients treated with biological agents have a positive therapeutic history for purines (corticodependent and corticorezistant severe illness), it is not clear whether the event is specific to this class of drugs or may be considered as a side effect of purine treatment. Conclusions on the adverse effects of biological therapy are still a subject for debate. Experts have tried to answer these questions by multicenter, randomized, case control, case studies (ex. reference studies for the validation of biologic therapy in IBD - ACCENT I and ACCENT II). In a series of 1107 patients with CD who received infliximab, 9 cases of lymphoma (0.81%) were found, percentage similar to that in the control group (CD patients not receiving infliximab) (16).

In the Mayo Clinic study conducted on 500 patients with IBD treated with infliximab, of 7 detected cancers (non-lymphomas) two were lung cancers, possibly due to infliximab treatment (17). In a Danish series of 561 patients with CD treated with infliximab for the 4 cancers reported no such association was found, the conclusion being that the treatment with infliximab is cancer-risk free (18). Similar conclusions come from a series of 2319 CD patients treated with infliximab: 30 patients (1.29%) developed cancer, percentage comparable with the general population and placebo group (19). A meta-analysis of 21 placebo-controlled trials including 5356 patients treated with biological agents concluded that cancer risk is not increased by biological therapy (20). An Italian study on 404 patients with CD treated with infliximab *vs.* 404 CD patients without infliximab demonstrated the similar malignancy rate in the both groups (9 vs. 7). The conclusion is that anti-TNF alpha agents seem not to increase cancer risk.

As to the relationship NHL-anti-TNF therapy the results are controversial. Also controversial is the response to the question whether the association of biological agents with purines increases the risk of lymphoma. In reference studies on this topic there are both pro and con opinions. In Britain, in a series of 6,000 patients with CD and 10,000 with UC treated with biological agents the risk of lymphoma was 1.2, comparable to that of general population (21). In Sweden, the study of 47,000 patients with IBD treated with biological agents showed a slight increased risk for NHL in those within the first 5 years of CD (22).

A Canadian study on 21,000 patients with CD showed a significantly increased

risk of NHL in men, independent of the use of purine immunomodulators (23). In the Mayo Clinic study the two NHL cases of the 500 patients treated with biologic therapy also received AZA (8). The study of 3,493 patients with rheumatoid arthritis treated with biological agents without AZA identified 10 cases of NHL and no case in the control group (24). In the study on 8,905 patients with IBD treated with biological therapy, 11/13 patients with NHL were exposed to purines (25). Hepatosplenic T-cell lymphoma is a rare type of peripheral NHL, 200 cases being described in the literature most in patients on immunosuppressive therapy (post-transplant, HIV). For IBD, 8 cases of hepatosplenic T-cell lymphoma in young men (12-31 years) with CD, treated with infliximab and purines, have been reported. And with adalimumab treatment 9 NHL cases were reported in patients with CD treated with purines (26). Experts concluded that there is risk for NHL in young men (20-54 years) on biologic therapy and prior exposure to purines.

IBD AND EXTRACOLONIC CANCERS

Patients with IBD are at high risk for extracolonic cancer, especially those with CD. In a series of 6027 patients with IBD (2857 CD and 2672 UC) extracolonic cancer rate was 690/100,000, higher than in general population. In CD cancer rate is significantly higher than in general population, compared with UC where the risk is similar to that in general population (27). Extraintestinal cancers are localized in the liver and bile ducts, with the same risk from CD and UC, in case of associated of primary sclerosing cholangitis (28). Breast, prostate, and lung cancers in patients with IBD have the same risk as in the general population (29).

In recent years, an increased risk for cervical dysplasia associated with IBD was noticed, but it has been suggested that this risk is high in patients receiving immunomodulation therapy. However, there are few studies reporting data on the risk of cervical abnormalities in patients treated with immunosuppressants. Presumptions of therapeutic risk for cervical dysplasia in IBD are based on theories related to inadequate surveillance of human papillomavirus (HPV) infection in patients treated with immunomodulators and reactivation of latent virus in patients treated with anti-TNF. IBD patients treated with immunomodulators (AZA, MP, MTX) have an increased risk for abnormal Pap smears compared with healthy people. Regarding therapies with biological agents (anti-TNF), a recent study showed that infliximab does not increase the risk for abnormal Pap smears. Also, combination regimens of medicines prescribed for patients with IBD (oral or intravenous steroids, 5ASA and various combinations of infliximab, AZA and 6-MP) have no significant contribution to cervical abnormalities. Certain conditions, such as multiparity, prolonged use of oral contraceptives, number of sexual partners, smoking, and Chlamydia infection are considered additional risks associated with developing cervical cancer. The recommendation is that tests for Papilloma virus to be run before starting immunemodulatory and biological therapy, which, by way of action, can activate HPV (30).

CONCLUSIONS

Data in the literature on the theoretical potential for malignant extracolonic and lymphoma developments, practically con-

firmed in small number of IBD patients on treatment with immunomodulators and biological agents, are considered sufficient to promote strategies for selecting and monitoring these patients at risk for malignant complications with various locations.

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NEWS

FIBROUS DYSPLASIA - A BENIGN BONE DISEASE WITH RARE POTENTIAL FOR MALIGNANT TRANSFORMATION

Fibrous dysplasia (FD) is an uncommon bone disease that has a rare but clear potential for malignant transformation. The diagnosis is usually not difficult given the symptoms, radiology, and histology. The histologic picture is classically of low to moderately cellular fibrous stroma surrounding irregularly shaped bone trabeculae without osteoblastic rimming, which matches the benign appearance on radiology. The gene involved in pathologenesis is a subunit of G-protein receptors, found on chromosome 20. Recent innovation in molecular pathology has helped us understand the mechanism of the disease, pertaining to cAMP and WNT/b-catenin. The treatment of FD is limited to maintenance of maximum bone density via diet, exercise, and therapeutic medications, with many patients also choosing to avoid substances that lower bone density, such as caffeine and nicotine. Oftentimes, surgical reinforcement is needed for bowing deformities and fractures when they occur. Currently, there is no therapy for preventing disease advancement or for malignant transformation. (Riddle ND, Bui MM. Fibrous dysplasia. *Arch Pathol Lab Med*. 2013;137:134–138).

Doina Butcovan

CLINICAL – IMAGING ASPECTS OF YOUNG PERMANENT TEETH TRAUMAS AND THE ETHIOPATOGENIC MECHANISMS INVOLVED

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CLINICAL – IMAGING ASPECTS OF YOUNG PERMANENT TEETH TRAUMAS AND THE ETHIOPATOGENIC MECHANISMS INVOLVED (Abstract): Dental trauma occurring to children and teenagers all over the world represents a serious issue in Public Health. **Aim:** This present study wants to investigate the etiology and the environment in which the dental trauma occurs and also wants to establish a connection between dental trauma and social-economic status. **Material and methods:** The study was made to collect information about dental trauma on human subjects involving 372 children and teenagers, both female and male, between 8 and 20 years of age. The data obtained from the clinical and radiological exams for each patient have been registered in a special conceived register, which represented a stage of the study. **Results:** The frequency of dental trauma varied from 62.1% for males to 37.9% for women. Most of them have suffered from dental trauma between the age of 14 and 16 (30.1%), and a few between 18 and 20 years (2.2%). Dental trauma has occurred most frequently in school, during sports lessons, followed by those in public places like the street (23.1%), from which 17.1 % have been associated with bicycle accidents, 3.5 % with scooter accidents and 2.5% with car accidents. Children and teenagers who live in areas with a low social economic level have been the fewest to seek medical attention due to difficult access to medical services. **Conclusions:** Overall, this study wanted to present the importance of knowing the frequency of dental trauma in children and teenagers and to point out the need of promoting medical education to parents regarding the means they can use to reduce the risk factors associated with dental trauma. **Keywords:** DENTAL TRAUMA ETIOLOGY, YOUNG PERMANENT TEETH, PREVENTION, CLINICAL AND IMAGING ASPECTS.

Dental trauma is acknowledged as a serious matter of Public Health amongst children and teenagers. These lesions can occur at young ages and are irreversible, painful; they involve high costs and complex treatments.

It has been proved that dental trauma has a psychological and social impact on children (1). Epidemiological studies have reported that one third of preschool children have suffered from trauma involving temporary dentition and a quarter of them have suffered from trauma involving the permanent dentition (2). According to the studies made by Andreasen et al. (3), dental trauma occurs frequently in childhood and adoles-

cence and statistics show that two thirds of the children have suffered from dental trauma before the adult period. Recent studies (1, 2, 4) on children between 11 and 13 years of age have reported a prevalence of 6.4% in South Africa, 11.2% in Malaysia, 12.8% in Nigeria, 13.1% in Turkey, 35% in Thailand, and 36% in Brazil. Dental trauma is continuously growing amongst children and teenagers due to their more frequent involvement in sports with contact, accidents, domestic fights and falls (4, 5). Few studies have evaluated the social economic indicators. An Australian study has presented an effect of the social economic status upon dental trauma as a result of sporting events (6, 7). On the other hand, Fakhruddin et al. (8) found a prevalence of dental lesions in the Canadian population of 11.5 % but with no statistic association between dental trauma and the parents' level of education, the family income.

The purpose of this study was to collect information about dental trauma of young permanent teeth amongst children and teenagers in Iasi. The specific objectives were to evaluate the epidemiological profile, the place of occurrence, the connection between the social economic status and these dental traumatic lesions.

MATERIAL AND METHODS

The study was made on human subjects involving 372 children and teenagers from both rural and urban environments in the district of Iasi, with ages between 8 and 20 that have come for treatment in the Prevention Clinic of the Faculty of Dental Medicine, University of Medicine and Pharmacy "Grigore T. Popa" - Iasi between 2008 and 2011. Each of these patients or their parents has signed an informed consent after the purpose and importance of this study had been explained to them.

The identification of the dental lesions was made by a team of assistant researchers and the main researcher in standard conditions. The diagnosis criteria used in this study has been set in detail before the beginning of the study. For each consult, the examiner wore gloves and used a sterile kit with a dental probe and a dental mirror. X-rays for the dental lesions were made for only 161 patients, using a digital roentgen device.

The data obtained after the clinical and radiological examination have been registered in a special chart for each patient in order to be able to evaluate statistically the parameters of interest.

RESULTS

All 372 patients that have been treated for dental lesions have agreed to participate in this study. The frequency of trauma of the young permanent teeth is twice higher in boys than girls, due to sports and games that are more violent for boys.

Most patients that have suffered from dental trauma have between 14 and 16 years of age and a few between 18 and 20 years. In this last interval, the dental traumatic lesions occur because of traffic accidents (tab. I).

In the etiology of trauma are involved numerous factors. Most important seem to be sports accidents, followed by traffic accidents, those due to aggressions , accidental falls and last but not least physical abuse (tab. II).

In tab. III it is shown that dental trauma is located most frequently at the frontal teeth because they are situated in such a way that they are exposed more at vulnerable agents. This explains the vestibular movement of the frontal teeth and the hypotonic of the upper lip.

TABLE I

The frequency of reported injuries to young permanent teeth by age group and sex

Age group (years)	Total		Male		Female	
	no	**%**	**no**	**%**	**no**	**%**
8 – 10	58	15.6	31	53.4	27	46.6
10 – 12	73	19.6	42	57.5	31	42.5
12 – 14	106	28.5	57	53.8	49	46.2
14 – 16	112	30.1	89	79.5	23	20.5
16 – 18	15	4.0	7	46.7	8	53.3
18 – 20	8	2.2	5	62.5	3	37.5

TABLE II

Frequency of various causes of dental trauma

Causes of dental trauma	no	%
Traffic accident	86	23.1
Sport at school	249	66.9
Falls and collisions	29	7.8
Physical abuse	8	2.2

TABLE III

Frequency of young permanent teeth that had a traumatic dental injury

Involved tooth	n	%
Maxillary		
Right lateral incisor (# 11)	43	12.8
Right central incisor (# 12)	98	29.1
Left central incisor(# 21)	137	40.7
Left lateral incisor (# 22)	59	17.5
Mandibulary		
Right lateral incisor (# 42)	12	13.6
Right central incisor (# 41)	31	35.2
Left central incisor (# 31)	36	40.9
Left lateral incisor (# 32)	9	10.2
Total	**425**	**100**

Out of all the patients, 78.2 % come from urban environment, 21.8 % from the rural one. The number of urban patients has been higher due to the superior level of living and easier access to medical service.

Traumatic lesions affect most frequently the central maxillary incisors. The patients with high over jet are more willing to have dental trauma. Raising the ove jet from 0-3 mm to 3-6 mm doubles the extend of the dental lesion, and a over jet of 6 mm triples the severity of the dental trauma. This is way the dental occlusion is a very important factor in producing traumatic

lesions. Most patients have had a traumatic occlusion (80.1 %) and only 19.9% of them have presented with normal occlusion.

The most frequent symptom was pain, reported by 48.8% of the patients. This was followed by physiognomic disorder (16.5%), dental mobility (11.4%) and coronary stains (16.0). Abrasion and contusion are present in a small percent (7.2%).

After the clinical and radiological exams performed on this human specimen, we have concluded that most patients had simple coronary fractures (43.3%), complicated coronary fractures (18.3%), coronary-root fractures (14.0%), fissures (10.4%) and root fractures only (9%). Dental dislocation has been present in 5% of the cases. In fig. 1, 2 we can observe two clinical cases that came and have received treatment in our clinic.

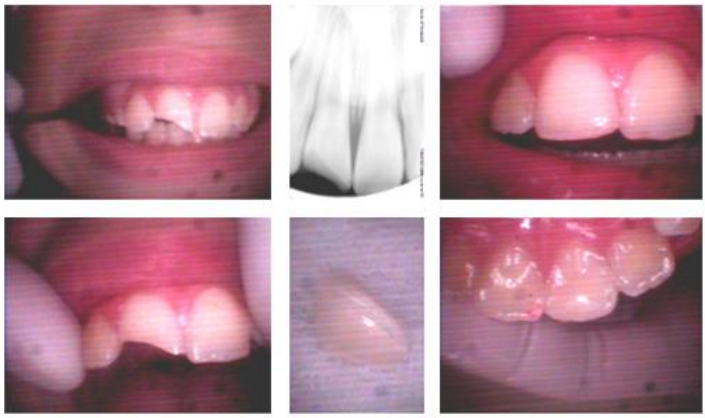

Fig. 1. Uncomplicated crown fracture.

Fig. 2. Extrusive dislocations.

DISCUSSION

The patients that have taken part in this study can be considered a representative population with ages between 8 and 20 years, but they do not represent the whole population of children and teenagers from Iasi district. The absence of signs and symptoms in the moment of the examination, in certain circumstances like dental dislocation and root fractures as well as the presence of the X-rays only for 161 patients, have reduced the estimation of dental trauma. This impediment has been shown in other similar studies made by other investigators.

These dental lesions affect 20-30 % of the population of the world regarding permanent dentition. They have psychological,

economical, functional and esthetic consequences. With such frequency of trauma, prevention has become the main target in Dental Traumatology.

Many epidemiological studies (9) regarding traumatic lesions have analyzed their etiology even though only a few have offered details regarding measures of precaution that should be taken. These studies made us understand better the clinical and biological aspects of the evolution and treatment of these pathological entities.

The statistic results are very similar to those studies made on the same subjects. The frequency of dental trauma in this study is twice as big for male subjects (62.1%) than female ones (37.9%). Furthermore, Glendor (10) have noticed a report of 2.5/1 in favor of the boys.

Most traumatic lesions have involved central maxillary incisors. This conclusion is the same in other studies as well and is explained by the anterior possition of these teeth on the dental arch (8, 9, 10).

A total of 66.9 % of the subjects participate regularly in a sports team, but none of them wear protection equipment during the sports activity. The reason for not using this equipment is the leak of information. This state's clearly the need of promoting public health in education institutions. The human subjects had been chosen because they are submitted to a high risk of developing dental trauma.

CONCLUSIONS

By promoting public health we must state the importance of the psycho-social impact on day to day life of children and teenagers that have suffered a dental trauma.

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ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND PULMONARY HYPERTENSION

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ANGIOTENSIN CONVERTING ENZYME INHIBITORS AND PULMONARY HYPERTENSION (Abstract): **Aim:** To evaluate the efficacy of angiotensin converting enzyme inhibitors Enalapril, Fosonopril and Moexipril on mean and systolic pulmonary artery pressure. **Material and methods:** The study included 111 patients with chronic obstructive bronchopneumopathies associated with arterial hypertension and mild to moderate heart failure (NYHA I-II class). The patients were examined at baseline and after 60 days of treatment. The central hemodynamic parameters and mean and systolic pulmonary artery pressure were studied by Doppler echocardiography (Phillips HD11XE). The pulmonary artery was viewed by left parasternal approach – cross section of the aorta. The following were measured: pulmonary artery flow acceleration and deceleration time (pafAT/ pafDT), right ventricular izovolumetric relaxation time (IVRT), mean and peak pulmonary blood flow velocities, right ventricular/pulmonary artery systolic pressure (RV/ PASP). The mean pulmonary artery pressure (MPAP) was estimated based on pulmonary flow acceleration time and right ventricular ejection time. **Results**: Following the treatment with angiotensin converting enzyme inhibitors a positive dynamics of SF pattern close to the normal one – a domelike contour with the maximum almost in the middle of diastole was noticed. **Conclusions**: the quantitative estimation revealed the considerable reduction in pulmonary artery systolic pressure (PASP) (from 46.3 ± 3.3 mmHg at baseline to 32.1 ± 2.6 mmHg after treatment, $p < 0.01$) and MPAP – from 26.7 ± 3.2 mmHg at baseline to 23.2 ± 2.6 mmHg after treatment, $p < 0.01$. **Keywords**: ANGIOTENSIN CONVERTING ENZYME INHIBITORS, CHRONIC OBSTRUCTIVE BRONCHOPNEUMOPATHY, PULMONARY HYPERTENSION, HEART FAILURE.

The cause of the disease has not been proven so far, but vasoconstriction represents an important pathogenic link of PH (1, 2, 3, 4).

Rennin-angiotensin-aldosterone system (RAAS) is important in maintaining vascular tone. The main role of RAAS dysfunction in systemic arterial hypertension (SAH) is well accepted, whereas in pulmonary hypertension it is less obvious.

MPAP > 60 mmHg revealed an increase in serum renin activity (SRA), aldosterone (AS), vasopressin (VP), converting enzyme activity, and AII concentration. Evaluating RAAS related to SRA, it was observed: RAAS increase in patients with PPH is an

adjunct to the increase in SRA, AII, AS and VP levels. Future research of the role of RAAS in the appearance and progress of PH could determine the strategy and a standardized treatment for PH in patients with obstructive lung disease (5, 6, 10, 11). The role of ACEIs in preventing pulmonary hypertension was demonstrated (e.g. use of Captopril and Cilazapril in hypoxic pulmonary hypertension led to hypertrophic changes in tunica media of pulmonary veins (12, 13, 14). The vasodilating effect of ACEIs might be less important than the humoral activity. Here, smaller doses of ACEIs may cause the blockade of RAAS and activate bradykinin to form L-arginine/ nitric oxide, and, it would not lead to systemic hypotension, thus being beneficial in patients with PH. Patients with PH presented a significant activation of RAAS which correlates with pulmonary artery pressure and severity of heart failure. The use of ACEIs in the treatment of patients with PH needs to be re-evaluated.

This study was aimed at assessing the efficacy of ACEIs Enalapril, Fosonopril and Moexipril on mean and systolic pulmonary artery pressure in patients with chronic obstructive bronchopneumopathy (COBP) in association with arterial hypertension (AHT) and mild to moderate heart failure (HF) NYHA I-II class.

MATERIAL AND METHODS

Group I: 61 patients (40 men and 21 women), aged between 18 and 65 years (mean age 46.1 ± 1.3 years) with moderate COBP (Tiffeneau index < 70%; 50% ≤ VEMS < 80%) associated with AHT and mild to moderate heart failure (NYHA I-II class). The patients were assessed at baseline and after 8 weeks of treatment with Enalapril (Ednyt, Geden Richter, Hungary), 10-40 mg once daily (mean dose 21.0 + 4.3 mg). *Group II*: 26 patients (12 men and 14 women, mean age 56 ± 0.9 years) with similar forms of COBP, treated with Fosinopril (Monopril, Bristol Myers Squibb, UK), 5-10 mg once daily (mean dose 7.3 ± 0.5 mg). *Group III*: 24 patients (8 men and 16 women, aged 54.4 ± 6 years) treated with Moexipril (Moex, *Schwarz Pharma*, Germany) 7.5 mg once daily – initially and after 60 days of treatment.

The central hemodynamic parameters and mean and systolic pulmonary artery pressure were studied by Doppler echocardiography (*Phillips* HD11XE), according to the traditional Teccholtz method (2, 3, 4). The pulmonary artery was viewed by left parasternal approach – cross section of the aorta. The sample volume was situated at the level of pulmonary artery distal the pulmonary valve or in the cone ejection of the right ventricle close to the pulmonary valve (thus highlighting the turbulent post valvular flow sample). The following were determined: pulmonary artery flow acceleration and deceleration time (pafAT/ pafDT), right ventricular izovolumetric relaxation time (IVRT), mean and peak pulmonary blood flow velocities, right ventricular/pulmonary artery systolic pressure (RV/PASP). To determine quantitative ratios we used Bernoulli's modified formula (3): $P = 4V^2$, where P – pressure gradient, V – flow velocity, m/ sec.

The pulmonary artery systolic pressure in PA (SPPA) was calculated upon the formula derived from L. Burstin's nomogram (7, 8, 9):

$$PAP = TIR + HR - 107.5$$

where TIR – RV izovolumetric relaxation time, m/ sec, HR-heart rate, beat/ min.

MPAP was estimated based on pulmonary flow acceleration time in the ejection

tract of the right ventricle (9): Log 10 (mPAP) = 2.8 AT/RVET + 2.4, where AT – pulmonary flow acceleration time (m/sec), RVET – right ventricle ejection time (m/sec).

RESULTS AND DISCUSSION

After the immediate test with Enalapril (5 mg) the hypertensive action of the preparation began within 1.5-2 hours, the maximum effect – over 4-6 hours, and duration of the action – 24-26 hours. After two months of Enalapril treatment, a significant decrease in SBP by 11.4% and DBP by 9.5% was noticed, $p<0.05$ (tab. I).

All patients had normal indices of left ventricular myocardial mass (< 120 g/ m^2), right ventricular telediastolic diameter (2.64 ± 2.015 cm), and right ventricular anterior wall thickness (5.77 ± 0.22 mm). The baseline qualitative estimation of SF at the pulmonary valve orifice and right ventricular outflow tract showed the predominance of typical forms of pulmonary hypertension (two waved, triangular or mixed). After treatment with Enalapril, the dynamics of the systolic flow pattern close to normal – domelike contour with the maximum almost in the middle of diastole was noticed. The quantitative assessment revealed the considerable reduction in PASP level (from 46.3 ± 3.3 mmHg at baseline to 32.1 ± 2.6 mmHg after treatment, $p<0.01$) and mPPA (from 26.7 ± 3.2 mmHg at baseline to 23.2 ± 2.6 mmHg after treatment, $p < 0.01$ (tab. II).

TABLE I

Dynamics of SBP, DBP and HR parameters after treatment with Enalapril

Ratios	SBP, mmHg	DBP, mmHg	HR, beat/ min
Baseline	172.5 ± 5.9	108.2 ± 3.9	75.1 ± 1.5
After treatment	137.5 ± 5.1**	87.2 ± 2.4**	71.5 ± 1.5

*p<0.05; ** p<0.001

TABLE II

Changes in pulmonary hemodynamic parameters after treatment with Enalapril

Ratios	PASP mmHg	TAPF, m/ sec	TIR, msec	TPR, dyn·cm·sec^{-5}
Baseline	46.3 ± 3.3	96.7 ± 2.5	78.6 ± 1.7	386.5 ± 22.7
After treatment	32.1 ± 2.6**	126.1 ± 3.0**	61.4 ± 2.9*	230.4 ±12.0*

In parallel, a significant reduction in total pulmonary resistance (TPR) and total vascular resistance (TVR): 386.5 ± 22.7 *vs.* 230.4 ± 12.0 dyn·sec·cm^{-5}, $p<0.05$ and 3200.4 ± 54.0 *vs.* 1850.5 ± 75.9 dyn·sec·cm^{-5}, $p<0.05$ were noticed. HR values were actually the same during the research (from 75.1 ± 1.5 beat/ min at baseline to 71.5 ± 1.5 beat/ min after treatment, $p>0.05$). Data analysis revealed a tendency towards the shortening of systolic acceleration time SAT) (< 100 msec) in the presence of pulmonary hypertension (SPPA > 32 mmHg) that can be used as a predictive criterion in the early stages of obstructive pulmonary disease. After treatment with Moexipril there was a statistically significant

reduction in SBP and DBP (by 12.9% and 10.2%, respectively) associated with a decrease in SPPA (42.1 ± 1.1 *vs.* 28.2 ± 0.8 mmHg, p<0.01), MPPA (from 28.0 ± 4.5 mmHg at baseline to 23.4 ± 2.1 mmHg after treatment, p<0.05), and TPR (424.5 ± 25.0 *vs.* 226.6 ± 15.5 dyn·sec·cm^{-5}, p<0.05) were also found (tab. III, IV).

TABLE III

Changes in pulmonary hemodynamic parameters after treatment with Moexipril

Ratios	PASP, mmHg	TASF, msec	FRI, msec	TVR, dyn·cm·sec^{-5}
Baseline	42.1 ± 1.1	104.4 ± 7.5	51.5 ± 2.2	2900 ± 33.2
After treatment	28.2 ± 0.8*	138.1 ± 10.0*	47.7 ± 1.7	1750 ± 57.4 *

TABLE IV

Dynamics of SBP, DBP and HR parameters after treatment with Moexipril

Ratios	SBP, mmHg	DBP, mmHg	HR, beat/ min
Baseline	160.9 ± 8.2	98.0 ± 3.4	98.2 ± 4.4
After treatment	140.1 ± 3.0*	88.1 ± 2.7*	86.1 ± 3.3 *

The patients treated with Fosonopril presented a significant reduction in TAS by 14.1%, TAD by 12.4% (p<0.01), and cardiac frequency by 10.1% (p<0.01). Also noticed was a significant decrease of SPPA from 35.3 + 3.07 mmHg to 23.1 + 2.64 (p < 0.01), APPA (from 28.0 ± 4.5 mmHg at baseline to 23.4 ± 2.1 mmHg after treatment, p<0.05), and TPR (424.5 ± 25.0 *vs.* 226.6 ± 15.5 dyn·sec·cm^{-5}, p<0.05). There was an inversely proportional correlation between TAPF and SPPA (tab. V).

TABLE V

Changes in pulmonary hemodynamic parameters after treatment with Fosinopril

Ratios	PASP mmHg	TAPF msec	TPR dyn·cm·sec^{-5}
Baseline	38.3 ± 3.1	112.4 ± 7.3	424.4 ± 16.6
After treatment	27.1 ± 2.6**	142.1 ± 6.5**	226.6 ± 15.5

* p < 0.05; ** p < 0.01

Thus, Fosinopril proved to be efficient in reducing SPPA and MPPA in patients with chronic obstructive bronchopneumopathies in association with AHT and moderate heart failure.

CONCLUSIONS

Angiotensin converting enzyme inhibitors reduce significantly after two months of treatment the systolic and mean pulmonary artery pressure and mean arterial pressure in patients with chronic obstructive broncho-

pneumopathies associated with AHT and mild to moderate heart failure. The qualitative and quantitative estimation of the systolic flow pattern at the orifice of pulmonary valve and across the right ventricular outflow tract allows the early diagnosis of pulmonary hypertension in patients with COBP. Right ventricular hypertrophy is a late and unnecessary sign of central and pulmonary hemodynamic disturbance in patients with chronic obstructive bronchopneumopathies and arterial hypertension; the key to establishing and creating the changes in the pulmonary hemodynamics is obviously the stable increase of systolic and mean pressure in the pulmonary artery.

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MEDICAL AND ETHICAL DILEMMA IN BRAIN DEATH

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MEDICAL AND ETHICAL DILEMMA IN BRAIN DEATH (Abstract): For centuries, death has been defined, medically speaking, as the irreversible cessation of breathing and of nervous and cardiac activity. What radically changed this definition was the introduction of the concept "brain death" in 1968, by the"Ad Hoc Committee of the Harvard Medical School". According to it, the irreversible coma was associated with brain death and considered to be a criterion for the diagnosis of the deceased individual. The evergrowing need for transplant organs (provided this respects the dead honor rule, stipulating that organs can't be harvested unless someone is deceased) lead to making arbitrary decisions regarding the establishment of the exact time of death during the process of „losing life". What actually triggers the controversy related to the concept of brain death is the dilemma of associating this concept with that of biologic death or death of the person, the difference between the two being made by whether the mental characteristics are accepted or not in defining and individualizing the death of the human being. Given these circumstances, a dilemma appears- that of defining the death of the individual: we define death, as it has been for centuries, as the moment when the cardio-respiratory function no longer exists, which leads to the loss of tens of thousands of lives that might have been saved through transplant. Yet, this may lead to manipulating the border between life and death, with the risk of trespassing each individual's right to life.
Key words: BRAIN DEATH, ETHICS, IDENTITY.

Defining the death of a human being in a unitary and generally valid way is as relative and difficult as defining life itself, for the former depends directly on the latter, both relating to a sum of multiple, various ways of manifestation. Death is taken into account in terms of ways of seeing the world, or the individual, and it is reflected and conditioned by social organization, tradition, religion and so on.

Defining death also depends on the value system specific to each field of activity, the medical definition itself being influenced by philosophy, theology, scientific discovery and not to forget, political and social priorities, etc.

From a philosophical perspective, defining death depends on the one providing the definition. According to Hegel, the main figure of idealism during the 19th century philosophy, "death is the triumph of species over individual, thus underlining the benefits of the individual disappearance on the progress of the entire mankind" (1).

Epicurus stated that "...Death is nothing for us, for when we exist, it doesn't, and

when it comes up, we no longer exist. The whole of good or evil is given by sensations, and death is the very absence of such." This materialistic approach is based on the assumption that death is a spontaneous ending of our sensuous experience and, thus, a form of non-existence. Therefore the matter is not of transition, but simply an abrupt ending (2).

Camus, an important name for existentialism, asserts that every man is free to give meaning to his own death, an idea set on the borderline between idealism and materialism. Although hesitating to answer fundamental questions regarding the clash between life and anything that might come after, such a thesis emphasizes the very act of dying, a gesture that becomes fundamentally important in the man's interior and individual search (1, 3).

According to the Orthodox Church, death is, first of all, a spiritual phenomenon that stands for the primordial dismissal, the man's separation from Life, therefore God himself, as a result of disobedience. Physical death is the derived effect of spiritual death and it involves the separation of the soul from the body, the first step to going back to the Fountain of Life. This is why the patristic tradition calls the dissolution of the body "God's benefaction towards man, so that evil will not linger forever after" (4, 5).

Legally speaking, death is associated with the suspension of the individual's civil status, thus making him no longer be a subject to law.

Relating to physiological mechanisms, life represents the sum of the physical, chemical and mechanic actions through which the body accomplishes its functions: metabolism, reproduction, excitability. On this background, death may be seen as a process that brings these functions to an end, as a consequence of complete and definitive disappearance of input, transportation and use of oxygen at cell level (6).

Anthropologists define human life as a sum of three levels, interconnected and hierarchically placed: organic/ physiologic, psychic and social. The life of the organism is based on the integration and coordination of body functions necessary to maintain the organism as a whole. Psychic life depends on the physiologic one and, in the case of the adult, conscious man; it contributes to the perception, integration and reaction of the individual. Social life definitely requires for the previous two levels, and it represents the person's integration and status. Given this definition, one must sense the difference between the death of the biological organism and that of the person, the latter being the cessation of all three levels, the climax of which is the social life, places at the top of the hierarchy (7, 8).

THE CONCEPT OF BRAIN DEATH

If so far as the last few decades, death was the interruption of all three levels of life, the development of medical technology allowed that physiologic life to be maintained in situations where the psychic and social levels no longer exist. Still, there remains the question of life and death coexisting within the same body. If we are to speak only physiologically and to analyze death as a process generally known by means of the phrase "the cells are born together and die separately", then the answer is affirmative. Purely from a biological point of view, the individual's death is defined as disintegration of the body as a whole, but after its moment, certain organs and systems may still keep traces of life for different periods of time, depending on how long they can survive without oxygen

(for instance, the nervous cell dies within the first 3-5 minutes, whereas hair and nails may still keep a lively aspect for days) (9). Practically, however, medicine's technical possibility to maintain the cardiac and respiratory functions- thus to insure the physiological level, despite the brain's stopping its activity, and leading to an impossible social life- widely opened the door to new dilemmas that continue to be active topics for debates. The starting point was the necessity to find a new way of defining death, adequate for the remarkable progress in medicine. It is due to this progress that in some cases the traditional criterion of defining death, which is the cessation of heart and respiratory activity, has become inadequate and impractical. Suddenly, the medical world finds itself caught in its own virtuousness (10). The way out, not completely though, seems to be the introduction of the term brain death. Ever since this moment, in 1968[1], the concept of brain death triggered a multitude of medical, ethical, judicial and social problems. The fact that its introduction lead to such controversy on all aspects for over 40 years shows that a unique answer and solution are mere illusions (11).

As well as the attempt to solve the issue of defining death on the new background created by the progress in the reanimation field, the concept of brain death came up just in time to allow harvesting for transplant organs. The connection between the two events is more than obvious (12).

Were we to follow the temporary evolution of organ transplant and defining brain death, it would be clear that their development was parallel, as a consequence of progress in science and medical technology, but without any mutual conditioning. It was only in the '60s, when the first successful transplants are performed, using organs from dead donors, that the connection between the two is noticed (13).

The introduction of the brain death concept one year after the first successful heart transplant was considered by some authors to be the identification of a utilitarian solution meant to solve a problem both medical and social that was becoming ever more acute-the reduced number of transplant organs. However, this approach carried the risk of attempting for a definition of death according to necessity and not to scientific truth. Furthermore, there was the risk of transforming patients in a state of brain death into sheer instruments for reaching other people's targets.

Initially, brain death was the equivalent of the death of the entire brain. Subsequently, some authors affirmed that the death of the brain stem equals brain death since it involves the disappearance of the integrative functions (respiration, cardiac activity), in which case the cortical activity is no longer sustainable. As a consequence of the organ crisis, the notion of imminent cerebral death is introduced, in which are included those who do not meet all the criteria for brain death (13, 14).

Imminent brain death is characterized by complete loss of cortical functions and progressive weakness of brain stem functions. It is considered that they represent potential organ donors, for they are in a state of profound coma, as a consequence of massive and irreversible cerebral lesions of unknown origin; they depend on mechanic ventilation and have at least 3 abol-

[1] The concept of Cerebral Death was elaborated in 1968 by the Ad Hoc Committee of the Harvard Medical School. The criteria offered by this committee were three clinical signs: absence of visible reaction to external stimuli, absence of breathing and of spontaneous muscular movement for at least 1h, absence of reflexes (pupil, cornea, etc) and a paraclinical sign: the flat EEG line for at least 10 minutes.

ished brain stem reflexes (15).

Each of these concepts was supported by some researchers and fought by others with more or less relevant arguments.

BRAIN DEATH - THE CONTROVERSY CONTINUES…

Those who support the concept of whole brain death consider the brain to be the integrative unity of the organism. Its death will shortly lead to the disintegration of the human body as a whole and to the inevitable settling of death. This argument implies that heart failure is inevitable and that it happens soon after the ending of brain activity. Despite all this, in the case of some brain dead patients, a part of the integrative functions are kept (i.e. some automatic movements due to medullar reflexes).

In this context, the brain is simply a modulator, and not the central integration unity. Also, the patients with high cervical lesions have the same lack of integrative unity and yet they are not declared deceased. All the more so, brain death doesn't actually stand for the death of the entire brain, since research has shown that some brain dead patients recorded maintained functionality of certain brain areas, such as the secretion of antidiuretic hormone. Those who support the concept of brain death do not deny this reality, but demonstrate that the areas still functional are not essential for carrying out the brain's integrative functions (13).

The assumption that heart failure is unavoidable soon after brain death contradicts clinical experience, as there have been cases when spontaneous heart blocking in brain dead patients occurred no sooner than days or weeks after the end of cerebral activity (16).

The ones supporting the concept of brain stem death start from the idea that death is the irreversible loss of consciousness and of the capacity to breathe, shortly followed by the cessation of spontaneous heart beats.

Showman attacks this concept, claiming that the anatomic substrate necessary to express consciousness is maintained in the case of patients with massive, irreversible lesions of the brain stem, but with the integrity of brain hemispheres. Anatomic integrity is an argument for maintaining the brain functions; therefore one cannot have the certainty of lack of consciousness. In order to support this argument and to contradict the identification of the brain stem death with the death of the organism, there are patients in a vegetative state, with loss of consciousness, and patients with high cervical lesions, lacking the ability to spontaneously breathe; however, neither the former nor the latter are considered deceased (15, 17).

Higher brain death represents the irreversible loss of consciousness, considered to be the major attribute of human life. It is associated with the notion of imminent brain death, defined as the total loss of cortical functions, coming from the progressive weakness of the brain stem functions. In order to measure the degree brain affection, the FOUR (Full Outline of Un Responsiveness) score is used; it is calculated based on eye movement responsive to stimuli, motor response, brain stem reflexes and spontaneous breathing. Those who stand against this concept show that imminence is not similar to the event per se, and thus, the vegetative patients are not considered deceased, although they suffer from loss of consciousness (18, 19).

Out of all these concepts that came to be as a consequence of the need to identify the optimum moment for harvesting viable

organs for transplant, only the one of whole brain death is accepted in most countries; it is considered that just the patients with both the brain stem and the cerebral hemispheres destroyed are actually in a state of brain death and can thus be declared dead.

Despite all this, from a physiological point of view death is a process that cannot be reduced to one moment only. Thinking of death as a progressive process, between the emergence of brain death and the ending of all functions with the loss of the integrity of organs, we can at the most appreciate how far a certain moment is compared to the beginning or the ending of this process (20).

Referring to this aspect, Shewmon even builds a parallel from the point of view of life and the individual's identity; one between the period of time stretching from brain death and organism death and the time covered by the fetus from the moment of conceiving until birth (15).

Vital functions do not all collapse at the same moment, since in ICU (intensive care unit) some of them can be artificially maintained as a result of the development of resuscitation techniques and also that of life support. This is what makes the outline of clear and valid criteria for declaring the time of death very difficult.

The increasing need for transplant organs on the one hand, and the possibility of harvesting organs exclusively from deceased-declared persons, on the other hand, have forcefully lead to making arbitrary decisions regarding the setting of the exact time of death along the process of "losing life".

Seeing death as a process, harvesting organs from people in stages preceding death (but before all the criteria for its diagnosis are met) is one of the key elements of the ethical debate concerning the establishment of the exact time of death. It thus becomes essential to protect the person that is still alive, for the harvesting vital organs actually represents the moment of death (21).

In terms of these medical concepts and ethical principles, two polar opinions have been structured. On the one hand, cerebral death is seen as an artificially created term, requested by the increasing need for transplant organs; this term cannot be fully identified with the death of the individual. On the other hand, the artificial support of the respiratory and cardio-circulatory functions for a limited period of time in the case of a patient diagnosed with cerebral death does not mean his survival, but merely creating the conditions necessary for the harvesting of transplant organs from a corpse (5, 22).

This controversy has been lasting for over 40 years, in which time some of those who advocate for one of the opinions (for instance, Shewmon initially defended the central role of the brain and later on changed his position to that of considering the clinical signs of cerebral death as potential effects of serious lesions, including medullar shock). All these lead to yet another argument for the fact that there is no unique answer to this matter.

However, society holds its reserves in considering someone in a state of brain death as actually dead, mainly because of the light this casts upon the clear definition of death (22). Were we to consider organ transplant, ceasing the medical care of someone considered dead might lead to the impossibility of harvesting certain organs such as the heart, which might cause prejudice to potential receivers and deprive them of a surviving chance. This approach also has implications from the point of view of human dignity (at least for what it was, if not for what it is at that precise moment!),

given that the patient in the state of cerebral death is declared dead, whereas his body is still kept alive, deprived both of the rights of a living person and those of a deceased. The artificial support of vital functions is obtained by means of aggressive treatments aiming exclusively at preserving the organs to be transplanted to another person/ other people. The one who actually benefits this medical maneuver is not the one is the state of brain death, with no chance of surviving, but the one who might receive his organs. In this case, we might consider the idea of an abuse or of exploitation of a defenseless body to the benefit of another individual.

Art. 3 from the universal declaration of human rights guarantee each human being the right to life; in the case of euthanasia we can even speak of a human right to death. What can be appealed to in the case of a neither dead nor alive human ? Does a cerebrally dead still have the right to an identity ? (23).

Taking into account the fact that brain death and somatic death may occur in different moments in the case of the same patient and the fact that between the end of cardio-respiratory activity and that of cerebral activity there may be a period varying from hours to days, it is essential to accurately decide when the person can be declared dead, with all the ensuing consequences.

In Romania, judicially speaking, death is defined in the 95/ 2006 Act referring to health reform, where the matter of organ transplant is mentioned and brought under regulation. In title VI chap. II regarding "Donation and donor of organs, tissues and human cells", art 147, align. 1, the deceased donor is defined as the person that was found to have ceased cardio-respiratory activity, nonresuscitable and irreversible (somatic death), and in align. 2 of the same article, the deceased donor with cardiac activity is defined as the person that was observed to have irreversible cessation of all brain functions (brain death) (24).

It is thus decided that organs may be harvested both from those in a state of brain death and from the ones with nonresuscitable and irreversible cardio-respiratory cessation, as they are declared dead.

In the light of these aspects, we can conclude that both medically and legally, brain death is considered to be a person's time of death. This conclusion influences all the judicial consequences as to the ending point in the civil capacity (25).

Another issue rising from the acceptance of brain death as the end of someone's life is identity. Personal identity was defined keeping in mind two aspects: physical continuity (same body, same brain) and psychological continuity (same memories, same character). J. Locke says that the second perspective is necessary, considering memory and consciousness to be definitive for man, and Bernard Williams claims that body identity is a condition necessary for the idea of identity. The moral argument may lead only to the human being dignity that needs to be respected and that should be protected by the right to identity. Once the individual has lost the functions that define a human being- feelings, consciousness, memory etc- it is only moral to have his dignity respected, if not for what he is at the very moment, at least for what he was all his life (25).

CONCLUSIONS

Making the difference between the death of a person (also at a mental and social level) and the death of the biological

body may lead to a different interpretation concerning the definition of death and therefore of the moment it is generated, according to the criteria is uses. The concept of cerebral death keeps triggering controversy, despite the fact that in the case of the traditional definition of death, as the irreversible cessation of the cardio-respiratory activity, establishing the time of death raises no medical, ethical or legal issues. Medically speaking, the biological organism is still alive as long as the heart continues to beat, conditioned, however, by artificial respiration. Nonetheless, according to the definition of the human being from a legal and ethical perspective, a person is declared deceased when all the levels definitive of his or her life no longer exist. In these situations, it is also impossible to preserve the biological level unless the essential biological functions are artificially supported. The controversy of brain death actually springs from the identification of this concept with that of biological death of the person; what differentiates the two is made by whether the mental features in the definition and individualization of the human life and death are accepted or not.

Regardless of which of the two opinions we advocate, we are facing a dilemma, for they are two indisputable facts: on the one hand, organ transplant helps save lives, and on the other no one can be killed and neither can his death be rushed by or for harvesting organs. All this considered, we return to the dilemma of defining the death of the individual: we define death, as it has been done for hundreds of years, as the moment of cessation of cardio-respiratory function, which will lead to the loss of many lives that might be saved by transplant or we go on manipulating the borderline between life and death, with the risk of defying the right to life.

ACCREDITATION

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NEWS

CEDRUS DEODARA NEEDLES – ANTIBACTERIAL ACTIVITY AGAINST FOOD-BORNE BACTERIA

Cedrus deodara, also known as Himalayan cedar, has been widely used as food and drug in China. The needles have nutritional value due to a high content in proteins, vitamins and minerals. This study reports the antibacterial activity of a water-soluble extract from needles on five food-borne bacteria (*Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*). The extract showed a potent antibacterial activity with minimum inhibitory concentrations (MICs) from 0.78 to 12.5 mg/ml; the minimum bactericidal concentrations (MBCs) were in the range of 1.56-25 mg/ml. The mechanism of action was investigated by transmission electron microscope; changes of external morphological features of *Staphylococcus aureus* (broken cell walls and membranes, release of cytoplasm) were revealed. The main constituent in the extract, shikimic acid, was isolated and was found to be responsible for the antibacterial activity of the extract. Taken together, these results suggest that *Cedrus deodara* needles are an important source of natural antibacterial agents with potential uses in both food and pharmaceutical industry. (Zeng W-C, He Q, Sun Q, Zhong K, Gao H. Antibacterial activity of water-soluble extract from pine needles of *Cedrus deodara*. *Int J Food Microbiol* 2012; 153: 78-84).

Anca Miron

EFECTUL STIMULĂRII ELECTRICE FUNCȚIONALE ASUPRA PERFORMANȚELOR MOTORII LA PACIENȚII PARKINSONIENI

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FUNCTIONAL ELECTRICAL STIMULATION EFFECT ON THE MOTOR PERFORMANCES IN PARKINSONIAN PATIENTS (Abstract): Parkinson's disease (PD) is a neurodegenerative disease involving dopaminergic, cholinergic, glutaminergic, serotoninergic and noradrenergic neurons, having as clinical manifestations both motor and non-motor symptoms. Functional electrical stimulation (FES) is a complementary therapy that can improve the motor disabilities (i.e. bradykinesia, hypokynesia and the motor block/ "freezing"). **The aim** of this study is to assess, by clinical scales and tests, the influence of FES on motor symptoms in Parkinsonian patients and to establish the differences in motor learning with healthy subjects. **Material and methods:** 26 PD patients and 15 healthy volunteers were included in the study. The two groups of subjects were clinically assessed at two different points in time: before and after ten consecutive days of FES exercise for 30 min/day on the most affected/ less skilful upper and lower limb. **Results:** All of the clinical tests revealed bilaterally improvements in both groups, mainly in PD patients group, even if the FES application was unilaterally. **Conclusions:** Unilateral peripheral electrostimulation improves bilaterally motor performances due to an interhemispheric transfer called "cross education". This phenomenon seems to be more pronounced in Parkinsonian patients because of the alteration of cortical inhibition. **Key words:** PARKINSON'S DISEASE, FUNCTIONAL ELECTRICAL STIMULATION, MOTOR PERFORMANCES, CORTICAL INHIBITION

Boala Parkinson (BP) este o maladie neurodegenerativă multifocală progresivă, caracterizată prin simptome motorii şi nonmotorii, care pot duce în timp la un grad important de dizabilitate (1). Procesul degenerativ afectează în special neuronii dopaminergici din substanța neagră mezencefalică şi nucleii bazali. Evoluția bolii cunoaşte trei stadii (preclinic, premotor şi motor), din care ultimul este patognomonic (2). Afecțiunea devine manifestă când numărul de neuroni dopaminergici ajunge la circa 20%, iar cantitatea de dopamină scade la jumătate față de normal. Simptomele motorii pot fi ameliorate prin tratament dopaminergic, dar şi prin metode terapeutice complementare: stimulare cerebrală profundă (DBS), stimulare magnetică repetitivă (rTMS), stimulare electrică funcțională (FES). Există numeroase studii referitoare la stimularea cerebrală (electrică sau magnetică), însă datele privitoare la aplicațiile FES în boala Parkinson sunt puține (3).

Studiul de față îşi propune evaluarea

efectului stimulării electrice funcționale asupra bradikineziei şi a fenomenului de *"freezing*" (blocaj motor) la un grup de pacienți parkinsonieni, respectiv stabilirea diferențelor de învățare motorie în raport cu un grup de subiecți sănătoşi.

MATERIAL ŞI METODE

Studiul este de tip analitic intervențional - trial clinic controlat randomizat. În lotul test au fost incluşi 26 de pacienți parkinsonieni, recrutați din Clinica de Neurologie a Spitalului Clinic de Recuperare Iaşi în perioada ianuarie 2011-ianuarie 2012. Lotul martor a fost alcătuit din 15 voluntari sănătoşi (V). Atât subiecții bolnavi, cât şi cei sănătoşi, au fost informați de posibilitatea participării la studiu şi şi-au exprimat acordul prin semnarea consimțământului aprobat de Comisia de Etică a Spitalului.

Selecția pacienților s-a realizat pe baza unor criterii de includere, şi, respectiv, de excludere.

Criterii de includere:

- vârsta între 30 şi 85 de ani;
- boală Parkinson idiopatică, stadiile I-III Hoehn-Yahr, controlată medicamentos; fază "on" la momentul examinării;
- status cardio-vascular şi neurologic stabil;
- nivel cognitiv suficient de bun pentru a înțelege principiul de funcționare şi modul de aplicare a dispozitivelor de stimulare electrică funcțională.

Criterii de excludere:

- parkinsonism atipic, secundar sau din alte boli neurodegenerative;
- patologie cerebrală şi/sau medulară cu interesarea sistemului piramidal şi/sau extrapiramidal;
- epilepsie netratată sau refractară;
- *pacemaker* cardiac;
- leziuni ale nervului radial şi/sau sciatic popliteu extern (SPE);
- afecțiuni osteo-articulare sau dermatologice la nivelul antebrațului şi gambei propuse pentru FES;
- boli cardio-vasculare grave;
- demență.

Într-o primă etapă, fiecărui participant la studiu i s-a întocmit o fişă cu următoarele date: sex, vârstă, membrul superior (MS) cel mai afectat (în cazul pacienților) sau MS mai puțin abil (în cazul voluntarilor).

Evaluarea inițială a pacienților cu BP s-a realizat prin stabilirea stadiului Hoehn-Yahr, aprecierea nivelului de independență în activitățile din viața cotidiană pe scala Schwab & England, evaluarea mersului prin scorul Tinetti Gait, aprecierea stării generale pe scala Webster Rating şi a statusului motor prin scorul UPDRS motor (4).

La membrele superiore s-au aplicat *testul de închidere şi deschidere a pumnului* (numărând de câte ori pe minut subiectul execută aceste mişcări) şi *nine hole peg test-ul/9-HPT* (măsurând timpul în care subiectul umple 9 găuri cu 9 cuie de lemn şi apoi le scoate).

La membrele inferioare (MI) s-au observat inițierea şi fluența mişcării utilizând *5m-timed-up-and-go test (5m-TUG)*; din poziția şezând, subiecții au fost invitați să se ridice de pe scaun, să parcurgă distanța de 5 metri, să se întoarcă şi să revină la poziția inițială. S-au notat următorii parametri: viteza de mers (= 10 m/timp); indicele energetic [PCI= (FC în mers - FC în repaus)/viteza x 60m/min, unde PCI= Physiological Cost Index, FC=frecvența cardiacă]; lungimea medie a pasului (P= 10 m/nr. paşi); cadența (=nr. paşi/timp). Aceleaşi teste au fost aplicate şi voluntarilor sănătoşi.

După această evaluare, subiecții din cele două loturi au urmat un program de FES

timp de 10 zile, câte o şedinţă de 30 min/zi, la MS cel mai afectat/mai puţin abil şi la MI de aceeaşi parte. Electrozii au fost plasaţi pe traiectul nervului radial la MS şi pe traiectul nervului peronier comun (SPE) la MI. În vederea stimulării s-au utilizat două tipuri de aparate: Odstock Microstim 2v2 pentru MS şi Odstock Dropped Foot Stimulator (ODFS3) pentru MI, procurate de la centrul specializat din Salisbury, Anglia, şi avizate de Ministerul Sănătăţii din România. Parametrii curentului utilizat au fost: amplitudinea= 10-100 mA, reglabilă, cu 1 kΩ impedanţă de ieşire, durata pulsului= 300 μs pentru Microstim 2v2 şi 350 μs pentru ODFS3, frecvenţa stimulării= 40 Hz, forma de undă= asimetrică bifazică.

La sfârşitul celor 10 zile de antrenament, participanţii au fost reevaluaţi prin aceleaşi scale şi cu aceeaşi baterie de teste, iar valorile obţinute s-au comparat cu cele măsurate înainte de stimulare.

Prelucrarea statistică a datelor s-a realizat în programul SPSS 17 aplicând testele statistico-matematice p, χ^2, t.

REZULTATE

Ponderea subiecţilor de sex masculin a fost uşor mai crescută în lotul de parkinsonieni (57,7%), însă distribuţiile de frecvenţă nu prezintă diferenţe semnificative faţă de lotul voluntari sănătoşi (33,3%) (χ^2=4,49; GL=3; p=0,214). Pe cazuistica studiată nu s-au evidenţiat diferenţe semnificative între sexe ale vârstei medii. Totuşi, vârsta medie a lotului de pacienţi parkinsonieni a fost semnificativ mai mare faţă de lotul de voluntari sănătoşi (p=0,012) (tab. I).

TABELUL I

Indicatori statistici ai sexului şi vârstei pe loturile studiate

Lot	Sex (F/B)	N	Vârsta medie (ani)	Deviaţie standard	Eroare standard	Diferenţe între sexe	Diferenţe faţă de voluntari
Voluntari sănătoşi	F B Total	10 5 15	59,60 53,20 57,47	11,25 14,27 12,21	3,56 6,38 3,15	t=0,953 p=0,358	-
Pacienţi parkinsonieni	F B Total	11 15 26	66,45 66,40 66,42	7,79 10,75 9,43	2,35 2,77 1,85	t=0,014 p=0,989	t=2,626 p=0,012

Influenţa stimulării electrice funcţionale asupra scorurilor diferitelor scale se poate observa în fig. 1. FES nu a modificat *stadiul Hoehn-Yahr* (I-III) de evoluţie a bolii la nici unul dintre pacienţi, media lotului fiind 2,2. Valorile medii obţinute pe *scala Schwab & England* (0-100%) au crescut semnificativ după stimulare electrică funcţională de la 73% la 80% (t=2,16; p<0,05). *Scorul mediu Tinetti Gait* (0-12) a înregistrat o uşoară creştere după FES de la 7,6 la 9,1 (t=1,91; p>0,05). Valorile medii obţinute pe *scala Webster Rating* (0-30) au scăzut după FES de la 14,2 la 11, însă descreşterea nu a fost semnificativă din punct de vedere statistic (t=1,97; p>0,05). Scorul mediu UPDRS motor (0-108) a înregistrat o uşoară descreştere după FES, de la valoarea medie de 37,9 la 31 (t=1,71; p>0,05).

Modificările parametrilor clinici la testele aplicate membrelor superioare. Înainte de FES, la MS cel mai afectat, valorile medii la testul de închidere-deschidere a pumnului au fost semnificativ mai reduse la parkinsonieni comparativ cu voluntarii sănătoşi (tab. II).

După stimulare, viteza s-a îmbunătățit cu 37,8%, apropiindu-se de valorile înregistrate la voluntari (la care a crescut cu 16,5%). La MS controlateral nu s-au înregistrat diferențe statistic semnificative, nici înainte nici după stimulare, iar creşterea după FES a valorilor medii a fost de 18% la pacienții cu BP şi de 6,3% la subiecții sănătoşi.

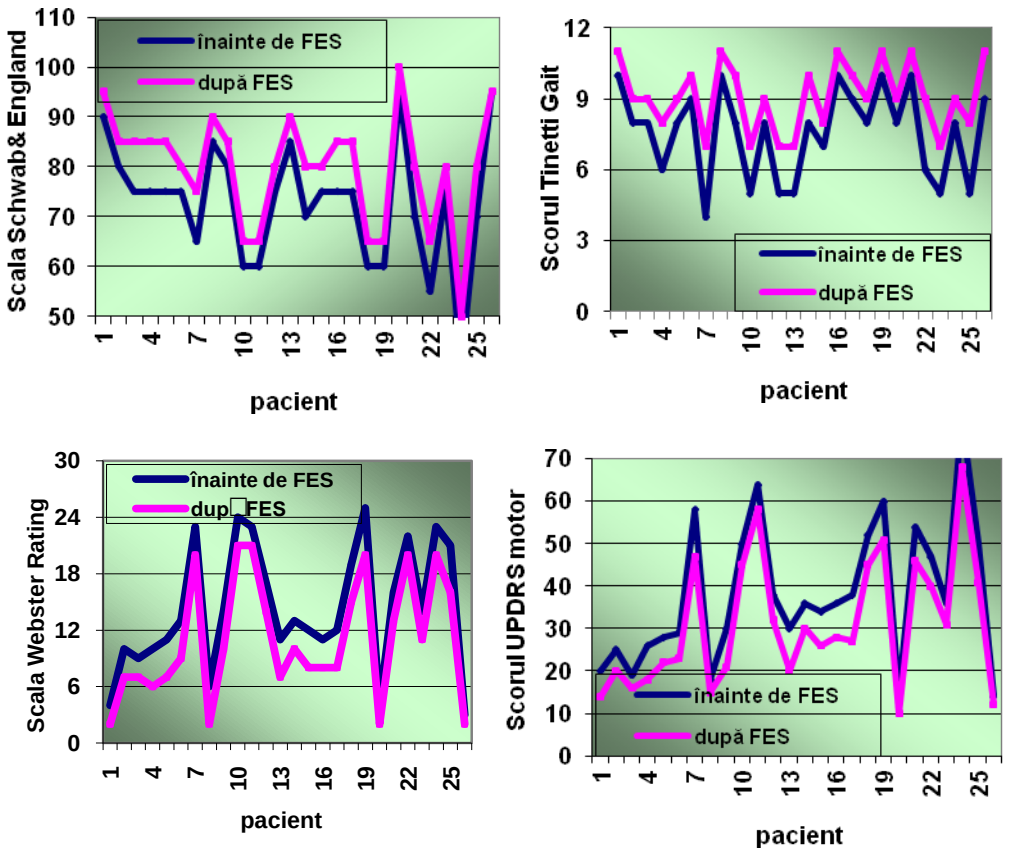

Fig. 1. Evoluția valorilor individuale în funcție de scala de evaluare

TABELUL II

Testul de închidere-deschidere a pumnului

Loturi		N	MS cel mai afectat/ mai puțin abil			MS controlateral		
			Medie (/min)	Deviație standard	Eroare standard	Medie (/min)	Deviație standard	Eroare standard
Înainte de FES	BP	26	50,96	17,67	3,46	60,00	18,31	3,59
	V	15	65,33	16,30	4,21	70,87	19,06	4,92
După FES	BP	26	70,24	20,65	4,51	70,81	16,99	3,71
	V	15	76,08	18,06	5,21	75,33	20,63	5,96

Valorile medii obținute la testul 9-HPT au fost semnificativ mai mari la pacienții cu BP comparativ cu subiecții sănătoşi, atât înainte cât şi după FES. Totuşi, prin stimulare, timpul de realizare a testului a scăzut cu 29,7% pentru MS stimulat şi cu 18,4% pentru MS controlateral (în raport cu 8,2% şi, respectiv, 6,7% la voluntari) (tab. III).

Parametrii înregistrați la testul de mers au cunoscut o ameliorare post-stimulare mai evidentă în cazul pacienților parkinsonieni, cu excepția lungimii pasului care a crescut mai mult la subiecții sănătoşi (fig. 2).

TABELUL III

Nine Hole Peg Test (9-HPT)

Loturi		N	MS cel mai afectat/ mai puțin abil			MS controlateral		
			Medie (sec)	Deviație standard	Eroare standard	Medie (sec)	Deviație standard	Eroare standard
Înainte de FES	BP	26	47,87	36,69	7,19	35,15	18,31	3,59
	V	15	25,20	3,68	0,95	25,13	3,04	0,79
După FES	BP	26	33,67	11,65	2,54	28,69	6,48	1,41
	V	15	23,13	1,55	0,45	23,46	2,70	0,78

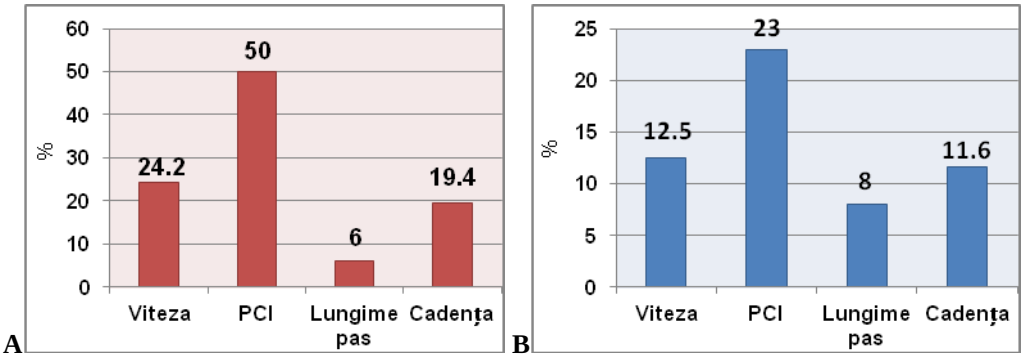

Fig. 2. Evoluția indicatorilor statistici la 5m-TUG, după FES
A.la pacienții parkinsonieni, B. la subiecții sănătoşi

DISCUȚII

În concordanță cu statisticile din literatura de specialitate, în lotul de pacienți parkinsonieni vârsta medie a fost peste 60 de ani (66,4 ani), cu o uşoară predominanță masculină (57,7%).

Testele clinice au pus în evidență o ameliorare semnificativă ($p<0,05$) a bradi- şi hipokineziei la pacienții cu BP şi o îmbunătățire a performanțelor motorii la subiecții sănătoşi după stimulare electrică funcțională. Creşterea vitezei de realizare a testelor a fost bilaterală în toate loturile, mai ales în grupul cu BP, deşi stimularea a fost unilaterală.

Studii recente de rezonanță magnetică funcțională (fMRI) au arătat că aplicarea electrostimulării pe muşchii extensori şi flexori ai pumnului la voluntarii sănătoşi determină activarea cortexului motor pri-

mar controlateral, a ariei primare somatosenzoriale şi a cortexului premotor controlateral, precum şi a cerebelului ipsilateral, a ariei secundare somatosenzoriale bilateral, a ariilor motorii suplimentare şi a cortexului cingulat anterior bilateral (5). Mai mult decât atât, se pare că FES este capabilă să modifice excitabilitatea la nivelul conexiunilor interemisferice şi implicit echilibrul excitație-inhibiție (6). Totodată, antrenamentul fizic al unui membru se însoțește de mărirea forței de contracție în membrul opus, neantrenat, prin diminuarea inhibiției corticospinale şi existența unui proces de transfer interemisferic, cunoscut sub numele de "cross education" (7, 8). Deşi au efecte supraspinale diferite, stimularea electrică şi kinetoterapia pot creşte, ambele, forța de contracție la nivelul muşchiului omolog din membrul controlateral (8).

Perioada de linişte corticală, un marker al inhibiției intracorticale obținut prin stimulare magnetică transcraniană, este mai scurtă la parkinsonieni comparativ cu subiecții sănătoşi, durata sa fiind mai mică pe emisfera opusă hemicorpului mai afectat (9). Inhibiția interemisferică şi facilitarea intracorticală sunt de asemenea reduse la pacienții parkinsonieni (10). Probabil că alterarea circuitelor inhibitorii constituie substratul ameliorării bilaterale în urma FES a statusului motor la pacienții cu BP.

CONCLUZII

Cercetarea noastră a urmărit evaluarea aportului stimulării electrice funcționale în ameliorarea simptomelor motorii din boala Parkinson. Aplicarea unilaterală a stimulării neuromusculare a determinat o creştere a mobilității membrelor stimulate, dar şi a membrelor controlaterale, nestimulate. Acest efect a fost mai important în cazul pacienților parkinsonieni comparativ cu voluntarii sănătoşi.

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TESTUL NEUROPAD® ÎN EVALUAREA PICIORULUI DIABETIC

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NEUROPAD® TEST IN EVALUATION OF DIABETIC FOOT (Abstract): **Aim.** Diabetic neuropathy (DN) is the main cause of foot ulceration. One of the earliest modifications is the loss of normal sudomotor function, a sign of autonomic neuropathy. The aim of this study is to evaluate sudomotor dysfunction using a new tool (Neuropad®) in diabetic patients with foot ulceration. **Material and methods.** We included 58 diabetic patients with foot ulceration admitted in 2010 in the Clinical Center for Diabetes, Nutrition and Metabolic Diseases. We collected data regarding age, gender, type and duration of diabetes, glycated hemoglobin (HbA1c), history of amputations and duration of hospital admission. Peripheral neuropathy was evaluated through the Neuropathy Disability Score (NDS) and autonomic neuropathy was tested through Neuropad®. **Results.** 95% of patients had NDS $\geq$ 6, among which 76% had also positive Neuropad® test. Patients with both peripheral and autonomic neuropathy had longer duration of diabetes (p=0.027) but similar HbA1c as patients with only peripheral neuropathy (p=0.09). The former also had longer duration of current admission (p=0.022) and a higher percentage of these patients had history of amputations (p=0.041). **Conclusions.** Neuropad® is a reliable, easy to use test for the diagnosis of autonomic neuropathy, which puts patients at greater risk for foot ulceration and amputation

Neuropatia diabetică (ND) are un important impact negativ asupra calității vieții şi supraviețuirii la pacienții cu diabet, iar componenta sa autonomă (NDA) este una dintre complicațiile cronice cele mai puțin diagnosticate şi înțelese (1). Evaluarea afectării nervoase în ND este importantă pentru a defini grupurile aflate la risc de a dezvolta pierderea sensibilității, durere şi ulcerații la nivelul piciorului. Teste clinice simple pentru evaluarea deficitelor neurologice includ scorul de neuropatie (Neuropathy Disability Score – NDS) (2), pragul de percepție vibratorie sau testarea cu monofilamentul Semmes-Weinstein pentru a defini neuropatia severă şi deci riscul de ulcerație (3).

NDA poate fi evidentă clinic sau de natură subclinică şi poate afecta unul sau mai multe organe sau sisteme, fiind cardiovasculară, gastrointestinală, sudomotorie, oculară etc. (4). Manifestările clinice de NDA sunt evidente după o perioadă îndelungată de evoluție a diabetului, în timp ce disfuncția subclinică poate exista chiar la 1 an de la diagnostic la pacienții cu diabet zaharat (DZ) tip 2 (5). În general, NDA centrală este evaluată prin cercetarea variabilității frecvenței cardiace, iar cea periferică prin

teste ale funcţiei sudomotorii. Simptomele NDA sudomotorii sunt reprezentate de hiperhidroză în partea superioară a corpului (legată uneori de alimentaţie, fiind cunoscută sub denumirea de „transpiraţie gustativă") şi reducerea secreţiei sudorale, uneori până la anhidroză, în jumătatea inferioară, intoleranţă la cald. Afectarea autonomă sudorală la nivelul picioarelor va duce la piele uscată, producerea de fisuri, ceea ce reprezintă un factor de risc pentru ulceraţii ulterioare la acest nivel (6).

Testele utilizate pentru evaluarea funcţiei sudomotorii relevă extensia, distribuţia şi localizarea deficitului activităţii simpatice colinergice, dat fiind faptul că fibrele postganglionare simpatice ce inervează glandele sudoripare secretă acetilcolină. Aceste teste includ testul cantitativ sudoral axono-reflex (quatitative sudomotor axon reflex test - QSART), amprenta sudorală, testul termoreglării (thermoregulatory sweat test – TST) şi reacţia simpatică la nivelul pielii. QSART implică iontoforeza unui agonist colinergic pentru a măsura răspunsul sudomotor mediat de reflexul de axon. Amprenta sudorală necesită existenţa unui mulaj de plastic sau silicon ce captează secreţia sudorală ca răspuns la iontoforeza unui agonist colinergic. TST evaluează distribuţia sudoraţiei printr-o modificare de culoare a unui indicator aplicat pe piele după expunerea la raze infraroşii. Reacţia simpatică la nivelul pielii sau potenţialul autonom periferic de suprafaţă generat de glandele sudoripare poate fi măsurat cu ajutorul electrozilor conectaţi la un electromiograf (7). Toate aceste teste necesită aparatură complexă şi scumpă, fiind dificil de utilizat de rutină.

Ca instrument de screening al disfuncţiei sudorale dispunem la ora actuală de testul comercial Neuropad®, recent propus ca o metodă simplă de diagnostic a disfuncţiei sudomotorii şi implicit a NDA la nivel periferic, la pacienţii cu DZ (8), cu reproductibilitate foarte bună (9). Consensul de la San Antonio (10) recomanda efectuarea testelor pentru NDA cardiovasculară, considerând că doar acestea erau standardizate şi potrivite pentru screening-ul şi monitorizarea progresiei NDA. Mai recent, Asociaţia Americană de Diabet (ADA) recomandă şi examinarea funcţiei sudomotorii pentru detecţia NDA (11). Testul Neuropad® este un plasture impregnat cu soluţie de clorură de cobalt, care îşi schimbă culoarea în funcţie de starea de hidratare a zonei de tegument pe care este aplicat, de la albastru la roz, dacă sudoraţia şi deci funcţia simpatică colinergică este normală (8). Aplicarea se realizează pe o zonă de tegument curată, în condiţii de confort termic, iar citirea virajului culorii se face după 10 minute. Testul poate fi autoadministrat de către pacient (ceea ce reprezintă un avantaj) şi se recomandă a fi efectuat cel puţin odată la 6 luni. Un rezultat anormal al testului impune evaluarea detaliată a celorlalţi factori de risc. (6).

Scopul acestui studiu a fost de a evalua prezenţa disfuncţiei sudomotorii printr-un instrument nou - plasturele Neuropad® la pacienţii cu DZ şi cu ulceraţie pe fond neuropat.

MATERIAL ŞI METODE

Lotul de studiu a fost format din pacienţii internaţi în Centrul Clinic de Diabet, Nutriţie şi Boli Metabolice pe parcurul anului 2010 cu diagnostice în relaţie cu "piciorul diabetic". Au fost excluşi din studiu pacienţii cu diagnostic de arteriopatie obliterantă a membrelor inferioare.

Pentru fiecare pacient s-au înregistrat următoarele date: sex, vârstă, tipul de diabet zaharat, durata diabetului, valoarea he-

moglobinei glicate (HbA1c), istoricul de amputaţii, durata actualei spitalizări.

Neuropatia periferică a fost diagnosticată pe baza scorului de neuropatie (Neuropathy Disability Score - NDS) ≥ 3. NDS este un sistem de clasificare a neuropatiei periferice care se bazează pe suma unor semne şi simptome prezentate de pacient la nivelul ambelor membre inferioare: reflexele achiliene, percepţia vibraţiei evaluată cu diapazonul, sensibilitatea la durere şi sensibilitatea la temperatură cu Tip-therm.

Componenta de neuropatie vegetativă a fost testată cu Neuropad®. Pacienţii au fost aşezaţi în decubit dorsal, fără pantofi şi şosete pentru cel puţin cinci minute înainte de testare. Plasturele cu cobalt a fost aplicat la nivelul capului metatarsianului unu sau doi. Atunci când pe locul de amplasare exista piele hipercheratozică sau o ulceraţie, locul de aplicare a fost schimbat cu capul celui de al 5-lea metatarsian. După zece minute a fost evaluat rezultatul: lipsa de schimbare de culoare de la albastru la roz, sau schimbare incompletă (albastru şi roz mixt) au fost considerate a corespunde unui test de screening pozitiv, însemnând pierderea sudoraţiei şi, prin urmare, disfuncţie autonomă. Dimpotrivă în cazul în care cardul a devenit complet roz, testul a fost considerat normal.

Analiza statistică a folosit testul $\chi 2$ pentru variabile categoriale şi testul t Student pentru variabilele continue.

REZULTATE

În anul 2010, în Centrul Clinic de Diabet, Nutriţie şi Boli Metabolice au fost internaţi 90 de pacienţi cu diagnostice în relaţie cu "piciorul diabetic". Dintre aceştia, 32 prezentau diagnosticul de arteriopatie obliterantă a membrelor inferioare. Astfel, pe baza criteriilor de includere şi excludere, s-a format un lot de studiu de 58 de pacienţi, dintre care 43 sunt bărbaţi (74%). Vârsta medie ± DS în lotul studiat este de 60.2 ± 4.1 ani. Doar 5 pacienţi prezintă DZ tip 1 (8.6%), restul având DZ tip 2. Durata medie a bolii a fost de 13.9 ± 2.6 ani, iar media HbA1c a fost de 8.7 ± 1.1 %. 95 % dintre pacienţi au prezentat un scor de neuropatie (NDS) ≥ 6. Dintre aceştia, 42 de pacienţi (76 %) au prezentat test Neuropad® anormal (pozitiv). De asemenea 2 din cei 3 pacienţi cu scor de neuropatie (NDS) < 6 au prezentat test Neuropad® pozitiv. În total, 44 de pacienţi (75.8%) au prezentat test Neuropad® pozitiv, implicit neuropatie autonomă.

Am analizat 2 subloturi de pacienţi: sublotul A, format din pacienţi care prezentau numai neuropatie periferică, evaluată prin scor de neuropatie (NDS) ≥ 6, fără neuropatie autonomă (test Neuropad® normal), şi anume 21% dintre pacienţi (12 pacienţi) cu sublotul B, format din pacienţi care prezentau ambele forme de neuropatie, şi anume 72.4% dintre pacienţi (42 pacienţi).

Pacienţii din sublotul B au prezentat un scor de neuropatie (NDS) statistic semnificativ mai mare decât pacienţii din grupul A (p=0.043). De asemenea, pacienţii din sublotul B au prezentat o durată a diabetului statistic semnificativ mai mare (15.4 ± 2.1 ani) decât pacienţii din sublotul A (11.8 ± 2.5 ani), p=0.027. Nu au existat diferenţe semnificative statistic în privinţa valorilor HbA1c între cele 2 subloturi (p=0.09). Un procent semnificativ statistic mai mare dintre pacienţii din sublotul B au prezentat istoric de amputaţii (34.7%), faţă de pacienţii din sublotul A (30.8%), p=0.041. De asemenea, durata spitalizării a fost semnificativ statistic mai mare la pacienţii din sublotul B (15.3 ± 1.03 zile) faţă de pacienţii din sublotul A (11.2 ± 1.6 zile), p=0.022.

DISCUȚII

Neuropatia periferică (senzitivo-motorie şi vegetativă) este principala cauză a ulcerației la picior la pacienții cu DZ. Una dintre primele sale manifestări este pierderea sudorației normale la picior, care are ca rezultat uscarea pielii şi riscul de fisură. Funcția sudomotorie are un rol protectiv deosebit de important, iar prezența NDA la nivel periferic, caracterizată prin anhidroză, reprezintă o verigă fiziopatologică importantă în apariția ulcerațiilor (12). Disfuncția sudomotorie subclinică poate să fie detectată precoce în DZ, chiar şi la subiecții cu viteză de conducere nervoasă normală (13). În consecință, identificarea pacienților la risc pentru ulcerații la nivelul piciorului utilizând metode simple şi de încredere are o relevanță clinică deosebită.

Quattrini et al. (14) au comparat rezultatele evaluării piciorului diabetic prin Neuropad® şi prin teste obiective ale neuropatiei somatice şi autonome, inclusiv densitatea intra-epidermală a fibrelor nervoase în biopsia cutanată, obținând că răspunsul anormal la testul Neuropad® s-a corelat cu NDS, sensibilitatea termică, cu simptomele de neuropatie şi cu variabilitatea frecvenței cardiace. De asemenea, densitatea fibrelor nervoase a fost semnificativ mai redusă la pacienții cu test Neuropad® anormal. Autorii concluzionează că testul Neuropad® este o metodă simplă pentru screening-ul pacienților cu ND.

Studiul lui Tentolouris et al. (15) a găsit că un test Neuropad® anormal se corelează cu prezența ulcerațiilor la pacienții cu diabet, sugerând că acest test poate fi inclus printre testele screening de prezicere a ulcerației piciorului. Şi în studiul nostru, majoritatea pacienților au prezentat funcție sudomotorie anormală (75.8 %). De asemenea, într-un alt studiu s-a arătat că 95% dintre pacienții cu ulcerații au prezentat anhidroză, evaluată prin Neuropad®, date similare cu evaluarea prin reacția simpatică la nivelul pielii, şi că valoarea NDS a fost mai mare la pacienții cu ulcerații (16). Valoarea predictivă a testului Neuropad® pentru ulcerații este similară cu utilizarea monofilamentului, percepția vibratorie şi NDS (15). Un alt studiu efectuat pe 154 pacienți cu DZ tip 2 în care ND a fost diagnosticată clinic utilizând NDS, a arătat că sensibilitatea pentru acest diagnostic a fost mai mare cu testul Neuropad®, comparativ cu pragul de percepție a vibrațiilor, dar specificitatea a fost mai mare în cazul celui de al doilea test. Astfel, testul Neuropad® pare a fi un instrument suplimentar util de diagnosticare a neuropatiei, deosebit de potrivit pentru screening şi auto-examinare, în DZ tip 2 (17). Mai mult decât atât, s-a demonstrat că testul Neuropad® a fost capabil să separe pacienții în grupuri cu diferite profile de risc general, inclusiv vârstă, durata DZ, prezența bolii vasculare coronariene şi/sau cerebrale, nefropatie şi retinopatie, precum şi să stratifice pacienții în grupuri cu diferite stadii de ND, evaluată prin simptome, diagnostic, NDS (18).

O potențială limitare clinică a testului Neuropad® ar putea fi reprezentată de intervalul de 10 minute necesar pentru citirea rezultatului. Un studiu recent sugerează că intervalul minim de timp pentru a defini un test Neuropad® ca fiind anormal poate fi 530 secunde (19). Ziegler et al. (20), într-un studiu recent, a găsit că în primul an de la diagnosticul de DZ, valoarea cut-off de citire a Neuropad® la 10 minute oferă o sensibilitate relativ mare şi o specificitate modestă pentru polineuropatie distală simetrică (PDS) şi pentru disfuncția de fibre mici, fiind potrivit ca instrument de screening, mai mult decât punctele de cut-off de

citire la 15 şi 20 minute. Spre deosebire de aceste date, un alt studiu demonstrează că valoarea cut-off de 15 minute, comparativ cu 10 minute, a arătat o specificitate mai bună pentru NDA cardiovasculară şi PDS, fără a diminua sensibilitatea, autorii concluzionând că este un instrument fiabil de diagnostic pentru ambele afecţiuni, deşi numai de precizie moderată, şi că extinderea perioadei de observaţie la 15 minute oferă o mai mare utilitate de diagnosticare (21). Papanas et al. (22) a evaluat relaţia dintre Neuropad® şi NDS la pacienţi cu DZ tip 2, ajungând la concluzia că timpul necesar pentru virajul culorii poate indica stadiul PDS, efectuându-se astfel un triaj al pacienţilor ce necesită îngrijiri de specialitate. Acelaşi autor (23), într-un studiu prospectiv, a găsit că dintre pacienţii cu DZ tip 2 cu NDS normal, dezvoltarea de neuropatie este semnificativ mai frecventă la cei cu Neuropad® anormal. Aceste rezultate sugerează un potenţial de utilitate al Neuropad® pentru diagnosticul precoce al neuropatiei în DZ tip 2.

Totusi, avantajul practic evident al acestui test este că pacientul poate să îl efectueze singur, inclusiv la domiciliu şi, mai mult decât atât, vizualizarea unui rezultat anormal va creşte gradul de conştientizare al riscului de ND şi ulceraţie (24).

În ciuda evoluţiei tratamentului prin implicarea echipelor multidisciplinare, ratele amputaţiilor majore rămân mai mari decât se doreşte, cu costurile aferente exorbitante. Pentru reducerea acestora este necesar să se crească efortul pentru prevenirea ulceraţiilor şi să se focalizeze atenţia pe prima etapă a cascadei evenimentelor prin detectarea precoce a neuropatiei periferice (25).

Mai multe studii au confirmat sensibilitatea testului cu monofilamentul Semmes-Weinstein în detectarea neuropatiei periferice. Este unanim acceptat că este cel mai simplu mod de testare şi de asemenea reproductibil (26). Cu toate acestea, este o evaluare care necesită colaborarea pacientului şi poate fi influenţată de către examinator, având o interpretare subiectivă. În schimb, testul Neuropad® este un mod obiectiv de testare, reproductibil şi uşor de executat. După cum a postulat Risse (27), cu cât fibra nervoasă este mai subţire, cu atât este mai precoce afectarea sa, iar, cum fibrele nervoase vegetative sunt nemielinizate, şi deci mai subţiri decât fibrele senzoriale, vor fi primele afectate. Acest lucru poate explica detecţia mai precoce a pacienţilor cu neuropatie periferică cu ajutorul testului Neuropad®, comparativ cu rezultatele obţinute cu monofilamentul Semmes-Weinstein, care aparent poate reprezenta un alt avantaj al primului.

CONCLUZII

În lotul studiat 95% dintre pacienţi au prezentat neuropatie periferică senzitivo-motorie (NDS≥6). La 76% dintre aceştia a fost găsită şi neuropatie autonomă, detectată prin test Neuropad® pozitiv. În plus 2/3 din pacienţii fără neuropatie periferică au prezentat neuropatie autonomă.

Pacienţii cu neuropatie periferică şi autonomă au avut o durată a diabetului mai mare, un istoric mai important de amputaţii şi un interval mai lung de spitalizare faţă de subiecţii care au prezentat numai forma periferică de neuropatie.

Având la dispoziţie testul Neuropad®, uşor de utilizat, am putut analiza şi componenta autonomă a neuropatiei, de obicei neobiectivată, şi astfel am putut individualiza un sublot de pacienţi la risc mai mare de a dezvolta ulceraţii cu o evoluţie mai îndelungată.

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NOUTĂȚI

DEFICITUL DE VITAMINA D ŞI ASTMUL LA COPIL

Astmul bronşic dezvoltat în copilărie are o prevalenţă crescută la nivel global. Printre factorii de risc este acceptată asocierea dintre existenţa unui teren atopic, expunerea la alergenii din mediu şi istoricul familial de boală alergică. Studii recente au sugerat că deficitul de vitamina D poate de asemenea predispune la fenotipul alergic. Vitamina D este un important modulator al sistemului imun şi reglator al proceselor de proliferare şi diferenţiere celulară. O serie de studii epidemiologice au arătat că deficitul de vitamina D este legat de creşterea incidenţei şi severităţii astmului la copii. În acest context, un grup de cercetători au desfăşurat un studiu în India având ca scop măsurarea nivelului seric al vitaminei D la copii astmatici şi compararea cu cel determinat la copii sănătoşi. Părinţii au fost chestionaţi despre: datele demografice, dieta copiilor şi aportul de vitamină D. La copii au fost determinate valorile serice pentru: vitamina D, calciu, fosfor, fosfatază alcalină, magneziu, creatinină şi hormon paratiroidian. Rezultatele au arătat că 68,1% dintre copiii astmatici au deficit de vitamina D, cu proporţii ale deficitului mediu şi sever semnificativ mai mari decât la copii sănătoşi. Istoricul familial pozitiv de deficit de vitamina D şi astm bronşic a fost semnificativ mai mare la copiii astmatici. Deficitul de vitamina D şi astmul au asociat, de asemenea, valori serice reduse ale fosforului şi magneziului, dar niveluri crescute ale fosfatazei alcaline serice şi IgE. Concentraţiile serice ale calciului, creatininei şi hormonului paratiroidian au fost similare la asmatici şi non-asmatici. Prin analiza statistică a rezultatelor cercetătorii au stabilit că deficienţa de vitamina D a fost cel mai puternic predictor al astmului la copil (OR 4.82; 95% CI 2.41-8.63, p <0,001) asociată cu prezenţa altor factori de risc: istoricul familial de astm şi de deficit de vitamina D şi concentraţii serice ridicate ale IgE. (Bener A, Ehlayel MS, Tulic MK, Hamid Q. Vitamin D Deficiency as a Strong Predictor of Asthma in Children. *Int Arch Allergy Immunol*. 2011:168-175).

Cătălina Luncă

FOCUS ON QUALITY OF LIFE AND COSTS IN PATIENTS WITH IRON DEFICIENCY AND HEART FAILURE

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FOCUS ON QUALITY OF LIFE AND COSTS FOR IRON DEFICIT HEART FAILURE PATIENTS (Abstract): Chronic heart failure is an important health problem worldwide. Despite the reduction in mortality associated with the newest medical and interventional treatments, the quality of life remains an important concern for patients as well for doctors. **Aim:** to quantify the quality of life and the financial burden in patients with heart failure and iron deficiency. **Material and methods:** Patients with heart failure and iron deficiency were included in the study. Personal medical data were collected, and the patients completed the Kansas City Cardiomyopathy Questionnaire (KCCQ), which is a validated questionnaire for anemia associated with heart failure. The financial costs of hospital admission were evaluated. **Results:** A statistically significant moderate direct correlation between calcium channel blockers treatment and the scores in KCCQ was found. Also, a statistically significant, indirect correlation was shown between anticoagulant treatment and quality of life. As to the financial burden, the average cost per admission was 3,641.75 RON ± 1,881.32 RON (783.17 euro ± 404.58 euro). **Conclusions:** Anticoagulant treatment in patients with heart failure and iron deficiency was associated with reduced quality of life. Calcium channel blockers were associated with improvement of the quality of life in this category of patients. **Keywords:** HEART FAILURE, IRON DEFICIENCY, QUALITY OF LIFE, MEDICAL COSTS.

Chronic heart failure is associated with significant morbidity and in advanced stages has a higher mortality than many cancers (1). Current studies reveal an improvement in survival determined using evidence-based drugs and devices (2). Despite these results on mortality, many patients give equal or more importance to quality of life (QoL) when compared with length. QoL in patients with heart failure is importantly reduced compared with general population and with patients with other chronic diseases (3). Heart failure is responsible for enormous health costs and resource utilization. In United Kingdom this pathology accounts for 5% of emergency medical admissions to hospitals and is the primary diagnosis associated with hospital stay in German (4). Several studies support the importance of minimizing recurrent admissions as primary measure for reducing the economic burden of heart failure (5, 6). In this context, improvement of QoL and reduction of symptoms should be an im-

portant goal for clinicians.

Iron deficiency associated with heart failure leads to deterioration in QoL especially due to physical limitations in daily activities and a rise in hospital admission frequency (7). Iron deficiency is associated with impaired exercise capacity and fatigue in healthy populations (8). There is a growing interest to explore the determinants of QoL that could lead to interventions for improving of patient wellbeing.

In heart failure, patients receive treatment with the same classes of drugs for long periods of time. We understand the effects of this treatment on heart problems, but can this treatment influence differently the patients which associate iron deficiency? Could QoL be influenced by this treatment? We conducted a prospective study in patients with heart failure and iron deficiency to determine if the treatment used for heart failure could influence the QoL and its financial implications.

MATERIAL AND METHODS

An observational prospective study was conducted to evaluate different classes of drugs that could influence the QoL in patients with heart failure and iron deficiency.

Patients admitted to our Internal Medicine Clinic, diagnosed with heart failure (with reduced, mid-range and preserved ejection fraction) according to the diagnostic criteria of the latest European Society of Cardiology guidelines (9) and with associated iron deficiency (defined as serum iron <60 μg/dL with ferritin <100 μg/L or 100-300 μg/L with transferrin saturation <20%) were included in the study.

Detailed information on the drugs used before admission and past medical history were recorded for each patient.

Patients with positive fecal occult test or with pre-existing or newly diagnosed conditions that could lead to anemia were excluded. The patients with chronic blood loss (recurrent epistaxis, hematuria, menometrorrhagia, gastrointestinal bleeding), neoplasia, inflammatory disease, pregnancy, recent surgery, anemia that required blood transfusion were excluded from our analysis.

Blood samples for laboratory tests were collected early in the morning after overnight fast. After inclusion, each patient completed the Kansas City Cardiomyopathy Questionnaire (KCCQ). This is a 23-item self-administered questionnaire and is a disease-specific measure that quantifies some clinically relevant domains of patient health: physical limitation, symptoms (frequency, severity, changes over time), quality of life assessment in heart failure, social function, self-efficacy and knowledge. All these scales can be combined into a single, overall summary score. Scores range from 0 to 100, higher scores indicating fewer symptoms and better QoL. This is a validated instrument to evaluate the QoL in patients with heart failure and anemia (10).

Finally, as a major public health issue, the financial costs of hospital admissions were of interest. Variables like the length of hospital stay and in hospital costs were analyzed in a subgroup of 20 patients. All costs are in RON (the currency of Romania) and were converted to euros (2018 exchange rate using the National Bank of Romania inflation calculator).

The study design was approved by the Research Ethics Committee of the Iasi "Grigore T. Popa" University of Medicine and Pharmacy and by the Ethics Committee of the "Sf. Spiridon" County Clinical Emergency Hospital. All participants were informed about the study protocol and signed the informed consent.

Data are presented as mean ± standard deviation (SD) or as percentages.

Point biserial correlation was used to investigate the relations between certain nominal variables.

Means between groups were compared using Mann Whitney U test for two samples. The statistical software used was *IBM Statistics SPSS 20* for Windows. All tests were two-tailed and a p-value <0.05 was considered statistically significant.

RESULTS

Included in the study were 125 patients who met the inclusion criteria and completed the KCCQ. Of these 54,68 % were female, 45,31% male and the mean age was 73.18 (range 42-98) years. Based on the type of heart failure, 21.87% were categorized as reduced ejection fraction, 22.65% as mid-range ejection fraction and 38.28% as preserved ejection fraction. According to New York Heart Association (NYHA) classification, 45.6% were in NYHA class II, 52% in NYHA class III and only 2.4% of the patients were NYHA class IV. The main characteristics of patients are presented in table I.

TABLE I

Descriptive statistics of the patients included in the study

Parameter	Mean	SD	95% Confidence Interval	
			Lower Bound	Upper Bound
Hemoglobin (g/dL)	12.37	1.62	11.94	12.96
Hematocrit (%)	38.65	6.07	37.58	39.72
MCV (fl)	86.11	8.78	84.57	87.65
MCH (pg)	27.7	2.58	27.25	28.16
MCHC (g/dL)	31.95	1.31	31.72	32.18
Iron (µg/dL)	38.33	12.05	32.37	39.84
Ferritin (ng/mL)	86.51	97.21	69.37	103.65
NT-proBNP (pg/mL)	4730.72	6681.87	3391.09	6070.35
Urea (mg/dL)	48.92	18.78	45.58	52.26
Creatinine (mg/dL)	0.99	0.34	0.93	1.05
eGFR (mL/min/1.73m²)	71.28	22.43	67.34	75.22
C-reactive protein(mg/dL)	2.41	5.48	1.38	3.45
LVEF (%)	49.79	13.47	44.34	48.82

MCV-mean corpuscular volume; MCH-mean corpuscular hemoglobin; MCHC-mean corpuscular hemoglobin concentration; NT-proBNP - N terminal pro brain natriuretic peptide; eGFR-estimated glomerular filtration rate; LVEF-left ventricular ejection fraction; SD-standard deviation

The patients were divided into groups according to the treatment followed before admission. The percentage of patients using different drugs is shown in fig. 1.

To investigate the correlations between different classes of drugs and QoL, point biserial correlation was used. A significant moderate direct correlation between calcium channel blocker (CBB) treatment and the scores on KCCQ related to symptoms was found (tab II).

A statistically significant, indirect correlation was found between anticoagulant treatment and QoL (r -0.2, p 0.02), social limitation (r -0.28, p 0.001) and combined

scores (r -0.23, p 0.01; r -0.17, p 0.05).

No other significant correlations were found between beta blockers, angiotensin converting enzyme inhibitors (ACEI) or antiplatelet treatment and the KCCQ scores.

In the subgroup of patients evaluated for in-hospital costs, the average hospital stay was 8.75 ± 4.54 days and the average cost per admission was 3641.75 RON ± 1881.32 RON (783.17 euro ± 404.58 euro). All financial data were certified by the Hospital Financial Department and were collected at discharge according to the national economic regulations.

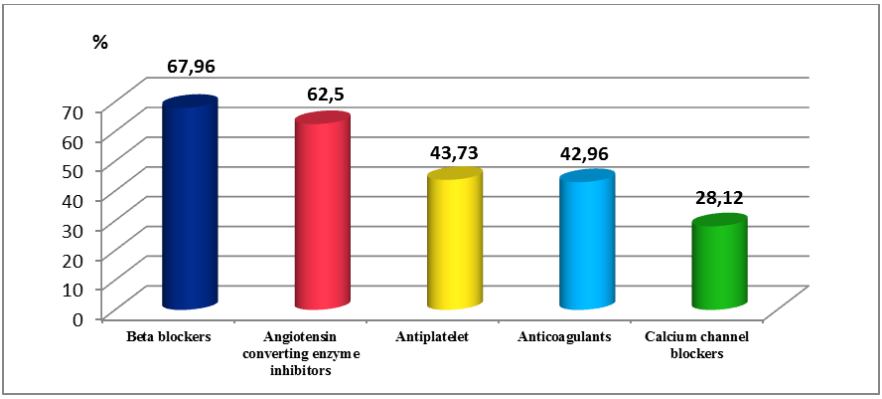

Fig. 1. Percentage of drugs used by patients before admission

TABLE II

Correlation between drugs used by patients and quality of life

Parameter	Beta blockers		CCB		ACEI		Antiplatelet		Anticoagulants	
	r	p value	r	p value	r	p value	r	p value	r	p value
Physical limitation	-0.14	0.11	0.17	0.05	0.07	0.43	0.07	0.44	-0.16	0.06
Symptom stability	-0.12	0.15	0.00	0.98	-0.16	0.07	-0.12	0.18	0.06	0.49
Symptom frequency	-0.1	0.23	0.22	0.01*	0.02	0.77	0.13	0.14	-0.07	0.4
Symptom burden	-0.3	0.74	0.23	0.01*	0.07	0.42	0.12	0.16	-0.18	0.04
Total symptom score	-0.92	0.31	0.24	0.007*	0.07	0.4	0.15	0.08	-0.18	0.03
Self-efficacy	0.01	0.83	0.12	0.16	-0.05	0.54	0.13	0.15	-0.13	0.12
Quality of life	-0.1	0.23	0.13	0.14	0.01	0.88	0.09	0.28	-0.2	0.02*
Social limitation	-0.05	0.55	0.18	0.04*	0.09	0.28	0.19	0.03	-0.28	0.001*
Overall summary score	-0.09	0.28	0.21	0.01*	0.05	0.57	0.14	0.1	-0.23	0.01*
Clinical summary score	-0.12	0.17	0.21	0.01*	0.06	0.48	0.17	0.19	-0.17	0.05*

CCB-calcium channel blockers; ACEI-angiotensin converting enzyme inhibitors;
r-Pearson correlation coefficient; *p<0.05

DISCUSSION

This study shows that the treatment used for heart failure has an influence on QoL of patients. It appears that symptoms and social limitation were improved in patients receiving CCB. One of the explanations for this result is the fact that most of the patients included in this analysis (38%) had heart failure with preserved ejection fraction.

CCB do not have a specific indication in heart failure, so there are no studies aimed at evaluating QoL in this category of patients. A research, involving hypertensive patients, showed that patients treated with CCBs have a better QoL (11).

It was proved that iron deficiency has a negative impact on QoL independent of the presence of anemia (12). This effect is determined mostly by physical limitation in daily activities, secondary to cardiac and skeletal muscle dysfunction (13). It is possible that CCBs determine vasodilation which exceeds the negative effect of iron deficiency. However, these hypotheses need to be proven in large randomized studies.

According to our results, anticoagulant treatment has a negative effect on QoL in patients with heart failure and iron deficiency. The correlation was moderate statistically significant. Patients with an indication for anticoagulant treatment were expected to be in a more advanced stage of heart failure, and, therefore, their QoL was reduced. Furthermore, patients with anticoagulant treatment could have intermittent digestive blood loss and, therefore, a higher degree of iron deficiency that could explain these results.

It has already been shown that anticoagulant treatment determines a reduction in QoL especially at the beginning of treatment. Qol is more influenced by other drugs that could increase the anticoagulant effect (14).

However, the anticoagulant therapy is indicated for the prevention of embolic events, so this advantage exceeds the impact of QoL.

We could not find an improvement on QoL in patients receiving beta blockers or ACEI treatment, but this result is in accordance with other larger studies (15,16).

The unpredictable clinical course of heart failure has a significant socioeconomic impact. A compensated heart failure results in improvement in QoL and large cost savings. Our study emphasizes the necessity of assessing the financial aspects of patients with heart failure admitted to Romanian public hospitals. Further efforts are needed to establish proper measures of prevention of cardiac disease and reducing hospital readmissions.

There are some limitations of the present study. First, the number of patients included in the analysis is limited but, considering the inclusion and exclusion criteria, we believe that it is eloquent for the investigated disease. In addition, we excluded patients who could have another cause of iron deficiency except heart failure, this way limiting possible biases. The questionnaire being self-administered, the responses could be influenced by the social status of the patients.

CONCLUSIONS

Our study revealed that the group of patients associating iron deficiency could benefit from CCB treatment. Anticoagulants can reduce QoL. These results are important for personalizing the treatment

for heart failure according to the presence of other comorbidities (i.e. iron deficiency) with respect to optimal QoL and balanced financial costs.

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BOALA ARTERIALĂ PERIFERICĂ NOU DIAGNOSTICATĂ LA PACIENTUL CU RISC CARDIOVASCULAR

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NEWLY DIAGNOSED PERIPHERAL ARTERIAL DISEASE IN PATIENTS WITH CARDIOVASCULAR RISK FACTOR (Abstract): The gold of this study is to investigate the utility of ankle-brachial index (cut-off value < 0,90) as an indicator of peripheral arterial disease in a group of patients hospitalized in the Medical Clinic and to assess the prevalence of risk factor and associated comorbidities in patients with low index. **Material and methods:** A total of 202 patients older than 50 years with established cardiovascular or cerebrovascular disease or at least one cardiovascular risk factor (smoking, diabetes, dyslipidemia and hypertension) underwent ankle-brachial index measurement for this study. Demographic data and cardiovascular risk factor were analyzed. We assessed medical history, symptoms and clinical examination. **Results;** In this study, a number of 72 patients (35.64%) were diagnosed with peripheral arterial disease. Our results indicated that advanced age, greater number of total risk factors and presence of peripheral arterial disease related physical findings were associated with increased likelihood of low ankle-brachial index. **Conclusions.** We should recommend ankle-brachial index as screening test in pacients older than 50 years, in the presence of at least one cardiovascular risk factor, for early detection of peripheral arterial disease and systemic atherosclerosis, to improve prevention and early aggressive treatment. **Key words:** PERIPHERAL ARTERIAL DISEASE, ANKLE-BRACHIAL INDEX, RISK FACTOR, EPIDEMIOLOGY

Boala arterială periferică (BAP) constituie un capitol important în patologia cardiovasculară, datorită incidenţei ridicate şi problemelor complexe de diagnostic şi tratament pe care le ridică. BAP este un marker al aterosclerozei, care asociază frecvent determinări vasculare şi în alte teritorii: cardiovascular, cerebrovascular sau renal.

Studiile epidemiologice au estimat că prevalenţa BAP poate fi de până la 10% în populaţia generală şi de două ori mai mare la cei cu vârsta peste 70 de ani. Mai mult, la fiecare pacient diagnosticat cu BAP simptomatică, există aproximativ 3 la 4 pacienţi nediagnosticaţi, cu BAP asimptomatică.

Pacientul cu BAP are un risc de deces în următorii 10 ani de 6 ori mai mare decât populaţia generală, un risc de infarct miocardic fatal de 4 ori mai mare şi un risc de accident vascular cerebral, inclusiv atac ischemic tranzitor, de 2 - 3 ori mai mare.

Excluzând pacienţii cu ischemie critică a membrelor inferioare, pacienţii cu BAP au o incidenţă anuală de 2 - 3% a unui infarct miocardic non-fatal şi un risc de 2 –

3 ori mai mare decât populaţia generală de a dezvolta angină pectorală. Morbiditatea şi mortalitatea la 5, respectiv 10 şi 15 ani sunt de aproximativ 30%, respectiv 50% şi 70%, la aceşti pacienţi.

Valorile incidenţei şi prevalenţei arteriopatiei obliterante sunt dificil de stabilit întrucât această afecţiune cuprinde şi un stadiu asimptomatic. Aceasta poate fi descoperită cu ocazia unei evaluări generale a unui pacient asimptomatic sau a unuia cunoscut cu afectare aterosclerotică manifestă într-un alt teritoriu arterial (1, 2, 3).

Scopul acestui studiu este de a investiga utilitatea indicelui gleznă-braţ (IGB<0,90) ca indicator al BAP la o populaţie internată în Clinica Medicală şi de a evalua caracteristicile indivizilor cu IGB scăzut în raport cu sexul, vârsta şi prezenţa unei boli cardiovasculare sau cerebrovasculare cunoscute sau a unor factori de risc cardiovascular.

MATERIAL ŞI METODE

Au fost incluşi în studiu 202 pacienţi internaţi, în perioada februarie – mai 2011, în Clinica I Medicală Cardiologică, cu vârste cuprinse între 50 – 89 ani, care nu au fost diagnosticaţi anterior cu BAP, în prezenţa unei boli cardiovasculare sau cerebrovasculare cunoscute sau a cel puţin un factor de risc cardiovascular major (fumat, diabet, HTA, dislipidemie).

Au fost considerate criterii de excludere BAP cunoscută, refuzul pacientului şi condiţiile ce necesită spitalizare pentru afecţiuni ameninţătoare de viaţă sau neoplasme.

Au fost urmărite şi corelate datele demografice, comorbidităţile asociate, prezenţa factorilor de risc cardiovascular, examenul clinic sugestiv pentru BAP (absenţa pulsului la artera pedioasă sau tibială posterioară, prezenţa unui suflu arterial sau a unor tulburări trofice cutanate) şi simptomatologia clinică (asimptomatici, simptomatici – cu claudicaţie intermitentă şi simptomatici sever – cu durere de repaus şi/sau tulburări trofice cutanate prezente, interpretate în contextul comorbidităţilor – insuficienţă venoasă cronică, polineuropatie diabetică) (fig.1).

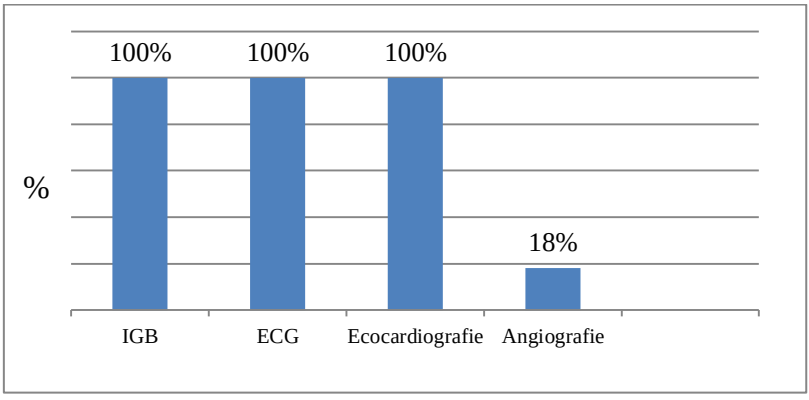

Fig. 1. Protocolul de urmărire – Indicele gleznă-braţ (IGB), electrocardiograma (ECG), ecocardiografia şi explorarea angiografică a arterelor membrului inferior.

Protocolul de urmărire a lotului de studiu a cuprins IGB, electrocardiogramă şi ecografie cardiacă efectuată întregului lot de 202 (100%) pacienţi şi explorarea arterială a membrelor inferioare la 36 (17,82%). La acestea s-au adăugat testarea

glicemiei, funcţiei renale (uree, creatinină, clearance de creatinină, acid uric), profilului lipidic (colesterol total, HDL-colesterol, LDL-colesterol, trigliceride), fibrinogenului şi hematocritului.

IGB este un indicator uşor de obţinut şi care se realizează cu costuri minime. El reprezintă raportul dintre tensiunea arterială sistolică (TAS) de la nivelul gleznei şi tensiunea la nivelul arterei brahiale. O valoare IGB<0,90 are o sensibilitate de 95% şi o specificitate de 99% pentru diagnosticul arteriopatiei obliterante a membrelor inferioare. Este metoda validată pentru screening la toate categoriile de pacienţi şi pentru diagnosticul iniţial al BAP, care depistează BAP semnificativă hemodinamic la pacienţi asimptomatici (2, 3).

Măsurarea IGB s-a realizat prin măsurarea TAS cu ajutorul unui tensiometru standard la ambele arterele brahiale şi la ambele artere pedioase şi tibiale posterioare după repaus de cel puţin 5 minute în decubit dorsal. La membrele superioare, TAS s-a măsurat în mod convenţional. TAS la membrele inferioare s-a evaluat prin plasarea unei manşete pe gleznă şi gonflarea sa peste valoarea TAS de la braţ, cu înregistrarea succesiv a primului semnal Doppler audibil la nivelul arterei pedioase şi tibiale posterioare cu ajutorul unei sonde speciale de 10 mHz. Pentru calculul IGB, s-a utilizat întotdeauna valoarea cea mai mare a TAS brahiale măsurate la nivelul membrului superior drept sau stâng, iar la membrul inferior valoarea cea mai mare dintre TAS la pedioasă şi tibiala posterioară (2).

Analiza statistică a fost realizată cu ajutorul programelor *SPSS 13.0* şi *Statistica 6.0* după prealabila introducere a datelor în Microsoft Excel. Pentru compararea valorilor medii am utilizat testul t Student şi ANOVA . Analiza corelaţiilor s-a efectuat cu ajutorul coeficientului de corelaţie Pearson. Datele au fost exprimate în valoare medie±deviaţie standard sau în procente. Semnificaţia statistică a fost luată în considerare pentru un nivel de $p<0,05$.

REZULTATE ŞI DISCUŢII

Cercetările noastre au fost motivate de apariţia studiului CAREFUL, singurul publicat anterior care a urmărit prezenţa BAP pe un lot de 530 pacienţi cu boală cardiovasculară sau cerebrovasculară cunoscută sau în prezenţa a cel puţin un factor de risc cardiovascular prezent, prin măsurarea IGB (4).

Lotul de studiu a cuprins 104 (51,49%) bărbaţi şi 98 (48,51%) femei, cu o medie de vârstă a lotului de 70,67±9,21 ani, fără diferenţe semnificative între cele 2 sexe, cu domiciliul preponderent în mediul rural 126 (62,38%) pacienţi.

Distribuţia pe grupuri de vârstă a evidenţiat că: între 50-59 ani au fost 32 (15,84%) pacienţi; 60-69 – 38 (18,81%); 70-79 – 102 (50,5%), iar între 80-89 – 30 (14,85%) pacienţi (fig. 2).

Au fost evaluate evenimentele aterotrombotice în cadrul lotului: antecedentele personale patologice (APP) cardiovasculare au fost prezente la 194 (96,04%) pacienţi, cerebrovasculare - 26 (12,87%), iar renale - 12 (5,94%) cazuri.

Evenimentele cardiovasculare au fost dominate de HTA pentru 70,51% cazuri (66 bărbaţi, 76 femei), urmată de fibrilaţia atrială la 35,62% (38 bărbaţi, 34 femei), iar cele cerebrovasculare de accident vascular cerebral în 7,81% cazuri (12 bărbaţi, 4 femei).

APP cardiovasculare au fost prezente în proporţii asemănătoare la cele 2 sexe (94,23% la sexul masculin versus 97,96% la feminin), în timp ce istoricul de boală cerebrovasculară a fost semnalat în propor-

ție mai mare în rândul bărbaților (19,23% la masculin versus 6,12% la feminin).

Au fost evaluați factorii de risc cardiovascular prezenți: dislipidemia a fost cel mai frecvent factor de risc prezent (58,42%), urmată de obezitate şi diabet, în proporții apropiate (39,6%, respectiv 37,62%), iar fumatul a fost implicat în 26,73% cazuri, îndeosebi bărbați care au fost mai frecvent diagnosticați cu diabet şi obezitate.

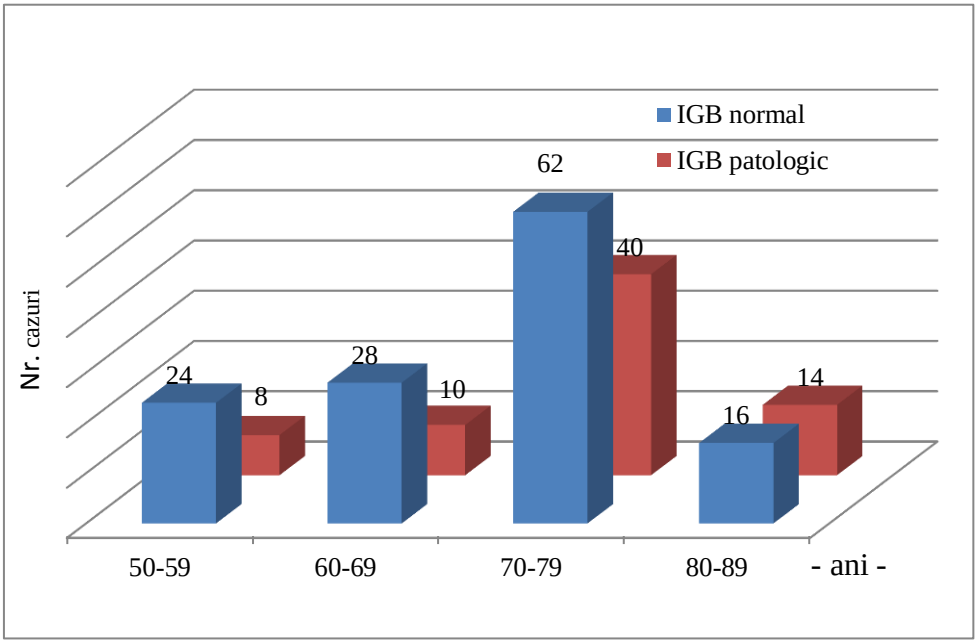

Fig. 2. Prezența bolii arteriale periferice repartizată pe grupuri de vârstă.

Din totalul pacienților incluşi în studiu, 52 (25,74%) au avut un singur factor de risc prezent; 68 (33,66%) – 2; 40 (19,8%) – 3; 18 (3,96%) – 4, iar 52 (25,74%) pacienți nu au avut nici un factor de risc prezent, dar au avut patologie cardiacă sau cerebrovasculară asociată.

În cadrul lotului studiat, BAP a fost descoperită la 72 (35,64%) pacienți prin determinarea IGB, considerând ca diagnostică o valoare IGB<0,90. Boala a fost mai frecvent evidențiată la sexul masculin (44 bărbați versus 28 femei, $p<0,05$) şi în decada de vârstă 70-79 de ani (55,6%), dar avem în vedere că aproape jumătate din lot se încadrează în această vârstă. BAP a fost diagnosticată într-o proporție de 19,4% în decada de vârstă 80-89 de ani şi a fost mai puțin frecventă în decadele 60-69, respectiv 50-59 ani (13,9%, respectiv 11,1%).

Studiul CAREFUL, realizat pe un lot de 530 pacienți cu aceleaşi criterii de includere ca şi studiul nostru, a evidențiat prezența BAP în 25,1% din cazuri, cu o prevalență mai mare, de 30% la vârstnicii peste 70 de ani, indiferent de factorii de risc cardiovascular asociati (4).

Incidența semnificativ crescută a BAP în cadrul lotului studiat comparativ cu datele din literatură este explicată, pe de o parte, de faptul că în România adresabilitatea

pacientului arteriopat la medic este tardivă, în stadii simptomatice de boală, dar şi de faptul că am inclus în studiu 22 de pacienţi cu tulburări trofice cutanate la nivelul membrelor inferioare de etiologie neprecizată în momentul internării, care s-au dovedit ulterior a fi de cauză vasculară, asociate sau nu cu neuropatie periferică.

Din totalul pacienţilor nou diagnosticaţi cu BAP, 18 (8,91%) au prezentat BAP simptomatică, acuzând claudicaţie intermitentă; 36 (17,82%) cu BAP simptomatică sever, prezentând durere de repaus şi/sau tulburări trofice cutanate şi 18 (8,91%) cu BAP asimptomatică, neavând simptome sugestive pentru BAP (fig.3).

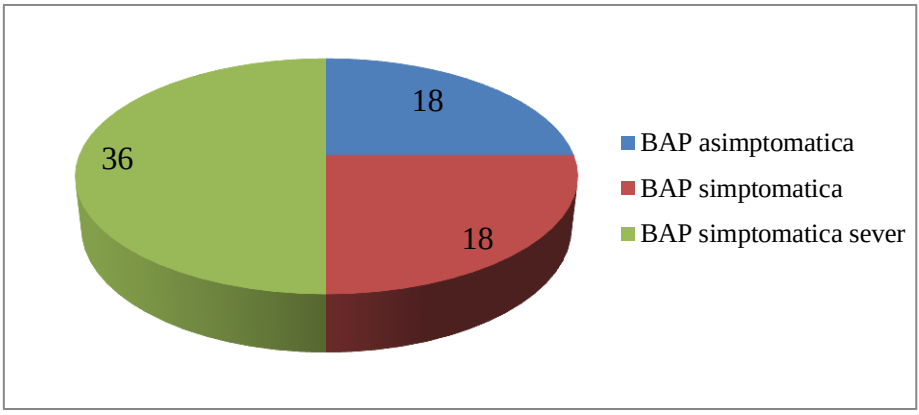

Fig. 3. Stadiul clinic al bolii arteriale periferice în relaţie cu examenul clinic şi simptomatologia pacientului.

Studiul PARTNERS realizat pe 6979 de pacienţi cu vârste peste 50 de ani sau în prezenţa unui factor de risc (fumat sau diabet) a urmărit capacitatea de depistare a BAP folosind IGB. Rezultatele arată un procent semnificativ de BAP de 29% (1865 pacienţi), dintre care 825 cu BAP izolată şi 1040 cu BAP asociată cu boală cardiovasculară. De remarcat, în studiul PARTNERS, că doar 8,7% din totalul pacienţilor identificaţi cu BAP au prezentat istoric de claudicaţie tipică (5).

În studiul actual, BAP simptomatică, cu istoric de claudicaţie intermitentă, a fost depistată în 8,91% cazuri, valoare similară cu studiile anterioare, care raportează valori între 5,3% - 18,9% pentru BAP simptomatică (6, 7, 8).

Din totalul pacienţilor luaţi în studiu, un număr de 10 (4,95%) au avut cel puţin o valoare IGB>1,3, valoare considerată fals supraunitară, apărute la pacienţi vârstnici şi diabetici, probabil datorate calcificării arteriale, dar care nu au fost incluşi în categoria BAP nou diagnosticată, procent uşor mai scăzut decât în studiile anterioare (5).

Din totalul pacienţilor diagnosticaţi cu BAP, 26 (33%) au prezentat BAP unilaterală, iar 48 (67%) bilaterală.

În studiul nostru, BAP a fost confirmată prin efectuarea IGB la toţi pacienţii care au prezentat suflu la ascultaţia arterelor femurale (6 pacienţi) şi tulburări trofice cutanate (22; eritem – 14; ulceraţii – 6; necroză – 4). S-a obţinut, de asemenea, confirmarea diagnosticului la peste 90% (58) din pacienţii care la examenul clinic au prezentat puls periferic absent la nivelul arterei pedioase

sau tibiale posterioare. Au fost doar 6 pacienți care au avut puls periferic absent, dar IGB>0,9 a infirmat diagnosticul de BAP, pacienții prezentând obezitate, edeme ale membrelor inferioare datorate insuficienței cardiace sau insuficienței venoase cronice.

Pentru diagnosticul de BAP sunt foarte importante anamneza dirijată cu descrierea claudicației intermitente sau a durerii de repaus şi examenul clinic al pacientului sugestiv pentru BAP, cu examenul arterelor membrelor inferioare şi depistarea leziunilor trofice cutanate, cu confirmarea ulterioară prin efectuarea IGB şi eventual explorare arteriografică, pentru stabilirea unei conduite terapeutice optime. Studiul actual confirmă, ca şi cele anterioare, importanța examenului clinic al pacientului, cu limitele induse de prezența comorbidităților asociate (5, 7, 9, 10, 11).

S-a efectuat arteriografie la 36 de pacienți cu IGB<0,9, reprezentând un procent de 50% din totalul pacienților diagnosticați cu BAP, aflați în stadiile Leriche IIB, III şi IV de boală. Confirmarea angiografică a BAP a fost obținută la toți cei 36 de pacienți explorați, dintre care 14 au prezentat leziune tip stenoză arterială, iar 28 tromboză arterială, cele mai multe localizări fiind la nivelul arterei femurale superficiale (32 pacienți).

CONCLUZII

Studiul arată o incidență crescută a BAP nou diagnosticate în rândul pacienților internați în Clinica Medicală, care au un eveniment aterotrombotic în antecedente sau cel puțin un factor de risc cardiovascular prezent. Rezultatele arată că vârsta înaintată, prezența unui număr mai mare de factori de risc şi constatarea unor semne clinice sugestive pentru BAP au fost asociate cu un IGB scăzut.

S-a evidențiat utilitatea în sistem screening a IGB la pacienții cu vârsta peste 50 de ani, în prezența a cel puțin unui factor de risc cardiovascular, pentru depistarea precoce a BAP şi a aterosclerozei sistemice, pentru îmbunătățirea prevenției şi un tratament agresiv precoce.

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NOUTĂŢI

RISCUL APARITIEI CANCERULUI LA PACIENTII CARE PRIMESC TRATAMENT CU STATINE

Statinele sunt prescrise pe scară largă în tratamentul dislipidemiei, aterosclerozei, şi pentru reducerea riscurilor cardiovasculare. Date ştiinţifice contradictorii au generat o dezbatere cu privire la asocierea utilizării statinelor cu riscul aparitiei cancerului. Unele studii au demonstrat că terapia cu statine este bine tolerată şi sigură, inclusiv în utilizarea pe termen lung, alte studii au contrazis această concluzie. Dar, toate aceste studii au avut o serie de limitări legate de numărul şi caracteristicile subiecţilor studiaţi, perioada scurtă de monitorizare, constrângerile de selecţie a pacienţilor şi rigiditatea protocoalelor de tratament tipice studiilor randomizate, controlate. Pentru a corecta aceste limitări, un grup de cercetători americani a efectuat un studiu de cohortă retrospectivă a incidenţei cancerului la persoanele în vârstă care au folosit şi care nu au folosit statine. Mai mult de 11 milioane de înregistrări medicale, din perioada ianuarie 1990- februarie 2009 au fost extrase din baza de date a General Electric Centricity. Prin metode statistice au fost selectate 45857 de perechi de pacienţi cu caracteristici similare pentru utilizarea statinelor. Studiul a demonstrat că incidenţa cancerului la pacienţii care au primit tratament cu statine a fost 11.37%, comparativ cu 11,11% la pacienţii care nu au primit acest tratament. Analiza Cox de regresie a arătat o rată a riscului nesemnificativă de 1,04 (interval de încredere 95%: 0.99 - 1.09). Curbele Kaplan-Meier pentru diagnosticul de cancer până la 10 ani nu au arătat diferenţe între cele doua loturi de pacienti. Concluziile acestei analize retrospective au fost că nu există risc semnificativ statistic crescut de cancer asociat cu statine (Claudio Marelli, Candace Gunnarsson, Susan Ross et al. Statins and risk of cancer, a retrospective cohort analysis of 45,857 matched pairs from an electronic medical records database of 11 million adult Americans. *J Am Coll Cardiol*. 2011; 58(5): 530-537).

Cătălina Luca

MONITORIZAREA INFECŢIEI CU *MYCOBACTERIUM TUBERCULOSIS* ÎN JUDEŢUL IAŞI, ÎN ANUL 2009

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MONITORING OF MYCOBACTERIUM TUBERCULOSIS INFECTIONS IN IASI COUNTY IN 2009 (Abstract): Tuberculosis is a major public health problem worldwide. Our study aimed to investigate epidemiological and demographic characteristics of tuberculosis infection and resistance to antituberculous drugs in Iasi County in 2009. **Material and method**: We have analysed the epidemiological parameters for 687 patients with pulmonary tuberculosis, new cases confirmed by microscopy and cultivation on Lowenstein Jensen. First and second-line antituberculous susceptibility testing was done for 130 strains, using agar proportion method and absolute-concentration method. **Results**: The number of new cases of tuberculosis was higher in rural areas and in males 41-50 years old (sex ratio=2.22). The proportion of positive cases in microscopy was 81.51%. Drug resistance was as follows: 16 isolates (12.3%) to isoniazid, 5 (3.84%) to rifampin, 2 (1.53%) to ethambutol, 2 (1.53%) to streptomycin and 4 (3.07%) were multidrug-resistant. **Conclusions**: In this study we found high resistance rates to isoniazid, streptomycin and ethambutol. This requires increasing efficiency of tuberculosis diagnosis and susceptibility testing. **Key words**: TUBERCULOSIS, DRUG RESISTANCE, MYCOBACTERIUM TUBERCULOSIS

Tuberculoza (TB) este una dintre problemele majore de sănătate publică pe plan mondial, Organizaţia Mondială a Sănătăţii (OMS) estimând că 32% din populaţia globului este infectată cu *Mycobacterium tuberculosis* (1). În ţările Uniunii Europene, în anul 2008, s-a raportat un număr de 315.285 cazuri noi de tuberculoză şi 45.448 decese prin tuberculoză, iar în România, în anul 2009 au fost diagnosticate 14.648 de cazuri noi de tuberculoză pulmonară dintr-un total de 21.457 cazuri înregistrate (2). Aceasta reprezintă o importantă problemă de sănătate publică nu doar datorită incidenţei crescute ci şi datorită dificultăţilor terapeutice şi a asocierii factorilor socio-economici dificil de controlat dar care constituie parte integrantă a managementului acestor cazuri.

Referitor la dificultăţile terapeutice, literatura de specialitate menţionează că tuberculoza multi-drog rezistentă (MDR TB),

definită prin rezistenţa cel puţin la izoniazidă (HIN) şi rifampicină (RMP) a atins în 2008 o proporţie de 6% din totalul cazurilor înregistrate în 25 de ţări europene, iar România a înregistrat cel mai mare procent MDR TB (14,7%), după cele raportate de Ţările Baltice (1,3). De asemenea, rezistenţa extensivă – XDR (rezistenţa la HIN, RMP, fluorochinolone şi cel puţin unul dintre următoarele droguri injectabile: amikacina, capreomicina sau kanamicina) îmbracă de asemenea proporţii îngrijorătoare la nivel european: 7,3% în 2008, când România a raportat 54 de cazuri (1).

OBIECTIVE

Acest studiu retrospectiv şi-a propus descrierea caracteristicilor epidemiologice şi demografice ale infecţiei tuberculoase în judeţul Iaşi şi pe cele legate de rezistenţa la antituberculoase. În acelaşi timp, acest studiu constituie o motivaţie pentru optimizarea metodelor de diagnostic în ceea ce priveşte raportul timp/beneficiu şi a mijloacelor de monitorizare a rezistenţei la antituberculoase, prin metode de înaltă sensibilitate şi specificitate.

MATERIAL ŞI METODĂ

Lotul de studiu a cuprins un număr de 687 de pacienţi cu tuberculoză pulmonară, cazuri noi, confirmate bacteriologic în perioada ianuarie-decembrie 2009 în cadrul Laboratorului Spitalului Clinic de Pneumoftiziologie Iaşi. Cazul nou este definit ca pacient care nu a primit niciodată tratament cu medicamente antituberculoase în asociere pe o perioadă mai mare de o lună de zile.

A fost urmărită repartiţia cazurilor pe vârstă, sex, mediu de provenienţă, sensibilitate la antituberculoase de prima şi a doua linie.

Diagnosticul de laborator a fost efectuat pe baza examenului microscopic al frotiurilor din probe clinice colorate Ziehl-Neelsen, urmat de izolarea, identificarea culturii bacteriene şi testarea sensibilităţii la antibiotice.

Izolarea tulpinilor de *Mycobacterium tuberculosis* a fost efectuată prin însămânţarea pe mediul Löwenstein-Jensen a probelor ca atare sau decontaminate chimic în funcţie de condiţia lor microbiologică. Identificarea de specie s-a bazat pe: caracterele microscopice în coloraţie Ziehl-Neelsen (acido-alcoolo-rezistenţa, dispoziţia în corzi), caracterele de cultură (viteza de creştere pe subcultură, aspectul coloniilor, pigmentogeneza), caracterele biochimice (producerea de catalază, producerea de niacină, nitrat reductază) (4).

Pentru un număr de 130 de tulpini izolate de la pacienţi nou diagnosticaţi a fost făcută testarea sensibilităţii la antituberculoase de prima linie (izoniazida, rifampicina, pirazinamida, etambutolul, streptomicina) şi a doua linie (etionamida, ofloxacina, kanamicina, capreomicina, acidul paraaminosalicilic, cicloserina), prin metoda proporţiilor (Canetti) şi metoda concentraţiilor absolute (Meissner) aşa cum stabileşte Programul Naţional de Control al Tuberculozei. Metodele de testare utilizate compară creşterea bacteriană de pe tuburile test (conţinând medicamente), cu cea de pe tuburile martor, după însămânţarea unui eşantion reprezentativ din populaţia bacilară de testat (4). Rezistenţa este definită prin prezenţa unei proporţii de mutanţi rezistenţi mai mare de 1% din populaţia martor.

REZULTATE

Dintre cei 687 de pacienţi, 474 (68,99%) au fost bărbaţi, iar în funcţie de mediul de provenienţă, am constat că 418 (60,84%) proveneau din mediul rural.

În ceea ce priveşte repartiţia cazurilor pe grupe de vârstă, grupa 41-50 de ani a fost cea mai afectată (198 de cazuri, 28,82%) şi s-a constatat un număr de 12 cazuri (1,74%) la copii (fig. 1).

Examenul microscopic a evidenţiat un număr de 560 de cazuri noi microscopic pozitive, reprezentând 81,51% din totalul de 687 de cazuri noi de TB înregistrate.

Rata de depistare a cazurilor noi pulmonare, microscopic pozitive a atins o valoare de 14,06% din 3982 cazuri suspecte examinate bacteriologic.

Dintre cele 687 cazuri noi pozitive în cultură, 127 au fost microscopic negative reprezentând o proporţie de 18,48%.

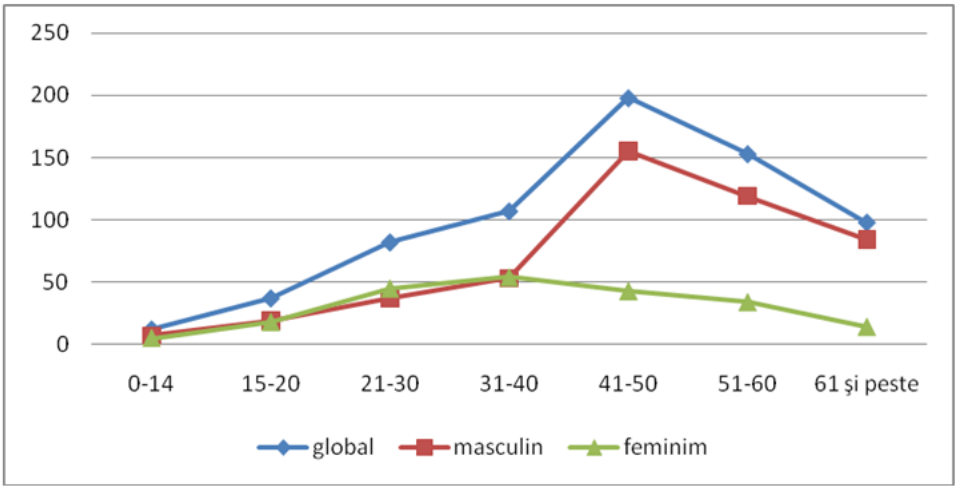

Fig. 1. Repartiţia cazurilor pe vârstă şi sex

În ceea ce priveşte sensibilitatea la antituberculoase, am testat 18,92% din totalul pacienţilor investigaţi, iar din cele 130 de tulpini testate, 25 de tulpini au fost monorezistente (19,23%), 16 tulpini au prezentat polirezistenţă (12,3%), iar 4 tulpini au fost MDR (3,07%).

Monorezistenţa la HIN a fost identificată la 16 tulpini (12,3%), pentru RMP la 5 tulpini (3,84%) iar pentru streptomicină (SM) şi respectiv etambutol (EMB) la 1,53% din cazuri (2 tulpini).

Polirezistenţa (definită ca rezistenţa la 2 antituberculoase cu excluderea asocierii HIN- RMP) a fost repartizată astfel: 15 tulpini au avut polirezistenţă la HIN şi SM (11,53%) iar o tulpină a prezentat polirezistenţă la HIN şi EMB (0,75%) (tab. I).

TABELUL I

Repartiţia tulpinilor funcţie de rezistenţă la antituberculoase de linia întâi

Rezistenţă	Nr. cazuri	Rata%
Monorezistenţă	25	19,23
HIN	16	12,3
RMP	5	3,84
SM	2	1,53
EMB	2	1,53
Polirezistenţă	16	12,3
HIN-SM	15	11,53
HIN-EMB	1	0,75
MDR	4	3,07

DISCUŢII

În 2009, în România a fost înregistrată o rată a incidenţei tuberculozei de 125 cazuri la 100.000 de locuitori, cifră care situează ţara noastră pe primul loc în Uniunea Europeană.

În ceea ce priveşte caracteristicile lotului remarcăm preponderenţa sexului masculin (68,99%), cu diferenţe statistic semnificative, majoritatea pacienţilor provenind din mediul rural (60.84%).

Rezultatele obţinute sunt concordante cu cele din literatură. În ceea ce priveşte repartiţia pe sexe, rezultatele obţinute arată că frecvenţa tuberculozei la bărbaţi este de 2,22 ori mai mare decât la femei, proporţie comparabilă cu cea menţionată în raportările OMS (sex ratio = 2,3) (3).

Procentul mai mare de cazuri noi de TB înregistrate în mediul rural comparativ cu mediul urban este justificat prin nivelul scăzut al condiţiilor socio-economice, educaţia sanitară deficitară şi accesul mai dificil la asistenţă medicală, în special în zonele defavorizate economic aşa cum este regiunea geografică de care aparţine judeţul Iaşi.

De asemenea, repartiţia pe grupe de vârste a arătat diferenţe între cele două sexe: cele mai multe îmbolnăviri apar la bărbaţi în jurul vârstei de 50 de ani, iar la femei la vârste mai tinere, în jur de 30 de ani. Această observaţie sugerează că transmiterea infecţiei în populaţie nu este pe deplin controlată (5).

Referitor la diagnosticul bacteriologic, proporţia cazurilor noi pozitive prin microscopie, raportată la toate cazurile noi pulmonare este peste 60%, ceea ce demonstrează că sunt respectate standardele de calitate în efectuarea acestuia (5). Rezultatele noastre nu au inclus cazurile noi de TB diagnosticate prin criterii clinice şi radiologice şi neconfirmate bacteriologic deoarece managementul acestor cazuri este independent de confirmarea bacteriologică.

Din analiza statistică a datelor remarcăm o importantă creştere a rezistenţei la antibiotice, concordantă cu evoluţia ratelor la nivel mondial. În evaluările OMS, România a raportat pentru cazurile noi de TB în 2008 o rată de rezistenţă la HIN de 8,7%, la RMP 4,9%, la SM 0,7% iar la EMB 1,2%. Procentul cazurilor noi de TB MDR a avut o evoluţie ascendentă de la 2,8% în 2005 la 4,3% în 2008 (3). Remarcăm că în studiul nostru ratele de rezistenţă au valori superioare celor din literatură, cu excepţia celor pentru RMP şi MDR. Acest aspect se corelează cu lipsa complianţei pacienţilor la schema terapeutică datorată atât aspectelor socio-economice (accesul scăzut la îngrijiri de sănătate, educaţie sanitară precară, lipsa suportului familial), cât şi a celor legate de durata mare a tratamentului şi a multiplelor reacţii adverse.

Cunoaşterea acestor date este importantă, deoarece monorezistenţa prelungeşte durata tratamentului antituberculos până la 12 luni în cazul rezistenţei la HIN şi până la 18 luni în cazul rezistenţei la RMP. Monorezistenţa la HIN sau SM este calea de achiziţie a rezistenţelor adiţionale. Rezistenţa la RMP este considerată predictivă pentru TB MDR. Costul mediu al unui tratament individualizat pentru MDR TB este de aproape zece ori mai mare comparativ cu tratamentul tuberculozei chimiosensibile, fiind estimat la 9.000 - 20.000 RON, în funcţie de schema terapeutică şi de durată.

CONCLUZII

Cu toate că la nivel mondial se desfăşoară, prin intermediul OMS, programe de supraveghere şi control al tuberculozei, rata incidenţei globale a infecţiei rămâne cres-

cută (137 de cazuri la 100.000 de locuitori, 2009), ceea ce impune intensificarea eforturilor de depistare a cazurilor noi şi de optimizare a metodelor de diagnostic, tratament şi supraveghere. Creşterea ratei depistării cazurilor noi se poate realiza prin introducerea de noi metode de diagnostic, cu sensibilitate comparabilă, dar care să ofere rezultatele într-un timp mai scurt decât metodele folosite în practica uzuală (microscopia şi cultivarea), aşa cum ar fi metodele de biologie moleculară.

Un alt aspect important al managementului cazurilor de TB îl constituie tratamentul instituit în momentul diagnosticului, dificultatea cea mai importantă în acest demers, fiind reprezentată de rezistenţa la antibiotice. Creşterea ratelor de monorezistenţă remarcată în studiul nostru este predictivă pentru creşterea ratei de tulpini MDR, deşi în momentul actual aceasta nu constituie un pericol imediat pentru regiunea noastră.

Evitarea extinderii circulaţiei tulpinilor MDR, corelată semnificativ cu eşecul terapeutic şi creşterea incidenţei TB, poate fi realizată prin introducerea în practica uzuală a metodelor de identificare rapidă a MDR, cum ar fi identificarea genelor de rezistenţă în genomul bacterian.

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ROLUL VIDEOCAPSULEI ENDOSCOPICE ÎN BOALA CROHN SUSPECTATĂ

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THE ROLE OF CAPSULE ENDOSCOPY IN SUSPECTED CROHN'S DISEASE (Abstract): Capsule endoscopy (CE) revolutionized the small bowel examination. We emphasize the role of CE in suspected Crohn's disease, studying 24 investigations performed in the Institute of Gastroenterology and Hepatology. The global diagnostic yield was 54%, the specific diagnostic yield for Crohn's disease was 37,5%, the specificity was 100% and the retention rate was 4,1%. For maximum accuracy and efficiency, unitary administration and diagnostic criteria are needed, and for minimising the risk of complications, specific prior investigations would be suitable. **Key words:** SUSPECTED CROHN'S DISEASE, CAPSULE ENDOSCOPY

La ora actuală nu există un test unic, standard, de diagnostic pentru boala Crohn, acesta bazându-se pe o combinaţie de elemente clinice, biologice, endoscopice, radiologice şi anatomopatologice. Clasic, imagistica bolii Crohn a fost reprezentată de endoscopia convenţională şi metodele radiologice de examinare a intestinului subţire (1). Videocapsula endoscopică (VCE), dar şi apariţia enteroscopiei cu balonaş au revoluţionat imagistica intestinului subţire, aducând contribuţii majore în diagnosticul, clasificarea, decizia terapeutică sau aprecierea prognostică a bolilor inflamatorii intestinale (2).

Studiul de faţă îşi propune să analizeze rolul videocapsulei endoscopice în evaluarea pacienţilor cu suspiciune de boală Crohn.

MATERIAL ŞI METODĂ

Am analizat pacienţii investigaţi cu VCE pentru suspiciunea de boală Crohn în Institutul de Gastroenterologie şi Hepatologie Iaşi în perioada 2004-2010.

Am analizat datele demografice ale pacienţilor, durata de la debutul simptomelor, tabloul clinic, investigaţiile anterioare, rezultatele la examinarea cu VCE, complicaţiile acesteia, evoluţia ulterioară şi confirmarea diagnosticului pacienţilor pe parcursul a minimum şase luni de urmărire.

REZULTATE

În perioada studiată, dintre cele 164 de investigaţii cu VCE efectuate în Institutul de Gastroenterologie şi Hepatologie, suspiciunea de boală Crohn a constituit indicaţia de administrare a VCE în cazul a 24 de pacienţi.

Date demografice. Şaisprezece pacienţi (66,7%) au fost de sex masculin şi 8 pacienţi (33,3%) de sex feminin. Vârsta s-a încadrat între 19 şi 55 de ani, cu o vârstă medie de 36 +/- 12 ani. Repartizarea pe grupe de vârstă a fost următoarea: sub 20 de ani: 1 caz, între 20 - 30 ani: 7 cazuri, între 30 - 40 ani: 6 cazuri**,** între 40 - 50 ani: 7 cazuri, peste 50 ani: 3 cazuri.

Caracteristici clinice. Suspiciunea s-a bazat în primul rând pe tabloul clinic, caracterizat de: durere abdominală**,** diaree cronică**,** scădere ponderală sau status subnutriţional**.** În unele cazuri s-au adăugat simptome sau semne suplimentare, precum: leziuni perianale, sindrom subocluziv în antecedente, masă abdominală palpabilă, artralgii, febră.

Tablou biologic. Au fost urmăriţi următorii parametri: anemie, markerii inflamatori precum viteza de sedimentare a hematiilor (VSH), proteina C reactivă (CRP).

Istoricul bolii a măsurat o medie de 8 +/- 3 luni. De menţionat că doi pacienţi prezentau antecedente semnificative, de enterectomie în urmă cu şase, respectiv zece ani.

Investigaţii imagistice anterioare. Toţi cei 24 de pacienţi au efectuat anterior administrării VCE, colonoscopie, în cinci cazuri completată de ileoscopie, nediagnostice. Tranzit baritat de intestin subţire a fost efectuat la trei pacienţi, un pacient prezentând aspect inflamator combinat cu leziuni ulcerative, un pacient prezentând doar aspect de afectare inflamatorie, iar un pacient neavând leziuni. Tomografia computerizată abdominală a fost efectuată la şapte pacienţi (29,1%), descriind leziuni ridicând suspiciunea de boală Crohn de intestin subţire la şase pacienţi (25%).

Criterii de excludere. Nu au fost investigaţi cu VCE pacienţii care au prezentat la examinări imagistice anterioare aspecte de stenoză sau strictură a tractului digestiv sau care prezentau anamneză sugestivă de sindrom subocluziv. Totodată, pentru a elimina rezultatele fals pozitive, a fost efectuată anterior administrării VCE o anamneză amănunţită privind medicaţia AINS; nici unul dintre pacienţi nu a avut istoric de consum de AINS în luna premergătoare evaluării cu VCE.

Rezultatele investigaţiei cu VCE

Pacienţilor le-a fost administrată videocapsula M2A PillCam SB2 Given Imaging, Yoqneam, Israel. Pregătirea a constat în ingestia a 2 litri de polietilenglicol (PEG) în seara dinaintea examinării, cu post minimum 8 ore înaintea investigaţiei. Ingestia capsulei s-a produs fără incidente.

Rata de vizualizare a intestinului subţire. În 17 (70.8%) cazuri examinarea intestinului subţire a fost completă, în timp ce în 7 (29.2%) cazuri capsula nu a atins cecul după cele 8 ore de tranzit. Cauzele au fost: viteza de tranzit lentă în 6 (25%) cazuri şi existenţa unei stenoze ileale împiedicând progresia capsulei într-un caz (4,1%), în care s-a înregistrat şi retenţia capsulei cu intervenţie chirurgicală consecutivă.

Leziunile au fost împărţite în: **leziuni minore (**congestie izolată, eroziuni sau ulceraţii în număr mai mic de trei, limfangiectazii, angiodisplazii) care nu au permis un diagnostic specific şi l**eziuni majore (**minimum trei ulceraţii sau ulcere, stenoze ulcerate, fistule, cazurile cu mucoasă cu vili şterşi, serată şi cu leziune protruzivă, parazitoză), care au permis formularea unui diagnostic endoscopic specific, şi anume: suspiciune de boală Crohn necomplicată

(trei cazuri), boală Crohn complicată cu stenoze (patru cazuri), boală Crohn complicată cu fistulă (două cazuri), boală celiacă necomplicată (un caz), suspiciune de boală celiacă complicată (un caz), modificări inflamatorii probabil postoperatorii (post-enterectomie) într-un caz, parazitoză intestinală (un caz). Unsprezece pacienți nu au beneficiat de un diagnostic în urma efectuării videocapsulei. La aceştia, tranzitul intestinului subțire s-a încadrat în cele 8 ore de înregistrare.

Randamentul diagnostic al VCE. A fost formulat un diagnostic la 13 din cei 24 de pacienți, randamentul diagnostic global al VCE fiind de 54%. Nouă (37.5%) pacienți din totalul celor 24 au fost diagnosticați cu boală Crohn. Au mai fost descrise elemente patologice, dar fără semnificație diagnostică, la trei pacienți (congestie parcelară a mucoasei, limfangiectazii duodenale la un pacient, diverticuli la un pacient, angiodisplazii la un pacient). Nu au fost găsite leziuni la un număr de 9 pacienți.

Complicații. S-a înregistrat un caz (4,1%) de retenție a capsulei, la un pacient cu multiple ulcere etajate şi stenoze ulcerate. Retenția a antrenat un sindrom subocluziv care a necesitat intervenție chirurgicală.

Urmărirea pacienților a durat între 6 luni şi 7 ani. Diagnosticul de boală Crohn a fost confirmat la toți cei nouă pacienți, fie chirurgical, fie prin evoluția favorabilă la tratamentul medical specific (derivați de 5-ASA, imunomodulatori sau agenți biologici). Pacientul suspectat de boală celiacă complicată a fost diagnosticat în urma enteroscopiei cu biopsie cu limfom B, evoluând ulterior nefavorabil. Pacientul cu aspect sugestiv de boală celiacă necomplicată a efectuat serologie şi biopsie duodenală cu confirmarea diagnosticului, evoluând favorabil la regim fără gluten**.** Pacientul cu parazitoză intestinală a efectuat tratament antiparazitar, cu normalizarea hemoglobinei şi ameliorarea simptomatologiei digestive.

DISCUȚII

A fost analizat un grup de 24 de pacienți suspectați de boală Crohn, pe baza unor criterii clinice, biologice şi/sau imagistice, cărora li s-a administrat VCE de intestin subțire după excluderea contraindicațiilor. Şaisprezece pacienți (76,7%) îndeplineau, opt din 24 pacienți (33,3%) nu îndeplineau criteriile Conferinței Internaționale de Capsulă Endoscopică de suspiciune a bolii Crohn (un simptom digestiv plus încă cel puțin un element dintre următoarele: fie un simptom extradigestiv - febra, artralgii, eritem nodos/pioderma, colangita sau CSP, fie rezultat imagistic anormal, fie markeri inflamatori). Rata de diagnostic a fost semnificativ mai mare la pacienții cu criterii ICCE față de cei care nu le îndeplineau (75% versus 12,5%), asemeni altor studii (3,4). Aceasta susține utilitatea algoritmului în păstrarea raportului cost-eficiență.

Randamentul diagnostic global al videocapsulei a fost de 54%, iar randamentul în boala Crohn suspectată a fost de 37,5%, ceea ce încadrează seria noastră către limita inferioară a valorilor din literatură, încadrate între 33% şi 70% (5). Explicațiile pot fi: laxitatea criteriilor de administrare a videocapsulei (inclusiv la cerere) şi neincluderea în calcul a cazurilor în care s-au descris leziuni certe, dar care nu explicau simptomatologia (limfangiectazii, polipi duodenal, angiodisplazie). Includerea acestora ar fi însumat 17 cazuri cu leziuni diagnostice, corespunzând unui randament

mai mare, de 70.8%. La 11 (45,8%) din pacienții explorați, videocapsula fie a evidențiat leziuni minore, fie nu a descris nici o leziune. Evoluția clinică ulterioară a fost favorabilă sub tratament simptomatic sau specific pentru tulburări funcționale intestinale. Astfel, acest procent nu trebuie privit ca risipă de resurse, rezultatul negativ evitând costuri exploratorii ulterioare.

Videocapsula este o tehnică pur vizuală; nu există posibilitatea unor manevre externe care să ajute vizualizarea sau a prelevării de biopsii sau efectuării de acte terapeutice. Acestea ar putea constitui factori de influență negativă asupra randamentului diagnostic şi asupra specificității metodei.

Specificitatea VCE a fost de 100%, la toți pacienții suspectați în urma investigației cu VCE cu boală Crohn fiind confirmat ulterior diagnosticul, prin chirurgie şi examen histopatologic (trei pacienți) sau prin evoluția clinică (şase pacienți). Nu există un standard diagnostic pentru boala Crohn. Mow (6) a propus un minim de 3 eroziuni sau ulcerații, iar Voderholzer (7) minimum zece leziuni aftoide. În prezent, cea mai largă utilizare are propunerea lui Mow, adoptată şi în studiul de față. Leziuni minore precum edemul vililor, eritem parcelar, hiperplazie limfoidă, limfangiectazii, deşi considerate în unele studii ca manifestări precoce ale bolii Crohn, nu au fost considerate diagnostice. Specificitatea maximă se poate explica prin rigurozitatea în interpretarea leziunilor evidențiate şi formularea diagnosticului de boală Crohn pe bază vizuală.

Pacienții cu diagnosticul confirmat clinic au fost urmăriți o perioadă suficientă de timp (minimum 6 luni). Astfel, a fost evitat riscul diagnosticelor fals pozitive.

În studiul nostru, 29% din examinări au fost incomplete, dar toate s-au înregistrat între pacienții cu leziuni, putând atrage atenția asupra unor factori de influență negativă asupra vitezei de progresie a videocapsulei precum stenozele, cu acumulare de lichid la nivel suprajacent.

Complicațiile au fost reprezentate de un caz (4,16%) de retenție a capsulei, care a necesitat intervenție chirurgicală, în a zecea zi post-ingestie. Frecvența retenției depinde de populația studiată (8), prin indicația de administrare a videocapsulei şi selecția pacienților, variind între 0 şi 21%, cu incidență globală de 1-2% (9-11). Rata de retenție din studiul nostru se corelează celei din boala Crohn suspectată, care variază între 0 şi 5% (11) Clasic, boala Crohn suspectată constituia o contraindicație pentru videocapsulă, prin potențialul de impactare. Recent multiple studii susțin utilitatea videocapsulei şi în suspiciunea de boală Crohn (12-14), păstrând prudența prin efectuarea anterioară de investigații care să excludă obstrucția.

CONCLUZII

Randamentul diagnostic al videocapsulei de intestin subțire administrată în cazurile suspectate de boala Crohn intestinală a fost de 37.5%. A fost înregistrată o rată a complicațiilor de 4.16%. Pentru un diagnostic de acuratețe maximă, este nevoie de stabilirea unor criterii unitare şi bine definite. Pentru a creşte randamentul diagnostic şi cost-eficiența, este nevoie de aplicarea unor criterii de selecție mai riguroase, iar pentru a minimaliza rata complicațiilor, se impune prudență în administrarea videocapsulei in prezența suspiciunii de stenoză intestinală, cu efectuare prealabilă de alte investigații noninvazive care să excludă obstrucția.

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PULMONARY REHABILITATION IN COPD PATIENTS. OBSERVATIONAL STUDY

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PULMONARY REHABILITATION IN COPD PATIENTS-OBSERVATIONAL STUDY **(**Abstract): The **aim** was to analyze the effects of an 8-week pulmonary rehabilitation (PR) program on quality of life (QoL), physical performance (6MWD value - six-minute walk test) and pulmonary function in patients with mild and moderate COPD. **Material and methods:** 23 subjects with COPD with FEV1≥50% were assigned to a clinic treatment group (n=12) or a home exercise group (n=11). Subjects in the first group received supervised exercise program over a 6-week period after 2 weeks inpatient PR program. Subjects in the second group received the same exercise program initially in hospital, followed by 6 weeks of home exercises, reinforced by a clinic visit at 2 weeks. Measured outcomes were the 6MWD, the generic QoL scale St. George's Respiratory Questionnaire Disease (SCRQ) and statistical analysis by ANOVA test. **Results:** Both groups showed clinically and statistically significant improvements in 6MWD and SCRQ scores at 8 weeks; improvements were still evident in both groups at 3 months. By 8 weeks, SGRQ scores had improved in both groups with 28% and 24%; 6 MWD had improved with 13% and 5%, and important differences were observed in the mean changes of 6MWD: 56.42 m in the first group, 17.7 m in the second group. At 3 months, the results of both groups were substantially and about equally improved over baseline measurements. **Conclusions:** Subjects in the first group were more satisfied with the overall outcome of their rehabilitation treatment compared with subjects in the second group. **Keywords:** PULMONARY REHABILITATION, COPD, QUALITY OF LIFE, PHYSICAL PERFORMANCE, PULMONARY FUNCTION.

Chronic Obstructive Pulmonary Disease (COPD) is "a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases" (1). COPD patients must face limitations in daily activities and reduced quality of life (QoL) caused by dyspnea, airflow limitation, skeletal muscle dysfunction and comorbidities (1, 2, 3, 4, 5). The major

goals of stable COPD management are to reduce symptoms, increase participation in daily living activities and improve health-related quality of life (HRQoL) (1, 2, 3, 6). The pharmacological treatment of COPD is main target is to reduce the degree of airflow limitation and there is evidence that FEV1 has a relatively poor correlation with symptoms, HRQoL and daily functioning (7, 8, 9).

American Thoracic Society (ATS) and European Respiratory Society (ERS) recommendations for the management of COPD are to use of pulmonary rehabilitation programs (PRPs) for all groups of risk of the illness (A, B, C, D), especially self-management techniques to improve exercise tolerance and HRQoL, and to reduce symptoms like dyspnea and fatigue (1, 2, 10, 11).

PRPs have been shown not only to improve exercise capacity and health status and to reduce dyspnea and but also to diminish the use of healthcare resources (2, 6, 12-18).

We carried out a comparative study single blind to analyze the effects of an 8 weeks rehabilitation program on dyspnea, HRQoL, physical performance and pulmonary function in patients with mild and moderate COPD (19, 20).

The aim of the study was to evaluate functional capacity and health status at 8 weeks of physical treatment and at 3 months after conclusion of PRP in patients regularly followed in respiratory health services.

MATERIAL AND METHODS

All patients with a confirmed diagnosis of COPD with FEV1>50% admitted to a PRP at Physical Medicine and Rehabilitation Department, Hospital No. 2, Craiova, between January 2018 and May 2018 were recruited (tab. I). The diagnosis of COPD was made according to GOLD definition and classification (1, 7, 9). All patients received pharmaceutical treatment according with COPD GOLD stage. The respiratory specialist oversaw confirming the diagnoses, treatment and verifying exclusion criteria.

PRP of mild/moderate COPD patients included: the patient selection and initial evaluation, the goal developing, the ensuring the smooth running of the program, the evaluation of patient outcomes, the performing of long/term follow-up.

On admission, patients were excluded from this study if they had a diagnosis of other pulmonary disease, with unstable cardiac conditions, neuromotor limitation or COPD patients with a recent exacerbation (in the preceding 4 weeks).

TABLE I

The main characteristics of the study patients

	Study group 1 (G1)	**Study group 2 (G2)**
No of patients	12	11
Gender	10 M / 2 W	8 M / 3 W
Mean age	46 (±8) years old	48 (±7) years old
Own place	7 V, 5 T	6 V, 5 T
Smokers	13	10
Tobacco index (mean)	23 PA	26 PA
Onset of COPD (mean)	3.2 years	4.4 years
FEV 1 initial	65.5 %	67.82 %
W = women, M = men, V = village, T = town		

Study design

The study design as a flow diagram: pre-to-post comparisons were made in those patients (fig. 1).

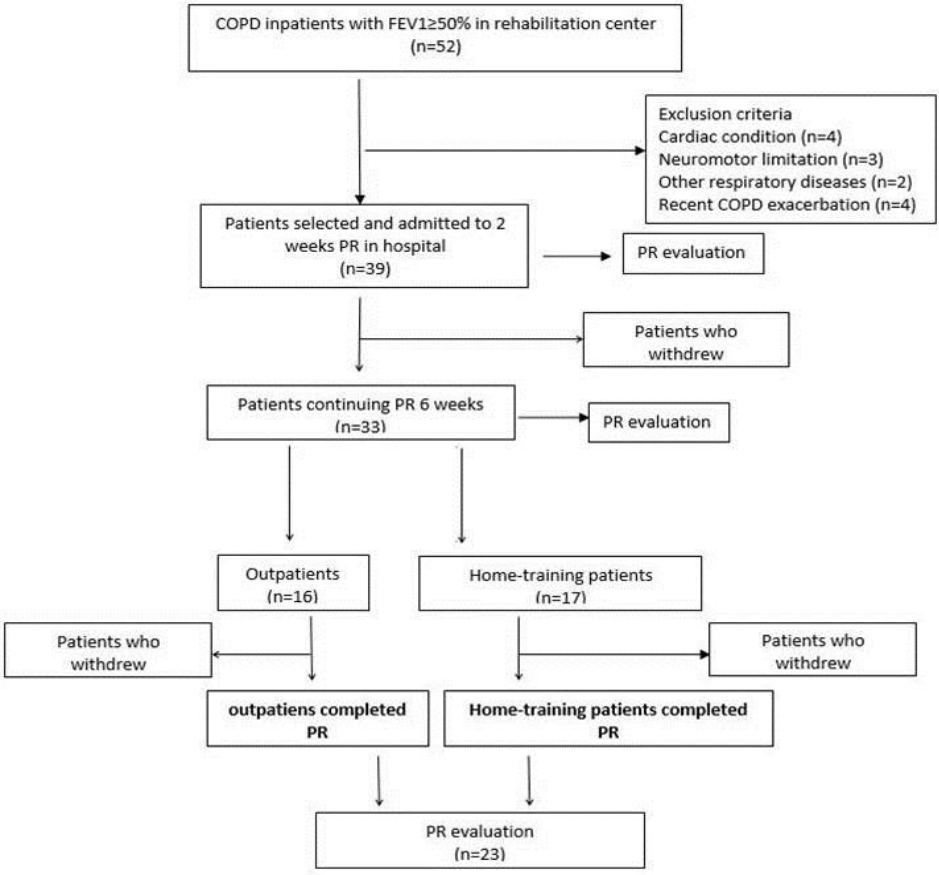

Fig. 1. A flow diagram of study design

The patients with mild and moderate COPD were begun 8 weeks of PRP in the hospital. Sessions were supervised by a rehabilitation therapist. According with patient's preference and to the probability that patient maintain the exercise at home, patient was randomized in two study groups. Both groups started PRP in hospital for 2 weeks. After, the study group (G1) - received supervised exercise in hospital (outpatient PRP). The second group of patients (G2) received the same exercise program initially - in hospital, followed by 6 weeks of PRP at home residence, reinforced by a clinic visit at 2 weeks.

We performed a complete history, physical examination, chest radiography, laboratory and pulmonary function data. The studied parameters were:

- dyspnea - using Medical Research Council (MRC) scale (16);
- pulmonary lung function: FEV1 and FEV1/FVC % using a spirometer (Vitalograph) connected to a computer, for a data analysis (1,8,9);
- exercise capacity: six-minute walking distance (6 MWD) according to the ATS Guidelines (17);

- QoL using St. George's Respiratory Questionnaire Disease (SGRQ) - a standardized, self-administered questionnaire for measuring impaired health and perceived HRQL in airways disease (21, 22).

All evaluations were performed by the pulmonary physician, without knowing the location of the PRP. Patients were able to perform the inpatient (2 weeks) and outpatient (6 weeks) RPPs. The size of group at any session was limited to 5 patients.

The rehabilitation methods we used were: monitoring pharmacotherapy, education 15 minutes daily sessions (breathing retraining, energy conservation, nutritional and medication education, smoking cessation, teaching the patients how to cope with feelings of dyspnea), physical training and educational and psychological management. The second part was devoted to physical training, preceded by Jacobson's technique, for twice-daily, 3 days a week, for eight weeks, 30-minute sessions, at 75 - 80% of the load achieved on the incremental pre-exercise test carried out at enrollment, and consisted of:

- respiratory muscle training - inspiring against a closed glottis, pursed lips breathing, expiratory abdominal augmentation and synchronization of thoracic and abdominal movements;
- modified proprioceptive neuromuscular training (four periods with 5 minutes of repetitive large muscle dynamic exercise separated by 1 minute with mild exercise), including stretching as part of the warm-up period and of the cool-down, respectively;
- complete lower extremity aerobic training on a cycle ergometer (Aerobic 2100) / the morning session; training workload was assessed daily in the inpatients and weekly in the outpatients.

After 2 weeks of inpatients PRP, the patient of G1 continued physical therapy in hospital 3 days a week like outpatient PRP. The patient of G2 continued PRP at home.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy Craiova and was carried out in concordance with the World Medical Association Declaration of Helsinki (2000). We have obtained informed consent from our patients for the treatment and for their medical data to be used in a study.

Statistical analysis

Data are presented as mean ± standard deviation for continuous variable and percentages for categorical variables. The difference between means was determined using Student's t-test. Differences parameter values between groups were tested by ANOVA test. A p-value <0.05 was considered statistically significant. The statistical analysis was performed using *SPSS 8.0* for Windows; SPSS Inc., Chicago, IL, SUA and applied according to the current methodology.

RESULTS

After 8 weeks of PR all subjects reduce dyspnea - we obtained a fall in breathlessness mean score, had a significant, albeit small, improvement in mean FEV1, extended exercise endurance (6 MWD increased significantly), enhanced HRQoL (we considered 4 points as minimal clinically important difference as stated by Paul Jones), reduced use of pharmacological agents (tab. II).

For G1 we did not identify any significant difference between the mean values for FEV1 measured at three points (p ANOVA = 0.759> 0.050). Spirometry showed at all patients, especially in younger, small increases in FEV1 after 8-week

inpatient PR. At 3-month FEV1 increased at 67.83 %. For G2 we did not identify any significant difference between the mean FEV1values at three points (p ANOVA=0,913>0,050); spirometry showed small increases in FEV1 after 8-week PR (FEV1=69.05%) compared to initial (FEV1=68.8%). For FEV1 we have identified no statistically significant difference between G1 and G2 (tab. II).

TABLE II

Results of PRP in the two-study group

Parameter	Study group	Baseline				After 8-week rehabilitation				After 3 months			
		Mean	Std.dev.	CI.95%	p	Mean	Std.dev.	CI.95%	p	Mean	Std.dev.	CI.95%	p
FEV1 % predicted	G1=12	65.50	7.62	60.66-70.34	0.466	66.58	7.90	61.56-71.60	0.447	67.83	7.47	63.09-72.58	0.766
	G2=11	67.82	7.32	62.90-72.73		69.09	7.58	64.00-74.18		68.73	6.68	64.24-73.21	
MRC scale score	G1=12	1.67	0.89	1.10-2.23	0.10	0.92	0.79	0.41-1.42	0.103	0.92	0.79	0.41-1.42	0.360
	G2=11	0.73	0.68	0.27-1.19		0.45	0.44	0.16-0.75		0.64	0.62	0.22-0.75	
Well-being (SGRQ total score)	G1=12	31.50	6.50	27.37-35.63	0.376	22.58	7.28	17.96-27.21	0.636	24.00	6.94	19.59-28.41	0.428
	G2=11	28.36	9.91	21.70-35.02		20.91	9.39	14.60-27.21		26.36	7.07	21.61-31.12	
6 MWD (m)	G1=12	434.08	30.83	414.50-453.67	0.253	490.50	43.26	463.01-517.99	0.012	500.25	45.91	471.08-529.42	0.003
	G2=11	407.82	70.44	360.50-455.14		425.55	68.82	379.31-471.78		416.09	71.16	368.29-463.90	

For G1, we obtained a fall in breathlessness mean score (MCR scale) with 45% of the initial score after 8 weeks; we identified a significant difference at the boundary between the MRC mean values to three times (p ANOVA = 0.049 <0.050). In the G2, MCR mean score decreased with 38% after 8 weeks, but at 3 months was decreased only with 12%, without significant difference between the mean values (p ANOVA = 0.553> 0.050). We have identified a statistically significant difference only for the initial investigation (p = 0.010 Student <0.050), between G1 and G2.

For G1, we identified a significant difference between the mean values measured for SGRQ at three points (p ANOVA = 0.007<0.050). At the end of PRP there was an improvement in health status in both groups. At 8 weeks in G1, mean SGRQ score increased with 28% and with 24% in 3 months after PRP compared by baseline (tab. II). On the contrary, in G2, if at 8 weeks SGRQ score was better that before PRP with 26%, there were changes in total SGRQ score, with a tendency to get worst at 3 months after PRP, when the mean SGRQ score was increased only with 7%

compared by baseline (tab. II). Comparing the results between G1 and G2 we have not identified at any of the three points, any statistically significant difference.

After PRP, in the G1, mean 6MWD was clinically and statistically significant increased at 8 weeks (+13%) (tab. II). We identified a significant difference between the mean 6MWD values at three points for G1 (p ANOVA = 0.001<0.050). In G2, mean 6MWD was increased at 8 weeks (+17.7m =5%) compared with initial values. After completing PRP inpatient, 6MWD was increased only with 2% compared with baseline (tab. II). For G2 did not identify any significant difference at three points (p ANOVA = 0.840> 0.050). Comparing the results between the two groups we have not identified at baseline, a statistically significant difference, but the investigation of the second and the final difference between the two groups became statistically significant difference (the end p Student = 0.003, the intermediate p Student = 0.012).

Although all parameters studied had a favorable evolution, when we performed linear regression equation for the mean initial values and intermediary, respectively final values of the studied parameters, we found that initial value are predictive for both intermediary and final values only in G1 (fig. 2, 3, 4).

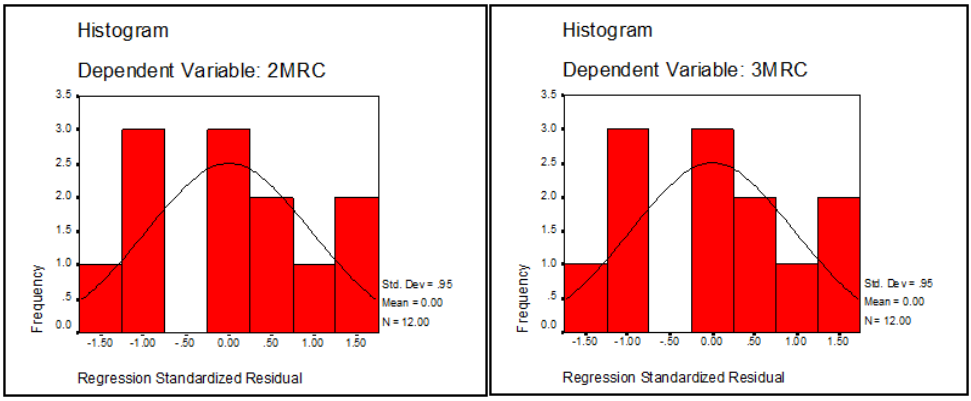

Fig. 2. The frequency graphs of linear regression equations for MRC

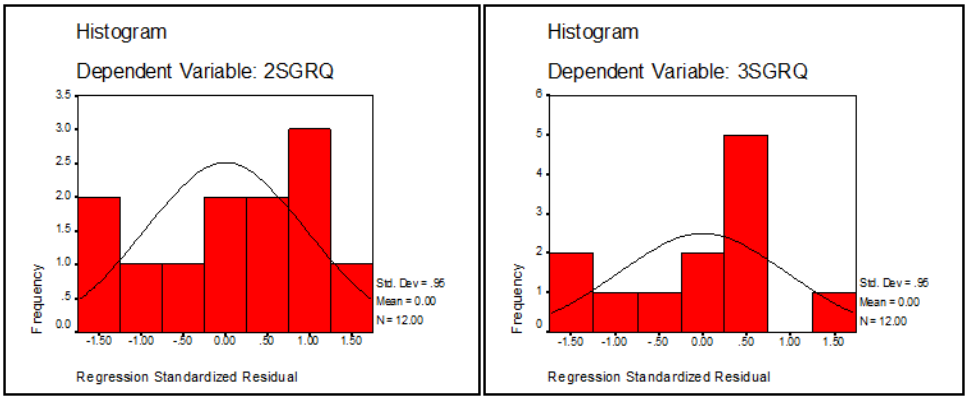

Fig. 3. The frequency graphs of linear regression equations for SGRQ

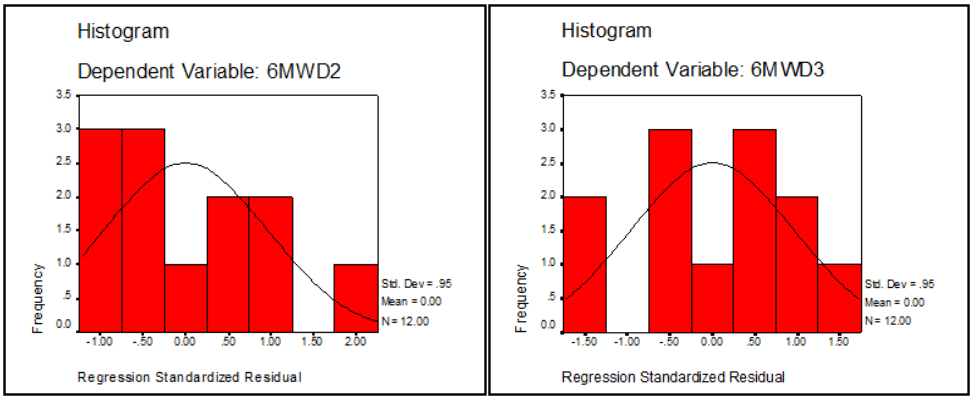

Fig. 4. The frequency graphs of linear regression equations for 6MWD

DISCUSSION

The benefits of the PRP in the management of patients with chronic respiratory disease already known and well-established (1, 2, 3, 6-12, 14, 15), were also observed in this study. The studies published so far in the literature show the positive effects rehabilitation has in patients with moderate to severe (23, 24), very severe COPD (25) or comorbidities (26).

The present study highlights the benefits of PR in patients diagnosed with COPD with FEV1> 50% without comorbidities, rehabilitation initially performed under hospital conditions, then at home. In both groups, after PRP, patients showed improvement in health status and but not in functional capacity.

Studied patients have carried out a PRP during two weeks in hospital treatment, followed by six weeks at home (G2) or hospital (G1). 2017 GOLD appreciates the minimum duration of an effective PRP is at least 6 weeks, the effectiveness increases with increasing duration (1). Our program duration was greater than the minimum recommended two weeks of GOLD 2014 and the British Thoracic Society (6 weeks) (27). Compared with other studies (24, 25), the duration of the program is average, and there are programs for 4 weeks or three years (23), in moderate-severe COPD (24), and 26 weeks for patients with severe ventilatory dysfunction (25). Other studies evidenced that longer programs seem to enhance long-term effects (23, 28).

Initiation of the program was carried out under hospital conditions, which is considered necessary given the fact that patients have never carried out such a program and requiring the careful monitoring of respiratory function during exercise. Subsequent completion (out-patient and home-training) was done to capture the effects of PR in the two situations. We mention that carrying home-training program was accepted in patients who did not have easy access to the out-patient services. In the literature we didn't find any studies we could refer to, the more often patients performing an outpatient PRP (24, 25, 29).

The limited number of patients compared to other studies (25, 30, 31), is explained by the inclusion only patients with FEV1>50%, without comorbidities and poor adherence for this form of non-pharmacological treatment. We do not have an explanation for the poor adherence of G2 to the PRP, as both groups were similar in terms of baseline clinical and demo-

graphic characteristics and similar PRP benefits in both groups (tab. I). Translation of the acquired skills to patient's lifestyle may be prevented by different barriers: lack of self-confident, cognitive impairment and behavioral issues, lack of motivation and the presence of physical barriers at home or in the community (25, 32).

The G2 poor adherence to follow-up testing could be explained by the fact they were also less compliant to exercise. A possible explanation for the worst long-term results with patients that did cycling during PRP, could be the difficult maintaining this exercise regularly at home and consequently they became less active.

We mention that most patients with COPD have a respiratory function with a severe or very severe reduction (FEV1 < 50%), highlighting the absence or ineffectiveness of programs for early detection of smoking-induced pathology or respiratory hazards. Most patients included in the study came from rural areas.

Unlike other studies in which correlations are made between respiratory function parameters (29, 30, 31), in our study we followed the recovery program effects on pulmonary function, exercise capacity (6MWD), dyspnea (MRC) and quality of life (SGRQ) after 6 weeks of rehabilitation and three months after the end of the program. In our study we didn't set out to achieve a target intensity of 80% of peak work rate but strived that in the first 2 weeks of the rehabilitation program, patients would learn a correct respiratory pattern (4 stage), with physical effort during exhale.

For FEV1 parameter we cannot appreciate an improvement percentage, given the irreversible character of the lesions and respiratory function degradation in COPD, regardless of the therapeutic method applied.

6MWD values were in the concordance with the literature (29, 31). The improvement of 6MWD after the rehabilitation was higher compared with other published results (29,31). This is because our patients perform daily physical exercises; most of them come from rural areas. Also, our patients were complaining to proprioceptive training.

Like other literature data (33) our results confirmed that regular aerobic exercise is associated with minimal increase in overall health status and walking self-efficacy, expressed through 6 MWD, as well as a reducing progression of dyspnea, especially during activities of daily living effort.

After 12 weeks the results of PR program are decreasing, similar another publication (34), perhaps due to limiting interest for constant and daily proprioception training. As limitations of this study we can consider the small number of patients, the short duration of follow-up phase, compared with other studies (35, 36) and lack of comorbidities. Poor adherence has a detrimental effect on morbidity, mortality and health care resources (37).

In appropriate future, PRP in Romania should become a current medical option in all disabled patients, so the mentioned limits will be eliminated.

CONCLUSIONS

The results of the present study show that the proprioception is fundamental for sensory/motor control over thorax joint stability, so our proprioceptive neuromuscular training offers optimal control of respiratory movements and is essential in patients with COPD.

Combining in-patient / out-patient or in-

patient / home-training is an optimal way in which the patient with COPD with FEV1>50% improve their exercise capacity and Qol.

Based on the results of this study, there is a need for more research on this subject. We consider that chest stretching and adapted neuromuscular training represent two important educational needs of an individual COPD patient management.

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CONSIDERAŢII ASUPRA ETIOLOGIEI INSUFICIENŢEI RENALE ACUTE LA COPIL (STUDIU PE 110 CAZURI)

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CONSIDERATIONS BASED ON THE ETIOLOGY OF ACUTE RENAL FAILURE IN CHILDREN (STUDY ON 110 CASES) (Abstract) : The Article highlights the fact that without a homogeneous definition of acute renal injuries of adults and infants, for the last 5 years a new classification system has appeared. The RIFLE pediatric criteria are looked over, validated in 2007 which allow a better IRA classification at infants and allow comparisons between the intensive care units and also allow assumptions on the predictions of the disease. The list of certain causes of IRA at infants is rendered, the genetic background of children with IRA risk is highlighted, the necessity of reevaluating and criteria of evaluating renal functions is underlined. The etiology of renal failure was studied on 110 infants interned on a period of 5 years (2003- 2008) in 2 centers with different profile : The Clinic Of Pediatric Nephrology "Sf. Maria" Iaşi and the Intensive Care Compartment of The Pediatric Hospital "Sf. Ioan" Galaţi.
Keywords : ACUTE KIDNEY INJURY, CHILD, ACUTE RENAL FAILURE

Insuficienţa renală acută, cunoscută în prezent ca leziune renală acută, rămâne o problemă semnificativă în pediatrie, prin evoluţia sa, de multe ori devastatoare.

Incidenţa acestei afecţiuni este în creştere şi etiologia sa în ultima decadă s-a schimbat prin predominanţa bolilor renale de cauza multifactorială, în general la copiii polispitalizaţi (1, 2, 6). Există factori genetici care predispun anumiţi copii la leziune renală (polimorfismul genei enzimei de conversie, polimorfismul genei factorului de necroză tumorală, a interleukinei-1, 6, 10, variaţiile genetice ale proteinei de şoc termic 72) (2).

Este în general acceptat că leziunea renală acută este caracterizată prin insuficienţa rinichiului de a regla homeostazia adecvată a fluidelor, electroliţilor şi acido-bazică, prin creşterea producţiei de azot în asociere cu creşterea creatininei serice (la valori mai mari cu 50% faţă de nivelul bazal) şi, în multe cazuri, cu reducerea concomitentă a diurezei.

În literatură există peste 30 de definiţii, multe dintre ele bazate pe nivelul creatininei serice (2,3,4). Concentraţia creatininei serice poate avea variaţii mari, în funcţie de vârstă, sex, masă musculară, metabolismul muscular şi starea de deshidratare (3,5,7). Ea poate să nu fie crescută până când aproximativ 50% dintre nefroni au fost pierduţi.

La pacienţii cu leziune renală acută, schimbările precoce ale funcţiei renale, manifestate prin creşteri mici ale creatininei serice ce reflectă o leziune renală semnificativă şi

TABELUL I
Criteriile pediatrice RIFLE

Criteriul	Estimarea Cl. la creatinina	Diureza
Risk	Cl.creat ↓ cu 25 %	< 0.5 mL/ Kg/h pentru 8 h
Injury	Cl.creat ↓ cu 50 %	< 0.5 mL/ Kg/h pentru 16 h
Failure	Cl.creat ↓ cu 75 % Cl.creat < 35 mL/min/1.73m^2	< 0.3 mL/ Kg/h pentru 24 h sau anurie pentru 12h
Loss	Insuficienţă persistentă > 4 săpt	
End stage	Insuficienţă persistentă > 3 luni	

care se asociază cu o înaltă morbiditate şi mortalitate. Creatinina serică nu este un indicator fidel al funcţiei renale şi din cauza altor comorbidităţi la pacienţii trataţi precum şi datorită creşterii sale tardive, ceea ce face aproape imposibilă stabilirea debutului insuficienţei renale acute (2, 5, 10, 11). Întârzierea diagnosticului determină întârzierea terapiei. Este ştiut faptul că restabilirea fluxului sanguin renal previne instalarea leziunilor renale ireversibile şi, deci, în multe cazuri diagnosticul precoce poate schimba cursul bolii.

Lipsa unei definiţii uniforme si multidimensionale (2, 8, 10, 11) a făcut ca insuficienţa să fie recunoscută ca o leziune renală semnificativă iar întârzierea tratamentului şi incapacitatea de a generaliza rezultatele unui singur studiu să ducă la necesitatea unei clasificări sistematice.

Noul sistem de clasificare (4, 15) intitulat criteriile RIFLE (R - risc pentru disfuncţie renală ; I - injurie, leziune renală ; F - failure, insuficienţa funcţiei renale ; L - loss, pierderea funcţiei renale ; E - end stage kidney disease, stadiul final al bolii renale) a fost propus pentru standardizarea clasificării insuficienţei renale acute la adulţi, în 2004 de grupul ADQI (Acute Dyalisis Quality Initiative).

Criteriile RIFLE au arătat, prin studii multinaţionale şi multicentrice, predicţia indiferent de durata internării, cost, morbiditate şi mortalitate (2, 4).

Criteriile Pediatric RIFLE, folosite pentru clasificarea mai bună a insuficienţei renale acute reflectă cursul leziunii renale la copiii admişi în unităţile de terapie intensivă (4, 6).

Criteriile RIFLE pediatrice au apărut pentru o mai bună caracterizare a leziunii renale şi au fost validate la copii, permiţând comparaţii între centre. Criteriile RIFLE au fost utilizate de AKIN, un grup de nefrologi pentru adulţi, nefrologi pediatri, reanimatori şi terapeuţi organizaţi într-o societate interesată de cercetări în domeniul insuficienţei renale, accesul fiind facilitat internaţional, interdisciplinar şi prin colaborare între societăţi. În 2007 s-a modificat scorul RIFLE pediatric, introducându-se predicţii ale creşterii costurilor, creşterii duratei de spitalizare, mortalităţii şi necesitaţii terapiei de substituţie renală la pacienţii pediatrici din unităţile de terapie intensivă. Stabilirea criteriilor p-RIFLE implică studii prospective multicentrice, similare cu ale grupului registrului de prospectivă pediatrică pentru terapia de substituţie renală (7,8).

Folosirea formulei lui Schwartz pentru estimarea leziunii renale acute este mai adecvată pentru pacienţi peste 30 de ani. A fost stabilită nouă ecuaţie Schwartz pentru determinarea GFR la copii (=0,4 x T/ creatinină serică).

În prezent este necesară revizuirea ghidurilor şi criteriilor pentru evoluţia funcţiei renale, inclusiv Pediatric RIFLE.

Este nevoie urgent de utilizarea unor

TABELUL II
Etiologia leziunii renale acute
(după Sharon Phillips Andreoli. *Pediatric Nephrol*. 2009, 24, 253-263)

Tipul	Etiologie
Leziunea prerenală	Scăderea volumului intravascular "adevărat" Scăderea efectiva a volumului intravascular
Boli renale intrinseci	Necroza tubulara acuta (nefropatia vasomotorie) Agresiunea hipoxic-ischemică Indusa de droguri Mediata de toxine - toxine endogene: hemoglobina, mioglobina - toxine exogene: etilenglicol, metanol Nefropatia urică şi sindrom de liza tumorala Nefrita interstițială: - indusă de droguri - idiopatică Leziuni vasculare: - tromboza arterei renale - tromboza venei renale - necroza corticala - SHU Hipoplazia ± displazie - idiopatică - expunerea la droguri nefrotoxice
Uropatiile obstructive	Obstrucție pe rinichi unic Obstrucție bilaterală ureterală Obstrucție uretrală

noi marcheri sensibili si specifici (9) pentru leziunea renală acută: NGAL, molecula 1 de leziune renală (kim 1), interleukina 18, cystatin C. Aceştia apar la scurt timp după producerea leziunii şi este posibil ca valorile lor să fie folosite în timpul leziunii iniţiale şi în perioada de evoluţie a insuficienţei renale. Bazându-ne pe diferenţierea expresiei biomarcherilor este posibil ca aceştia să facă distincţia între variate tipuri şi etiologii ale insuficienţei renale acute.

Apariţia biomarcherilor precoce de leziune renală şi noua clasificare sunt necesare pentru iniţierea strategiei terapeutice de succes în insuficienţa renală.(2, 9)

În ultima decadă s-a schimbat atât epidemiologia insuficienţei renale acute, cât şi accesul la tratament, au apărut tehnologii rafinate şi echipament de monitorizare complex, precum şi modificări în structura etnică a populaţiei (12,13).

Cele mai frecvente cauze sunt enumerate în tabelul următor (2).

MATERIAL ŞI METODĂ

S-a urmărit structura etiologică a leziunii renale acute la 110 pacienţi de vârsta pediatrică internaţi pe o perioada de 5 ani în două spitale cu profil diferit:

- Clinica de Nefrologie Pediatrică Sf. Maria, Iaşi – lotul A, 51 de cazuri;

TABELUL III
Etiologia insuficienţei renale pe cele 110 cazuri

Etiologie	Nr. cazuri	Procente
Ischemie prerenală	44	40
Suferința organică multiplă	19	17
SHU	12	11
Intoxicații	11	10
Uropatii obstructive	7	6
Rinichi polichistic	2	1.8
Boli renale primitive	5	4.5
Litiaza	3	2.7
Şoc hemoragic	5	4.5
Rabdomioliză	1	0.9
Limfom malign	2	1.7

TABELUL IV
Diagnosticul conform criteriilor p-RIFLE

Nr. crt.	Criteriul	Număr de cazuri	Procente %
1.	R	38	35
2.	I	31	28
3.	F	41	37

- Compartimentul de Terapie Intensivă Pediatrică a Spitalului "Sf. Ioan", Galaţi – lotul B, 59 de cazuri.

Copiii au fost diagnosticaţi conform criteriilor RIFLE pediatrice, determinându-se Cl. la creatinina după formula lui Schwartz modificată. În 62 de cazuri a fost greu sau imposibil să se demonstreze oliguria sau anuria (diureza <500 ml la copii mari sau <1 ml/kg/h la copii mici si sugari), astfel că în numai 48 de cazuri a fost posibilă monitorizarea diurezei. O motivaţie ar fi dificultatea de recoltare a urinei la grupa mică de vârstă, lipsa unor dispozitive colectoare, evoluţia rapidă şi diagnosticul tardiv al bolii.

Anamneza, examenul fizic şi examenele de laborator (inclusiv examenul de urină şi examenele imagistice) au stabilit etiologia insuficienţei renale.

REZULTATE

Se observă predominanţa cauzelor prerenale, urmate de insuficienţa organică multiplă şi sindromul hemolitic uremic.

Grupul intoxicaţilor a cuprins: etilenglicol 1 caz, algocalmin 2 cazuri, paracetamol 1 caz, ciprofloxacin 1 caz, aspirina 1 caz, ciuperci 1 caz, substanţe detoxifiante 1 caz, nitriţi 1 caz.

Se observă la acest lot că predomină copiii în stările critice, în special cei cu insuficienţă organică multiplă, SHU şi intoxicaţiile. Insuficienţa organică multiplă s-a complicat în 9 cazuri cu insuficienţă renală acută şi în trei cazuri insuficienţa renală acută a evoluat spre insuficienţă organică multiplă.

În al doilea lot cea mai obişnuită cauza de leziune renală acută în copilărie este depleţia volemică şi aceasta poate fi prevenită, prin diagnostic şi tratament precoce.

Am observat asocierea cu un mare număr de comorbidităţi, mai ales la grupa a doua, în cazul sindroamelor de deshidratare.

Din totalul de 110 cazuri, la 25 de cazuri etiologia leziunii renale acute a fost multifactorială, agresiunea hipoxic-ischemică şi nefrotoxicitatea având o contribuţie importantă.

TABELUL V
Etiologia in lotul A

Etiologie	Nr. cazuri	Procente
Ischemie prerenală	9	8.1
Suferința organica multiplă	12	10.9
SHU	11	10
Intoxicații	9	8.1
Uropatii obstructive	4	3.6
Rinichi polichistic	1	0.9
Boli renale primitive	3	2.7
Litiază	1	0.9
Şoc hemoragic	2	1.7

TABELUL VI
Etiologia in lotul B

Etiologie	Nr. cazuri	Procente
Ischemie prerenală	35	31
Suferința organică multiplă	7	6.3
SHU	1	0.9
Intoxicații	2	1.7
Uropatii obstructive	3	2.7
Rinichi polichistic	1	0.9
Boli renale primitive	2	1.7
Litiază	2	1.7
Şoc hemoragic	3	2.7
Rabdomioliză	1	0.9
Limfom malign	2	1.7

Am constatat următoarele comorbidităţi :

- Sindrom Down 3 cazuri,
- Hidranencefalie 1 caz,
- Mielingomielocel 2 cazuri,
- Hidrocefalie 2 cazuri,
- Keilognatopalatoschizis 2 cazuri,
- Malformaţii cardiace 5 cazuri,
- Paralizie cerebrala infantila 3 cazuri,
- Microcefalie 2 cazuri,
- Mucoviscidoza 1 caz.

În ceea ce priveşte evoluţia 20 de cazuri au decedat şi la 15 cazuri evoluţia a fost spre boală renală cronică. Mortalitatea a fost mai mare în lotul B (16 cazuri), dar decesul s-a produs de obicei prin altă cauză decât cea renală. Ştiut fiind că acei copii care au suferit o leziune renală au riscul mai târziu de a dezvolta o boală renală cronică, toţi pacienţii din loturile noastre au fost chemaţi la control clinic, al tensiunii arteriale şi al examenului de urină pentru decelarea microalbuminuriei, dar s-au prezentat numai 25 %.

DISCUŢII

Global, leziunea renală acută are o contribuţie semnificativă la morbiditatea şi mortalitatea copiilor în stadii critice, în special la cei cu insuficienţă organică multiplă. Distribuţia epidemiologică a leziunii renale acute pediatrice variază în funcţie de tipul instituţiei care raportează rezultatele (11, 12, 13). Unele unităţi, conţinând un mare număr de traume chirurgicale, cum ar fi chirurgia cardiacă, raportează un număr mai mare de pacienţi pediatrici cu leziune renală decât centrele mai puţin dezvoltate (6, 8). Hui-Stickle şi colab. au arătat o creştere dra-

matică a leziunii renale acute pediatrice (18).

Înainte de 1990, cauzele cele mai frecvent citate erau : sindromul hemolitic uremic, *sepsis*-ul şi arsurile (2). Acum în ţările dezvoltate cea mai frecventa cauză este insuficienţa organică multiplă, (incluzând bolile congenitale cardiace), necroza tubulară acută, medicamentele nefrotoxice şi *sepsis*-ul (14, 16). În ţările dezvoltate SHU continuă să fie raportat ca una din primele cauze ale insuficienţei renale pediatrice.

Alte cauze de leziune renală, cum ar fi glomerulonefrita rapid progresivă pot prezenta leziune renală rapid evolutivă spre boala renală cronică.

Mai multe boli renale, cum ar fi : SHU, purpura Henoch-Schonlein şi uropatiile obstructive, asociate cu displazie renală, pot prezenta leziune renală cu funcţie renala normală sau aproape normală. La copii, funcţia renală poate fi puţin deteriorată, ducând la insuficienţă renală cronică după mai multe luni sau după mai mulţi ani, uneori la vârsta de adult.

Dacă în lotul nostru de 110 de copii predomină cauzele prerenale, urmate de *sepsis* şi de SHU, în raportul lui Bunchman- 2001, pe 226 de copii dializaţi acut, cauzele cele mai frecvente au fost bolile congenitale de cord, urmate de necroza tubulară acută şi de *sepsis* (17).

Raportul lui Goldstein din 2005 arată că cele mai frecvente cauze de IRA au fost în necroza tubulară acută şi medicaţia nefrotoxică iar bolile renale primitive apar doar în 7% din cazuri (18).

Două studii din regiuni diferite din Kenya au raportat de asemenea predominanţa etiologiilor prerenale la copil (2).

CONCLUZII

Cu toate progresele obţinute în terapia intensivă şi în terapia de substituţie renală IRA rămâne o boala cu o înaltă mortalitate.

Este o entitate comună în centrele de urgenţă, medicilor din aceste unităţi revenindu-le un rol decisiv în recunoaşterea precoce, în prevenirea leziunilor iatrogene şi în modificarea cursului bolii.

Există o strânsă relaţie între insuficienţa multiorganică şi mortalitatea intraspitalicească din bolile severe. O abordare mai dinamică a stării critice de boală, având ca scop identificarea pacienţilor cu risc, înainte de a se instala disfuncţia organică este, în mod cert, mai puţin costisitoare decât încercarea de a salva pacienţii *in extremis*.

Un index de prognostic uşor de folosit în camerele de gardă poate înlesni intervenţia precoce, cu o evoluţie mai bună şi cu un cost mai redus.

Recunoaşterea promptă şi la timp a bolii precum şi îndrumarea spre medicul nefrolog a fost postulată ca având drept rezultat, în multe cazuri, vindecarea pacienţilor cu IRA.

Este necesară revizuirea ghidurilor şi a criteriilor pentru evoluţia funcţiei renale.

Este nevoie de utilizarea unor marcheri sensibili şi specifici pentru leziunea renală, evaluările lor putând fi folosite în timpul agresiunii iniţiale dar şi în perioada de evoluţie a bolii, realizând distincţia între variate tipuri şi etiologii ale bolii.

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STROKE - A POSSIBLE ADVERSE EFFECT OF COMBINATION THERAPY WITH PARITAPREVIR/RITONAVIR/OMBITASVIR + DASABUVIR. CASE REPORT

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STROKE-A POSSIBLE ADVERSE EFFECT OF COMBINATION THERAPY WITH PARITAPREVIR/RITONAVIR/OMBITASVIR + DASABUVIR. CASE REPORT (Abstract): The development of direct-acting antiviral agents against hepatitis C virus has revolutionized the management and control of this important liver disease. Combination therapy with paritaprevir/ritonavir/ombitasvir + dasabuvir is considered one of the most effective and well-tolerated regimens used to treat chronic hepatitis C, with response rates over 95%. Ease of administration, excellent tolerance, and the absence of severe side effects have made treatment of chronic hepatitis C possible even in patients with severe fibrosis and in those with comorbidities. Insomnia, headache and fatigue are the most commonly reported side effects, the serious adverse events being uncommon. We will report a case of stroke as a possible serious adverse effect during treatment with paritaprevir/ritonavir/ombitasvir + dasabuvir (PrOD) in a 61-year-old male, former smoker, without cardiovascular history, in whom a conflict situation resulted in a hypertensive episode accompanied by paresthesia in the upper and lower right limbs, vertigo, equilibrium disorder, which turned out to be a stroke. **Conclusions**: Because the reported case may be a rare serious effect during the treatment with parita previr/ritonavir/ombitasvir + dasabuvir in a patient with hepatitis C virus (HCV) cirrhosis and without a cardiovascular history, careful neurological monitoring is recommended during this therapeutic regimen. **Keywords**: PARITAPREVIR, RITONAVIR, OMBITASVIR, DASABUVIR, STROKE, CHRONIC HEPATITIS C.

Current treatment of hepatitis C virus infection is based on various oral regimens with direct-acting antiviral agents (DAAs) that have high rates of sustained virologic response (SVR), very good tolerability, low rates of serious adverse events or treatment discontinuation, and require a shorter treatment duration than interferon and ribavirin therapy (1).

New recommendations for the treatment of HCV infection consist of drug combinations and therefore efficacy and safety are assessed especially in patients using combination therapy, making the obtained data difficult to interpret for only one drug (2).

Paritaprevir/ritonavir/ombitasvir + da-

sabuvir stops HCV multiplication and infection of new liver cells. Ombitasvir and paritaprevir block two proteins essential for virus multiplication. Ritonavir acts as a potentiator and prolongs the action of paritaprevir in the body. Dasabuvir (250 mg) stops viral multiplication and infection of new liver cells.

Both paritaprevir/ritonavir/ombitasvir and dasabuvir are pan genotypic DAAs and are administered according to a protocol for 3 months in viral hepatitis C patients with or without cirrhosis (1, 3).

The most common side effects of these combined therapies are: insomnia (30%), headache (24%), and fatigue (20%). The serious adverse events have been reported during or after the treatment (4, 5).

We present a case of stroke as a severe and rare adverse event in a 61-year-old patient. Data related to the history of the disease, laboratory examinations, investigations and monitoring are presented. The patient has consented to the use of the data for scientific purposes.

CASE REPORT

A 61-year-old male patient, Caucasian, retreated, without significant heredocolateral history, residing in an urban area was diagnosed with chronic HCV infection in 2005. He received treatment with peginterferon α-2a + ribavirin for 4 months in 2006, being declared non-responder. He presented to the Iasi Clinic of Infectious Diseases in June 2017 for clinical and biological evaluation and investigations aimed to assess the opportunity of PrOD therapy.

The patient said he quit smoking 2 years ago and had no personal or family history of disease.

He had no specific complaints except fatigue. Physical examination revealed a mild hepatomegaly (about 3 cm below the costal margin) and normal blood pressure. Laboratory investigations at the initiation of therapy revealed AcHCV - present, viral load (HCV RNA) - 1690,000 IU/mL, FIBROMAX-F4 A3 S1 N1 H0, leukocytes = 9370/mm^3, hemoglobin = 18.6 g/dL, GGT = 91 IU/l, INR 1.53, ALAT = 53 IU/L, ASAT = 54 IU/L, bilirubin = 0.87 mg%, cholesterol = 153 mg%, serum albumin = 4.83 g/dL, AFP = 3.80 IU/l (N <5).

Abdominal ultrasound: no ultrasound suspected macronodules, calcification of 8 mm in segment VII, subcapsular, absence of ascites.

Cardiology consultation revealed arterial pressure (AP) 120/70 mmHg, electrocardiogram - sinus rhythm, 86 beats/min, no ischemic changes. The diagnosis at the initiation of antiviral therapy was compensated HCV LIVER CIRRHOSIS, Child score 5A.

In September 2017, PrOD therapy was initiated, at which time the liver function tests revealed ALAT 85 IU/L, ASAT 83 IU/L, total bilirubin 1.25 mg/dL.

Three weeks after the initiation of the therapy he experienced right hemi body paresthesia, vertigo, balance disorder, accompanied by a hypertension crisis (AP = 200/100 mmHg), for which he presented to the Focsani Hospital. Following the consultation, he was sent home with a decreased value of AP (170/80 mmHg), but he didn't mentioned a medication to be continued in ambulatory.

After 4 weeks of treatment the patient presented for a check-up and in view of obtaining the second prescription of the recommended medication, when it was found that the therapy was well tolerated, liver function tests returned to normal (ALAT = 27 IU/mL, ASAT = 33 IU/mL, total bilirubin = 0.7 mg/dL), and physical examination and blood pressure were with-

in normal range. He didn't declared the previous episode of hypertension.

After 5 weeks of treatment with PrOD, the patient presented to the "Prof. Dr. N. Oblu" Emergency Clinical Hospital from Iasi with the same manifestations as those experienced during the previous episode; he was admitted for 5 days, period during which DAA treatment was discontinued. The native cerebral computed tomography (CT) scan was normal, and the psychological consultation indicated an anxiety-depressive syndrome for which psychiatric consultation was recommended.

The diagnosis at discharge from the "Prof. Dr. N. Oblu" Emergency Clinical Hospital from Iasi was: left hemiparesis, minor recurrent ischemic accidents in the right carotid system, anxiety-depressive syndrome, HCV cirrhosis. Anticoagulation/antiplatelet treatment, Holter EKG monitoring, contrast enhanced cranio-cerebral magnetic resonance imaging (MRI) and follow-up visit one month after discharge were recommended.

The psychiatric consultation before the discharge from the neurology hospital made a diagnosis of severe depressive disorder and treatment with bromazepam and tianeptine sodium was recommended.

The patient returned to the Clinic of Infectious Diseases in good general state, with no neurological disturbances (motor deficit, speech disorders). Giving the fact that the first treatment was not completed (the length of the treatment should be 12 weeks and he only had 4 weeks), at his insistent request, PrOD therapy was resumed, in order to achieve a sustained virological response. Holter ECG monitoring was performed and it did not reveal changes in heart rate, and blood pressure levels within normal range.

Three days after the resumption of DAA therapy, unsystematic muscle contractions (upper and lower limbs) and cutaneous hyperesthesia occurred. Viral load (HCV RNA) was undetectable and the liver function tests were normal.

The next day, the general state of the patient had worsened: motor deficit in the right hemi body and speech disorders. The permanent discontinuation of PrOD therapy was decided, and the patient was referred for neurological reassessment, a diagnosis of right hemiplegia and stroke in the left superficial sylvian territory being made. After 6 months of rehabilitation therapy, the patient could rise to an erect position and walk possible intermittent spastic on the right side, right hemiparetic motor deficit, predominantly brachial, normal deep tendon reflexes in the upper and lower right limbs, but viral load (HCV RNA) became detectable, and ALT and ASAT levels remained normal.

DISCUSSION

Stroke occurred in a 61-year-old patient with advanced compensated liver disease (HCV liver cirrhosis) without a cardiovascular history, former smoker, on the 5th week of treatment with paritaprevir/ ritonavir/ombitasvir + dasabuvir. Worth mentioning is the early response to antiviral therapy (undetectable viral load at 4 weeks), but with a documented relapse 6 months after discontinuation of the therapy administered for 6 weeks. This is the only case that developed a severe cerebrovascular adverse effect of the 680 PrOD-treated patients in the Iasi Hospital for Infectious Diseases between 2016 and June 2018. Though, it should be mentioned that is possible that the stroke could have been an independent event, with no connection to the oral antiviral treatment.

In the literature (4, 6), serious adverse

events reported during treatment included isolated episodes: stroke, myocardial infarction, liver decompensation, acute kidney failure due to dehydration, anxiety, aggravation of psoriasis, hypokalemia, gastroenteritis, colitis.

In an analysis (7) of direct-acting antivirals (DAAs) for chronic hepatitis C conducted in 2017 by the Cochrane Hepatobiliary group (including 138 trials randomizing a total of 25,232 participants), 5 cases of severe cerebrovascular adverse events (stroke, cerebral ischemia, cerebrovascular accident) were recorded.

In a large cohort of 1008 patients with HCV genotype 1b compensated cirrhosis treated with PrOD + ribavirin for 12 weeks, Trifan *et al.* (8) reported only 1 case of stroke in the group of patients aged over 70, which required treatment discontinuation. Recently, Kaneko (5) reported severe cerebrovascular adverse events (including subarachnoid hemorrhage and cerebral hemorrhage) in 2 patients receiving Sofosbuvir- ledipasvir and Zaid (4) in one patient during combined sofosbuvir- daclatasvir therapy. More importantly, treatment discontinuation was very infrequent, even in large cohort of DAA therapy in solid organ transplant recipients highlighting the marked improvement in treatment tolerability in an interferon-free era (9).

Among HCV-infected subjects, the presence of steatosis was found to be an independent risk factor for carotid atherosclerosis and subsequently chronic HCV infection increases the stroke risk and should be also considered an important and independent risk factor (10). Our patient had a low degree of steatosis (S1) in the absence of a metabolic syndrome (serum cholesterol, normal blood glucose level), or a medication predisposing to drug interactions.

Reoccurrence of neurologic phenomena upon resuming antiviral therapy and their degree of severity during the second episode allows us to claim that PrOD therapy and the neurologic event are very likely connected.

From what we know up to now by studying the literature, no direct mechanism to explain stroke after therapy with DAAs was found (5), these being considered effective and safe, associated with a reduced number of serious adverse effects requiring therapy discontinuation.

CONCLUSIONS

Unexplained ischemic strokes may be considered serious adverse effects of antiviral therapy with DAAs, even in the absence of risk factors. As a result, careful neurological monitoring is recommended for HCV infected patients during treatment with this antiviral regimen or after discontinuation of therapy.

The presented case stands to highlight the possible occurrence of severe repetitive cerebrovascular events, during therapy with PrOD +/- ribavirin, events which require immediate discontinuation of treatment.

Antiviral treatment of advanced hepatitis C should no longer be necessary, HCV-infected individuals should be treated successfully before developing advanced fibrosis or cirrhosis.

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NEWS

CIPROFLOXACIN - ALLY OR ENEMY?

Aortic dissection is the most common fatal disease process of the aorta and is precede in most cases by the aneurysm formation. Ciprofloxacin is one of the most often used antibiotic and it is prescribed to treat a broad spectrum of bacterial infections, including infected aortic aneurysm. Results of recent clinical studies revealed that treatment with fluoroquinolone is associated with an increased risk of acute aortic events. In a recent study, Le Maire *et al.*, have induced a sporadic aneurysm formation in mice with high-fat diet and low-dose angiotensin infusion, the effects of ciprofloxacin on smooth muscle cells and frequency of acute aortic events were studied. The results were stunning, incidence of aortic dissection and rupture was almost 10 times higher in the group with ciprofloxacin and this outcome was linked to the increased level and activity of matrix metalloproteinase. Thus, this drug should be used with caution in patients with aortic dilatation and those at high risk for acute aortic events (Scott A. Le Maire, Lin Zhang, Wei Luo *et al*. Effect of Ciprofloxacin on Susceptibility to Aortic Dissection and Rupture in Mice. *JAMA Surg*. doi:10.1001/ jamasurg. 2018.1804).

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DATE EPIDEMIOLOGICE ASUPRA ŞOCULUI ÎN CAZUISTICA MEDICO-LEGALĂ

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EPIDEMIOLOGICAL DATA ON SHOCK IN Forensic MEDICINE (Abstract): The shock is a relative frequent diagnostic in forensic medicine. **Material and method**: We realized a statistical study, using 323 deaths through diverse shock typology, registered within the Forensic Medicine Institute of Iaşi in 2000-2009. The concerned variables were: the spread of cases on years, the clinical forms, age, sex, provenience environment, volemic feature, the survival time from the shock to the death, the spread of complications on the form of the shock and survival, the juridical way of death. Our data were introduced in the EXCEL database and statisticaly analyzed by SPSS program. **Results**: We obtained statistic data on shock typology, based on the survival time and based on the complications of shock types. **Conclusion**: Relevant statistic data were obtained on the relations between the factors that generated the shock, the complications induced and the shock types, related also to the juridical way of death.
Key words: SHOCK, EPIDEMIOLOGY, LEGAL MEDICINE, JURIDICAL IMPLICATIONS

Şocul poate fi definit ca un sindrom plurietiologic evolutiv, caracterizat printr-o tulburare hemodinamică gravă, cu hipoperfuzie tisulară, datorită reducerii volumului sanguin sau debitului cardiac, ceea ce conduce la un volum sanguin circulator efectiv inadecvat. Modificările care se succed în etapele patogenezei şocului pot fi reversibile, iar dacă factorii etiologici persistă acestea devin ireversibile cu moartea celulară consecutivă. Afectarea unui număr crescut de celule din organele vitale determină decesul individului chiar în condiţiile eliminării cauzei (1,2,3).

În cazuistica medico-legală, şocul este un diagnostic relativ frecvent ceea ce impune o definire exactă a tipului de şoc, stabilirea condiţiilor de apariţie şi derularea proceselor patologice care au condus la deces. Majoritatea studiilor din literatură cu privire la epidemiologia şocului au la bază diferite studii clinice din serviciile de medicină de urgenţă si terapie intensivă, în care poziţia principală o ocupă şocul septic (4,5). In Anglia 0,003% din totalul consulturilor spitalicesti sunt pentru soc, 72% din cazuri au fost spitalizate, numarul mediu de zile de spitalizare a acestora a fost 6,7 (Hospital Episode Statistics, Department of Health, England, 2002-2003).

Aducerea de date noi legate de acest subiect din cazuistica medico-legală este în opinia noastră o încercare dificilă prin faptul ca nu poate include toate cazurile de şoc, mai ales pe cele de şoc septic, care intră de obicei în cazuistica anatomoclinică.

În studiul de faţă am urmărit corelarea datelor privind factorii care au determinat decesul, complicaţiile apărute şi tipurile de şoc în relaţie şi cu forma juridică de moarte.

MATERIAL ŞI METODĂ

S-au luat în studiu un număr de 323 cazuri de deces prin şoc dintr-un număr total de 9489 autopsii efectuate la Institutul de Medicină Legală Iaşi, pe o perioadă de 10 ani între 2000-2009. S-a efectuat un studiu statistic privind repartiţia cazurilor pe ani, pe vârstă, pe sex şi formă clinică. S-au analizat timpii de supravieţuire în funcţie de tipul de şoc, global şi pe fiecare tip în parte. S-a efectuat o analiză statistică privind distribuţia complicaţiilor pe forme de şoc si supravieţuire. Datele obţinute au fost centralizate în baze de date EXCEL şi SPSS şi prelucrate cu funcţii statistice adecvate.

REZULTATE

În perioada analizată între 2000-2009, la Institutul de Medicină Legală Iaşi s-au efectuat un număr total de 9489 autopsii, iar dintre acestea 323 au fost cazuri de şoc care au reprezentat un procent de 3,4 % din totalul autopsiilor. În anul 2000 au fost înregistrate 905 cazuri din care 28 cazuri cu şoc, în 2001 - 877 cazuri din care 33 cazuri de şoc, în 2002 - 1027 cazuri, din care 34 cazuri de şoc, în 2003 - 918 cazuri din care 30 cazuri de şoc, în 2004 - 865 cazuri din care 34 cazuri de şoc, în 2005 - 874 din care 37 cazuri de şoc, în 2006 - 935 cazuri din care 34 cazuri de şoc, în 2007 - 898 din care 30 cazuri de şoc, în 2008 - 1072 din care 32 cazuri de şoc şi în 2009 - 1018 din care 31 cazuri de şoc.

Distribuţia cazurilor de şoc, pe ani de studiu, a fost bimodală, cu vârfuri de frec-

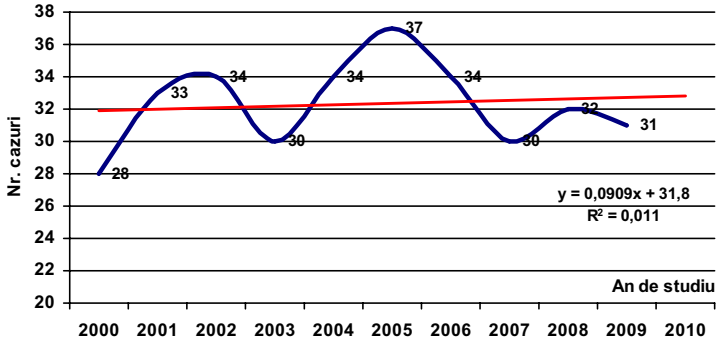

Fig. 1. Distribuţia cazurilor de şoc pe ani de studiu

TABELUL I

Repartiţia cazurilor pe ani şi tipuri de şoc

Anul	Nr. autopsii	Nr. cazuri de şoc	Toxicoseptic	Mixt	Traumatic	Hemoragic	Combustional	Cardiogen
2000	905	28	8	4	3	4	7	2
2001	877	33	9	5	4	5	8	2
2002	1027	34	9	4	6	5	7	3
2003	918	30	10	3	3	6	6	2
2004	865	34	8	7	3	5	8	3
2005	874	37	11	10	3	4	7	2
2006	935	34	10	9	2	5	6	2
2007	898	30	9	7	1	4	8	1
2008	1072	32	10	2	4	6	8	2
2009	1018	31	11	6	3	3	7	1
Total	9489	323	95	57	32	47	72	20

TABELUL II
Repartiţia cazurilor în funcţie de vârstă şi ponderea procentuală

Vârstă (ani)	Nr. cazuri	Procent
0-10	40	12,3%
10-20	27	8,35%
20-40	89	27,5%
40-60	110	34%
60-80	57	17,64%

venţă în anii 2002 (34 cazuri) şi 2005 (37 cazuri), observându-se o tendinţă uşor crescătoare ($y = 31,8 + 0,0909 x$)(fig.1).

Din numărul total de 323 decese prin şoc s-au evidenţiat următoarele formele clinice de şoc : toxico-septic - 95 cazuri, şoc mixt - 57 cazuri, şoc traumatic 32 cazuri, şoc hemoragic - 47 cazuri, şoc combustional - 72 cazuri, şoc cardiogen - 20 cazuri (tab. I).

În funcţie de vârstă s-au luat în considerare următoarele grupe : 0-10 ani - 40 cazuri, 10-20 ani - 27 cazuri, 20-40 ani - 89 cazuri , 40-60 ani - 110 cazuri, 60-80 ani - 57 cazuri (tab. II).

În funcţie de sex, cazurile au prezentat următoarea distribuţie : la sexul masculin 221 cazuri (68%), iar la sexul feminin 102 cazuri (32%)(tab. III).

În ceea ce priveşte mediul de proveniență, din cele 323 cazuri 214 au fost din mediul rural şi 109 din mediul urban (tab.IV).

În funcţie de componenta volemică, din numărul total de cazuri de şoc au fost 208 cazuri de şoc hipovolemic şi 115 cazuri cu şoc normovolemic (tab.V).

Timpul de supravieţuire de la momentul instalării şocului până la deces a conturat următoarele situaţii : supravieţuire până la 1 oră - 60 cazuri, supravieţuire până la 24 ore - 110 cazuri, supravieţuire între 1-3 zile 57 cazuri, supravieţuire între 3-10 zile 52 cazuri, supravieţuire între 10-20 zile 29 cazuri, supravieţuire între 20-60 zile 15 cazuri.

TABELUL III
Repartiţia cazurilor în funcţie de sex

Sexul	Nr. cazuri	Procent
M	221	68,4%
F	102	21,6%

S-au analizat timpii de supravieţuire în funcţie de tipul de şoc, global şi pe fiecare tip în parte (tab. VI, fig.2).

Pe tipuri de şoc, în funcţie de timpul de supravieţuire se remarcă puternice diferenţe semnificative :

- cel mai frecvent pacienţii cu şoc hemoragic sau mixt au decedat în primele 24 ore în comparaţie cu celelalte tipuri de şoc ;
- pacienţii cu şoc combustional prezintă letalitatea maximă în primele 3 zile ;
- pacienţii cu şoc toxicoseptic prezintă letalitatea maximă în primele 10 zile.

S-a analizat existenţa unei corelaţii statistic semnificative între fiecare tip de complicaţie apărută şi fiecare tip de şoc precum şi între complicaţie şi şoc în general (tab. VII, fig.3).

Din punct de vedere statistic, pe tipuri de şoc, în funcţie de afectare se remarcă :

- ponderea semnificativ mai mare a cazurilor cu afectare MSOF în cazul şocului toxicoseptic, mixt sau traumatic ($p = 0,00005$) ;
- ponderea cazurilor cu afectare renală a fost semnificativ mai crescută în cazul şocului hemoragic sau combustional ($p = 0,000006$) ;
- distribuţia cazurilor cu şoc cardiogen a fost semnificativ mai crescută la pacienţii cu afectare cardiacă ($p < 0,001$) ;

TABELUL IV
Repartiţia cazurilor în funcţie de proveniență

Mediul	Nr. cazuri
R	214
U	109

TABELUL V
Repartiţia cazurilor în funcţie de sex

Componenta volemică	Nr. cazuri
Şoc hipovolemic	208
Şoc normovolemic	115

TABELUL VI

Repartiţia cazurilor în funcţie de tipul de şoc

Forma clinică de şoc	Timp de supravieţuire						Nr. cazuri
	1h	1-24h	1-3zile	3-10zile	10-20zile	20-60zile	
Şoc mixt	8	46	3	-	-	-	57
Şoc traumatic	14	15	3	-	-	-	32
Şoc hemoragic	22	25	-	-	-	-	47
Şoc combustional	7	19	33	13	-	-	72
Şoc cardiogen	9	5	4	2	-	-	20
Şoc toxicoseptic	-	-	14	37	29	15	95
Total cazuri	60	110	57	52	29	15	323
Test χ^2 (GL=5)	73,59	111,16	57,18	63,57	95,92	37,75	
p	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**

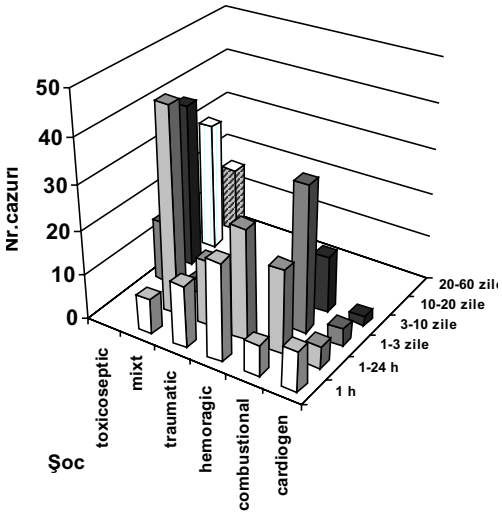

Fig. 2. Distribuţia cazurilor de şoc în funcţie de timpul de supravieţuire

- afectarea pulmonară, digestivă, hepatică şi CID, pe tipuri de şoc, prezintă distribuţii fără diferenţe semnificative din punct de vedere statistic.

În cadrul protocolului medico-legal, fiecare caz a fost clasificat în funcţie de forma juridică de moarte. Din cele 323 cazuri de şoc s-a constatat că 221 din cazuri sunt accidente, 60 cazuri au fost catalogate drept crime, iar suicidul s-a înregistrat într-un număr de 42 cazuri.

DISCUŢII

Din studiul de faţă rezultă că din numărul total de autopsii efectuate la IML Iaşi pe o perioadă de 10 ani, 3,4 % din decese au fost cauzate de diverse tipuri de şoc, procentul rezultat fiind în concordanţă cu

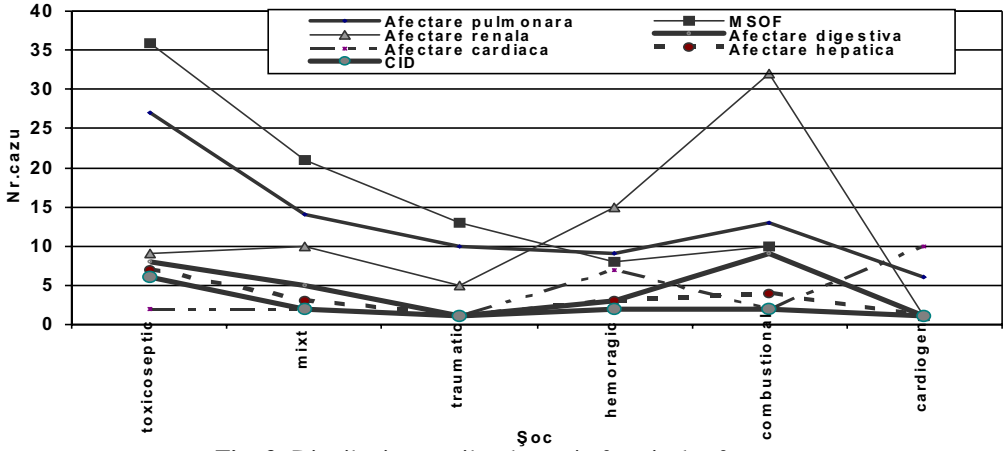

Fig. 3. Distribuţia cazurilor de şoc în funcţie de afectare

TABELUL VII
Repartiţia cazurilor în funcţie de tipul de complicaţii pe formele de şoc

Forma clinică de şoc	Afectare pulmonară	MSOF	Afectare renală	Afectare digestivă	Afectare cardiacă	Afectare hepatică	CID	Nr. cazuri
Şoc toxicoseptic	27	36	9	8	2	7	6	95
Şoc mixt	14	21	10	5	2	3	2	57
Şoc traumatic	10	13	5	1	1	1	1	32
Şoc hemoragic	9	8	15	3	7	3	2	47
Şoc combustional	13	10	32	9	2	4	2	72
Şoc cardiogen	6	-	1	1	10	1	1	20
Total cazuri	79	88	72	27	24	19	14	323
Test χ^2 (GL=5)	4,25	27,42	36,94	3,30	64,82	0,92	1,55	
p	0,513	**0,00005**	**0,0000006**	0,653	**<0,001**	0,969	0,907	

datele din literatură. Distribuţia bimodală, obţinută în cadrul studiului nostru, cu vârfuri de frecvenţă în anii 2002 şi 2005, ar putea fi legată de o frecvenţă mai crescută a tipurilor de şoc toxico-septic şi mixt în aceste perioade.

În studiul nostru, cel mai frecvent tip de şoc a fost cel toxico-septic, urmat de cel combustional, însumând peste jumătate din numărul de cazuri. Numeroase studii au demonstrat faptul că sepsisul sever este o cauza principală de deces la pacienţii în stare critică (6,7,8). Cauzele de deces după arsuri severe sunt variabile, însă literatura de specialitate incriminează drept cauză principală insuficienţa multiplă de organ, distribuţia cu exactitate a celorlalte cauze nefiind ştiută (9,10). Grupa de vârstă cea mai afectată de şoc în studiul nostru a fost la adulţii între 40-60 ani, care împreună cu grupa de vârstă 20-40 ani, totalizează aproape 2/3 din cazuri.

S-a constatat că sexul cel mai afectat a fost sexul masculin, în peste 2/3 din totalul cazurilor (11,12). În cadrul repartiţiei pe ani nu s-au observat variaţii semnificative ale prezenţei celor 6 tipuri de şoc. În funcţie de timpul de supravieţuire în funcţie de forma de şoc se remarcă diferenţe statistice puternic semnificative. Cel mai frecvent, decesul la pacienţii cu şoc hemoragic sau mixt s-a produs în primele 24 ore în comparaţie cu celelalte tipuri de şoc, în timp ce la pacienţii cu şoc combustional, vârful de letalitate a fost în primele 3 zile, iar la pacienţii cu şoc toxicoseptic în primele 10 zile (13,14). S-a observat că *perioada de supravieţuire* în cazul a diferite forme de şoc se limitează la maximum 3 zile pentru aproximativ 3/4 din totalul cazurilor, în concordanţă cu literatura şi rapoarte statistice ale departamentelor de sănătate din Anglia şi SUA (15,16).

Analiza statistică privind distribuţia complicaţiilor pe forme de şoc şi supravieţuire a relevat următoarele complicaţii generatoare de deces: insuficienţă multiplă de organ (MSOF), complicaţii pulmonare, complicaţii cardiace şi complicaţii renale, acestea fiind pe primele locuri. Mai rar au fost întâlnite complicaţii digestive, hepatice şi de tip CID (17). *Afectarea organică multiplă*, alături de afectarea pulmonară şi renală, atât ca leziuni iniţiale cât şi drept complicaţii apărute în evoluţie sunt legate de decesul

a 75% din cazuri (18). Cazurile de accident au predominat în cazuistica noastră reprezentând peste 2/3 din numărul deceselor prin şoc.

CONCLUZII

Studiul nostru este printre puţinele studii care realizează o cercetare amănunţită asupra epidemiologiei şocului în cazuistica medico-legală. Am obţinut date semnificative statistic privind relaţiile dintre factorii care au determinat decesul, complicaţiile apărute şi tipurile de şoc.

Ca interpretare medicolegală atrage atenţia faptul că cele mai severe forme de şoc se corelează cu forma juridică de moarte. Exista o corelaţie intre şocul hemoragic şi mixt şi accidentele rutiere.

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CLINICAL SIGNIFICANCE OF C-REACTIVE PROTEIN LEVELS IN PREDICTING RESPONSIVENESS TO IRON THERAPY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND IRON DEFICIENCY ANEMIA

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CLINICAL SIGNIFICANCE OF C-REACTIVE PROTEIN LEVELS IN PREDICTING RESPONSIVENESS TO IRON THERAPY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND IRON DEFICIENCY ANEMIA (Abstract): Iron deficiency anemia (IDA) has been recognized as a major cause of anemia in inflammatory bowel disease (IBD). Anemia has a significant impact on the quality of life of these patients and thus correction is very important. **Aim:** To determine if systemic inflammation (assessed by C-reactive protein [CRP]) can predict the response to iron therapy (oral versus intravenous). **Material and methods:** A seven-month prospective study (June-December 2016) conducted at the Iasi Institute of Gastroenterology and Hepatology. Sixty-three patients with IBD and IDA were assessed. Inflammation was defined as CRP levels > 4mg/L. Patients were divided as follows: 29 patients received iron based oral treatment (group A) and 34 intravenous therapy [IV] (group B). **Results:** Of the 29 group A patients 14 responded (11 had CRP levels < 4mg/L and 3 CRP levels > 4mg/L) and 15 did not respond (3 with CRP levels < 4mg/L and 12 with CRP levels > 4mg/L). As to the 35 group B patients, 25 responded (12 with CRP levels < 4mg/L and 13 with CRP levels > 4mg/L) and 9 did not respond (4 with CRP levels < 4mg/L and 5 with CRP levels > 4mg/L). Among iron tablet-treated patients, hemoglobin (Hb) increase was significantly slower in the high versus low CRP subgroup (2.8 vs 3.8 g/dL, p=0.04). Response to IV therapy was independent of inflammation. **Conclusions:** Patients with higher CRP levels achieved a lower Hb response with oral iron therapy. CRP may be useful in selecting the iron administration route as well as identifying IBD patients who can benefit from first-line IV iron therapy to improve their IDA. **Keywords:** INFLAMMATORY BOWEL DISEASE, IRON DEFICIENCY, ANEMIA, IRON THERAPY, C-REACTIVE PROTEIN.

Inflammatory bowel diseases (IBD) consisting of Crohn's disease (CD) and ulcerative colitis (UC) are conditions of the gastrointestinal tract characterized by peri-

ods of activity and remission, multifactorial etiology and underlying mechanisms that remain incompletely elucidated. Anemia is the most common extraintestinal complication (EIC) in IBD patients (1). Its major importance lies not only in the negative impact on the quality of life (QoL) of these patients, the increased number and duration of hospitalizations and healthcare costs, but also in its continuing underestimation in current clinical practice (2).

In IBD patients, anemia can have a variety of different causes etiologies of which the most important are iron deficiency anemia (IDA) followed by anemia of chronic disease (ACD) (3). For a correct management and approach, it is necessary to distinguish between them as the treatment is different.

IDA is known to be the most common type of anemia in IBD patients. In Romania, the prevalence of the different types of anemia in IBD patients is as follows: 78% - IDA, 15% - mixed anemia and 8.8% - ACD (4).

The main causes of iron deficiency in IBD patients include: chronic intestinal bleeding from inflamed mucosa, inadequate intake during active disease and/or impaired absorption (5, 6).

In IDA, serum ferritin is an indicator of iron storage. A level lower than 15 ng/ml for women or 30 ng/ml for men is characteristic for absolute iron deficiency and in the absence of chronic inflammation associated with low hemoglobin (Hb) for IDA. However, ferritin is also an acute-phase protein and in the presence of chronic inflammatory conditions, such as IBD, its levels may be increased if there is active disease even in patients with IDA (7).

Different biological markers have been used in IBD for various purposes. Of these, we mention C-reactive-protein (CRP), an acute phase protein with pentameric structure, produced by hepatocytes. CRP has been extensively studied over time: from correlating it with endoscopic activity (8, 9, 10), predicting relapse (11, 12), postoperative recurrence (13) and evolutive aspects (14) to predicting response to different treatments (15, 16).

CRP is used to screen for anemia in patients with IBD, along with full blood count and ferritin test, and a level > 5 mg/L is considered significant for systemic inflammation. According to the European Consensus recommendations, it should be measured every 3 months in patients with active disease and at 6, 12 months for those in remission or with mild impairment (3). In the presence of inflammation (CRP > 5 mg/L), ECCO guidelines recognize iron deficiency as a cause for anemia if ferritin level is < 100 ng/dL (17).

Studies have shown that, even when detected, IDA is rarely treated, and that it is usually managed with oral iron supplementation, without regard to other parameters besides Hb and clinical status, although it is known that 90% of the ingested iron remains unabsorbed and that chronic inflammation decreases iron absorption in the gut (18). The goal of treatment, which involves oral or intravenous iron, is to normalize the Hb levels, replenish iron stores and improve QoL (19, 20, 21).

The aim of this study was to determine if systemic inflammation, assessed by CRP, predicts the response to oral versus intravenous iron therapy.

MATERIAL AND METHODS

Between June and December 2016, we performed a prospective analysis of 63 patients with IBD and IDA admitted to the

Iasi Institute of Gastroenterology and Hepatology. Clinical and demographic parameters (age, gender, phenotype, disease duration, extension and activity) were recorded.

Anemia was defined as according to the World Health Organization (WHO): Hb <12 g/dL in women and <13 g/dL in men. The serum markers assessed at baseline were: Hb, erythrocyte indices, ferritin, transferrin saturation (TSAT), iron levels and CRP.

The studied population were patients diagnosed with UC/CD who had IDA (defined as: Hb <12g/dL in women and <13 g/dL in men, TSAT <20% and serum ferritin <100 ng/ml). We followed the recommendations of the European guidelines for the selection of the iron management method. In group A we included patients with mild anemia, inactive disease and who have not had a history of intolerance to iron. Patients with Hb <10g/dL, with active disease and previous intolerance to oral iron route were included in group B.

We divided the patients into two groups as follows: 29 patients were treated with iron-based oral treatment (group A) and 34 with intravenous (IV) therapy (group B). The oral treatment used was ferrous sulfate - 100 mg (FS) administered b.i.d. for 12 weeks and the IV one ferric carboxymaltose (FCM) - maximum 1,000 mg per infusion, weekly, until the patient's calculated cumulative dose had been reached.

Excluded were patients with co-existing causes of anemia: other gastrointestinal causes (hemorrhoids, gastroduodenal ulcers, B12 and folic acid deficiency), myelosuppressive medication (methotrexate and sulfasalazine), renal-urinary and genital causes or pregnancy in women.

Disease activity was determined using Ulcerative Colitis Disease Activity Index - UCDAI for UC and Crohn's Disease Activity Index - CDAI for CD. Inflammation was defined as CRP levels > 5mg/L. Hb level was measured at 4, 8 and 12 weeks. Ferritin and serum iron levels were also reassessed at 12 weeks. Response to treatment was defined as absence of anemia (Hb > 12 g/dL in women and 13 g/dL in men) at 12 weeks and increase in Hb level > 2g/dL at 4 weeks.

The collected data were analyzed using the *SPSS 23.0* software. We used Pearson's Chi-squared test to correlate inflammation at baseline (CRP > 4 mg/L) with response to iron supplementation (defined by the absence of anemia at 12 weeks), oral vs IV administration.

RESULTS

The study population comprised 63 patients with IDA and IBD: 44 with UC and 19 with CD. Demographic parameters were (tab. I): age (mean: 36.72 ± 6.28 years), gender (58.73% men, n=37; sex ratio: 1.42), smoking status (52.38%, n=33 - never smoked).

Duration of disease was 6.24 ± 3.28 years. In terms of disease activity: 33 (52.38%) had mild disease, 25 (39.68%) moderate disease, and severe disease activity in 5 (7.93%) cases.

Of those with CD, 16 (25.3%) had ileal disease, 26 (41.26%) colonic disease and 21 (33.3%) an ileocolonic location. Most of the CD patients had an inflammatory phenotype (73%, n=46), 19% (n=12) a stricturing phenotype and 6% (n=4) a penetrating one. Both, structuring and penetrating phenotypes were encountered in 2% of cases (n=1). On the other hand, 14 (22.2%) of the UC patients had proctitis, 39 (61.9%) left-sided UC and 10 (6.30%) an extensive UC.

TABLE I

Clinical and demographic characteristics of patients with IBD and IDA

	IBD and IDA n (%)	CD n (%)	UC n (%)	p value
Number of Patients	63 (100)	19 (30.16)	44 (69.84)	0.001
Men/Women	37 (58.7)/26 (41.2)	8 (42.10)/11 (57.89)	29 (65.90)/15 (34.09)	0.018
Mean age (years)	36.72 ± 6.28	31.52 ± 2.78	39.15 ± 3.84	0.002
Smoker/ Ex-smoker/ Non-smoker	17 (26.98)/13 (20.63)/ 33 (52.38)	8 (42.10)/ 3 (15.78)/ 8 (42.10)	9 (20.45)/ 10 (22.72)/ 25 (56.81)	0.001
Disease activity: mild/moderate/severe	33 (52.38)/ 25 (39.68%)/ 5 (7.93)	9(47.36)/9(47.36)/ 2(10.52)	24 (54.54)/16 (36.36)/ 3 (6.81)	0.073
CD location L1/ L2/ L3/ L4	N/A	16 (25.3)/ 26 (41.26)/ 21 (33.33)	N/A	0.081
CD phenotype B1/ B2/ B3	N/A	46 (73)/12 (19)/ 4 (6.34)	N/A	0.026
UC extension E1/ E2/ E3	N/A	N/A	14 (22.22) / 39(61.9) /10(6.30)	0.031

Mean baseline Hb level was 9.8 g/dL (range 7.5-10.7). In UC patients, anemia was predominantly found in case of extensive disease (39.7%, $p<0.05$) and in CD in patients with penetrating behavior (21.05%, $p<0.01$). Anemia was found more often in patients with severe disease activity (59.32%, $p=0.07$). There was no statistically significant correlation between disease duration and anemia.

Symptoms of anemia (fig.1) were found in 73% of the study patients that consisted in: fatigue (93.4%), reduced physical performance and cognitive function (84.7%), headaches (15.2%) and sleeping disorders (8.6%).

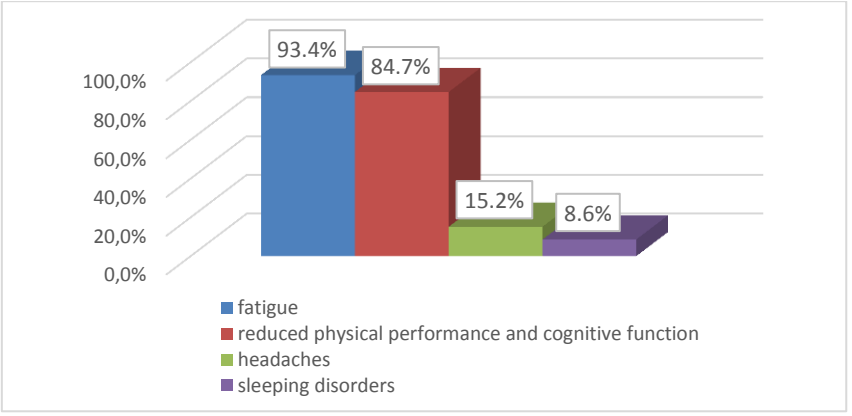

Fig. 1. Symptoms of IDA in the study population

Inflammation was defined as of CRP levels > 5mg/L. Twenty-three patients had 'high CRP levels' while 40 of them had 'low CRP levels' (fig 2.).

In group A (tab. II), Hb level at week 12 increased with: 2.8 ± 0.1 g/dL in those with 'high CRP' and 3.8 ± 0.2 g/dL in those with 'low-CRP'.

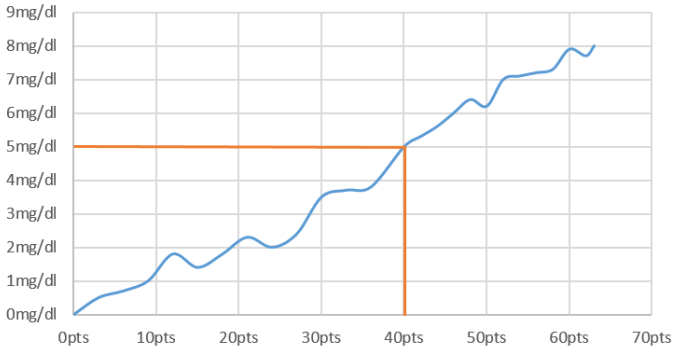

Fig. 2. Distribution of CRP levels (cut-off 5 mg/dL) among study patients

In those receiving IV iron therapy (tab. III), increases at week 12 were: 3.2 ± 0.1 g/dL and 3.6 ± 0.2 g/dL respectively.

Of the 29 group A patients (tab. IV) 14 responded (11 had CRP < 4mg/L and 3 CRP > 4mg/L) and 15 did not respond (3 with CRP < 4mg/L and 12 with CRP levels > 4mg/L).

As to the group B patients (tab. V), 25 responded (12 with CRP < 4mg/L and 13 with CRP > 4mg/L) and 9 did not respond (4 with CRP < 4mg/L and 5 with CRP > 4mg/L).

TABLE II

Increase in Hb levels (g/dL) in patients treated with oral iron

	Oral iron therapy		
	CRP > 4 mg/dL	CRP < 4 mg/dL	p value
Hb (g/dL) week 4 ± SD	1.9 ± 0.2	2.8 ± 0.2	0.001
Hb (g/dL) week 8 ± SD	2.6 ± 0.3	3.6 ± 0.3	0.029
Hb (g/dL) week 12 ± SD	2.8 ± 0.1	3.8 ± 0.2	0.043

TABLE III

Increase in Hb (g/dL) levels in patients treated with intravenous iron

	IV iron therapy			
	CRP > 4 mg/dL	CRP < 4 mg/dL	p value	
Hb (g/dL) week 4 ± SD	2.6 ± 0.2	2.8 ± 0.2	0.036	
Hb (g/dL) week 8 ± SD	2.9 ± 0.3	3.8 ± 0.3	0.048	
Hb (g/dL) week 12 ± SD	3.2 ±0.1	3.6 ± 0.2	0.001	

TABLE IV

The correlation between the response to oral iron treatment and CRP values

Iron based oral treatment		
Treatment response	Number of patients	CRP value
Positive	11	<4mg/L
	3	>4mg/L
Negative	3	<4mg/L
	12	>4mg/L

TABLE V

Correlation between intravenous iron response and CRP levels

IV iron therapy		
Treatment response	Number of patients	CRP value
Positive	22	<4mg/L
	3	>4mg/L
Negative	4	<4mg/L
	5	>4mg/L

Among iron tablet-treated patients, the mean Hb increase was significantly slower in the 'high' versus 'low' CRP subgroups (2.8 vs 3.8 g/dL, p=0.04). Response to IV therapy was independent of inflammation.

In our patients no SAEs were seen; gastrointestinal side effects were more frequent in group A vs. group B (vomiting: 3% vs 1.2%, diarrhea: 2.8% vs. 0.7% and nausea 2.6% vs. 0.8%) but there were no symptoms requiring discontinuation of treatment.

DISCUSSION

Although conducted on a relatively small number of patients, our study presents data consistent with those reported in the literature. As shown by Gasche *et al*. (22), Iqbal *et al*. (23), CRP is useful in selecting the iron treatment method.

In our study, we noticed that in patients treated with oral iron the response to treatment was in accordance with the baseline CRP level. In this group, increases in Hb levels were more significant in those with CRP < 4 mg/dL (week 4: 2.8 vs. 1.8 g/dL, week 8: 3.6 vs. 2.6 g/dL and week 12: 3.8 vs 2.8 g/dL, p=0.04).

It was noted, according to the increase in Hb levels, that irrespective of the form of administered iron, the results are relatively similar when the endpoint has been reached (week 12).

As well as in the study published by Schroder O. (24), in the IV group we noticed the lack of correlation between CRP level and Hb increase, suggesting no link with the degree of inflammation (week 12: 3.2 vs. 3.6 g/dL, p<0.001).

These findings indicate that the best choice in patients with IBD, IDA and elevated baseline CRP is intravenous iron.

In terms of adverse effects, as reviewed by a recent meta-analysis (25), the likelihood of severe adverse effects (SAEs) is higher in those receiving IV iron therapy while gastrointestinal effects are more commonly reported in those receiving oral treatment, which has also been seen in our patients.

CONCLUSIONS

Our study, through the above mentioned, contributes to the establishment of the right therapeutic strategy in patients with IBD and IDA. CRP, as an indicator of inflammation, predicts by its level (high or low) the response to iron treatment. Therefore, it may be considered a useful tool in selecting the most efficient method of iron administration and also in selecting the patients that can benefit from IV iron as first-line therapy.

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SEROPREVALENŢA ANTICORPILOR p-ANCA LA PACIENŢII CU RECTOCOLITĂ ULCEROHEMORAGICĂ ŞI LA RUDELE LOR DE GRADUL I

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p-ANCA PREVALENCE IN ULCERATIVE COLITIS PATIENTS AND FIRST-DEGREE RELATIVES (Abstract): Perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) are more common in patients with ulcerative colitis (UC) than in patients with Crohn's disease (CD), but its prevalence depends on the population being studied and the method employed for its detection. **Aim**: To determinate the prevalence of p-ANCA in ulcerative colitis (UC) patients and their first degree in NE Romania area. **Material and method**: In this study, we investigated the prevalence of p-ANCA as detected by ELISA, in the serum of 44 patients with UC and 22 first degree relatives. We also correlated the presence of this antibody with disease activity and extent, extraintestinal complications and therapy. 26 healthy individuals comprised the control group. **Results**: p-ANCA was detected in 11% of the patients with UC. Of the 5 p-ANCA positive UC patients, 1 case were found to have proctosigmoiditis, 1 cases had left-sided colitis and 3 cases had pancolitis. There was no correlation between the presence of this antibody and any of the studied clinical variables (extend disease, complication, family disease, surgery). No person of the first degree and control group presented positive test. **Conclusions**: The prevalence of p-ANCA in NE Romania patients with UC (11 %) is lower than that in the Western population. The negativity of p-ANCA in all first degree relatives of NE Romania UC patients should be further elucidated. **Key words**: pANCA, ULCERATIVE COLITIS, ELISA

Diagnosticul serologic din rectocolită ulcerohemoragică (RCUH) cuprinde o serie de anticorpi cu importanţă diferită în precizarea diagnosticului. Printre aceştia se numără şi anticorpii anticitoplasmă neutrofilică ANCA care au fost descoperiţi de mulţi ani ca markeri ai dezechilibrului imunologic în boala inflamatorie intestinală (BII), patternul pANCA - perinuclear ANCA fiind mai specifici pentru RCUH (1). Anticorpii ANCA reprezintă o familie de autoanticorpi din clasa imunoglobulinelor G şi M îndreptaţi împotriva unor antigene ţintă reprezentate de ganulaţiile azurofile ale polimorfonuclearelor (proteinaza 3 şi mieloperoxidaza). Determinarea acestor anticorpi se face prin imunofluorescenţă, sau, cu acurateţe sporită prin ELISA.

Utilitatea determinării ac pANCA este motivată din mai multe considerente:

- ca test adjuvant în precizarea fenotipului de BII (RCUH sau Boală Crohn),
- markeri pentru evoluţia bolii şi precizarea tratamentului.

Scopul acestui studiu a fost de a evalua semnificaţia seroprevalenţei anticorpilor pANCA la pacienţii cu RCUH din N-E României şi rudele lor de gradul I.

MATERIAL ŞI METODĂ

În studiu au fost incluşi pacienţi diagnosticaţi cu RCUH în Institutul de Gastroenterologie şi Hepatologie Iaşi şi rudele lor de gradul I. S-a constituit în acelaşi timp şi un lot martor din 26 subiecţi sănătoşi, comparabil ca distribuţie pe grupe de vârstă şi sex cu loturile studiate.

Pacienţii evaluaţi au fost grupaţi în 3 categorii :

- *grupul cu RCUH* include 44 pacienţi diagnosticaţi cu RCUH ;
- *grupul rudelor de grad I* al pacienţilor cu RCUH, 22 persoane ;
- *grupul de control* include 26 subiecţi sănătoşi cu distribuţie pe grupe de vârstă şi sex similară cu a pacienţilor din lotul de studiu.

Includerea pacienţilor în studiu s-a bazat pe următoarele criterii :

- *criterii diagnostice* definite pe baza datelor clinice, endoscopice, histologice, radiologice ;
- *criterii evolutive* - boală cu o durată de cel puţin 2 ani.

Severitatea bolii a fost apreciată în raport cu criteriile Truelove şi Witts cu definirea formelor uşoare, moderate şi severe de boală.

Evaluarea extinderii leziunilor inflamatorii s-a făcut prin examinare endoscopică cu încadrare în proctită (afectare rectală), colită stângă (afectare până la unghiul splenic al colonului), pancolită (afectare colonică întinsă ce depăşeşte unghiul splenic al colonului).

Criterii de excludere au fost pentru pacienţii care au primit terapie imunosupresivă considerată ca medicaţia susceptibilă de a modifica titrul ac pANCA, aspect care este însă controversat (2).

Din probele de sânge recoltate s-au efectuat testări serologice pentru evidenţierea pANCA, utilizându-se pentru determinare metoda ELISA.

Metoda de determinare

Pentru determinarea pANCA a fost utilizată metoda cantitativa ELISA, cu evidenţierea mieloperoxidazei (MPO) şi proteinaza 3 (PR3) ca antigenelor responsabile pentru pANCA. Metoda are la bază o reacţie indirectă cu fază solidă de tip sandwich, cu realizarea în ser a unui complex antigen--anticorp între enzimele IgG conjugate cu MPO PR3. Testările serologice s-au efectuat în cadrul Laboratorului de Microbiologie, Institutul de Sănătate Publică Iaşi (laborator autorizat în efectuarea testelor ELISA), cu analizor automat pentru ELISA, ETI-STAR, (DiaSorin Italia). Fiecare probă biologică reprezentată de serul provenit de la subiecţii incluşi în studiu a fost prelucrată în acord cu instrucţiunile specificate pentru metoda de determinare. Procedura de lucru presupune mai multe etape :

1. Se prepară tamponul de spălare şi diluentul pentru probe.
2. Se diluează probele. Probele de ser sau plasmă se diluează 1 : 100 cu diluant pentru probe (10 μL ser sau plasma + 990 μL diluant)
3. Se marchează pe foaia de lucru poziţia probelor în godeuri incluzând 6 godeuri pentru calibratori, 2 pentru Controalele Pozitive (PC) şi două pentru Controalele Negative (NC). Se poate opta şi pentru testarea probelor în duplicate.
4. Se aşează în holder numărul necesar de godeuri.
5. Se pipetează în godeuri câte 100 μL de Calibratori, controale şi probe diluate.
6. Se incubează 30 minute la temperatura camerei.
7. Se spală de 3 ori cu câte 300 μL de tampon de spălare.
8. Se pipetează câte 100 μL de Conjugat în fiecare godeu.
9. Se incubează 15 minute la temperatura camerei.
10. Se spală de trei ori ca în pasul 7.
11. Se pipetează în fiecare godeu câte 100

TABELUL I
Valori pANCA prin testare ELISA

Interpretare	Ac anti-PR3 (U/mL)	Ac anti-MPO (U/mL)
Normal	<5	<5
Crescut	>5	>5

µL de Substrat pastrând aceeaşi ordine în care s-a pipetat Conjugatul.

12. Se incubează 15 minute la temperatura camerei.
13. Se adaugă câte 100 µL de reactiv de stopare in aceeaşi ordine ca şi Substratul. Se aşteaptă 5 minute.
14. Se citeşte absorbanta fiecărui godeu la 450 nm dacă se foloseşte un singur filtru, sau la 450/ 600-690 nm dacă se foloseşte un cititor cu două filtre. Reacţia este stabilă pentru cel puţin 30 minute. Citirea densităţii optice trebuie făcută în acest interval de timp.

Valori probabile (aşteptate) sunt redate în tabelul I, cu recomandarea ca fiecare laborator să stabilească propriile limite pentru valorile normale sau patologice.

Evaluarea rezultatelor se face cu ajutorul curbei obţinute. Se recomandă curba cu 4 parametri pentru interpretarea rezultatelor.

Rezultatul pozitiv trebuie raportat la statusul clinic al pacientului. De asemenea, fiecare decizie pentru terapie se stabileşte individual.

Tehnica prezintă o serie de limite legate de calitatea preparării antigenelor, izolarea sau purificarea proteinelor citoplasmatice din neutrofilele umane şi modificările de conformaţie a epitopilor pentru antigen ocupaţi după aderarea la suprafaţă cu antigen.

REZULTATE

Din distribuţia cazurilor referitoare la extensia leziunilor inflamatorii la grupul pacienţilor cu RCUH s-a constatat afectare tip colită stângă în 50% cazuri, pancolită în 32% cazuri şi proctită în 18% cazuri. La acelaşi grup de studiu evaluarea severităţii bolii în raport cu criteriile Truelove şi Witts relevă forme uşoare la 41% pacienţi, forme mederate la 38% pacienţi şi forme severe la 21% pacienţi.

În grupul pacienţilor cu RCUH testul pANCA a fost identificat pozitiv în raport cu valoarea coutoff (> 5U/mL) la 5 pacienţi (11%). Majoritatea titrurilor au fost sub valoare coutoff de 5U/mL. Cel mai mare titru a fost de 17U/mL.

La grupul rudelor cu RCUH şi martori nu s-a înregistrat nici un test pozitiv pentru p ANCA (fig. 1).

Prezentarea pacienţilor cu RCUH şi pANCA pozitiv în raport cu datele demografice, vechimea bolii, extinderea leziunilor, stadiul de activitate este redat în tabelul II.

DISCUŢII

pANCA este raportată în literatură cu o prevalenţă variabilă la pacienţii cu BII. Pacienţii cu RCUH prezintă mai frecvent pANCA (între 33%-84%) comparativ cu boala Crohn (BC) unde pANCA apare la 15% din pacienţi şi caracterizează subgrupul de BC colonică (3,4). În studiul nostru prevalenţa pANCA

TABELUL II
Pacienţii cu RCUH şi pANCA pozitiv

Pacient	Sex	Vârstă	Vechimea bolii	Extensie	Severitate	Complicaţii, ME, chirurgie
1	M	46	3	Pancolită	S	Spondilită anchilozantă, colectomie, pouchită
2	F	25	5	Pancolită	S	Tromboflebită, anemie, megacolon toxic
3	F	34	2	Pancolită	M	CS
4	M	70	10	Colită stângă	M	Pseudopolipi
5	M	51	8	Proctosigmoidită	M	XXX

Legendă S=sever, M=mediu, CS=colangită sclerozantă, ME=manifestări extraintestinale

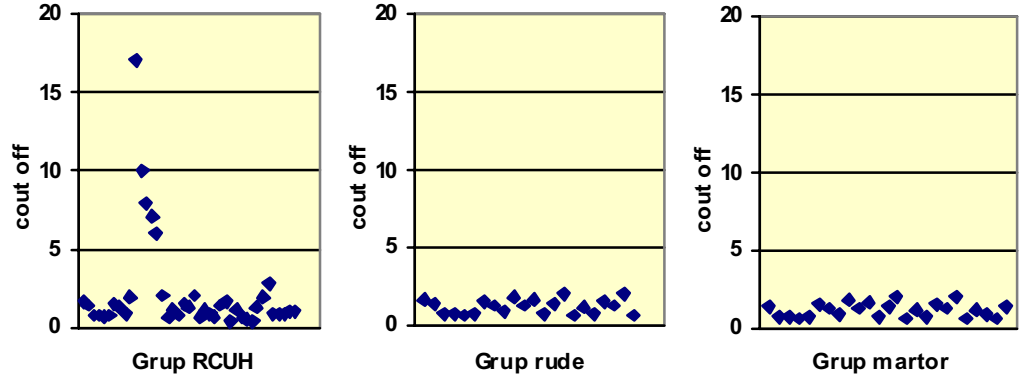

Fig. 1. Rezultate la testul pANCA în loturile de studiu

la pacienţii cu RCUH este mai mică comparativ cu rezultatele raportate de alţi autori. Din totalul pacienţilor testaţi doar 5/44 seruri (11%) au fost pozitive pentru pANCA aparţinând pacienţilor cu RCUH; pacienţii incluşi în grupul rudelor şi martori sănătoşi nu au prezentat teste pozitive. Determinările s-au făcut la pacienţii cu evoluţia bolii de peste 2 ani şi fără tratament imunomodulator care să modifice configuraţia bolii.

Diferenţe de prevalenţă a pANCA au fost constatate la mai multe serii de studii derulate în arii geografice diferite; ele pot fi atribuite atât particularităţilor etnice cât şi tehnicii de lucru utilizate. La pacienţii cu RCUH din Estonia (populaţie cu incidenţă mică a BII comparativ cu seriile europene), pANCA a fost detectată în 29/59 (49%) din cazuri cu RCUH, în 4 din 17 cazuri cu BC şi în 4% din 111 cazuri subiecţi sănătoşi în timp ce la populaţia din UK prevalenţa a fost de 42,4% (5). Pentru ambele boli nu se constată corelaţie între prezenţa de pANCA şi durata sau extensia bolii

Prezenţa pANCA cât şi titrul nu se corelează cu extensia bolii, severitatea puseului de activitate şi forma clinico evolutivă (6). Controverse ale relaţiei pANCA- stadiul de activitate al bolii rezultate din datele diferitelor studii, care raportează atât titruri înalte de pANCA la pacienţii cu RCUH activă, cât şi lipsa de corelaţie între statusul pANCA + şi boală activă, par a fi clarificate de datele unui singur studiu de urmărire pe termen lung a prezenţei şi variaţie titrului pANCA, care precizează absenţa corelaţiei între pANCA şi stadiul de activitate al bolii (1,7). Mai mult, posibilitatea de conversie din pANCA negativ în pANCA pozitiv şi viceversa nu se corelează cu activitatea bolii. În studiul nostru 4/5 pacienţii cu seruri pozitive pentru pANCA prezentau boala în stadiul de activitate şi leziuni extinse la nivelul colonului descendent sau pancolită. Având în vedere numărul redus de teste pozitive, rezultatele constatate nu pot fi considerate definitorii pentru corelaţia între pANCA şi boala activă. Cel mai mare titru de anticorpi a fost înregistrat la un pacient cu pancolită care a necesitat colectomie. În acest caz, tirul ridicat de pANCA a probat nu numai relaţia cu boala activă cu leziuni extinse, dar s-a dovedit şi predictiv pentru pouchita apărută la un an după intervenţia chirurgicală, care a necesitat reintervenţie chirurgicală.

Variaţiile de prevalenţă pot fi explicate prin implicarea factorilor tehnici utilizaţi în diferite metode de determinare (ELISA, imunofluorescenţă) (7). Metoda comună de determinare a anticorpilor pANCA este imunofluorescenţa indirectă (IFI), tehnica ELISA fiind mai recent introdusă. Variabilitatea rezultatelor între diferite laboratoare a impus standardizarea metodelor de determinare, în special pentru studiile comparabile. Dife-

renţele de rezultate între studii ar trebui atribuite nu numai metodelor variate de lucru, dar şi opţiunii laboratorul pentru valoarea de cutoff (valoarea prag de detecţie a antigenelor cu semnificaţie patologică), cu recomandarea ca fiecare laborator să stabilească limitele normale şi patologice (8). Încadrarea valorilor pANCA într-un model cantitativ este folosită în evaluarea clinică şi biochimică a pacienţilor cu RCUH, în estimarea corelaţiilor cu factorii de risc şi stabilirea asociaţiilor epidemiologice.

În studiul nostru serurile pANCA pozitive au fost evaluate prin metoda ELISA, tehnica sandwich, cu valoarea coutoff de 5 U/mL. Pozitivitatea pANCA la populaţia de rectocolitici din NE României trebuie interpretată în raport cu structura genetică a pacientului, natura antigenului împotriva căruia se dezvoltă anticorpii, natura florii intestinale a populaţiei din regiune.

Susceptibilitatea pentru BII poate fi influenţată de genele complexului major de histocompatibilitate HLA, implicate în mod regulat în răspunsul imunologic. În mod distinct a fost raportată asocierea antigenelor CMH clasa I şi II cu RCUH, statusul pANCA pozitiv fiind mai frecvent asociat cu HLA DR2 în timp ce pANCA – este asociat cu HLA DR4 (9). Medierea imunologică a fost un obiectiv urmărit în criteriile de includere a pacienţilor, încât cei care au urmat tratament imunomodulator nu au fost selectaţi.

Determinările HLA pentru compatibilitatea transplantului de organ relevă un mozaic genetic, astfel încât, în lipsa unor studii imunogenetice despre structura genetică a pacienţilor cu RCUH, nu pot fi făcute aprecieri care să demonstreze asocierea dintre pANCA şi HLA pentru populaţia de rectocolitici din această regiune a ţării.

Mai mult, din datele din literatura reiese că această asociere pANCA-markerii genetici nu este confirmată la toate populaţiile, ceea ce denotă o dependenţă mare a pozitivităţii testului de structura etnică a populaţiei. Existenţa unor variaţii regionale importante de tipul extremelor de prevalenţă menţionate în unele studii din Germania, pun sub semnul întrebării relaţia pANCA–HLA. De asemenea, este acceptată şi existenţa unor diferenţe între date complete despre structura genetică a populaţie, care nu permit aprecieri ale relaţiei pANCA şi haplotipul pacientului (10).

Creşterea prevalenţei pANCA la rudele pacienţilor cu RCUH, în special probanţii pANCA pozitiv, validată pe mai multe serii de studiu, susţine ipoteza de marker genetic al susceptibilităţii pentru RCUH (11,12).

În studiul nostru din 22 subiecţi testaţi, rude de grad I ale pacienţilor cu RCUH (copii, părinţi), nici un ser nu este pozitiv pentru pANCA (nici pentru rudele pacienţii pANCA pozitiv şi nici pentru rudele pacienţii pANCA negativ), titrurile de anticorpi fiind sub valoarea coutoff de 5U/l. Absenţa markerilor genetici la rudele pacienţilor cu RCUH, sugerează o structură genetică diferită a populaţiei din regiunea de NE a României. Acest aspect poate reprezenta o particularitate regională, a cărei confirmare necesită analiză în cadrul unor studii suplimentare.

Specificitatea pANCA este dată de prezenţa a doua antigene majore proteinaza 3 (PR3) şi mieloperoxidaza (MPO) şi de un set de antigenelor minore reprezentate de : catepsina, elastaza, lactoferina, lizozimul, proteine bacteriane implicate în creşterea a permeabilităţii. În RCUH antigenele majore sunt rar detectate şi la titruri joase, în timp ce antigenele minore sunt mai frecvent descrise (13). În studiul nostru frecvenţa redusă şi titrurile joase ale ac pANCA rezultate prin determinarea ELISA, pot fi datorate specificitatii kiturilor pentru antigenele majore MPO şi PR3. Din grupul antigenelor minore, lactoferina reprezintă antigenul majoritar. Este cunoscut faptul ca lactoferina umană prezintă o secvenţa comună, de 68%, cu lactoferina din laptele de vacă, cu posi-

bilitatea unei reacţii încrucişate între anticorpii umani şi lactoferină (3). Alimentaţia copilului din primul an de viaţă (naturală sau artificială) pe un sistemul imun imatur, poate fi implicată în apariţia de anticorpi împotriva lactoferinii. Istoricul pozitiv de alimentaţie naturală prelungită până la 1 an, constatat la majoritatea pacienţilor cu RCUH, ar putea argumenta prevalenţa pANCA redusă. De asemenea, este cunoscută şi acceptată coexistenţa diferiţilor antigeni în acelaşi ser, dar numai testele cantitative antigen specific pot defini specificitatea antigenică a pacientului cu RCUH, creând şi premizele unei mai bune corelaţii între titru de anticorpi şi activitatea bolii. Faptul că titrul pANCA s-ar putea corela în principal cu antigenele majore, testarea, prin specificitatea kiturilor, nu permite analiza relaţiei între activitatea bolii şi titrul antigenelor specifici populaţiei cu RCUH din NE României al cărui spectru antigenic nu este cunoscut.

Rolul bacteriilor în patogeneza BII este susţinut de rezultatele studiilor experimentale pe modelul animal, dar nici până în prezent nu a fost identificat un germene specific. Alterarea florei intestinale a fost bine demonstrată la pacienţii cu RCUH. Această ipoteză este susţinută de faptul ca modificări ale concentraţiei florei intestinale se asociază cu scăderea potenţialului protectiv al lactobacilului şi bifidobacteriei (14). Modificarea în timp a frecvenţei RCUH poate fi şi consecinţa modificărilor florei intestinale. Faptul că pANCA reacţionează cu antigenele bacteriene vine în sprijinul ipotezei mimetismului antigenic investigat pentru autoanticorpii direct îndreptaţi asupra lactoferinii, care posedă o secvenţă comună cu proteina de şoc microbiană (15). Nu sunt excluse variaţii de microfloră gastrointestinală umană pentru populaţia din NE României, dacă se are în vedere frecvenţa mare a enterocolitei la populaţia pediatrică. În Estonia a fost comparată microflora bacteriană prin coproculturi prelevate de la copii cu vârstă până la 1 an cu cei din Suedia. Cauza diferenţei de microfloră bacteriană cercetată la cele 2 populaţii rămâne necunoscută. Microflora curentă în populaţia din Estonia este similară cu cea din Suedia unde RCUH este mult mai frecventă, diferenţele fiind atribuite implicării altor cauze (16).

Studiul nostru suportă rezultatele şi pentru un caz de RCUH asociat cu colangită sclerozantă, dar titrul nu se corelează cu activitatea sau extensia bolii. Similar cazului cu pouchită, asocierea pANCA-RCUH-CS vine ca o confirmare a implicării factorului imunogenetic în patogeneza bolii.

CONCLUZII

Frecvenţa mică a anticorpii pANCA la pacienţii cu RCUH din NE României le atribuie un rol limitat în diagnosticul serologic al bolii. Din cauza sensibilităţii reduse, determinarea uzuală a ac pANCA nu este utilizată. Testele pozitive influenţează probabilitatea distincţiei între RCUH şi BC. Titrul acestor anticorpi nu se corelează cu activitatea bolii şi pare stabil în timp.

Probabilitatea susceptibilităţii genetice a RCUH, demonstrată prin frecvenţa mare a bolii la rudele de grad I, reprezintă un punct de interes pentru majoritatea cercetătorilor. Predispoziţia genetică a bolii la rudele de gradul I, verificată prin testări serologice imune, nu se confirmă pentru pacienţii din această regiune a ţării, iar rezultatele testărilor pANCA efectuate la pacienţii cu RCUH din N-E României prezintă valori mult mai mici faţă de datele din literatură. Interpretarea conjugată a frecvenţei reduse a BII familiale şi seroprevalenţa ac pANCA relevă aspecte care argumentează constatările noastre. Nici unul dintre pacienţii cu istoric pozitiv pentru BII nu a prezentat pANCA +.

Considerăm aceste aspecte o particularitate regională, care necesită o documentare susţinută şi extinsă la un număr cât mai mare de pacienţi în cadrul unor studii suplimentare.

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OUR EXPERIENCE IN THE SURGICAL TREATMENT OF ACETABULAR FRACTURES USING "SPRING PLATE" TECHNIQUE

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OUR EXPERIENCE IN THE SURGICAL TREATMENT OF ACETABULAR FRACTURES, USING SPRING PLATE TECHNIQUE (Abstract): Fractures of the posterior wall, with comminution, are often hard to reduce and stabilize due to the small fragments that are involved, so the "spring plate" technique was developed**. Aim:** To show the advantages and the results we obtained in treating the posterior wall fractures using "spring plate" technique. **Materials and methods:** Included in our study was a number of 21 patients with an average age of 42 years, which had fracture of the acetabular posterior wall or acetabular fracture associated with posterior wall fracture. They were treated by open reduction and internal fixation with one or two spring plates and one or two reconstruction plates overlapping them. **Results:** Postoperative X-rays revealed that anatomic reduction was obtained in 15 cases (72%), was acceptable in 3 cases (14%) and imperfect in 3 cases (14%). Clinical outcomes were very good in 16 cases (76%), acceptable in 4 cases (19%) and poor in one case (5%). **Conclusions:** Using spring plate technique in comminuted posterior wall fracture proves to offer a good stability for small fragments, eliminates the risk of articular penetration of the screws and offers the possibility of early recovery. **Keywords:** SPRING PLATE, ACETABULAR FRACTURE, POSTERIOR WALL.

The fractures of the posterior wall of the acetabulum account around 30% of all acetabular fractures (1), but often they are associated with other acetabular fractures. They are produced by high energy trauma (traffic accidents mainly) (2), but also by low energy trauma in obese patients or persons with osteoporosis (3).

By the 1970, the treatment for these fractures was nonsurgical, having relatively good results in 50-60% of cases (3, 4); then, the studies by Matta (2) and Mayo (5) have demonstrated the superiority of surgical treatment of these fractures, with excellent results in 80-90% of cases, even delayed acetabular fractures have better clinical outcomes after surgical treatment (6).

The surgical treatment is indicated in fractures with step-off greater than 2 mm (7) and depends on the size of fracture site and hip stability (8).

In posterior wall fracture with small fragments, anatomic reduction and internal fixation with reconstruction plate or interfragmentary screw can prove to be very difficult (1), associated with an increased

risk of articular penetration of screws, so Mast *et al.* (9) suggests the use of a plate perpendicular on the bone fragments, covered by the reconstruction plate molded along the posterior column of the acetabulum.

The aim of this study was to demonstrate the advantages and our short-term results of treating the posterior wall fractures by using spring plate technique.

MATERIAL AND METHODS

Between January 2015 and December 2017, 45 patients with acetabular posterior wall fractures were admitted and treated surgically in the Orthopedics and Traumatology Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital, Iasi and "Mavromati" County Emergency Hospital, Botosani. Based on the admission criteria (comminuted posterior wall fracture or other acetabular fractures associated with comminuted posterior wall fracture and minimum of one-year follow-up), 21 patients were included in our study.

Distribution by gender showed a higher proportion of male patients (90%), the gender ratio being M/F 9.5/1. Distribution by age group revealed a peak in the age group 30-40 years, the average age was 42 years old (range 19-79 years). The left acetabulum was more often affected: 14 (77%) cases *vs.* right one: 7 (33%) cases. The mechanism of the injury was high velocity trauma in 90% of the cases (car accident in 12 (57%) cases and falls from height in 7 (33%) cases) and only in 2 (10%) cases of low velocity trauma (fall from ground level).

According to Letournel classification, 9 (43%) cases were classified as posterior wall fracture, 9 (43%) cases as transverse+ posterior wall fracture and 3 (15%) cases as both columns with comminuted posterior wall fracture. The associated local injuries were: posterior hip dislocation in 8 (38%) cases, marginal impaction in 4 (19%) cases, intraarticular loose body in 2 (10%) cases and osteochondral femoral head lesion in 4 (19%) cases. 9 cases had sciatic nerve palsy associated.

Emergency reduction and skeletal traction was applied to patients who had hip dislocation. The period from the trauma to the surgery was on average 7 days (3-21 days) depending on the general status of the patient.

Preoperative imaging included anteroposterior X-rays, oblique Judet views (fig. 1) and CT scans of the pelvis (fig. 2). The indication for surgical treatment was given by hip instability, involvement of over 40% of the posterior wall (fig. 2), step-off greater than 2 mm, intraarticular loose body and marginal impaction.

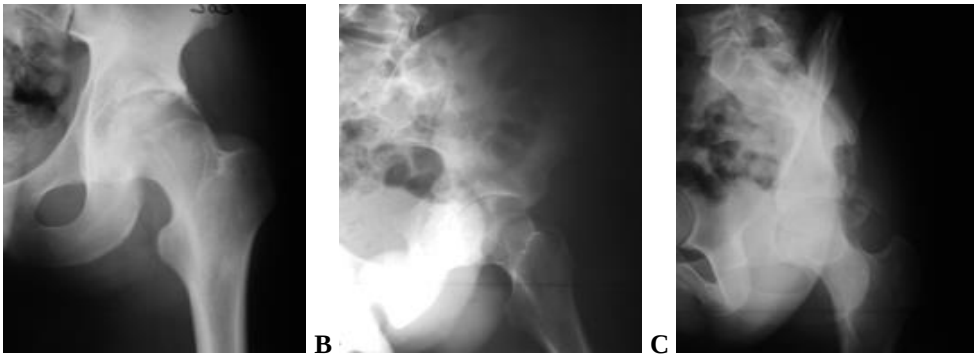

Fig. 1. Hip X-Ray - posterior wall fracture:
AP view **(A),** external oblique view (**B**), internal oblique view (**C**)

For all patients we used a Kocher Langenbeck approach with the patients positioned in prone position in 19 (90%) cases and lateral decubitus in 2 (10%) cases. Open reduction and temporary stabilization of the posterior wall fragments with Kirschner wires was performed. For the definitive internal fixation, we used spring plates made out of one-third tubular plate (fig. 3 A, B) placed with spikes to the border of posterior wall. In 18 (86%) cases we stabilized the plate with an additional screw in the posterior column and in 3 (14%) cases the pressure of the reconstruction plate offered enough stabilization. The number of spring plates used depended on the area and number of fragments that could not be stabilized by other methods, so we used one spring plate in 18 (86%) cases and 2 plates in 3 (14%) cases. The spring plates were overlapped by one preshaped reconstruction plate along the posterior column of the acetabulum in 15 (71%) cases and 2 preshaped reconstruction plate along the posterior column and posterior wall of the acetabulum in 6 (29%) cases. The plates were preshaped on plastic coxal bones (fig. 3 C, D). Intraoperative fluoroscopic images were used to confirm the reduction and position of the plates and screws.

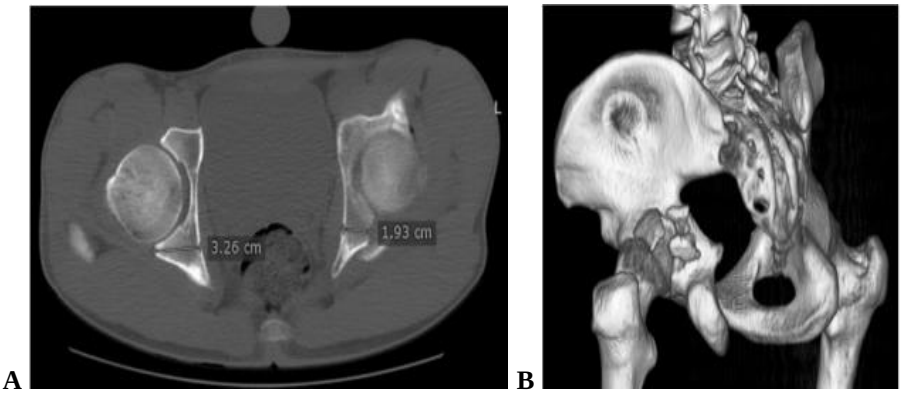

Fig. 2. 2D CT axial incidence and measurement of the posterior wall – 40% **(A)**, 3D image **(B)**

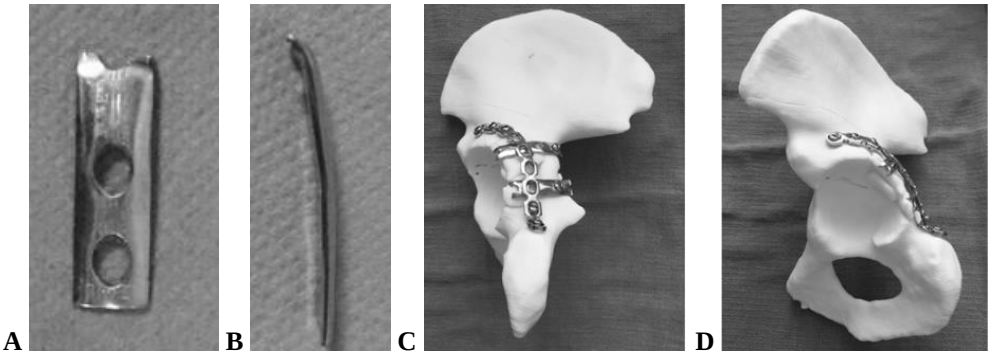

Fig. 3. "Spring plate" made out of one-third tubular plate (photos: **A** - anterior view, **B** - profile); Placement and positioning of the plates on the 3D printed plastic coxal model (photo images: **C** - posterior view, **D** - profile view)

The mean duration of surgery was 2.46 hours (2-4 hours). Blood loss during surgery was on average 500 ml (250-1750 ml). The average duration of postoperative admission was 8 days (4-28 days). Pre- and postoperative prophylaxis of deep vein thrombosis consisted in the administration of low molecular weight heparin, antibiotic prophylaxis was made with III generation cephalosporin, one dose intraoperatively, no prophylactic treatment of heterotopic ossifications was administered.

Follow-up was performed at 45 days, 3, 6 and 12 months. These consisted of physical examination based on the Merle D'Aubigné-Postel score and the radiological findings were graded by Matta criteria (2). Heterotopic ossifications were graded using the Brooker classification (10).

RESULTS

Postoperative X-rays (fig. 4) showed that anatomical reduction was obtained in 15 (72%) cases, was acceptable in 3 (14%) cases and imperfect in 3 (14%) cases. The imperfect results were obtained in the cases of both columns fractures. Clinical outcome was very good in 15 (72%) cases, acceptable in 3 (14%) cases and poor in 3 (14%) cases.

At 45 days follow-up, in one (5%) case we found loss of reduction with subluxation of the hip. Clinically, at 12 months follow-up, walking was completely normal in 14 (67%) cases, a slight limp in 4 (19 %) cases and walking with one crutch in 3 (14%) cases. Hip joint mobility was very good in 14 (67%) cases, good in 4 (19%) cases and poor in 3 (14%) cases. Also, we noticed lack of hip pain in 15 (72%) cases, mild pain in 3 (14%) cases and moderate pain in 3 (14%) cases. Radiologic results at one-year follow-up showed: preservation of initial reduction in 20 (95%) cases; heterotopic ossification Brooker type I in 3 (14%) cases and type II in 1 (5%) case; 2 (10%) cases of hip arthritis; no case of femoral head avascular necrosis.

No case of infection, deep vein thrombosis and non-union was recorded so far. No difference in the surgical result between sexes, the part of injury, age and the position on the surgical table.

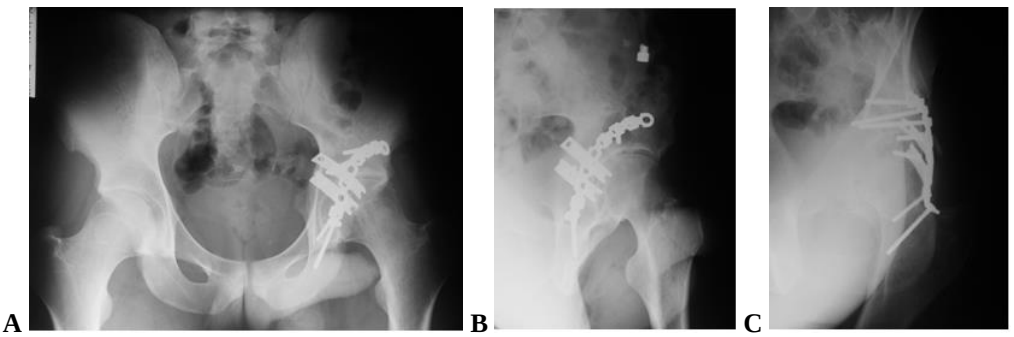

Fig. 4. 12 months follow-up: **A** - anteroposterior view**, B** – external oblique view, **C** – internal oblique view.

DISCUSSION

Despite the fact that posterior wall fractures are easy to treat, even with a 94–100% anatomical reduction rate, the percentage of excellent and good hip function scores represent only 68-82% (1, 2, 11). The difference between the radiological and clinical result can be explained due to comminution, marginal impaction and chondral lesions (12). In our series we obtained anatomical

and acceptable fracture reduction (according to Matta score) with very good and acceptable clinical score (according to Merle D'Aubigné-Postel score) in 86% of cases. This higher clinical score that we obtain could be the result of the short follow up period but is still an encouraging score.

The major challenge in the treatment of comminuted posterior wall fractures is reduction and stabilization of small fragments, because these fragments cannot be stabilized by interfragmentary screws due to their small size and/or the risk the screws to penetrate the joint surface. To solve this problem, Mast *et al.* (8), described the "spring plate" technique using a plate placed perpendicular to the fracture covered by a posterior column reconstruction plate. A biomechanical study showed that large size fragments of posterior wall fractures can be stabilized with 2 or more screws but when comminution occurs 1 screw and a reconstruction plate is needed (13). Goulet *et al.* (14) demonstrated in his biomechanical study that internal fixation with interfragmentary screws is weaker than internal fixation with reconstruction plate and the combination of the two techniques being the most rigid in transversely comminuted fractures and that internal fixation with reconstruction plate versus reconstruction plate associated with spring plate showed no increase in rigidity but seems to be better in concentrically comminuted fractures

The clinical studies that used only screw fixation in cases with one large fragment or sufficiently large fragments obtained good results, but they don't describe the minimum size of the fragment or size of the comminution (15, 16).

The spring plate technique is not so rigid as the association between interfragmentary screws and reconstruction plate, but is rigid enough (12, 14). Richter *et al.* (17) sustained that the spring plate acts as dynamic buttress, it is solid enough for fixation of small fragments, can be used alone without other implants reducing the necessary dissection and is ideal for fixing small periarticular fragments which cannot be fixed by screws. Using this technique, Ebraheim *et al.* (12) had good results and the fixation was stable enough for early range of motion and weight bearing.

The plates used as spring plate can be made out of one-third tubular plates, most often used, or are prefabricated (12, 17). Ziran *et al.* (18) suggested the use of a "T" plate (used for distal epiphyseal radius internal fixation) as a spring plate. Other types of plates used, like "cervical vertebral plates" have the advantage of increased screw density (19); locking reconstruction plates with the advantage of monocortical screws in the danger zone (20).

Although the number of patients is not great, we believe that this technique of internal fixation using one-third tubular plates as spring plate, is the best for comminuted posterior wall fractures even though it does not provide the best rigidity but enough stability and by the buttress effect during mobilization and loading helps modeling the acetabular joint surface. This technique does not require any extra special instrument, guides, larger dissection and is easy to learn and apply.

Heterotopic ossification Brooker type I and II were present in 4 cases (19%) and we did not do any prophylaxis. This percentage is lower or the same with other studies (12, 21) that used prophylaxis of heterotopic ossification.

Hip arthritis was present in 2 cases (10%), higher than other studies, were the rate of this complication is 4-7.5% (11, 22). The arthritis occurred in one case were the reduction was lost and one case were the fracture involved both columns and comminuted posterior wall fracture. Loss of

reduction was reported up to 26% (23) which seems very high compared to our 1% but is a small series and we selected only the cases treated using spring plate technique. The outcome of both columns fracture with and without posterior wall fracture was reported to be the same (24), so it seems that our hip arthritis was not correlated to this injury.

CONCLUSIONS

We believe that internal fixation of comminuted posterior wall fractures using the "spring plate" technique provides sufficient rigidity to allow rapid mobilization, eliminates the risk of joint penetration of the implants and by the "buttress" effect helps shape the joint surface. It is a simple, easy to learn and to use technique.

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NEWS

HPV AND BREAST CANCER

Studies evaluating the presence of HPVs in breast cancer have generated considerable controversy. Investigating a relationship between HPV and breast cancer is a valid hypothesis for a number of reasons, such as the exposure of the mammary ducts to the external environment via the nipple areola complex that could provide an entry point for HPV infection. Also, most breast cancers originate from mammary duct epithelia. Di Lonardo *et al*. (1992) were the first to report the positive relationship between HPV and breast cancer, by demonstrating the presence of HPV in 29% of breast carcinoma. Since the first discovery, several studies have investigated and detected over 60% HPV in breast cancer tissue. On the contrary, opposing studies were reported the absence of HPV DNA in breast cancer and suggested it is improbable that HPV is associated with the development of breast carcinomas. In the current study, tissue specimens from a total 110 fresh abnormal breast tissues (including cancerous and non-cancerous) from women aged 25-82 years old were examined for the presence of DNA of 12 HR-HPV types using type-specific PCR. Sequencing was performed on PCR products which confirmed the amplification of HPV DNA. The BLAST analysis revealed 99% and 98% concordance of the HPV DNA sequences in breast tissue samples tested with the reference sequences for high risk HPV types. Additionally, the data obtained from the sequencing are consistent with PCR results and this further validates our findings on the detection of HPV DNA. The results of high-risk HPV typing showed that HPV-DNA was found in 42% of abnormal breast tissue specimens and 31% of benign and normal breast tissue specimens. The presence of high-risk HPV viral sequences and the expression of viral protein in malignant breast tissue highlight the importance of further studies to investigate and assess the possible viral mechanism in the pathogenesis of breast cancer. (Salman NA, Davies G, Majidy F, *et al*. Association of high-risk human papillomavirus and breast cancer: A UK based study. *Sci Rep*. 2017; 7: 43591).

CHEST TRAUMA: THE DROP THAT FILLED THE GLASS

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CHEST TRAUMA: THE DROP THAT FILLED THE GLASS" (CASE REPORT) (Abstract): We present the case of a 50-year-old patient, smoker, alcohol user, with untreated stable angina pectoris who presented to the emergency department with severe chest pain which started about 4 hours earlier following a trauma by the fall of a solid object on his thorax. The anamnestic context requires the exclusion of a chest wall lesion, but at the same time it raises the problem of a heart lesion. Posttraumatic osteoarticular and pleuropulmonary lesions were not confirmed. The ECG pattern of acute anterior wall ST-segment elevation myocardial infarction and significant increase of highly sensitive troponin I (hs-TnI) drew our attention. Emergency echocardiography revealed anomalies of segmental left ventricular wall motion, moderate systolic dysfunction, and excluded other cardiac lesions. Primary percutaneous coronary intervention with drug-eluting stent is performed for thrombotic occlusion in the mid-segment of the left anterior descending coronary artery. The course was not complicated, but the left ventricular systolic dysfunction persisted. The presented case is particular due to the low prevalence of the posttraumatic acute myocardial infarction, mechanism of production, and diagnostic and therapeutic management issues. **Keywords**: POSTTRAUMATIC ACUTE MYOCARDIAL INFARCTION, CHEST TRAUMA, PRIMARY PCI.

The incidence of cardiac traumas caused by anteroposterior chest compression is increasing. Of these, contusions are much more frequent than cardiac injuries which often remain undiagnosed. The most common cardiac complications are arrhythmias, myocardial or valvular rupture, cardiac tamponade, and myocardial ischemia. Traumatic myocardial infarction caused by cardiac contusions is a rare but often fatal complication. The mechanism of production is both through direct myocardial injury and coronary artery injury. The prompt differential diagnosis and appropriate emergency management are essential for a favorable prognosis.

CASE REPORT

A 50-year-old patient, smoker (4-5 cigarettes per day) and a chronic alcohol user presented to the emergency department for severe, constrictive chest pain radiating posteriorly which started about 4 hours before presentation. These symptoms occurred after a chest trauma caused by the fall of a large solid object on his chest during work (he is a forester). The patient had no significant family history. He was

diagnosed with stable angina pectoris four years earlier but did not receive treatment. The anamnestic information about trauma required the exclusion of a chest wall lesion, but at the same raised the problem of a cardiac lesion. Objective examination did not reveal ecchymoses or rib fractures, lung sounds were normal, elements confirmed by the normal appearance of chest X-ray (fig. 1).

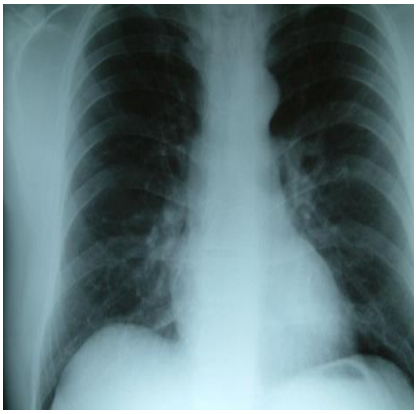

Fig. 1. Posterior-anterior chest X-ray. No bone and pleuropulmonary lesions, normal cardiothoracic ratio

The patient was hemodynamically stable (blood pressure of 140/80 mmHg, a resting heart rate of 64 beats per minute), no organic heart murmurs were detected, and radial pulse was symmetrical. The surprise but also the defining element for the diagnosis was the ECG suggestive of extensive acute anterior wall ST-segment elevation myocardial infarction (STEMI) (fig. 2).

The diagnosis of STEMI was supported by the prolonged chest pain and positive hs-TnI (2.4 ng/mL, normal values < 0.016 ng/mL). Emergency echocardiography provided consistent data for the positive diagnosis by highlighting akinesia of the apical half of the anterior wall and interventricular septum with moderate systolic dysfunction (EF of 35%). Echocardiography did not reveal acute valvular lesions, pericardial effusion and aortic dissection as secondary injuries to chest trauma. The patient was referred for primary percutaneous coronary intervention (PCI) (fig. 3A). The thrombotic occlusion was detected in the mid-segment of the left anterior descending artery (LAD). PCI with drug-eluting stent was performed successfully (fig. 3B) and TIMI 3 flow was achieved.

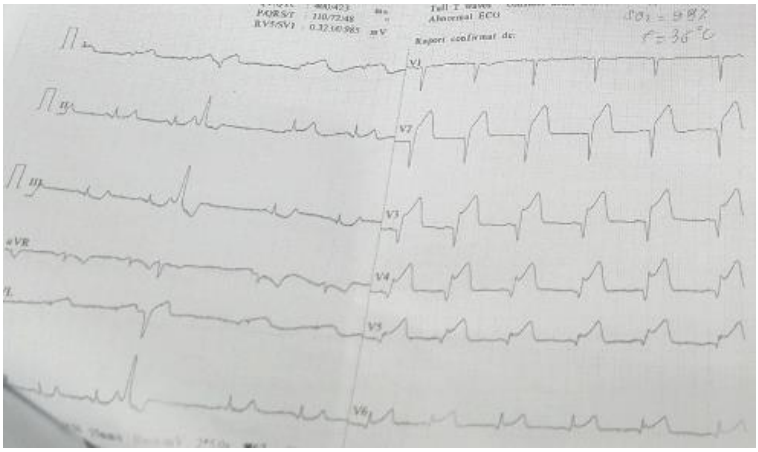

Fig. 2. ECG - extensive acute anterior wall acute myocardial infarction

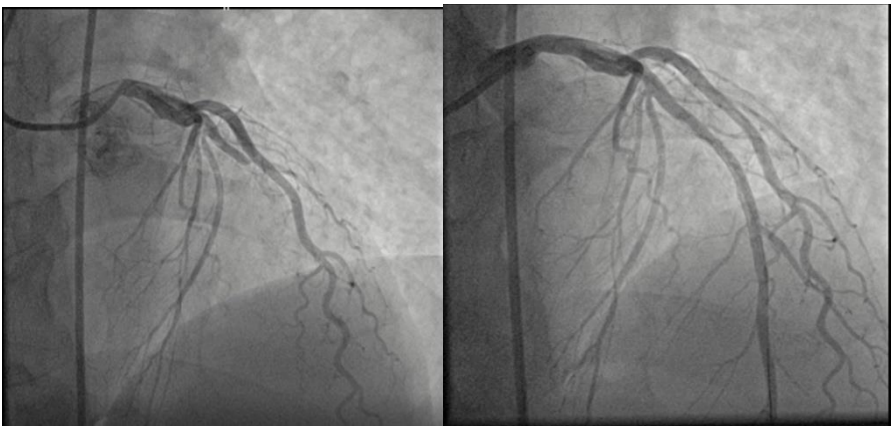

Fig. 3. A. Coronary angiography-right anterior oblique view

Thrombotic occlusion in the mid-segment of LAD. **B**. Post-stenting appearance

The course after revascularization was not complicated, the patient being hemodynamically stable, with no residual pectoral angina and no arrhythmias. ECG pattern was suggestive of subacute extensive anterior STEMI (fig. 4), and before discharge of chronic anteroseptal myocardial infarction.

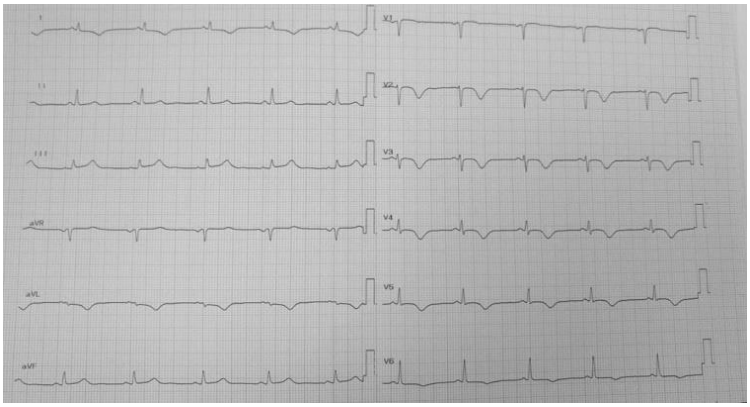

Fig. 4. ECG post PCI.
Appearance of subacute extensive anterior myocardial infarction

After revascularization, the balance of cardiovascular risk factors is completed. Total cholesterol and HDL-cholesterol were within normal ranges (200 mg/dL and 69 mg/dL respectively), but LDL-cholesterol above target values in a high cardiovascular risk patient (110 mg/dL), triglycerides 110 mg/dL, blood glucose level 94 mg/dL, creatinine 0.78 mg/dL were found. Worth mentioning was the persistence of echocardiographic features of systolic dysfunction (ejection fraction - EF of 35%). The patient was discharged with the recommendation of avoiding intense physical activity and high temperatures, eat a moderate sodium diet, and quit

smoking and drinking. Dual antiplatelet therapy with Aspirin 75 mg/day and Ticagrelor 90 mg b.i.d. for 12 months, Atorvastatin 80 mg/day, supplemented with the treatment of cardiac failure - Ramipril 5 mg/day, Bisoprolol 2.5 mg/day, a fixed-dose combination of furosemide and spironolactone 50/20 mg/day were recommended. A follow-up evaluation was scheduled at 3 months.

DISCUSSION

Cardiac injury after chest trauma is not very common but can be life-threatening. The prevalence is about 5-15% of chest traumas, 15% of them being documented at autopsy, but most of them remain undiagnosed. The most frequent cardiac complications are myocardial necrosis, arrhythmias and pericarditis or tamponade, frequently produced after road accidents (1). Acute myocardial infarction occurs in 5% of patients with blunt chest trauma, but it is associated with a significant risk of morbidity and mortality (2). The most frequent causes of coronary occlusion reported in the literature are *de novo* stenosis by external compression and subintimal edema, subintimal hemorrhage, spasm, thrombosis or rupture of a preexisting atherosclerotic plaque, compression by epicardial hematoma. Coronary artery laceration and dissection were also described after chest traumas (3, 4).

The most frequently involved vessel is the anterior descending artery, due to its anatomical position, being located proximal to the thoracic wall, as in the presented case. The right coronary artery is particularly vulnerable at its origin especially due to acceleration/deceleration injury. The circumflex artery and the left main trunk are rarely involved (5, 6) (tab. I).

TABLE I
Frequency of coronary artery involvement in chest trauma

Anterior descending artery	76%
Right coronary artery	12%
Circumflex artery	6%
Left main trunk	1%

The mechanisms by which posttraumatic cardiac injuries are produced are not very clear. Compression of the anterior thoracic wall along with the deceleration shear forces would be responsible for the arterial intimal injury (7, 8).

Our patient was a smoker and reported a history of untreated angina pectoris, which is likely to cause atherosclerotic lesions. However, *in situ* thrombosis cannot be excluded. Literature data mention the production of myocardial infarction several hours after trauma (9), as in the presented case. Patients with myocardial contusions may describe angina pain not relieved by analgesics or dyspnea, but these symptoms are nonspecific.

The investigation of blunt cardiac injuries always starts with a 12-lead ECG, a sensitive method considered the best predictor of diagnosis. In fact, 40-83% of patients with myocardial contusions have abnormal ECG. Therefore, ECG is a class I recommended diagnostic test for blunt cardiac injury (10). If ECG is abnormal, the cardiac enzymes (troponin I/T) can be measured in dynamics, although their positive predictive value is low in case of trauma. However, the association between normal troponin I/T and normal ECG at 4-6 hours after chest trauma rules out myocardial contusion (11, 12). For this reason, the determination of cardiac enzymes is considered a test with negative predictive value. According to the diagnosis algorithm proposed in the literature (13), if ECG and

troponin are abnormal another diagnostic method is required.

Echocardiography is considered the most useful and cost-effective (class II recommendations) because it can directly confirm the type of structural lesion and the aortic dissection (13). In some situations, transesophageal echocardiography is superior (13). In the presence of segmental wall motion abnormalities, the ECG changes are suggestive for STEMI and enzymatic dynamics confirms its diagnostic value. Cardiac enzymes have no high specificity in case of trauma, but correlated with ECG and echocardiography and, last but not least, with history may be valuable for diagnosis (11). The most useful one remains hs-TnI, levels above 1 ng/mL supporting a diagnosis of myocardial infarction in the presence of regional wall motion abnormalities. Finally, if ECG is abnormal and echocardiography shows regional wall motion abnormalities and a decreased EF, a coronary angiogram has to be performed (13). It can determine whether ECG and enzyme changes are caused by myocardial contusion *per se* or by the myocardial infarction secondary to a coronary artery injury.

Only if coronary angiogram is normal, nuclear imaging (SPECT, PET) may be indicated. The presence of concurrent perfusion and metabolism defects can make a diagnosis of myocardial infarction (14). To accurately identify the mechanism of myocardial infarction, advanced intracoronary imaging methods such as optical coherence tomography or intravascular ultrasound, unavailable in the presented case, may be used (15).

The persistence of systolic dysfunction after myocardial revascularization in a patient with a single coronary artery lesion and TIMI 3 flow raises the problem of a preexisting cardiac damage, most likely of toxic etiology, given the positive history of alcohol consumption. On the other hand, myocardial contusion *per se* can directly affect the systolic function by compromising myocardial perfusion.

The optimal management of posttraumatic myocardial infarction is not well defined because of the relatively low incidence of these cases and difficulty in conduction large randomized trials. Therefore, the decision must be made on a case-by-case basis considering the fragile balance between bleeding and thrombotic risks. Thrombolytic therapy required when optimal timing for primary PCI was exceeded is contraindicated due to the hemorrhagic risk of associated lesions. The preferred strategy remains as for any STEMI an emergency coronary angiography and reperfusion therapy. Revascularization by primary PCI remains the best treatment option in patients without internal bleeding, because it allows blood flow restoration in the culprit artery affected vessel and is minimally invasive (5-7, 15). A review of literature based on PubMed data on the prevalence and treatment of myocardial infarction after blunt chest trauma reported 24 cases (17 men and 7 women, mean age 43.6 ± 12.6 years) in the last 5 years (17). Of these, 3 patients (12.5%) were treated medically, 2 patients (8.3%) with coronary artery bypass grafting, and 1 patient (4.1%) received systemic thrombolysis after prolonged resuscitation. Urgent PCI was performed in 10 subjects (41.6%): 5 patients received a drug eluting stent, 4 patients a bare metal stent, and in one case the implanted device was not specified. Thromboaspiration was performed only in two cases. In contrast, 8 patients (33.3%) re-

ceived delayed PCI (24 hours to 15 days after trauma). This strategy was primarily adopted to avoid serious or fatal bleeding complications, those patients requiring noncardiac surgery (17).

It is recommended that all patients with documented posttraumatic cardiac injury should be reassessed after 3-6 months as late cardiac complications such as atrioventricular conduction disorders, cardiac rupture, heart failure, and pericarditis progressing to pericardial constriction may occur. As to the specific long-term treatment, there are controversies about the safety and usefulness of statin therapy in posttraumatic myocardial infarction (18). Thus, the administration of statins should be decided according to the cause and mechanism of myocardial infarction.

CONCLUSIONS

The possibility of coronary artery injury should be considered in all patients with anterior chest pain following blunt chest trauma, because late diagnosis and management can lead to serious complications or even death. Posttraumatic myocardial infarction remains a challenge because it raises the problem of the mechanism of production and has management particularities. The sequence ECG - cardiac enzymes – echocardiography - coronary angiography must be used according to the patient to differentiate the role of a blunt cardiac injury from a myocardial infarction secondary to a coronary lesion. Interdisciplinary collaboration is recommended, the interventional cardiologist being indispensable in management decisions.

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NEWS

BALOXAVIR MARBOXIL: THE NEW INFLUENZA DRUG ON THE MARKET

Annually, influenza viruses have a major impact on global health, causing 3-5 million severe cases of disease and 1 million deaths. Although vaccines for influenza are available, poor annual vaccine coverage, low immunogenicity in the elderly population, and occasional vaccine mismatches means that they do not deliver adequate levels of protection. For this reason, influenza antiviral drugs are essential and are used for both treatment and prophylaxis. For the first time in nearly 20 years there is a new class of antiviral drug for influenza. The latest approved antiviral is baloxavir marboxil (trade name, Xofluza) which targets the endonuclease function of the viral PA polymerase subunit and prevents the transcription of viral mRNA. The most promising aspect of this new drug is its pharmacology which allows for effective treatment of influenza A or B virus infection with just a single dose. A clinical trial showed greater reductions in viral loads with baloxavir marboxil treatment compared with oseltamivir, although no difference in the time to alleviation of symptoms between these two drugs. Baloxavir marboxil was well tolerated with only 4.4% of patients reporting minor adverse events related to the trial regimen, such as diarrhea and nausea. A similar number of patients reported the same adverse events for the placebo (3.9%) and oseltamivir (8.4%) groups, mitigating any concern that these events are unique to baloxavir marboxil. Similarly, only a few non-serious adverse events were reported during phase I and phase II trials with baloxavir marboxil, including a phase I trial looking for adverse events in 18 healthy adults treated with combinations of baloxavir marboxil and oseltamivir. Future developmental milestones for baloxavir marboxil include approval for use in children under age 12, and in patients with risk factors for influenza complications. A phase III clinical trial to address the latter is underway (CAPSTONE-2). Also, the efficacy of baloxavir marboxil and NAI combination therapies and the ability to improve clinical outcome when starting treatment after 48 hours has yet to be evaluated in a clinical trial. With this new class of influenza drug comes exciting prospects for combination therapy with the neuraminidase inhibitors which may help to abate concerns about the development of resistance (O'Hanlon R., Shaw ML. Baloxavir marboxil: the new influenza drug on the market. *Current Opinion in Virology* 2019; 35: 14-18).

CLINICAL AND ECHOCARDIOGRAPHIC PROFILE IN PATIENTS WITH END STAGE LIVER DISEASE

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CLINICAL AND ECHOCARDIOGRAPHIC PROFILE IN PATIENTS WITH END STAGE LIVER DISEASE (Abstract): The purpose of our study was to describe and better understand the clinical aspects of cirrhotic patients from a cardiologic perspective, to observe the correlations between clinical and echocardiography findings and to evaluate the prognostic impact of cardiac involvement in the end stage liver disease. **Material and methods**: We enrolled for this study a number of 83 patients that were devised according to MELD and Child-Pugh score. All study participants underwent a complete clinical examination and were assessed by echocardiography focusing on chambers dimensions, walls thickness, E/A ratio, left ventricular ejection fraction and pulmonary artery systolic pressure (PAPs). **Results:** The most frequent cardiac sign was edemas (34.9%), which was not correlated with the MELD score (p=0.118) but had an influence on the mortality rate (p=0.043). PAPs was higher among patients with edemas (mean value 29.34 mmHg; p=0.001), dyspnea (34 mmHg; p=0.012) and heart murmurs (36 mmHg; p=0.001) also if left atrium dilation was present (28.81±10.02 *vs.* 22.13±8.95, p=0.002). **Conclusions:** The echocardiographic parameters assessed in cirrhotic patients were not predictors of mortality and were not influenced by the severity of liver cirrhosis. **Keywords:** CIRRHOTIC CARDIOMYOPATHY, LIVER TRANSPLANT, PULMONARY ARTERY HYPERTENSION, LIVER CIRRHOSIS, ECHOCARDIOGRAPHY.

The close connection and interaction between the heart and the liver have been known for many years. The complex relationship between the two organs can be classified into several categories: heart diseases affecting the liver, liver diseases affecting the heart, including hepatopulmonary syndrome and portopulmonary arterial hypertension, and conditions affecting both the heart and the liver by having common etiological factors (1).

The end stage of liver disease has a systemic impact that negatively contributes to the patient prognostic. Cirrhotic cardiomyopathy (CCM) is defined as reduced contractility in condition of stress, diastolic dysfunction (DD) and associated electrophysiological abnormalities. Liver cirrhosis

is characterized by marked peripheral vasodilatation and a hyperdynamic circulation. In this hemodynamic context CCM is often subclinical and it is very easily overlooked. However, the cardiovascular events have a major contribution on morbidity and mortality associated with liver transplantation, being the third leading cause of death in liver recipients (2). Thus, we highlight the importance of early diagnosis of CCM by routine evaluation of the cardiovascular function in cirrhotic patients.

The aim of the study was to assess the echocardiographic parameters on cirrhotic patients according to the severity of the disease.

MATERIAL AND METHODS

Study population

The study was conducted as a cross-sectional analysis over a three-year period (2016-2019) and included patients with liver cirrhosis admitted in a tertiary center. A total of 83 cirrhotic patients (62 males) aged between 26-75 years, that had undergone a complete echocardiographic examination were evaluated. We excluded the cirrhotic patients with pre-existing cardiovascular disease. A close follow-up was performed and the mortality was checked during the first 12 months.

The diagnosis of cirrhosis was based on clinical, hemodynamic, biochemical parameters, ultrasound imaging. All the patients had a detailed medical history, physical examination, blood pressure measuring, electrocardiogram recording and echocardiography evaluation. Cardiac examination and echocardiography were performed by a single experienced cardiologist. The severity of liver disease was appreciated using Child - Pugh (CP) and Model of End-Stage Liver Disease (MELD) scores.

We excluded patients who refused to have a complete cardiological evaluation, patients with pathological conditions that prevented a correct echocardiographic examination (thoracic surgery, extreme obesity, severe osteo-articular chest pain), patients with neuro-psychiatric pathologies or special socio-cultural beliefs who refused a physical exam or who didn't sign the written consent.

The study was conducted in accordance with the Helsinki Declaration and all patients were consecutively enrolled to the study after providing written informed consent that was initially approved by the local Ethics Committee.

Echocardiographic parameters analysis

Transthoracic echocardiography was performed in all the patients. Measurements were made in M mode according to the European Society of Echocardiography. The following parameters were determined: diastolic diameter (Dd), systolic diameter (Ds), thickness of the walls. Ejection fraction (EF) was the most widely used parameter of global left ventricular systolic function. It was calculated using end-systolic and end-diastolic volumes, by the formula: $EF = (Dd^3 - Ds^3) \times 100 / Dd^3$ and we considered normal value EF between 60-80%. The visual estimation of the EF (eye balling) was used in parallel with the determinations using the diameters without significant differences. Diastolic function was appreciated in our study in 2D Echo and M Mode (dimensions and function of LA and LV, LV - wall thickness, the pattern of the interventricular septum (IVS) movement). Non-invasive assessment of DD was classically based on the echocardiographic analysis of mitral inflow pattern using pulsed-

wave Doppler. The impaired relaxation pattern is characterized by a decrease in E wave velocity, prolongation of E-wave deceleration time, and an increase in A wave velocity resulting in an inverted E/A ratio (< 1).

Statistical analysis

Statistical analysis was carried out using the *SPSS 20.0* software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation or number of cases with percentage, for continuous and ordinal variables. Cross-tabulation and Pearson Chi-Square test were used for describing the relationship between two categorical variables. The one-way analysis of variance (ANOVA) was used to determine the significant differences between the means of continuous variables and an independent categorical variable. For all data, a two-sided p value < 0.05 was considered statistically significant.

RESULTS

The study included 83 patients with liver cirrhosis, 56 patients in the age group of >50 years, the majority of patients being males (62 patients). In our study the etiology was mostly viral (virus B -6 patients; virus C - 29 patients, virus B+D - 23 patients) and alcoholic (16 patients, alcoholic and viral C -4 patients). We also had 5 patients with autoimmune etiology and one patient with cryptogenic cirrhosis. 31 patients (37. 35%) were Child-Pugh score C, and 32 patients (38.56%) Child-Pugh score B. The mean MELD score was 15.9±8.97, ranged between 7-30. Most of the patients (72.29%) were included on the transplant waiting list and 13 patients (15.67%) were transplanted, while 17 patients died (20.49%) during the study. The mortality rate was higher among patients with MELD score ≥ 20 (46.2%, p=0.002) and Child-Pugh class C (35.5%, p=0.008). All data can be found in table I.

TABLE I

MELD score correlations with echocardiographic parameters

Parameter	MELD<10 (n=17)	MELD=10-19 (n=40)	MELD≥20 (n=26)	p
EF (%), mean ± SD	56.47±12.76	59.85±10.45	63.92±7.24	0.060
LV Dd (mm), mean ± SD	47.94±8.25	49.00±7.30	47.88±6.76	0.795
IVS (mm), mean ± SD	11.12±1.55	10.71±1.29	10.96±1.37	0.551
LVPW (mm), mean ± SD	10.24±1.25	10.18±0.98	10.81±1.92	0.180
PASP (mmHg), mean ± SD	24.59±11.81	25.23±10.06	26.81±9.01	0.744
LA diameter (mm), mean ± SD	45.76±9.47	48.35±8.51	46.15±8.21	0.462
E/A ≤ 1, n, (%)	13 (76.5)	17 (42.5)	8 (30.8)	**0.010**
E/A >1, n, (%)	4 (23.5)	23 (57.5)	18 (69.2)	**0.010**
Viral etiology, n, (%)	15 (88.2)	28 (70.0)	14 (53.8)	**0.050**
Alcoholic etiology, n, (%)	2 (11.8)	5 (12.5)	8 (30.8)	0.056
Alcoholic and viral etiology, n, (%)	0 (0)	2 (5.0)	3 (11.5)	0.077
Mortality rate, n, (%)	2 (11.8)	3 (7.5)	12 (46.2)	**0.002**

Abbreviations: EF-ejection fraction; LV Dd- left ventricle diastolic diameter; IVS-interventricular septum; LVPW- left ventricle posterior wall; PAPS- pulmonary arterial systolic pressure; LA- left atrium; SD-standard deviation.

The echocardiographic assessment revealed the presence of LV hypertrophy in 27 patients (32.54%) with a moderate degree (LVPW between 12-13mm and IVS between 12-14mm). There were not significantly differences in the IVS or LVPW thickness between the MELD and CP subgroups. The LV Dd was abnormal in 15.6% of patients (between 59-70mm on males and 53-70mm on females), indicating a dilated cardiomyopathy. The most frequent etiology among patients with dilated LV was viral (42.9%-viral, 21.4% - toxic, 28.6% - viral and alcoholic), but the viral cause did not have a considerable influence on LV diameter. The alcoholic etiology was present in only 21.4% of the patients with dilated LV, in comparison with 17.4% patients without dilated LV (p=0.052). The LV dilation was associated with atrial fibrillation (AF) (21.4% *vs.* 4.3%, p=0.050) and a lower LVEF (49.43±17.51 *vs.* 62.67±6.36, p=0.001), but did not have an influence on the mortality rate (21,4% *vs.* 20,3%, p=0,693). The values of LV Dd did not significantly differ between the MELD (p=0.795) or CP class (p=0.682) subgroups. Also, 78,6% of patients with dilated LV associated a dilated LA (p=0.024). Table 1 shows MELD score correlations with echocardiographic parameters.

The LA was dilated in 43 patients (51.81%), without being influenced by the etiology or the liver disease severity, with similar values of LA diameter among MELD (p=0.462) and CP class (p=0.130) subgroups. Dilated LA was associated with significantly higher values of PASP (28.81±10.02 *vs.* 22.13±8.95, p=0.002) and IVS thickness (11.26±1.30 *vs.* 10.46±1.33, p=0.007), but without correlation with the mortality rate (18.6% *vs.* 22.5%, p=0.738). AF was with predilection more present in patients with dilated LA (p=0,004). The E/A ratio did not differ significantly depending on the LA dilation (p=0.45).

In our study group, 55 patients (66.27%) presented valvular regurgitations (mitral, tricuspid, aortic, pulmonary), the most frequent being mitral regurgitation in 45 patients, followed by tricuspid regurgitation in 39 patients.

Pulmonary artery hypertension (PAH), as PASP >35mmHg, was present in 41 patients (49.40%). PASP was significantly more elevated among patients with edemas (29.34 mmHg; p=0,001), dyspnea (34 mmHg; p=0.012) and heart murmurs (36 mmHg; p=0.001). The PASP did not correlated with CP score (r= +0.033; R^2=0.0011; p=0.769) or MELD score (r= +0.038; R^2=0.0015; p=0.730). Also, there was not any statistically association between the value of PASP and mortality rate (p=0.606) or cirrhosis etiology (p=0.402).

The EF ranged between 15-80% with an average value of 60.49 ± 10.30%. 59 patients (71.09%) had a normal EF. A systolic dysfunction with an EF ≤50% was identified in 11 patients (13.26%), while 12 patients (14.46%) from the study group presented a hyperkinetic heart with EF ≥80%. The values for EF were slightly higher in the MELD≥20 (p=0.060) and CP class C subgroups, but without significantly differences (p=0.183).

The E/A ratio was >1 in 45 patients (54.22%) and ≤1 in 38 patients (45.79%) from the study group. E/A ratio >2 suggestive for a restrictive diastolic pattern was not identified in our patients. The E/A ratio ≤1 was more frequently present in patients with MELD score<10 compared with the two others MELD subgroups (76.5% *vs.* 42.5% *vs.* 30.8%, p=0.010). On the contrary, the E/A ratio >1 prevailed in cases with

MELD scores >20 (23.5% *vs.* 57.5% *vs.* 69.2%, p=0.010). The mortality rate was not influenced by the E/A ratio (26.3% *vs.* 15.6%, p=0.664).

The heart rate (HR) ranged between 50-110 beats/min, the average HR was 70.82 ± 11.23, 77 patients from the total group study were in sinus rhythm (92.8%), 7 patients (8.45%) had atrial tachycardia, only 6 patients were on AF (7.2%) and 7 patients (8.45%) had premature ventricular contraction. There was observed an indirect of moderate intensity correlation between the values of HR and EF (r= -0.286; p=0.009), 28.6% patients associated elevated HR with reduced EF.

DISCUSSION

The complex pathophysiological changes that take place along with liver cirrhosis also have an impact on the cardiovascular system in the form of CCM. The diagnosis of CCM involves an impaired contractile responsiveness to stress, DD and electrophysiological abnormalities in the absence of known cardiac disease. By its effects, according to recent studies, this entity was associated with poor survival outcome independently of liver disease severity, being an important prognostic factor, which brings into focus the need for a positive diagnosis at the earliest possible stage (3).

Due to marked peripheral and splanchnic vasodilatation in the context of increased production/activity of vasodilator factors and decreased vascular reactivity to vasoconstrictors, cirrhotic patients may display low BP with a bounding, raised pulse and symptoms of reddish skin, palmar erythema. In early stages, the reduction in systemic vascular resistance is compensated by an increase in cardiac output and activation of vasoconstrictor systems which may keep the effective arterial blood volume and the arterial blood pressure in the normal range (4, 5).

The echocardiographic exam included measurements of heart chambers, the evaluation of systolic and diastolic function and the quantification of PASP. The end-stage liver disease is known to induce remodeling of the heart with structural changes: LV hypertrophy, LV dilation, LA enlargement (6-8). In the current study, LV hypertrophy was present in only 27 patients, and we did not obtain a correlation between the thickness of IVS or LVPW and MELD/CP scores. The discrepancy between our study results and previous studies may be explained by the small number of cases, larger studies being needed for a more precise correlation.

The DD often present in cirrhotic patients may cause an increase in LA pressure or volume overload which may lead to LA remodeling and enlargement (9). Being related with a higher risk of AF and cardiovascular events, the high prognostic value of LA enlargement in population without cirrhosis is already known (10-14). Hence, we assessed the LA size for predicting outcome in patients with end stage liver disease. Our study showed that patients with LA dilation had higher rates of AF and greater values of PASP. Even if in our cases the mortality rates were not influenced by the LA size, maybe due to the relatively small study group and limited follow up time, the presence of AF, PAH and dyspnea in these patients may predict a negative outcome. The LA enlargement prognostic significance in cirrhosis is supported by a study that identified dilated LA as a predictor of hepatopulmonary syndrome (15). Also, another study published by Merli *et al*. concluded there is an influ-

enced 2 years survival among liver cirrhotic patients by LA volume and LV mass (16). The importance of routine evaluation of the LA size, which reflects the cumulative effect of LV filling pressure over time, may be even greater than the assessment of diastolic function by Doppler parameters that may be altered by transient changes in loading conditions.

End stage liver disease may frequently associate DD. The pathogenesis may involve the sodium and fluid retention present in cirrhosis and modulation collagen configuration, followed by a combination of mild myocardial hypertrophy, fibrosis, and subendothelial edema (17-19).In our study we evaluated the diastolic function by trans mitral Doppler echocardiography, as an easily accessible diagnostic tool. There is a lot of scientific data supporting the significant relation between DD and survival rates in end stage liver disease (20, 21). Our results showed contrary to our expectation, a MELD score ≥20 associated with an E/A ratio >1, and a linear decrease in the E/A ratio (≤1) and the MELD score. From these data, we can't exclude the presence of DD in patients with end stage liver disease, as E/A ratio >1 may reflect a normal ventricular relaxation and diastolic function, but also a moderate DD in pseudo normal phase. If the latter is the case, than the grade of DD was significantly influenced by the advanced liver disease, results consistent with the larger studies on this topic (22). However, for more accurate results the preferred imaging techniques are tissue Doppler echocardiography and cardiac magnetic resonance imaging (20).

Most often, due to reduced afterload, the systolic ventricular function is normal at rest, being evident only in condition of physical or pharmacological stress (23-25). Most of the patients from our study group had a normal EF, only a minority of cases presenting impaired systolic function. However, for a better appreciation of subclinical systolic dysfunction the most sensitive and accurate method is 2D speckle-tracking strain analysis which was not available at the moment of our study (26).

PAH may be a common complication in the end stage liver disease, part of the portopulmonary hypertension (POPH) syndrome. The main pathological abnormality consist in progressive pulmonary vascular remodeling due to multiple changes present in cirrhosis (27-29). In the present study, almost half of the patients associated PAH (49.40%), but this parameter was not correlated with the severity of liver disease or mortality rate. Other studies also confirmed that the severity of liver disease may not be a factor of influence for the PASP (30).The clinical signs of right-heart failure may be an essential clue of PAH. All of our patients that presented high PASP values associated clinical manifestations like dyspnea, edemas, heart murmurs. The presence of edemas was also associated in our patients with higher mortality rates, which may be an indirect outcome predictor. The measurement of PASP is particularly important in patients undergoing liver transplant (LT), as the perioperative mortality rate is higher in patients associating PAH or POPH. Also, these patients are candidates for vasomodulator therapy which may improve long term survival (31, 32).

Our study has several limitations. Although the LA diameter is more widely employed in clinical practice, the measurement of LA volume is a more reliable estimator of LA size and a more accurate predictor of cardiovascular events. For a proper evaluation of systolic LV function

in cirrhotic patients the recommendations are to study the EF after an induced stress, and by 2D speckle tracking strain analysis as well. Also, the diastolic dysfunction may be better appreciated if the tissue Doppler is applied.

CONCLUSIONS

In the setting of end stage liver cirrhosis, the coexisting cirrhotic cardiomyopathy may have an impact on the overall prognosis and liver transplant success. Thus, the screening and monitoring of the cardiac function in cirrhotic patients is mandatory, even if they are asymptomatic. The echocardiographic assessment may bring valuable information concerning the impact of the disease on cardiovascular system. In our study, the echocardiographic parameters were not predictors of mortality and were not influenced by the severity of liver cirrhosis.

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ASPECTE PRACTICE ALE SEPSISULUI CU POARTA DE INTRARE ORALĂ

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PRACTICAL ASPECTS REGARDING ORAL SEPSIS (Abstract): Infections of the oral cavity are an important morbidity problem due to their frequency, their pluribacterial ethiology as well as their potential severe evolution, especially with the immunodepressive. The odontogenic infections are considered to be the cause of 40% of the deep throat infections. The infections of the submentonier space occur through direct extension from the mandibular incisors, those of the sublingual space start from the anterior mandibular teeth and those of the retrofaringian space start from the mandibular molar teeth. **Material and method**: The present study included 24 pacients diagnosed with oral sepsis in Iaşi Infectious Diseases Clinic. We analysed the ethiology and the clinical response to the administered antibiotherapy. **Results**: In several immunodepressed patients (with diabetus melitus, neutropenia, liver disfunction, carcinomas, tuberculosis) was noted sistemic evolution of odontogenic infection and they mainly came from the Surgery Clinics, where surgery was performed for the drainage of collections. Etiology was confirmed în 8 cases reflecting normal oropharyngeal flora. These infections were treated with antibiotics în synergistic combinations (betalactamines, quinolones, Cloramfenicol) with good results în 19 cases. **Conclusion**: Infections of the oral cavity are an important morbidity problem due to their frequency, their pluribacterial origin as well as their potential severe evolution, especially in immunodepressed patients. **Key words**: ORAL INFECTIONS, ORAL SEPSIS

CORRELATIONS BETWEEN GRAYSCALE AND HISTOPATHOLOGICAL PROPRIETIES OF CAROTID ATHEROSCLEROTIC PLAQUE

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CORRELATIONS BETWEEN GRAYSCALE AND HISTOPATHOLOGICAL PROPRIETIES OF CAROTID ATHEROSCLEROTIC PLAQUE (Abstract): Identifying patients at risk for ischemic cerebral events is an important concern for vascular surgeons. Previous studies have identified the ultrasonic echo lucent plaques as vulnerable, with a higher probability to cause transient ischemic attacks (TIAs) or ischemic strokes. Grayscale median (GSM) is an ultrasound-based risk marker that measures atherosclerotic plaque echogenicity. Its value is still disputed in the literature. **Aim:** This prospective study aimed to determine if GSM is a good predictor for carotid plaque vulnerability. **Material and methods:** Included in this study were 49 consecutive patients with indication for carotid endarterectomy (CEA). Ultrasound scans were performed on admission and stenosis was measured. In all study patients GSM was measured on the ultrasound-acquired images. CEA was performed and the collected specimens sent for histopathologic examination. Ultrasound and histopathological findings were compared. **Results:** GSM measured after image normalization revealed a high heterogeneity of carotid plaques (median 48, range 8-130). Of the total 49 carotid atherosclerotic plaques examined 14 presented complications, being classified as unstable lesions, *American Heart Association* (AHA) type VI. GSM < 37 strongly correlated with lipid-rich necrotic core (LRNC), risk factor for plaque vulnerability. No correlation was found between GSM and any other histopathological findings (GSM vs type V/VI plaque ($p = 0.838$), GSM vs other histopathological markers of plaque instability such as ulceration ($p = 0.73$) and intraplaque hemorrhage ($p = 0.29$)). **Conclusions:** Although ultrasound remains the best non-invasive method for the assessment of carotid atherosclerotic plaques, GSM measurement can be time-consuming and has little additional value in assessing plaque vulnerability.
Keywords: GRAYSCALE, CAROTID ATHERO-SCLEROTIC PLAQUE.

The high rate of strokes, 1.4 million/year and consequently the high mortality of 1.1 million/year among Europe citizens is alarming (1, 2, 3). The most common cause of TIA or stroke is carotid artery and vertebral artery atherosclerosis, or thrombosis and the neurological deficit can occur suddenly, in "full health" (4). The

management of atherosclerotic carotid and vertebral artery disease is one of the most studied and discussed topic in the last years at each international vascular surgery meeting and was the topic of 2 major trials: The North American Symptomatic Carotid Endarterectomy Trial (NASCET) and The European Carotid Surgery trial (ECST) (5, 6). Identifying patients at risk for ischemic cerebral events is an important concern for vascular surgeons. Ultrasound is preferred for carotid artery imaging due to its accessibility, low cost and non-invasiveness (1). The association of B-mode and Doppler color flow imaging increases the possibility of quantifying the degree of stenosis (1, 7).

Previous studies have identified the ultrasonic echo lucent plaques as vulnerable and associated them with a higher probability of causing TIAs or ischemic strokes. GSM is an ultrasound-based risk marker that measures atherosclerotic plaque echogenicity (8, 9). Its value is still disputed in the literature. This prospective study aimed to determine if GSM is a predictor of carotid plaque vulnerability.

MATERIAL AND METHOD

Included in this study were 49 consecutive patients admitted to the Department of Vascular Surgery of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital between January 2015 and April 2018 with indication for CEA. There were 38 male and 11 female patients, median age 66 years old (range 54-80), 34 patients being over age 65 years at the time of diagnosis.

The patients were selected from the cases with a high probability of advanced internal carotid artery (ICA) lesion referred from neurology departments, and from those with peripheral vascular disease already admitted to our department who screened positive for significant carotid artery stenosis.

The inclusion criteria were asymptomatic ICA stenosis greater than 70%, recent history of transient ischemic attack (TIA) and ICA stenosis of 50-99% with a history of stroke maintaining self-sufficiency.

The exclusion criteria were autoimmune diseases, hypothyroidism, osteoporosis, hepatic insufficiency (GOT higher than 2.5 U/L), chronic renal disease (creatinine above 2.0 mg/dL), ischemic heart disease classes III, IV NYHA, atrial fibrillation, malignancies (10).

All patients underwent a new Doppler ultrasound examination using a Siemens X 700 ultrasound system. B-mode settings for carotid exploration were saved and used each time trying to ensure uniformity throughout the study (fig. 1).

Patients were examined supine with the neck rotated 45 degrees to the opposite side. A peripheral vascular transducer was used.

Carotid arteries were examined bilaterally, both in transverse and longitudinal planes (using an angle < 60°). Stenosis was measured according to the NASCET method and was calculated as the ratio between minimal residual lumen diameter at the stenosis as the numerator and the diameter of the disease-free ICA segment above the stenosis (where the vessel walls were approximately parallel) as denominator, in correlation with Intrastenotic flow (5, 7). The images were stored and transferred to a computer in order to calculate GSM for all study patients.

All images were normalized according to the method proposed by El-Atrozy *et al*. in order to eliminate gain-induced variability of echogenicity (9). The enhanced ultrasound images were processed using the computer software Adobe Photoshop and

selecting two reference points: blood (black) and adventitia (white) (fig. 2). The GSM of blood was set to 0, while the GSM of adventitia was set to 255. To determine the GSM for each carotid plaque the best segment for visualization was selected followed by the use of the histogram function of the software (9, 10) (fig. 3).

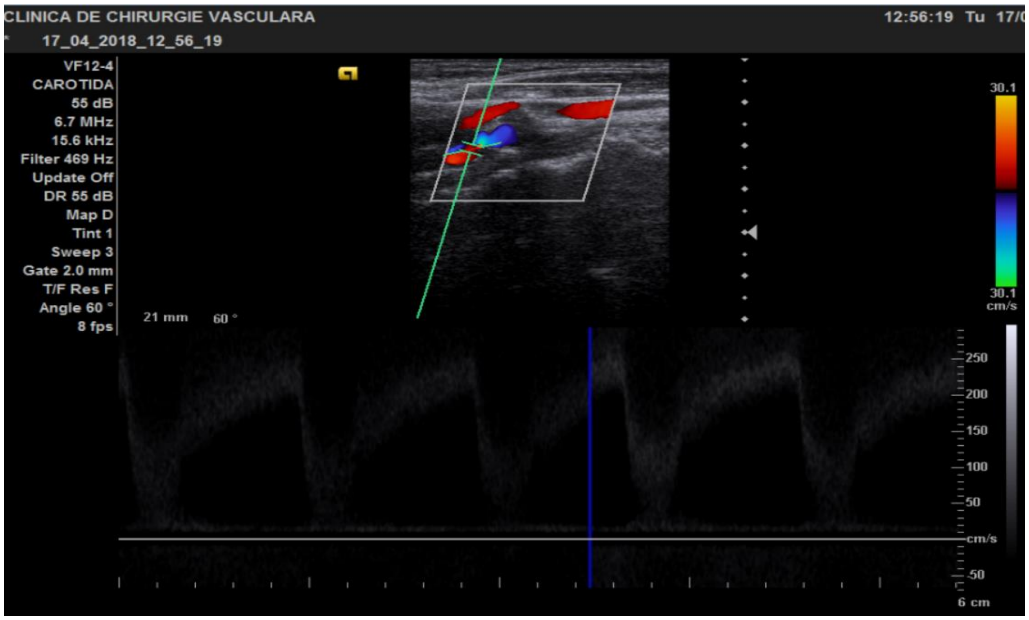

Fig. 1. Intrastenotic blood flow velocity at ICA bifurcation

Fig. 2. Post-normalization US image

Fig. 3. Histogram acquisition returning GSM value

All patients underwent carotid endarterectomy and patch angioplasty. Carotid endarterectomy specimens were processed by the standard paraffin embedded histology technique at the Histology Laboratory of the Iasi University of Medicine and Pharmacy. For each fragment, representative sections were selected and stained with

hematoxylin and eosin. The lesions were analyzed by two pathologists who classified the atherosclerotic plaques according to the AHA criteria (11).

The microscopic examination of carotid ATS plaques was focused on three main components, namely the cells (smooth muscle cells, macrophages and other leukocytes), extracellular matrix and intra- and extracellular lipids. Also determined was the presence or absence of the vulnerability factors (inflammation, LRNC, intraplaque hemorrhage or ulceration).

According to the histological criteria, the carotid ATS plaques belonged to two categories: stable, that includes type IV (atheroma) and type V (fibroatheroma) AHA lesions, and vulnerable, that comprises type VI AHA lesions (with erosions of the endothelium or fibrous cap, ulceration or intraplaque hemorrhage).

Statistical analysis was performed using *SPSS* software, Chi square descriptive test compared the ultrasound vulnerable plaques with GSM <=37 with the histopathologicaly-proven vulnerable plaques, AHA class VI.

RESULTS

Ultrasound scanning diagnosed 33 patients with 70-99% carotid stenosis, 14 patients with 60-69% and only 2 patients with 50-59% carotid stenosis, the last 2 patients having an indication for carotid endarterectomy due to recent symptomatology.

GSM measured after ultrasound image normalization revealed a high grade of heterogeneity of plaques. GSM ranged from 8 to 130, median 48 (tab. I).

Values in the first quartile ranged from 8 to 37. Unstable plaques were more heterogenic, with higher transparency and lower GSM than stable plaques. Quartile 25 included 13 plaques with increased ultrasound-based vulnerability and higher echo transparency. Quartile 50 and Quartile 75 included 36 echogenic, stable plaques according to ultrasound criteria.

TABLE I
GSM value quartile distribution

Min	Q25	Median	Q75	Max
8	37	48	59	130

Of the total of 49 carotid ATS plaques, 14 were classified as unstable AHA type VI lesions (fig. 4, fig. 5), with the following complications: intraplaque hemorrhage caused by the rupture of vessels in the granulation tissue of the atheroma in 2 cases, bleeding and ulceration in 5 cases and areas of erosion or irregular endothelial ulceration caused by plaque necrosis in 7 cases. The other 35 plaques were considered stable AHA type V lesions.

Inflammation and necrosis of the atheromatous core, considered risk factors for plaque vulnerability, were found in 17 and 13 lesions, respectively.

Dystrophic calcification was present in 39 of the 49 ATS lesions.

The plaques classified as vulnerable/stable by GSM and according to histopathological criteria were compared assuming that a lower GSM will be associated with histopathological plaque instability (tab. II).

We found no direct correlation either

between GSM (<=37 *vs.* >37) and type V or type VI plaque (p = 0.838), or the presence of each histopathological instability marker, namely ulceration (p = 0.73) and intraplaque hemorrhage (p = 0.29). Also, no statistically significant differences were found between GSM values and inflammation (p = 0.739).

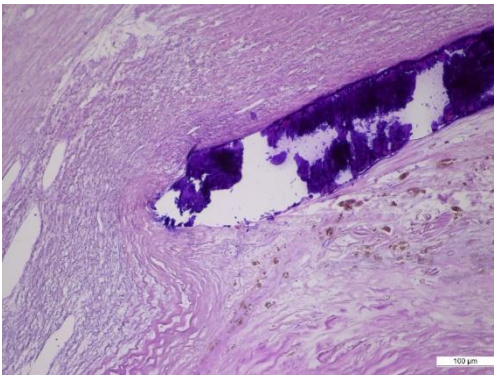

Fig. 4. Fibro lipidic plaque-compact calcification, destruction of internal elastic lamina (IEL) and marked compression of the media; adjacent to macrophage calcification loaded with hemosiderin pigment and neovascularization components, HEx100

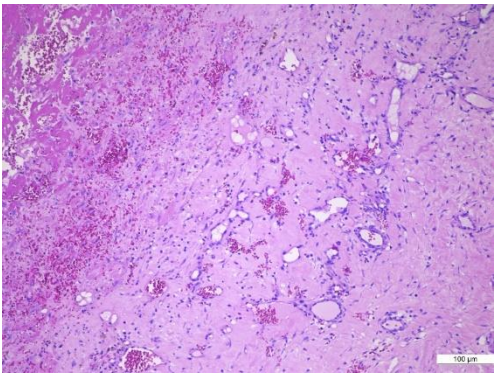

Fig. 5. Fibro lipidic plaque-intraplaque hemorrhage, numerous capillaries of neoangiogenesis, associated inflammatory infiltrate, HEx100.

TABLE II

GSM vs AHA plaque type - Cross tabulation

	Histopathological type		TOTAL
	Stabile	Vulnerable	
GSM>=37 0 →	26	10	36
GSM<37 1 →	9	4	13
TOTAL	35	14	49

GSM<37 strongly correlates independently with lipid-rich necrotic core, a morphological risk factor for plaque vulnerability. Of the 49 carotid plaques 13 showed signs of necrosis and 8 of these had GSM < 37 (p = 0.01).

DISCUSSION

Although the histopathologic examination is the "gold standard" for identifying and staging ATS lesions, this examination can be performed only postoperatively as confirmation based on the morphological substrate. In this context, a constant concern of specialists in vascular surgery is the implementation of alternative non-invasive diagnostic methods, which to allow the identification of vulnerable carotid ATS lesions. The rapid development of imaging techniques (Doppler ultrasound, carotid angiography, angio-CT, angio-MRI) has led to real progress, facilitating the primary

diagnosis and guiding the surgeon in making a therapeutic decision. Duplex ultrasound is "the firs line" imaging method for carotid artery (1). The Society of Radiologists recommended during the 2003 Ultrasound Consensus Conference that the final carotid artery ultrasound report should include velocity measurements, grayscale and color Doppler findings (12).

The relationship between echolucent plaque, plaque instability and presence of symptoms is proven (13). Echolucent plaques are more prone to rupture than dense fibrous echogenic plaques and are more common in patients with symptomatic cerebrovascular disease (14, 15).

Plaques with a higher GSM score are predominantly calcified plaques and are more stable and associated with a lower rate of cerebrovascular events (8). The GSM median of 48 and also the maximum at 130 show that most of our study patients had echogenic plaques. Also, in comparison with the results from other studies, our study showed higher median GSM values (8, 10). This is a consequence of the incidental discovery of carotid stenosis and that patients tend to present with more advanced disease or long after the symptomatic period (the first 6 months after an ischemic event). This fact is also confirmed by the predominant presence of AHA type V plaques in our study group.

Accumulation of lipid-rich necrotic debris and smooth muscle cells are present in advanced carotid plaques. Unstable plaques usually have a thin fibrous cap with a necrotic core situated juxta luminal. Our study supports a prior finding that lower GSM values are associated with lipid-rich necrotic core (16, 17). In presence of ulceration the necrotic core is exposed, the ATS plaque is vulnerable, predisposing to thromboembolic events.

However, full overlap of diagnostic imaging classes with histopathologic diagnosis classes has not been obtained, yet. Our study highlights the discrepancies between the classification of the plaques into the unstable, vulnerable versus stable categories by using ultrasound and histology, respectively. Our results demonstrate the limitation of ultrasound imaging for the assessment of plaque vulnerability. Despite the great benefits of ultrasound examination, as a non-invasive method, the vascular surgeon must be aware that GSM results cannot offer a perfect, point to point mirror of the morphological background that characterizes the plaques in their biological environment.

No carotid restenosis was detected during the first 12 months of ultrasound follow-up. The patients are still under surveillance, the follow-up aiming to detect the possible occurrence of restenosis at 24-year follow-up and to correlate the recurrence with GSM and histological characteristics.

CONCLUSIONS

High-grade carotid stenosis, GSM higher than 37, and AHA type V carotid ATS plaques predominantly identified in our study group show that patients tend to present with more advanced disease. GSM < 37 strongly correlates with intraplaque necrosis, but in the absence of other correlations with the histopathological vulnerable plaque characteristics it leads to the conclusion that although ultrasound remains the best non-invasive method for assessing the carotid ATS plaques, GSM measurement can be time-consuming with little additional value in evaluating plaque vulnerability.

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THE MANAGEMENT OF A HOSPITAL-ACQUIRED INFLUENZA AH_1N_1 OUTBREAK IN ONCOLOGICAL PATIENTS. A CASE SERIES REPORT

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THE MANAGEMENT OF A HOSPITAL-ACQUIRED INFLUENZA A H_1N_1 OUTBREAK IN ONCOLOGICAL PATIENTS. A CASE SERIES REPORT (Abstract): Despite prevailing concerns about the risk of influenza infection in immunocompromised patients, the actual rate and effect of infection in this population are not well characterized. We report a fulminant course of a hospital-acquired influenza AH_1N_1 infection in a public hospital for oncological patients. From a total of 60 persons (40 patients and 20 symptomatic health care workers - HCWs) screened by collecting nasal and pharyngeal swab samples, a number of 33 (24 patients and 9 HCWs) were found positive for respiratory viruses. Of them, 23 cases had influenza A virus (20 patients and 3 HCWs), all contacts with in-hospital influenza cases. The 4 first cases were diagnosed with influenza type A and subtype H_1N_1. In the epidemiological context we presumed all the other influenza A patients had the subtype H_1N_1. 9 influenza A patients were managed in the ICU: 5 patients for severe and 4 patients for mild/moderate ARDS. Oseltamivir treatment was instituted in all symptomatic patients and HCWs and in asymptomatic high-risk immune-compromised contacts. All ICU influenza patients and contacts were strictly isolated, had dedicated vaccinated HCWs wearing protective equipment and received oseltamivir treatment for prolonged periods until virus clearance or death. 5 patients died: 3 patients due to severe ARDS and intractable hypoxemia and 2 patients due to unrelated causes. The influenza-related death occurred in the first patients due to late diagnosis and late antiviral treatment institution. The epidemiological measures are described along with the hospital-acquired influenza outbreak course. **Keywords:** INFLUENZA, OUTBREAK**,** ONCOLOGIC.

The influenza virus is one of the most common human respiratory viruses associated with recurrent infections, which results in high population morbidity and, in the case of seasonal influenza, high mortality (1).

In March 2009, the influenza AH_1N_1 virus strain produced an outbreak of febrile respiratory infection worldwide and few months later World Health Organization declared influenza AH_1N_1 pandemic: more than 500,000 cases were reported in more than 214 countries and there were 18,849 deaths in that period (2).

High rates of influenza infection and

complications occur among people with impaired immune defense. Despite prevailing concerns about the risk of influenza infection in this type of patients, the actual rate and effect of infection in this population are not well characterized (3). Also, it is difficult to diagnose acute influenza infection in immunocompromised patients, who frequently develop neutropenic fever and are able to develop infections with almost any known pathogen. Doctors should stay alert to the possibility of influenza infection, especially in febrile immunocompromised patients during annual epidemic and should initiate antiviral treatment without delay (4). Influenza mortality is very high among this population, with reported case fatalities among cancer patients of 5 to 33% (5, 6, 7, 8).

CASE SERIES REPORT

We retrospectively recorded features of an influenza outbreak in the Regional Institute of Oncology (RIO) Iași, Romania during January-February 2018.

RIO Iași is a public 300 bed university affiliated hospital, dedicated to oncological patients and consists of 7 departments (Oncology, Hemato-oncology, Radiotherapy, Surgery, Anesthesia and Intensive Care and Palliation), some of them sharing the same floor. Patients are located in 1 to 4 bed wards. At the beginning of the influenza outbreak, 209 patients had long-term hospitalization. At that time, influenza viral infection was not reported in the city of Iași and our institution had no virus detection test, nor oseltamivir drug in the hospital's pharmacy.

Definitions - An **influenza A viral infection** was defined in a person, patient or health care worker (HCW), with an acute respiratory illness compatible with influenza A and confirmed by a positive polymerase chain reaction (PCR) test.

A **hospital-acquired influenza A viral infection** was defined in a person, patient or HCW, who developed symptoms 48 h following hospital admission or contact exposure in the hospital.

An **influenza A viral carrier** was an asymptomatic person with positive PCR test.

We used the Cepheid Xpert Xpress Flu/RSV assay, which is an automated, multiplex real-time, reverse transcriptase PCR assay intended for the in vitro qualitative detection and differentiation of respiratory viruses (influenza A, B, and respiratory syncytial virus).

From a total of 60 persons (40 patients and 20 symptomatic HCWs) screened by collecting nasal and pharyngeal swab samples, a number of 33 (24 patients and 9 HCWs) were found positive for respiratory viruses.

Of them, 23 cases were positive for influenza A virus, 2 influenza A and B virus, 6 influenza B virus, 1 influenza B and syncytial respiratory virus and 1 syncytial respiratory virus.

Out of the 23 influenza A cases there were 20 patients and 3 HCWs.

Out of the 2-concomitant influenza A and B cases: 1 patient and 1 HCW.

The infected HCWs consisted of 1 ICU nurse and 3 auxiliary ICU personnel, having mild/moderate illness treated at home, all contacts with the first ICU case (fig. 1).

Our first case was a 33-years-old male, diagnosed with a high-risk T cell acute lymphoblastic leukemia treated with induction chemotherapy in June 2017 and consolidation courses in September 2017. He was hospitalized from the 9th of January 2018 in Hemato-oncology Department for

continuation of consolidation chemotherapy and developed medullar aplasia on the 20th of January. On the 21st of January 2018 he was admitted to ICU for severe acute respiratory failure, 4 days after the onset of respiratory symptoms. Unlike the majority of immune-compromised patients admitted to our ICU with septic shock and multiple organ dysfunction, This patient had severe isolated acute respiratory distress syndrome (ARDS) caused by an interstitial pneumonia with negative results at direct microscopy, broncho-alveolar lavage cultures, broncho-alveolar lavage PCR for *Aspergillus spp.* and *Mycobacterium tuberculosis*. The clinical data and the negative respiratory cultures raised the suspicion of influenza infection. On the 25th of January „Dr. I. Cantacuzino" National Institute of Research-Development for Microbiology and Immunology, Bucharest confirmed the influenza type A subtype H_1N_1 infection. A hospital epidemiological alert was instituted considering the nosocomial type of the infection and the presence of high risk immunocompromised oncological patients.

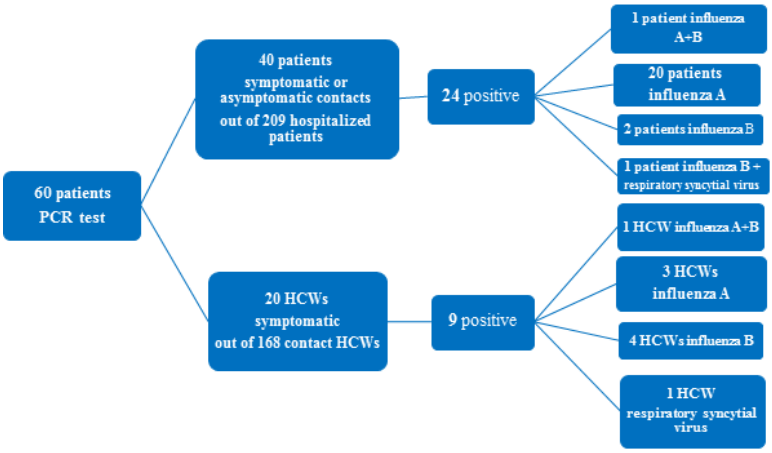

Fig. 1. Distribution of PCR tested cases

In the next 2 days we tested and diagnosed 3 other ICU influenza A H_1N_1 cases using remote (out-of-hospital) assays. The virus strain detection was performed only in the first four cases. Giving the epidemiological context, we considered the following detected influenza A viruses having the same subtype.

In response to the four potentially linked cases, an outbreak was declared. The epidemiological alert was upgraded, visitors' and students' access was denied, daily clinical examination of patients and HCWs for respiratory symptoms was performed, isolation of influenza patients and contacts were instituted, HCWs' appropriate hand hygiene and wearing surgical face masks all the time all over the hospital were mandatory. HCWs with symptoms were advised to stay off work until fully recovered. On the same time the hospital purchased rapid PCR tests for influenza detection and antiviral treatment (oseltamivir) in sufficient proactive supply. Oseltamivir treat-

ment was rapidly instituted in all symptomatic patients and HCWs and in asymptomatic high-risk immune-compromised contacts. The institution vaccination lists were checked and unvaccinated, asymptomatic HCWs received influenza vaccine. Hospital admission of patients scheduled for elective treatments or surgery were postponed.

The peak of newly diagnosed influenza A cases per day was on the 2nd of February 2018 (8 cases). The last influenza case was diagnosed one month after, on the 23rd of February 2018 (fig. 2).

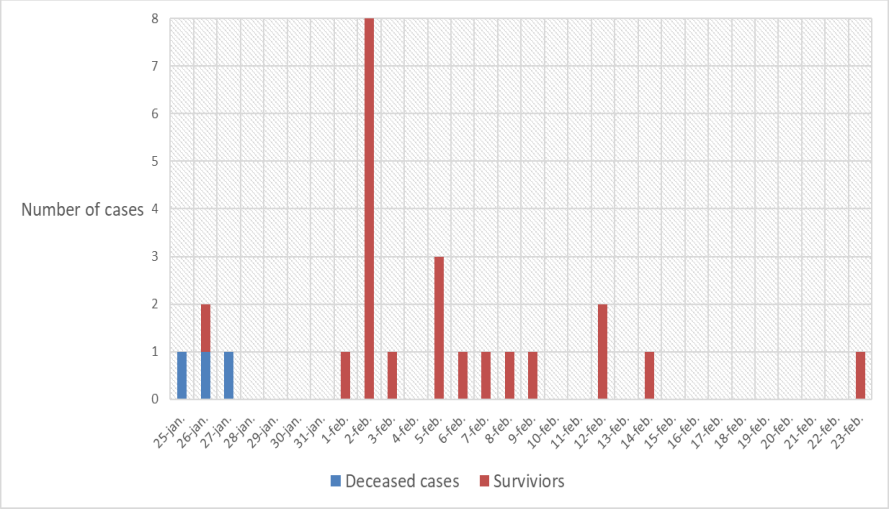

Fig. 2. Temporal dynamics of newly diagnosed influenza A cases and their influenza-related outcome

None of the infected patients and only 1 infected HCW had been previously immunized with the seasonal influenza vaccine.

A total of 9 patients with hospital-acquired influenza A were managed in the ICU, including the first case that was diagnosed: 5 patients for severe and 4 patients for mild/moderate ARDS (fig. 3).

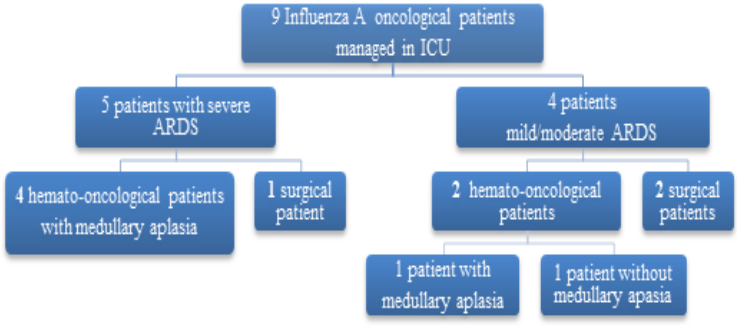

Fig. 3. Patients diagnosed with Influenza A managed in ICU

The patients with severe ARDS were: 4 medical (hemato-oncological) patients with post-chemotherapy medullary aplasia and 1 surgical patient with gynecological surgery. All cases needed ventilatory support: non-invasive ventilation (2 patients, worst PaO_2/FiO_2 54-94 mmHg, survivors) or invasive mechanical ventilation in prone position (3 patients, worst PaO_2/FiO_2 29-46 mmHg, deceased). Extracorporeal membrane oxygenation was not available in our ICU and the number of concomitant cases exceeded the capacity of available machines throughout the country. All ICU influenza patients and contacts were strictly isolated, had dedicated vaccinated HCWs wearing protective equipment and received oseltamivir treatment for prolonged periods until virus clearance or death. Mean duration of hospital oseltamivir treatment in survivors was 15.2 (10-21) days. Median ICU length-of-stay (LOS) was 13 (3-24) days in non-survivors and 15 (7-22) days in survivors. The 4 hemato-oncological patients with medullary aplasia had severe ARDS managed with non-invasive (1 patient survivor) or invasive mechanical ventilation (3 patients, non-survivors). All had the resolution of medullary aplasia during ICU stay. One single non-survivor had positive broncho-alveolar lavage cultures with *Pseudomonas aeruginosa* after 23 days of mechanical ventilation in prone position.

We performed PCR testing for virus clearance after resolution of respiratory symptoms. 3 patients out of 6 survivors had positive PCR when discharged from ICU and continued oseltamivir treatment until clearance.

The mortality rate of influenza A patients was 23% (3/13 positive symptomatic patients) due to severe intractable hypoxemia. 2 symptomatics (mild respiratory symptoms) contact patients died due to unrelated causes (1 patient due to septic shock and multiple organ dysfunction, peritonitis by ileal perforation post oncologic surgery for invasive synchronous colon neoplasm; 1 due to hemorrhagic shock in the context of myelo-monocitary chronic leukemia).

DISCUSSION

We describe the course of a hospital-acquired influenza A H_1N_1 infection outbreak in a group of immunocompromised patients in the Regional Institute of Oncology from Iași, Romania. Immunocompromised patients frequently develop neutropenic fever and are at risk of infection with almost any known pathogen, so the diagnosis of influenza infection can be difficult.

It is important to stress that at that time there was no outbreak of influenza in the general population and the rapid PCR diagnostic test was not available. It is very likely a single patient was the origin for influenza outbreak because screening test positive patients were from the same ward or wards who shared the same floor. Patients were housed in 2-4 beds wards and walked within the floor, increasing the opportunity to transmit infection. The clustering of initial cases suggests the Hemato-oncology Department as the source of outbreak and responsible for the secondary spread to the Surgery Department, with which it shares the same floor. As in the study of Drozd-Sokolowska *et al.* we could not establish the exact origin of infection: a patient could have been infected symptomatically or asymptomatically prior to admission and developed the disease (as a result of immunodeficiency or after chemo / radiotherapy) or could have been infected by an asymptomatic infected patient or HCW (4). The first 4 cases were diagnosed

at "Dr. I. Cantacuzino" National Institute in Bucharest, where the type A and subtype H_1N_1 were assessed. The other cases were diagnosed in our institution by PCR test, which detected only influenza virus type. In the epidemiological context we presumed all influenza type A patients had the subtype H_1N_1.Transmission of influenza virus occurs through aerosols, droplets or direct contact with hands and touch points. Patients, visitors and HCWs are usually involved in the transmission chain (9). We had only one influenza A and B infected patient, which was a symptomatic ICU contact patient, treated for influenza A with complete resolution of respiratory symptoms. Studies show that the co-infection with influenza A and B does not have a worse prognostic than mono-infection, this concomitant infection being mainly nosocomial (10).

Souza *et al.* showed that the median duration of viral shedding in a population of influenza A H_1N_1 infected cancer patients receiving immunosuppression was 23 days, with a range between 11-63 days (11). Among hospital admitted cancer patients with respiratory symptoms during times of an influenza epidemic, 21-33% might test positive for influenza (12). Our hospital hosts immune-compromised patients at high risk of developing severe forms of viral infection. This aspect raises another concern regarding the hospital's availability for rapid diagnosis testing. Could this allow sooner outbreak detection and limitation? At the beginning of outbreak, one deciding factor for spread and outcome was the lack of rapid diagnosis. The out-of-hospital and out-of-city confirmed diagnosis in the first 4 cases lasted 48 hours after suspicion, delaying the initiation of antiviral treatment. The facts that all these patients developed severe ARDS, 3 of them died and the nonsurvivors were only patients with delayed treatment outline the utmost importance of early diagnosis and early antiviral treatment in case of immunocompromised patients with type A influenza. Our conclusion is that influenza rapid diagnostic test should be readily available during the flu season and in hospitals with high risk patients.

After the first case diagnosis, all symptomatic influenza patients and asymptomatic PCR test positive contacts were treated with oseltamivir 150 mg/day. In immunosuppressed patients, antiviral treatment should be administrated independently of symptoms onset, unlike the general population, where its efficiency is questionable after the first 48 hours of symptoms (13). Considering the high incidence of severe forms in influenza infections and the high duration of viral shedding in immunocompromised patients, any oncological hospital should have the antiviral drug available at any time. In these patients, a 48-hour delay in receiving antiviral therapy is associated with an increased risk of developing pneumonia and greater mortality (14), as the case for our first 4 patients. We should also consider that in immunocompromised patients, symptoms are discrete or atypical, so hematologists should stay alert to suspicion of influenza infection, diagnose it early and initiate antiviral treatment without delay.

Complete recovery was considered at resolution of all influenza-related signs and symptoms, independently of the viral shedding (15). PCR retesting was made at complete recovery and before ICU discharge. 3 out of 9 ICU influenza patients were transferred on their wards with a positive PCR test. This raises another issue, concerning the isolation of these patients on their

wards and increased risk of viral transmission due to long viral shedding in immunocompromised patients.

A discussion should be made also concerning the quarantine state and measures implemented for containing and limiting the outbreak. In case of hospitals with patients at risk, as oncological hospitals, we should consider if implementation of quarantine should have been made earlier and when is the adequate moment of lifting this state. Considering that no other influenza A cases were declared at that time in our city, the hospital quarantine was instituted only after the diagnosis of the first case. But soon after, there was a rapid spread of the influenza infection through the whole country, therefore quarantine measures continued even 1 month after the diagnosis of the last case.

Efficacy of influenza vaccination in immunocompromised patients has limited and contradictory evidence. Although the immunogenicity of influenza vaccine is overall reduced, there is a trend of impaired humoral vaccine response in these patients. Despite the fact that the same immune dysfunction, that increases the risk and consequences of influenza infection might also compromise vaccine response and effectiveness, current evidence favor vaccination (8). But, despite this recommendation, rates of influenza vaccination remain low in general population and in cancer patients worldwide due to controversy regarding its effectiveness and safety and lack of physician's prescription. None of our influenza positive patients and just one of infected HCWs were previously vaccinated for seasonal flu. 2 review articles recommend vaccination every year, timed at more than 2 weeks before receiving chemotherapy or between chemotherapy cycles (16, 17). As a result of this outbreak and according to the latest guidelines for vaccination of the immunocompromised host (18), we intend to recommend vaccination as a standard policy to be implemented in our hospital for all immunocompromised patients and exposed HCWs during the season flu.

CONCLUSIONS

We describe the course of a hospital-acquired influenza A H_1N_1 infection outbreak in a group of immunocompromised oncological patients. Immunocompromised patients frequently develop neutropenic fever and are at risk of infection with almost any known pathogen, thus the diagnosis of influenza infection can be difficult. During the flu season and even out of epidemiological context suspicion of influenza infection should be considered in immunocompromised patients with respiratory symptoms. Rapid diagnostic tests and antiviral therapy should be available. Early diagnosis and early institution of treatment are determinants of influenza-related outcome. Epidemiological measures should be implemented in order to avoid and control the spread of infection within a highly predisposed community: the hospitalized immunocompromised patients.

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EARLY ENDOSCOPIC PHOTODYNAMIC DIAGNOSIS OF COLONIC LESIONS

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EARLY ENDOSCOPIC PHOTODYNAMIC DIAGNOSIS OF COLONIC LESIONS. (Abstract) **Aim:** The aim of the study is to improve the quality of early endoscopic verification of aplastic changes of the colonic mucosa by use of the photodynamic diagnosis method. **Material and methods:** The study is based on 2,817 video colonoscopies; bioptates were taken in 1,206 cases, representing 43% of the total number of endoscopic examinations. In 72 patients (44 males and 28 female) with neoplasia of the colon videocolonoscopic examination using the photodynamic diagnostics method was performed. **Results:** The results of endoscopic examination using the method of photodynamic diagnosis of 72 patients (44 male and 28 female) with neoplasia of the colon are presented. The method of photodynamic diagnostics is based on the differences of the autofluorescence spectra, which is characteristic for the unchanged mucous membrane of the colon (λ_{max}= 535-565 nm) and induced by the photosensitizer of fluorescence of morphologically atypical cells (λ_{max}=595-640 nm). **Conclusions:** The use of photodynamic diagnostics allowed to increase the accuracy, sensitivity and specificity of neoplastic processes of the colonic mucosa verification to 97.1%, 94.9% and 94.2%, accordingly, and the frequency of coincidence of endoscopic and pathohistological diagnoses 95.2%. Keywords: COLON, CANCER, NEOPLASM, DIAGNOSIS, ENDOSCOPY.

About 600,000 new cases of colorectal cancer are registered worldwide annually. In the general structure of all malignancies, the colorectal cancer possesses the fourth place in terms of morbidity and mortality with the average incidence (standardized indicator) of 13.0 cases per 100,000 population. Moreover, 44.5% of patients are diagnosed in advanced disease stages. Morphologically, the diagnosis was confirmed in 81.7%, with positive histologic findings in 78.5% of patients. About 20-35% of patients die of colorectal cancer during less than one year (1). The issue of colorectal lesions diagnostics in the early stages of disease remains relevant in modern coloproctology, considering the incidence of colorectal cancer, the presence of complications in treated patients and mortality rates among patients with this pathology (2-4), though screening techniques significantly improved the situation. Endoscopic verification of the pathological process is the "gold standard" in the diagnosis of pre-malignant diseases and malignant neoplasms of the colon (5-7). Only a visual

assessment of pathological changes of the intestinal mucosa in the early stages, even by an experienced endoscopic specialist can`t be considered sufficiently informative considering the minimal or unclear macroscopic changes in the structure of pathologically affected epithelium (8). On the other hand, endoscopic sampling of biopsy material from pathologically affected mucosa just under visual control has similar disadvantages, especially with spread mucosal processes (9). This is because, quite often, neoplastic cells have a small and multifocal mosaic type of localization within the pathologic area of the mucosa detected by endoscopist (9).

Mentioned above stimulates clinicians and researchers to find possible ways and techniques that make it possible to increase the recognition value of endoscopic diagnostics of precancerous diseases and malignant neoplasms of the colon in the early stages of development of the pathological process (2). Use of new physical approaches like laser-confocal endoscopy or chromoendoscopic techniques opens new horizons in colorectal diagnostics (10). Among them, use of fluorescent media for colorectal endoscopies is one of the promising.

The aim of the study is to improve the quality of early endoscopic verification of aplastic changes of the colonic mucosa by the use of the photodynamic diagnostics method.

MATERIAL AND METHODS

In total, 2817 video colonoscopies were performed during 2016-2019. Bioptates were taken in 1,206 cases, representing 43% of the total number of endoscopic examinations. In 72 patients (44 males and 28 female) with neoplasia of the colon videocolonoscopic examination using the photodynamic diagnostics method was performed. The mean age of the patients was 59.3±10.2 years. According to The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach and colon (11), 11 patients (15%) had a 0-Is type of neoplasia, 18 (25%) had 0-Ip, in 16 patients (22%) – 0-Isp. Non- polypoid neoplasms of type 0-IIa took place in 6 patients (8%), 0-IIb – in 2 (3%). LST-G (0-IIa+Is) neoplasms were diagnosed in 2 patients (3%), LST-NG (0-IIa) in 3 (4%). Type I polypoid cancers were found in 14 patients (20%).

The method of photodynamic diagnostics (FDD) is based on the differences of the autofluorescence spectra, which are characteristic for the unaltered colonic mucosa (λ_{max}=535-565 nm) (fig. 1) and the induced fluorescence of morphologically atypical cells with photosensitizer (λ_{max}=595-640 nm) (fig. 2).

For the photodynamic diagnostics we used the fluorescence diagnostics system SFD.M16-1S.2 manufactured by the Ukrainian National Technical University and the photosensitizer medium hypericin.

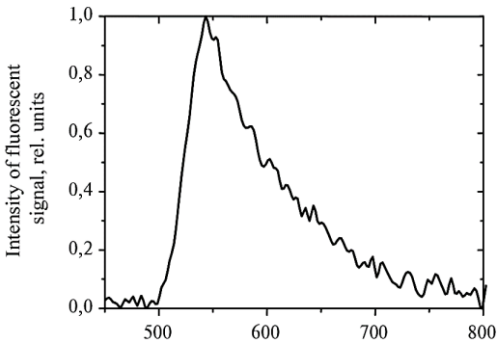

Fig. 1. Diagram of the fluorescence spectrum of the normal colonic mucosa.

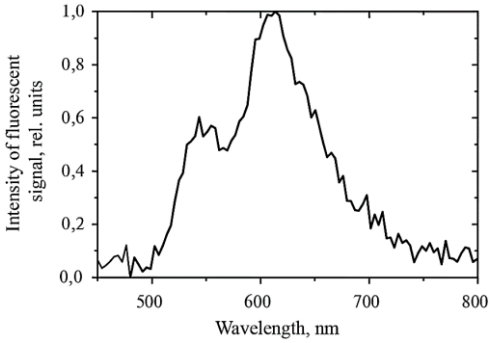

Fig. 2. Diagram of the fluorescence spectrum of the morphologically atypical cells.

The advantages of use of the photosensitizing medium are as follows:

- Oral administration of photosensitizer.
- Colonoscopy within 8-12 hours after the administration of the drug.
- The biological half-life of the drug is about 24 hours.
- The absence of the need for "shadow exposure" of the patient (staying in a darkened room) in the period after receiving a photosensitizer, that allows performing of examination in an outpatient conditions.

The advantages of use of fluorescent diagnostics system SFD.M16-1S.2 are:

- Optical radiation supply, excitation of fluorescence and collection of fluorescent signal through standard optical fibers that are compatible with the instrumental channel of serial endoscopes.
- The registration period of the fluorescence spectrum from the study area does not exceed 1-2 seconds, that does not fundamentally affect the total duration of traditional endoscopic examination.

Endoscopic examination was performed within 8-12 hours after patients receive two capsules of 8 mg hypericin. Its pharmacological action is based on the photosensitizing properties of hypericin, which selectively accumulate only in the dysplastic, metaplastic and tumor cells of the gastrointestinal mucosa and are retained in them during 4-12 hours after ingestion of the drug (2). During the video colonoscopy, the nature of the suspected pathologically affected mucosal area was visually assessed. From areas that had photosensitizer-induced fluorescence characteristics for morphologically affected cells biopsy material was taken.

For comparison, three groups of patients, comparative by age and nature of pathological changes of the colonic mucosa, whose biopsy material was collected only on the basis of visual assessment of the nature of pathological changes (28 patients), using the method of chromoendoscopy with indigocarmin solution (23 patients), and for assessment of neoplastic changes` nature in the colonic mucosa by narrow band imaging (NBI) video colonoscopy 32 patients were involved into the study.

In all cases, the final verification of neoplastic changes in the biopsy material of the colonic mucosa was performed by certified pathologist. Evaluation of the results of the study was made by comparing the data of the visual and morphological nature of pathological changes, namely the presence of dysplasia of different severity, metaplasia or malignant cells in biopsy material, obtained in the researched and control groups of patients, and calculation of statistical indicators, as sensitivity, specificity and accuracy of appropriate diagnostic methods.

RESULTS

The nature of pathology presented in patients of comparison groups is shown in first table. Analyzing the findings, it should

be noted, that endoscopic diagnostic techniques based on the use of a chromoendoscopy or NBI mode colonoscopy have a fundamental disadvantage of dependence of the result, as with conventional endoscopy, on the experience and professionalism of the physician performing the endoscopic examination.

TABLE I

The nature of neoplastic changes of the colonic mucosa in studied patients

Type of neoplasia	Colonoscopy, standard	Colonoscopy with chromoendoscopy	Colonoscopy in NBI mode	Colonoscopy with FDD	Total
0-Is	3 (11%)	3 (13%)	3 (9%)	11 (15%)	20 (13%)
0-Ip	5 (18%)	2 (9%)	6 (19%)	18 (25%)	31 (20%)
0-Isp	7 (25%)	4 (17%)	5 (16%)	16 (22%)	32 (21%)
0-IIa	2 (7%)	2 (9%)	4 (13%)	6 (8%)	14 (9%)
0-IIb	2 (7%)	2 (9%)	2 (6%)	2 (3%)	8 (5%)
LST-G	2 (7%)	1 (4%)	2 (6%)	2 (3%)	7 (4%)
LST-NG	0 (0%)	1 (4%)	1 (3%)	3 (4%)	5 (3%)
Cancer I	7 (25%)	8 (35%)	9 (28%)	14 (20%)	38 (25%)
Total	28 (100%)	23 (100%)	32 (100%)	72 (100%)	155 (100%)

In contrast, the method of photodynamic diagnostics enables real-time differentiation of the unchanged and pathologically affected areas of the gastrointestinal mucosa more objectively, and, accordingly, to detect neoplastic processes on the early stages reliably and in time, with the decision of further treatment. In this case, the degree of dysplastic changes of tumor has a direct dependence on the intensity of the fluorescent signal from the pathological area, which may be an additional factor in verifying the focus with the maximal pathological changes in the mucosa.

The statistics obtained during the study is shown in second table.

TABLE II

Comparative characteristics of methods for early diagnostics of aplastic processes in colon

Variable	Colonoscopy, standard	Colonoscopy with chromoendoscopy	Colonoscopy in NBI mode	Colonoscopy with FDD
Accuracy	71.4%	87.5%	93.7%	97.1%
Sensitivity	72.1%	89.7%	90.6%	94.9%
Specificity	65.7%	81.3%	83.7%	94.2%
Frequency of endoscopic and histological data congruence	68.5%	83.4%	87.3%	95.2%

DISCUSSION

Early diagnosis of colonic lesion is an important, though difficult issue (2, 3). This study shows benefits of the endoscopic photodynamic diagnostics of colonic lesions. While this method shows multiple benefits compared to standard endoscopy of confocal endomicroscopy (9), several short comes and limitations were revealed, similar to previously published papers (5, 10). Among the disadvantages of the photodynamic diagnostics method revealed during the study are the possibilities of influencing the quality of the fluorescence signal of the blood in the lumen of the intestine and the influence on the intensity and quality of the signal at a distance from the mucosa at which the fiber is located. The latter should be not more than within a few millimeters.

The technique of photodynamic diagnostics made it possible, during endoscopic examination in real time, to collect biopsy material precisely from focuses in which dysplastic, metaplastic or malignant cells are localized, even in the mosaic location within the pathological focus of the colonic mucosa for the further pathohistological examination of biopsies. The results indicate that photodynamic diagnostics can be considered as a highly informative screening method for early verification of neoplastic changes of the colonic mucosa.

CONCLUSIONS

The use of proposed photodynamic diagnostics allows to increase the accuracy, sensitivity and specificity of verification of neoplastic processes of the colonic mucosa to 97.1%, 94.9% and 94.2%, respectively. Photodynamic diagnostics is a modern, highly informative screening method of early endoscopic detection of aplastic colonic processes with the frequency of coincidence of endoscopic and pathohistological diagnoses 95.2%.

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NEWS

PEOPLE WITH PRE-EXISTING MENTAL HEALTH DISORDERS DURING THE COVID-19 PANDEMIC

Because of their life circumstances, people with pre-existing mental health disorders might have a higher risk of SARS-CoV-2 infection than those without mental health disorders. Risk factors for infection with SARS-CoV-2 and a severe course of COVID-19 include severe mental illness, alcohol or drug misuse, and homelessness, all of which are associated with other risk factors such as comorbid physical conditions. People with mental disorders are at increased risk of infections in general (and thereby potentially at increased risk of COVID-19) and are more likely to develop severe organ dysfunction and to die in ICUs than people without mental disorders. SARS-CoV-2 might also cause dysregulation of the stress system, which could contribute to the development or exacerbation of psychiatric disorders. Elderly people are at especially high risk of severe COVID-19 illness and mental-health-related consequences because they might already have some cognitive decline. Institutions can become epicenters for infection. Physical distancing can be challenging in these contexts, either because the nature of patients' conditions makes it difficult to manage (e.g., people with learning disabilities) or because of overcrowding (e.g., prisons). Increased death rates in assisted living facilities have been reported worldwide, especially among older people and people with learning disabilities. People with pre-existing mental health disorders have reported increased symptoms and poorer access to services and supports since the onset of the COVID-19 pandemic. Early discharge from psychiatric units and disruption of face-to-face psychiatric care have become common, the negative consequences of which could include relapse, suicidal behavior, lack of access to medical care, and social isolation. Quarantine and lockdown might particularly affect people with pre-existing mental health problems: increased symptoms of anxiety and depression, and high rates of post-traumatic stress disorder and insomnia have been reported (Moreno C, Wykes T, Galderisi S, *et al.* How Mental Health Care should change as a Consequence of the COVID-19 pandemic. *The Lancet Psychiatry* 2020; 7(9): P813-824).

SURGERY IN MESOPOTAMIA, EGYPT, AND CHINA

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SURGERY IN MESOPOTAMIA, EGYPT, AND CHINA (Abstract): The science of medicine has recorded a continuous evolution from the very beginning of its existence. In Mesopotamia, the health of the individuals was at the mercy of gods, and treatment was in close relation to astrology. Specialist have managed to retrieve a multitude of medical prescriptions and treatment techniques for various diseases, but no specific discovery has been made related to surgery. China practiced medicine focusing on the prevention of certain diseases, not on treating them. The best-known Chinese surgeon of the ancient era was Hua Tuo. His notoriety was conferred by the fact that he was the first Chinese physician to have used anesthesia during a surgical intervention. The Egyptian people left to posterity numerous papyri full of wise teachings and treatments for a multitude of diseases. The most interesting papyri in London is the one discovered in 1862, focusing on the topic of surgery. The science of medicine has recorded a continuous evolution from the very beginning of its existence. Each civilization has left us a heritage of a wealth of teachings, which led to medicine to be what it is today. It is impossible to establish who had the greatest contribution to the improvement of medical techniques, but it is especially important to use every research undertaken by ancient people. Although most civilizations have used magical techniques to treat the health of individuals, studies have shown that when the situation was serious, people have relied on mixtures of herbs to restore the vitality of our fellow human beings. **Keywords:** SURGERY, MEDICAL TECHNIQUES, ANESTHESIA.

As per the information available nowadays, the first decisive overcoming of the mythical-magical horizon by people involved in the healing craft occurred more or less simultaneously – two or three millennia ago – in several different regions of the Globe: in Greece, in India, and in China. Historians have still been debating passionately where exactly medicine shed its immemorial cloak of superstitions and if – at that far point in the past – certain influences may have been exercised in terms of medical practices and doctrines between the aforementioned areas of civilization (1).

The science of medicine has recorded a continuous evolution from the very beginning of its existence; the art of healing has represented a field of interest from time immemorial. History has shown that – whereas initially the surgical interventions were conducted brutally – as nations progressed, they developed a series of remedies capable of healing the human body. From magic techniques to natural remedies or drugs based on technological science,

the medical sector has been progressing constantly for thousands of years.

Mesopotamia was a territory occupied by modern-day Iraq, a part of western Iran, eastern Syria, and Kuwait; it represents a source of inspiration for the entire ancient world and for nations such as Jews, Egyptians, Persians, Phoenicians, and so on.

From an etymological perspective, the lexeme "Mesopotamia" means "the land between two rivers" and it refers to the well-known rivers Tigris and Euphrates. Specialists have managed to retrieve a multitude of medical prescriptions/recipes and treatment techniques for various diseases, but no specific discovery has been made related to surgery. Whereas some historians have argued that the lack thereof may mean the people in question did not practice such techniques, most experts do not agree with this opinion.

In this territory, medicine in practice had a mainly medical and religious nature (2). The health of the individuals was at the mercy of gods, while treatment was in close relation to astrology. Hammurabi's Code – considered the oldest law collection in the world – comprises a series of regulations for the medical sector. Therefore, should a physician treat a patient's lower limbs, the latter would have to pay five shekels; should the patient be the child of a nobleman, the price lowered to three shekels. Furthermore, concerning the ophthalmological treatments, in case of curing the eyeballs using a tool made of bronze, a physician would charge ten silver shekels. However, the writing included sanctions for practicians, too. In certain situations, a physician risked having their hands cut.

As I have mentioned above, in the Mesopotamian area there were no methods discovered directly attesting the use of surgical techniques, but archaeological finds suggest that it is impossible to deny such a reality. Thus, devices were used that seem to have derived from items normally belonging to a kitchen and made from bronze or wood. In addition, research have reported that the thorns of various trees were used by physicians to stitch the wounds. Bandages were also used at the time; they were even chosen for bone fractures.

Mesopotamian surgery seems to have had a conservative character; however, the outcomes of the procedures remain unknown to this day; there is no information related to anesthesia. Researchers suggest that wine, incense, or myrrh were used to soothe the patients' pain.

Before the year 3000 BC, Egyptian scholars kept the books for businesses, the government, and the society in their hieratical writings on the papyrus (they wrote a multitude a manuscript documents) (3). Under the influence of the Sumerian civilization, the Egyptian people left to posterity numerous papyri full of wise teachings, as well as graphical representations of various medical techniques. Among the best known, we mention here the Ebers Papyrus, the Kahun veterinarian Papyrus, the Kahun Gynecological Papyrus, the Berlin Papyrus, and the London Papyri.

Whereas the Ebers Papyrus has 110 pages and it comprises treatments for a multitude of diseases, one of the most interesting papyri in London is the one discovered by Edwin Smith in 1862, focusing on the topic of surgery. Specialists even consider it the oldest surgery treatise. This historical treasure is considerably different from any other writing discovered thus far. Organized rigorously, it contains a series of researchers mentioned for the first time in the history of mankind.

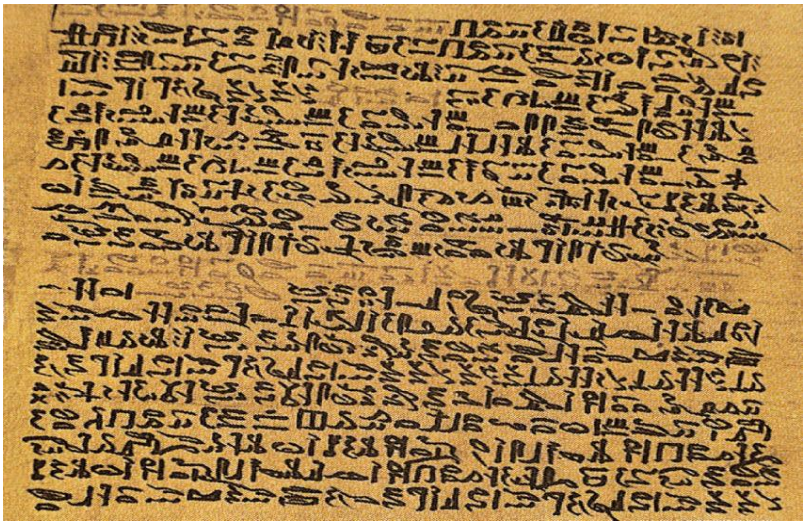

Fig. 1. The Ebers papyrus.
(Photo source: Ellis H, Abdalla S. *A History of Surgery*) (2)

The papyrus is structured on the part of the human body described; hence, the details comprise an individual's head, the chest area, and the spine. Therefore, 48 cases were described in the ancient writing, but only one mentions that the healing process involved magic. The other situations feature issues referring to certain lesions in the head area, to problems with the collarbone and the arms, to blood issues, to sternum, shoulder, or rib problems, and even to issues pertaining to the lower back area.

As for the underlying conversation in order to set a diagnosis, information may also be found among other nations of the ancient civilizations. Thus, it is worth highlighting a physician's triple mission: to assess what had happened in the past (medical history), to inquire about the present issue (current state), and to predict what would happen (prognosis) (4). Concerning the method used for examining a patient according to the Egyptian doctrine, the writing recommends a visual assessment of the person, in order to determine the type of the existing lesion. Surgeons asked various questions and then they told the patient to sit or lie in various positions or they asked the patient to perform certain movements, while the doctor assessed them both visually and olfactory, using various palpation techniques. Depending on the results of the aforementioned examination, the diagnosis fell into three categories. Hence, the first of them concerned the treatable problems; the second included diseases that a patient had "to fight"; the third comprised potentially incurable diseases, case in which the physician was exempted from any responsibility should death occur, given the seriousness of the situation.

The Egyptian era is one of the most interesting phases of universal medicine development and it was seminal in the enrichment of health and illness knowledge. Egyptians had a remarkable medical life, comprising various types of practices; they even had medical specialists and physicians with specific specialties.

Multiple sources attest to the existence of specialists in ophthalmology, dentistry, head problems, or even abdominal disorders, etc. (5).

The Chinese people are renowned for a wide array of scientific inventions that revolutionized the world. In this territory, Shen Nung developed agriculture, while Huangt Ti invented the ships, the bow and the arrows, music, and writing. The art of fishing and hunting was created by Fu Shi, while Yao and Shun outlined the calendar and the administration. Unlike other ancient civilizations, China practiced medicine focusing on the prevention of certain diseases, not on treating them. The first references strictly related to the field of surgery in this territory concern the Chou Dynasty, featuring treatments for swollen wounds, ulcers, and other diseases - traditional Chinese medicine books are known generically as Yijing (6).

The best-known Chinese surgeon of the ancient era was Hua Tuo. He penned several medical texts, and he was sent to prison by the emperor; his writings were entrusted to the prison guard. Unfortunately, they were burned when the famous physician died; his interventions were featured in various works written in that period. Besides the numerous patients healed, his notoriety was conferred by the fact that he was the first Chinese physician to have used anesthesia during a surgical intervention. It comprised a "tingling" powder combined with wine, which enabled the physician to operate on an individual and to remove the affected organs. Among his interventions, it is worth noting removing the liver, the spleen, or the intestines, as well as acupuncture or venesection procedures. His best-known procedures include the one on General Kuan Kung, who adamantly refused to be anesthetized. He had been hit by a poisoned arrow; when Hua Tuo arrived, he was playing chess and he continued the game while the physician was extracting the arrow and cleaning the wound (7).

As for his medical skills, legend says that Hua Tuo received the secrets of this art from two persons; he was advised not to ever discriminate between the rich and the poor, the young or the old, the lower or the higher social ranks (7).

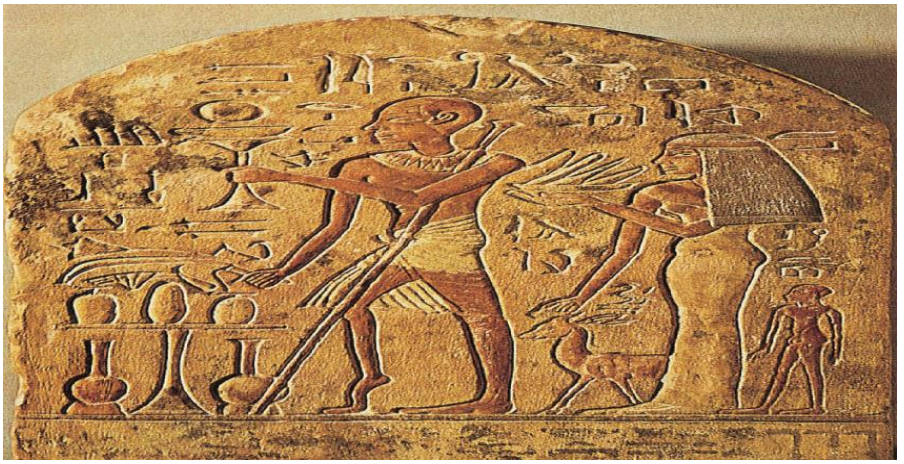

Fig. 2. An Ancient Egyptian stone relief showing a patient with obvious poliomyelitis. (Photo Source: Ellis H, Abdalla S. *A History of Surgery*) (2)

Medicine was closely connected to other fields; hence, philosophy helped people cure their illnesses without external interventions; human suffering was the source of inspiration for a rich argumentative material and even for valuable methodological suggestions (8). Each ancient civilization played an essential role in the development of the modern-day medical sector; it is thus impossible to determine who brought the most important contributions to this field. Nonetheless, certain historians argue that – whereas the Mesopotamians, Egyptians, and Chinese brought significant progresses – other civilizations, such as the Greek civilization, drew mainly on intuition, thus never managing to reach the phase of quantifying the phenomena they studied and to verify empirically the laws they managed to formulate (9). Due to the peoples who left us the legacy of numerous medical procedures, the progress recorded by modern surgery has been possible – is based on such ancient writings, abundant in useful teachings. Most of the times, the rapid pace of technology in our days makes us forget about the existence of the underlying principles in the art of healing. Without these principles, the science of medicine would have never made such tremendous progress.

CONCLUSIONS

The science of medicine has recorded a continuous evolution from the very beginning of its existence.

In Mesopotamia, the health of the individuals was at the mercy of Gods, and treatment was in close relation to astrology. Specialists have managed to retrieve a multitude of medical prescriptions and treatment techniques for various diseases, but no specific discovery has been made related to surgery.

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The Egyptian people left to posterity numerous papyri, full of wise teachings and treatments for a multitude of diseases. The most interesting papyri in London is that one discovered in 1862, focusing on the topic of surgery.

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150 YEARS SINCE THE BIRTH OF ERNEST JUVARA (1870-1933)

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150 YEARS SINCE THE BIRTH OF ERNEST JUVARA (1870-1933) (Abstract): Ernest Juvara was born in 1870, in Bârlad, Romania in a wealthy farmer family. He displayed some remarkable aptitudes and skills from early childhood. He finished his primary and high school studies at some of the most famous education institutions in Romania. In 1888 he is accepted at the Paris Medical School. There, Prof. Paul Poirier was an exceptional mentor for Juvara. From him, he learned the art of anatomic drawing. In 1895, Juvara defends his doctoral thesis in medicine and surgery and is awarded *"extrêmement bien"* by the Paris Medical School. In the same year he returns to Romania with Prof. Thoma Ionescu and begins his surgical and university career. During his stay in Paris, he managed to publish a successful anatomy book, personally decorated with multiple original anatomical drawings, and drew over 100 anatomy illustrations for demonstrations and anatomy treatise published by his mentor. His extraordinary surgical and scientific activity, organizer and inventor talent advanced him to the position of tenure professor at only 29 years of age. He is regarded as the founder of the Department of clinical surgery at Iași Faculty of Medicine and one of the orthopedics pioneers in Romania. Throughout his career he drew over 3,000 original anatomy illustrations and published over 230 articles and books. We pay tribute to one of the pillars of Romanian medicine, Ernest Juvara, a skillful surgeon, a talented professor, artist, and inventor, and especially a truly remarkable personality. **Keywords:** ERNEST JUVARA, HISTORY OF MEDICINE, ORTHOPEDICS, ROMANIAN MEDICINE.

Childhood and education

Ernest Juvara (fig. 1) was born on May 14th, 1870, in Bârlad, Romania. His father, Iorgu Juvara, was a wealthy farmer. During his childhood he was very shy, but this personality trait eventually worked in his

favor. His isolation from other children pushed him towards some remarkable activities for his age. One of his teachers, Professor Socrate Lalu, told the story of how Juvara completely disassembled and reassembled the first mechanical watch he received from his parents. The watch worked without an issue.

Aware of the remarkable aptitudes and skills he developed from early childhood, his parents sent him to the Romanian capital, Bucharest. Here he finished his primary school studies at "Urechia" Institute. He graduated from high-school at "Saint Sava" College – one of the most famous high-schools in Romania (fig. 2). According to the only 1880-1888 class book to survive the German bombardment of Bucharest in 1944, Juvara was not at the top of his class. Still, his physics and chemistry professor acknowledged his interest in applied sciences. In 1886, while still in high-school, Ernest Juvara was named laboratory preparer.

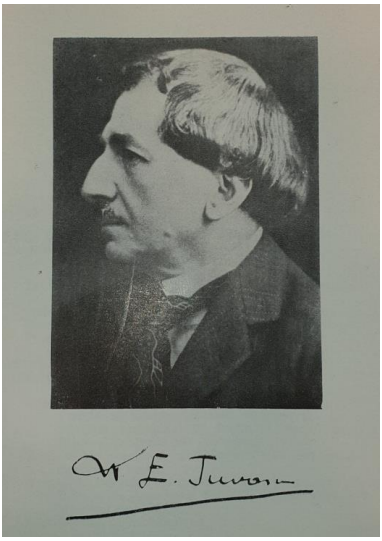

Fig. 1. Prof. Ernest Juvara and his signature
Image source: Fagarasanu I,
Ernest Juvara omul si opera, Romanian
Academy Publishing House, Bucharest, 1986
(1)

Fig. 2. "Saint Sava" National College, Bucharest (left) – founded in 1694 by Wallachian Prince and martyr, Constantin Brancoveanu (1654-1714) (right)

Student at the Paris Medical School

In 1888 he received his baccalaureate diploma in literature and sciences. In the same year, he is accepted at the Medical School in Paris. In that period, anatomy was taught by Prof. Louis Hubert Farabeuf (1841 – 1910). He often used drawings and chalkboard sketches, which inspired young Ernest Juvara to make use of his natural talent for drawing. At first, he tried to imitate his mentor and later even surpassed him. While the drawings were Prof. Farabeuf specialty, the practical demonstrations were Prof. Paul Poirier`s responsibility.

Even though, Juvara was learning from some of the best professors at the Paris Medical School, his appetite for scientific knowledge was not satisfied. He arranged a laboratory in his own home, where he continued his studies of anatomy, chemistry, and botany. At the same time, his chemistry professor, Armand Gauthier (1837-1920) offered him the position of laboratory preparer for three years (1890-1892).

Still, anatomy was his utmost passion. He was the favorite student of Prof. Paul Poirier (1853-1907) (fig. 3) who noticed his earnestness, dexterity in dissections and especially talent for anatomical drawing.

Prof. Poirier was a remarkable teacher who maintained an atmosphere of friendship in his laboratory. He often invited his students to bicycle and automobile races. This was the catalyst for Juvara who in later years was one of the first automobile owners in Romania. He remained for 5 years in Prof. Poirier`s laboratory where he met some of the greatest minds of the last century. This was a crucial decision for Juvara, who managed to meet here some of the most prominent Romanian doctors such as Thoma Ionescu (fig. 4) and Ion Cantacuzino (fig. 5).

Fig. 3. Paul Poirier c. 1893
Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

Fig. 4. Thoma Ionescu (1860-1926)
Image is in the public domain

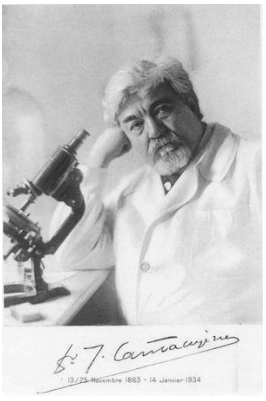

Fig. 5. Ion Cantacuzino (1863-1934)
Image is in the public domain

Passionate about the study and teaching of anatomy, in 1892, Juvara decided to write together with H. Friteau *"Quinze leçons d'anatomie pratique" (Eng. Fifty lessons of practical anatomy)*. This book contained 84 original drawings and sketches handcrafted by Ernest Juvara. The book was a great success. The two authors published five editions of this book. Later, it was even translated in German and Romanian.

In 1894, Prof. Poirier decided to publish a treatise of human anatomy. In this endeavor, he assigned the digestive tract chapter to Thoma Ionescu and the coordination of the manufacturing of all anatomical drawings to Ernest Juvara. Of his experience in Prof. Poirier's laboratory, Juvara will later write in his memoire:

"In Paris, under the influence of Farabeuf and Poirier and advised by artists such as Cuyer, Leuba, Devy and others, I caught the taste for scientific drawing. As chief of Prof. Poirier`s laboratory, I drew over 100 scientific drawings which still serve today for anatomy demonstrations at the Paris University. Later, I coordinated the manufacturing of the drawings for Poirier's anatomy treaty."

In 1895, Juvara defends his doctoral thesis in medicine and surgery *"Anatomie de la région ptérygo-maxilaire"* who was awarded *"extêmement bien"* by the Paris Medical School. Even before he finished the thesis, he was appointed assistant professor in the surgical clinic of Colțea Hospital in Bucharest and chief of the topographic anatomy laboratory, which was led by Thoma Ionescu. The two doctors were recalled in Romania through a special law in 1895.

Juvara left Paris after 8 years of studies, where he left a lasting impression on all his professors and collaborators. Prof. Poirier often said about him that he never had a more talented student than Juvara.

Return to Romania with Thoma Ionescu

He returns to Romania, together with his mentor, Thoma Ionescu in 1895. Here, Juvara began a double and tireless medical career. One was clinical surgery at Colțea Hospital in Bucharest, where he operated side by side with his mentor and the other as assistant professor at the Institute for Topographic Anatomy and Experimental Surgery where he worked every day from 2 P.M. until the evening.

As subdirector of the institute, Juvara firstly took care of its organization. He chose a new and courageous style for that time. In addition to the surgical clinic, the institute also contained a dedicated unit for research and experimentation, which was also divided in multiple departments: topographic anatomy, comparative anatomy, microscopic anatomy (physiological and pathological), physiology and experimental surgery. While he was occupied with organizing this structure, Thoma Ionescu succeeded in mustering some of the best and enthusiastic Romanian doctors for this new and exciting institute.

In 1897, Juvara publishes "*Lecțiuni de anatomie practică" (Eng. Lessons of practical anatomy)*. It was a follow-up to the successful French version he published in 1892. This book was again a great success in Romania. This motivated him to publish in 1899 a German version which now contained 183 drawings (fig. 6).

Ernest Juvara was always striving for better details in his drawings. In this endeavor, he decided to make use of photography - a new, emerging technology at the time. The first Romanian photo was shot only 50 years before in 1848. His technique

involved the hardening of the tissues using formaldehyde, sectioning, photography, and later drawing. This positioned him in the hall of Romanian pioneers of medical photography art alongside Gheorghe Marinescu (1863-1938).

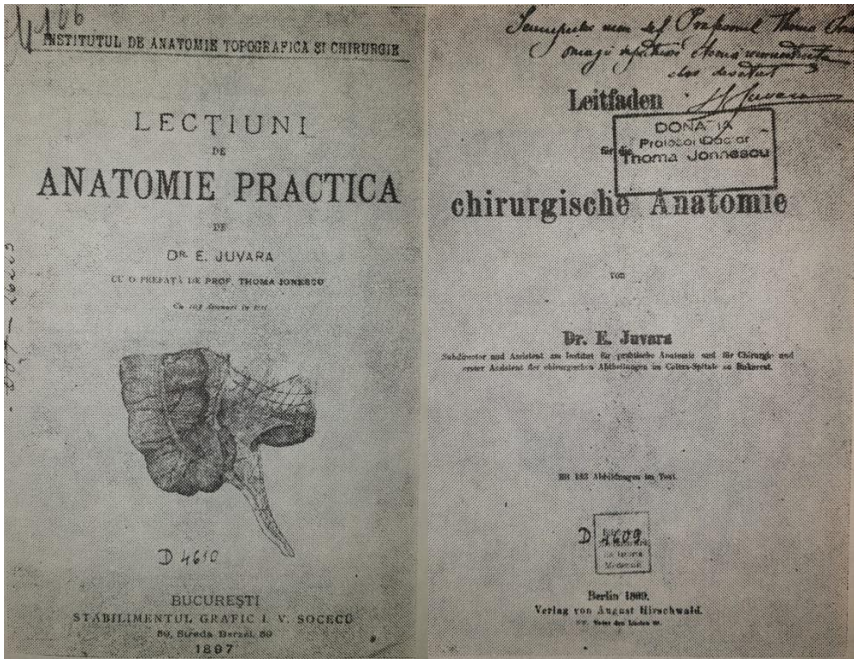

Fig. 6. First page of "*Lecțiuni de anatomie practică"* (Left) and *"Leitfaden fur die chirurgische Anatomie"* (Right)
Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

Juvara was an exceptionally talented surgeon, who created numerous procedures. Some of them were considered classic in the international scientific community. In 1897 he published *"Procedeele operatorii contra prolapsului complet al rectului si in particular despre un nou procedeu (Eng. Operative procedures against complete rectal prolapse and in particular a new procedure)"* in *"Revista de Chirurgie"*. Later, this became internationally known as the Juvara or Delorme-Juvara procedure.

He was also a prolific inventor. While under the tutelage of Thoma Ionescu, he invented multiple surgical instruments and sterilizing devices with Collin House and Adnet House in Paris and Lautenschläger House in Berlin (2).

Professor of topographic anatomy and clinical surgery at Iași

At Thoma Ionescu's initiative, Juvara applied to the position of associate professor of topographic anatomy in Iași. After passing the afferent contest, on November 1st, 1899 under Decree nr. 4414 of the Minister of Health, Juvara is named associate professor of topographic anatomy in Iași. His mentor continued to promote him in all university and academic circles. After just

5 years, on November 26th, 1904, Ernest Juvara became tenure professor of topographic anatomy and clinical surgery at Iasi Faculty of Medicine.

Under these circumstances, Juvara became tenure professor at just 29 years and founder of department of clinical surgery at Iași Faculty of Medicine. This was a well-deserved distinction for a man who had a thorough education in those two fields. After all, he was the pupil of Farabeuf and Poirier for 6 years and Thoma Ionescu for another 5 years.

As always, new beginnings are difficult. The new university department did not have an associated surgical department or even a basic laboratory. Even more so, there were no resources for course demonstrations, except cadavers who also were not stored properly.

After a short struggle, two years later, in 1906 a brand-new surgical department was established at "Saint Spyridon" Hospital in Iasi (fig. 7). Initially, only 20 beds were assigned, but it slowly grew in both size and renown. By 1910, the department had over 80 beds and Prof. Juvara performed over 5,800 surgeries.

Fig. 7. Saint Spyridon Hospital, 1845 Drawing by I. Rey *"Album de douze vues de la ville de Iassy"* Image in public domain

In those two years, he was not idle. He took upon himself the task of adorning the new university department with anatomical drawings, each piece individually handcrafted by himself. In 1900 he wrote about those drawings: *"they require a lot of my time, because I have to check them alone side by side with a cadaver and take notes that afterwards need to be scaled to larger dimensions on special paper and color them in order to highlight the most interesting part."*

His strenuous work was admired in 1906 at the National General Exhibition. Later, in 1912, when visiting the Iasi Faculty of Medicine together with King Carol the 1st of Romania, the famous German anatomist, Heinrich von Waldeyer (1836-1921), requested to be left alone to freely admire Juvara's work. This newly founded museum of surgical anatomy also contained numerous photographs of patients before the surgery, as he requested this to be standard procedure with every patient.

As a pupil of Thoma Ionescu, Juvara was the first to introduce rachianesthesia in

Iasi, without excluding the other types of anesthesia. One of his students, Ion Fagarasanu, writes: *"Personally, I have seen no complications or sequelae on the several thousand rachianesthesia performed in ten years, while I was an assistant to Juvara".*

His results were so impressive that one of his assistants, Dr. Alexandru Tzaicu (1883-1958) (fig. 8), decided to self-operate his inguinal hernia using this anesthesia method in 1909. This was the first self-operation in the world. Prof. Juvara assisted him with the rachianesthesia, while Dr. Tzaicu self-operated alone.

The experience was later published in 1911 in *"La presse medicale"* in 1911. He reported no incident, no unpleasant phenomena, or complications. As he personally writes, his motivation was to show that this kind of anesthesia is so *"[...] inoffensive that it can be performed with the patient in a sitting position, while with the cocaine anesthesia, even administered locally, is indicated that the patient must be laying horizontally. I had the conviction that the consciousness, intelligence, and the hand will not be affected by inferior injections. [...] I arranged the operating table in such a fashion that I could remain in a sitting position and have the certainty that I will remain this way no matter how anesthetized I will be".*

Ernest Juvara was the first to impose asepsis methods in Iasi and to highlight the importance of this practice to all his students. His intense clinical, surgical, didactic, and scientific activity catches the attention of the *Society of Physicians and Naturalists from Iasi* who elects him to be president in 1910. His work is recognized even by his French colleagues who appoint him corresponding member of the Paris Society of Surgeons (1911) and the National French Society of Surgery (1928).

Fig. 8. Dr. Alexandru Tzaicu self-operating his inguinal hernia, assisted by Prof. Ernest Juvara, 1909, Iasi, Romania

We list only some of his published work, while he was a professor at Iasi: *"Opération de la hernie inguinale par le procédé de Bassini"* based on a personal experience of 800 cases, *"De la création d'un vagin dans le cas de l'absence congénitale, par dédoublement de l'espace intervéssie-rectum, suivi soit d'autoplastie par manchon cutané", "Emasculation totale", "Pleurotomia cu rezectie de coastă sub lambou", "Evidement du plancher buccal"*, etc. *"Lucrari de terapeutică si clinică chirurgicală (Eng. Works of Clinical Surgery and Therapeutics)"* is his last book published while in Iasi. Grigore T. Popa, his successor, says about the deep admiration both staff and students had for Prof. Juvara *"Juvara made a cult out of being a professor and his students made a cult out of him".* Today, he is considered the founder of the modern school of anatomy and surgery in Iasi (3).

Return to Bucharest and the start of the Great War

In 1912, Ernest Juvara is transferred to Bucharest, at Filantropia Hospital as chief of the Department of Clinical Surgery. This transfer was just in time for the start of the Balkan Wars (October 8th, 1912 - July 18th, 1913). In 1913, he is mobilized by the Romanian Armed Forces in Bulgaria (fig. 9), where he offers his entire experience as organizer in the fight against a ferocious enemy: cholera. For the first time in Romanian history a large scale vaccination campaign is executed. In this endeavor, Juvara was responsible of organizing the hospitals where cholera patients were isolated.

Returned from this campaign, he continues his surgical and scientific activity at Filantropia Hospital. Aware of the imminence of the Great War, he is preoccupied with perfecting the rachianesthesia technique and with developing new surgical treatments of fractures. This motivates him to invent several exceptionally useful orthopedic devices described in his outstanding paper *"Tratamentul operator al fracturilor diafizelor prin metoda fixatorului extern (Eng. Surgical Treatment of Diaphyseal Fractures by External Fixator Method)".*

Fig. 9. Maj. Prof. Dr. Ernest Juvara in the Bulgarian Campaign (1913)
Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

The Great War begins in 1914 and Romania joins the forces of Antanta in 1916. The same year, Juvara is mobilized by the Romanian Army under the rank of Major. He is attached to the 4th Evacuation Hospital, which was under the command of the 4th Romanian Army. During the war he was dedicated body and soul to the wounded soldiers. He was later promoted to the rank of colonel (fig. 10).

Col. Dr. C. Giurea recalled *"I saw him during the war... both at Hangu and at Brosteni and Bicaz subsections, which he supervised. Many wounded owed their lives only to the tireless day and night work, peerless abilities, and devotion of Prof. Juvara. I*

often saw him, when his surgical work gave him respite, offering advice and helping with the repair of medical trucks' engines, in order to transport the wounded as soon as possible, inspiring the admiration of the mechanics and the respect of surgeons."

Fig. 10. Col. Prof. Dr. Ernest Juvara (red arrow) at the Red Cross Hospital in Botosani (1917). Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

At the end of the war, he returns to "Filantropia" Hospital, where he works until 1923 when he is transferred to "Brâncovenesc" Hospital in Bucharest. At the time, this hospital still existed and was considered the best hospital in Romania.

Here he organized the largest and most modern surgical department in Romania (fig. 11). It included over 120 beds. This clinic was later visited by the famous Prof. A. Hartmann (Paris), Prof. Fred H. Albee (New York).

Fig. 11. Prof. Ernest Juvara (red arrow) and his collaborators in the operating room at "Brâncovenesc" Hospital (1928) Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

Published work and distinctions

He continues his work in the laboratory as well. He edited a treatise of surgical anatomy meant to have 4 volumes, but in the end only 2 were published: Volume 1 - "Head and neck" (1924) and Volume 2 – "The limbs" (1925). For this project he created over 250 original color drawings, all individually handcrafted by himself. His work was so impressive, that in 1923 when Prof. Jean Louis Faure visited his clinic proposed to Prof. Juvara to donate all his drawings to the Paris Medical Academy.

After the war, his passion remained the orthopedics, especially osteosynthesis. Of his over 230 published works, more than a third are dedicated to osteoarticular pathology.

His student, Ion Fagarasanu, estimates that Prof. Juvara made over 3,000 anatomical drawings throughout his life, surpassing some of the most renown anatomists. This demonstrates how much time and effort he was prepared to invest in his art.

He was decorated with the Order of the Star of Romania, in the rank of Knight by King Ferdinand I of Romania and was elected President of the Romanian Society of Surgery between 1929-1930.

It is known that he had no love for distinctions and honorary functions. He avoided any initiative for promotions to any high-ranking positions. Even when proposals were spontaneously made from outside, he rejected them vigorously. This explains why he was never a Member of Parliament, dean, or rector of the University. Even the title of President of the Romanian Society of Surgery, of which he was a founding member and its first secretary, was awarded to him contrary to his wishes.

At only 63 years, in full health he suffered a tragic death. On May 5th, 1933, following an accident, he was electrocuted in the bath by the lamp he used to read with.

His work was never forgotten. He left behind a renowned Romanian medical school.

One year after his death, his colleagues created a memorial medal in his honor (fig. 12).

Fig. 12. Prof. Dr. Ernest Juvara memorial medal
Image used with the written agreement of the owner

In 1970, UNESCO celebrated a century since the birth of one the most brilliant medical minds Romania had to offer. Also, today his bust can be seen in bronze in the main hall of "Foișor" Clinical Orthopedic Hospital in Bucharest (fig. 13).

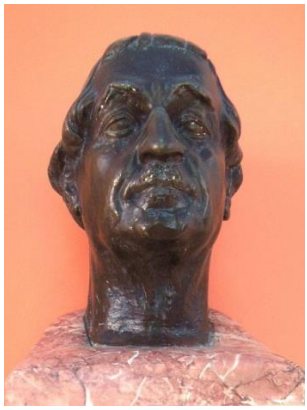

Fig. 13. Bust of Prof. Dr. Ernest Juvara
Image source: Cristian-Mihail Miehs; 20.11.2015
Image with CC-BY-NC 3.0 license

Non-medical interests and hobbies

Ernest Juvara was a founding member of the Romanian Automobile Club (1904). His great memories with Prof. Paul Poirier automobile racing in Paris grew into a real passion for automobiles and mechanical work. He was the first man from Iasi to ever have an automobile. It is even more impressive the fact that he ordered from abroad only the chassis and the engine. Everything else, he handcrafted himself (fig. 14).

He published numerous articles in the Romanian Journal *"Automobilul"* regarding different types of carburetors, often drawing related schematics (fig. 15). His passion for mechanics guided him to be a member of the technical commission of the Romanian Automobile Club.

He was in love with nature. He would often go on hikes or casual drives in the country. This, in combination with his passion for automobiles came in very handy during the Great War. Often, he would recommend shorter, less known routes for the ambulance drivers. Juvara was also a passionate hunter and owned an impressive firearms collection.

Fig. 14. Ernest Juvara with his own automobile (1906)
Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

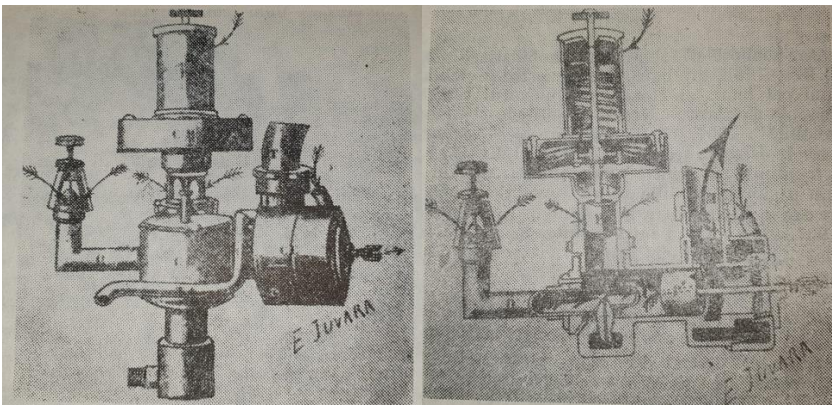

Fig. 15. A modified carburetor schematic by Ernest Juvara
Image source: Fagarasanu I, *Ernest Juvara omul si opera*, Romanian Academy Publishing House, Bucharest, 1986 (1)

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NEWS

MACHINE LEARNING IN FETAL CARDIOLOGY: WHAT TO EXPECT

Fetal echocardiography is used for the evaluation of the fetal heart. Due to increased bias, new technologies have emerged. Machine Learning (ML) is a computer science discipline focused on teaching a computer to perform tasks with a specific goal, without explicitly programing on how to perform it. In a recent review, Garcia-Canadilla et al. focus on the usefulness of ML in fetal cardiology. They cited a systematic review on the interobserver reliability between human and ML interpretation of fetal heart rate tracings, with no improve of the outcomes when ML was used. However, in another cited study, ML was successfully used in predicting fetal growth abnormalities in a cohort of more than 30,000 patients. An intelligent navigation method, FINE, developed by Yeo et al., can automatically obtain different sonographic views of the fetal heart and identify cardiac abnormalities. Concluding, ML for the assessment of the fetal heart can be successfully used for the identification of individuals with similar features, leading to a better standardization of fetal cardiac data, thus providing support for the clinical interpretation and taking a correct treatment decision. (Garcia-Canadilla P, Sanchez-Martinez S, Crispi F, Bijnens B. Machine Learning in Fetal Cardiology: What to Expect. *Fetal Diagn Ther* 2020; 47(5): 363-372)

VALUE OF DE LA TORRE-MONDRAGON PROCEDURE IN THE SURGICAL TREATMENT OF PATIENTS WITH HIRSCHSPRUNG'S DISEASE

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VALUE OF DE LA TORRE-MONDRAGON PROCEDURE IN THE SURGICAL TREATMENT OF PATIENTS WITH HIRSCHSPRUNG'S DISEASE (Abstract): Hirschsprung's disease (HD) is a congenital anomaly in which numerous improvements in terms of the level of knowledge and treatment options have been made. Along with modern diagnostic tools, like suction biopsy, a number of new surgical techniques were developed, such as the laparoscopic approach and trans anal endorectal pull-through. **Results:** This paper present our experience with De La Torre-Mondragon procedure, in a retrospective study of 28 HD cases. We focused on the value of rectal biopsy and water-soluble contrast enema as main diagnostic tools, the surgical technical details and the encountered complications - perianal dermatitis (10 cases), acute enterocolitis (14 cases), encopresis (4 cases), functional obstruction due to anastomosis in the transition zone (1 case), and mechanical obstruction (3 cases). **Conclusions:** We conclude that the De La Torre-Mondragon surgical technique is for us the procedure of choice in or common forms of HD. **Keywords:** HIRSCHSPRUNG'S DISEASE, CONGENITAL MEGACOLON, DE LA TORRE - MONDRAGON PROCEDURE.

Hirschsprung's disease (HD) is a congenital malformation affecting 1:5.000 live born infants, characterized by the absence of the ganglion cells in the myenteric and submucosal plexuses of the distal gut of variable length which results in absent peristalsis and chronic functional intestinal obstruction (1).

The advances in understanding the genetic and molecular mechanisms helped us understanding that it is a polymorphic, multifactorial disease with a defined multigenic etiology (2). Perturbation in the proper neural crest cell migration, proliferation, differentiation, survival, or apoptosis can all contribute to a dysfunctional enteric nervous system (3).

Regardless of the technique, the purpose of surgical treatment is the removal of the aganglionic bowel segment while keeping intact the anal sphincter and pull through of the ganglionic bowel with the preservation of fecal continence after surgery, without obstructive symptoms such as abdominal distention and postoperative enterocolitis.

The trans anal endorectal pull-through emerged as a need to evolve towards less invasive, single surgical correction of HD disease. At the same time, the age at the time of diagnosis has gradually decreased and in most cases the aganglionosis in-

volves only the rectosigmoid colon. This technique provides the advantages of a minimal access approach with shorter length of stay, shorter time to full feeding, less pain, and improved cosmesis with excellent outcomes (4).

The aim of this study was to present our experience with the implementation and surgical results in HD patients using the De La Torre-Mondragon technique (transanal endorectal pull-through).

MATERIALS AND METHODS

This is a 12-year retrospective, single surgical team experience. The medical records of 43 consecutive patients diagnosed and treated for HD from 2007 to 2019 were analyzed. The diagnosis was confirmed by submucosal rectal biopsy (either suction rectal biopsy or posterior rectal wall biopsy), histopathological examination revealing the absence of ganglion cells in the submucosal layer, and water-soluble contrast enema for the assessment of the anatomy and length of the aganglionic segment and the location of the transition zone. A total of 28 patients were diagnosed with the rectosigmoid HD and were treated using the one-stage transanal endorectal pull-through. The study was approved by the hospital ethics committee. The selected parameters where gender, age at diagnosis, age at surgery, associated malformations, imaging studies, clinical presentation, operative complications, surgical technique, histopathology report, and follow-up.

RESULTS

The study included patients aged 2 weeks to 5 years at the time of diagnosis. Five patients were operated during the neonatal period and 7 patients as infants. The majority were male (19 patients). No patient had a previous laparotomy. Associated malformations were cardiac malformations (9 patients), Down syndrome (5 patients), cleft lip (1 patient), and autistic spectrum disorder (1 patient).

The presenting symptoms included delayed meconium passage in the first 48 hours after birth (18 patients), abdominal distention and vomiting (11 patients), constipation alternating with episodes of enterocolitis (24 patients), and stooling requiring anal stimulation (5 patients). In 85% of cases, rectal exam showed an empty rectal ampulla with fecal impaction at the fingertip or of explosive evacuation stool on finger withdrawal. All patients were prepared preoperatively with 2-3 days of saline irrigations for emptying the impacted colon. The time between clinical diagnosis and surgery was 1 to 3 weeks.

In all cases, the HD diagnosis was made by submucosal rectal biopsy (suction biopsy in 9 patients) which showed absence of ganglion cells and hypertrophic nerve fibers in the Meissner's plexus with hematoxylin-eosin staining (figs. 1, 2). In 2 cases suction biopsy was not conclusive so it required repeated transrectal biopsy. The decision for endorectal surgical approach was made based on the radiologic appearance of the transition segment in the rectal, sigmoid or distal descending colon region with water soluble contrast enema (fig. 3). Intraoperatively, the selection of the colon level for pull through (fig. 4) was confirmed by frozen section biopsy which must show the presence of ganglion cells and normal size hypertrophic terminal nerves. In two cases intraoperative frozen section biopsy showed persistent transition zone which required further dissection and abdominal access. These patients were excluded from the study.

For surgery, all patients were placed in

dorsal decubitus position. A Lone-Star retractor was used to show the dentate line (fig. 5). Injection of saline/lidocaine/ adrenaline in the submucosa was used for hemostasis and to ease extra mucosal dissection. The mucosal incision was made 1.5 cm above the dentate line. The muscular cuff length was 3 to 5 cm with incision of the posterior wall. The operative time was influenced by age (lower in smaller age), difficulty of submucosal dissection due to the inflammatory process caused by recent enterocolitis, and prior posterior rectal wall biopsy. Oral feeding was resumed from postoperative day 1 with clear fluids and from day 5-7 with semiliquid. Patients were followed-up period 6 months to 5 years.

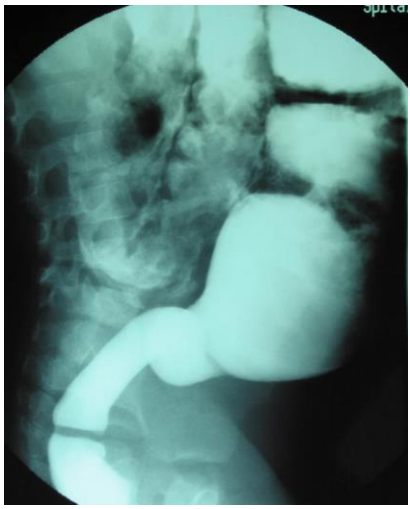

Fig. 3. Water soluble contrast enema showing illustrative aspect of recto-sigmoid aganglionosis.

Fig. 1. RBI suction rectal biopsy device

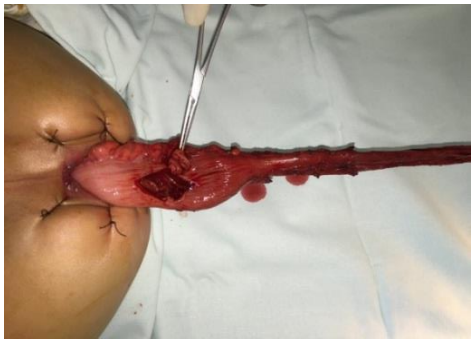

Fig. 4. Total layer biopsy for extemporaneous analysis.

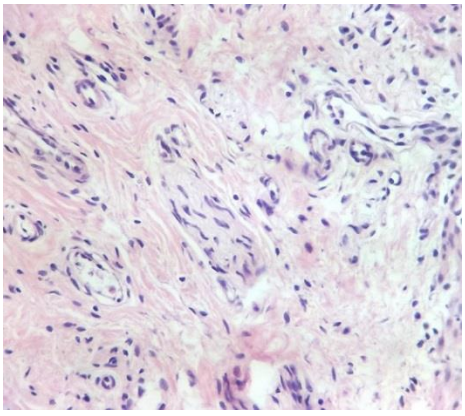

Fig. 2. Absence of ganglionic cells in submucous plexus and nervous fibers hypertrophy.

Fig. 5. Long-Star retractor.

Early postoperative complications included multiple daily soft stools with perianal dermatitis in 10 patients who were not toilet trained yet. The irritation was successfully managed with topical skin barrier products (PVM/MA Copolymers), and the number of daily stools decreased gradually to a normal of 2-3 per day starting with postoperative week 2 or 3. No cases of infection or anastomosis leakage were reported.

Long-distance complication included obstructive symptoms: episodes of acute enterocolitis in 14 cases with a median of 2-5 episodes per year and gradual decrease in the annual follow-up, 1 case of functional obstruction due to anastomosis in the transition zone, and 3 cases of mechanical obstruction (2 cases of stenosis at the anastomosis level and 1 case of stenosis due to the muscular cuff). All parents were taught before and after surgery how to recognize the early signs of enterocolitis and how to do saline irrigations. Only 30% (8 cases) required hospitalization for treatment of postoperative enterocolitis. All 3 cases with mechanical obstruction required redo surgery.

We also had 4 cases of encopresis but no cases of fecal incontinence. The encopresis cases were managed with bowel management: Senna laxatives for a median period of 6 months and hydrosoluble fibers as stool bulking agents. We believe that encopresis occurs in cases where the pull through colon has a degree of dilatation and is not efficient in complete emptying with each bowel movement.

All 6 patients with Down syndrome and autism had a slow postoperative recovery with longer periods of stooling problems of 3 to 6 months (multiple but small daily stools, difficulties in potty training) and more frequent (6-7/year), and severe episodes of acute enterocolitis which required hospitalization.

DISCUSSION

The experience of health professionals in highly specialized tertiary centers in rare congenital and acquired diseases (5,6) is an important factor for a better outcome.

In this study we present our cohort of patients with rectosigmoid HD who underwent one-stage transanal endorectal pull-through in our center. All patients' parents signed a comprehensive informed consent (7).

Complete transanal approach offers the same advantages as laparoscopic-assisted pull-through, namely reduced incidence of postoperative ileus, less postoperative pain, and early hospital discharge. The transanal route has the added advantages of limiting the risk of associated intraabdominal pelvic dissection, such as bleeding, injury to other organs, adhesion formation, better cosmetic results, and reduced cost compared with the laparoscopic-assisted approach and, also, no need for a temporary colic diversion (8, 9, 10). At the same, long-term complications can still occur including anastomotic or muscular cuff stricture, anastomosis of a twisted pull-through, anastomosis in transition zone, enterocolitis, constipation, incontinence, or soiling (11). In most cases, complications are related to the surgical technique and care in following the principles of anal canal dissection.

The age for surgical treatment has been controversial with initial more precaution regarding small age children. In the last years studies have shown that trans anal approach can be successfully and safely used as soon as the diagnosis is made, even in small newborns where the success of the

procedure lies in the magnifying loupes, meticulous dissection, and the fact that the neonatal pelvis is very shallow (4). In the postoperative period, they can develop obstructive symptoms and enterocolitis more frequently, which may be attributed to a functional obstruction caused by the inability of the internal anal sphincter to relax, particularly in not toilet-trained children (12, 13).

Suction biopsy is an easy, safe and efficient way to perform submucosal biopsy at bedside. The main problems are related to the insufficient specimen sample for the evaluation of ganglion cells in the submucosal layer or too close to the dentate line. Previous studies recommended delayed suction biopsy in preterm or low birth neonates, but others (14) showed 100% sensitivity and specificity of suction rectal biopsy for HD in late preterm neonates, small neonates who weighed less than 2,000 g at the time of biopsy including as low as 1,590 g.

Barium enema will show in 80% of HD patients an apparently "normal" size distal bowel with a transition zone and dilated proximal bowel (15). It can be used as a screening test in patients with constipation, but it can give false negative results (16, 17) especially in neonates where the dilated colon is not yet evident or in long segment aganglionosis.

Children with HD and Down syndrome are reported to experience a higher incidence and severity of Hirschsprung-associated enterocolitis. There are several potential factors that may contribute to this: delayed presentation related to the cognitive impairment in these patients which affects the child's ability to effectively communicate symptoms of pain or discomfort in combination with a delayed recognition by the caregiver of child symptoms, such as an episode of enterocolitis. Getting children with Down syndrome to comply with rectal irrigations might be less effective and result in an advanced stage disease at presentation, or the baseline dysfunctional cellular and humoral immune response seen in otherwise healthy patients with Down syndrome without HD may potentiate the infectious disease process in these cases, similar to colitis subsequent to other diseases (18, 19, 20).

Postoperative complications have a common spectrum with other surgical techniques (Soave, Swenson, Duhamel) and vary between different reporting groups (21). They can be grouped into obstructive (functional or mechanical) symptoms and incontinence. Functional obstruction is caused by anastomosis in the transition zone which highlights the need for intraoperative full- thickness frozen biopsy with evaluation of ganglion cells and absence of hypertrophic nerves (22). At the time of surgery, in not toilet-trained patients problems related to efficient anal sphincter relaxation due to retained aganglionic zone at the level of the anal sphincter were noticed, which can be managed with Botox injections (23).

After the surgical intervention, silver dressings can be used to cover the skin lesions in view of preventing bacterial infections (24), but also to reduce pain and accelerate healing (25, 26).

Mechanical obstruction can be caused by twisting the pull-through colon at anastomosis site, anastomotic stricture due to tension suture or poor vascularization, muscular cuff rolling if too long and not split in the posterior wall (27, 28).

In this group of patients, postoperative incontinence should not happen because the sphincter complex in HD patients is integral.

Close dissection with injury of the dentate line or aggressive stretching of the anal canal during surgery should be avoided.

In the long term, prognosis of these patients is very good but with a reported lower quality of life compared with healthy population and better than in those with severe forms (long segment aganglionosis) (29). Postoperative enterocolitis is an inevitable complication and consequently, education of the family is very important in recognizing the early symptoms of acute enterocolitis and performing rectal irrigation. This will avoid the late presentation, severity of symptoms, and the lengthy hospital stay.

CONCLUSIONS

The use of De La Torre-Mondragon surgical technique requires an accurate preoperative diagnosis, both of positive disease and anatomy of the transition zone. It is feasible and safe in young children when following the surgical principles for preserving continence and complete excision of the transition zone. The number of postoperative complications is low, but when they occur, reintervention or long-term care are required. Stooling problems and acute enterocolitis are complications common to all surgical techniques, some are inevitable and thus require family education and proactive measures.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SUBARACHNOID SPACE WASHOUT METHODS IN ANEURYSMAL SUBARACHNOID HEMORRHAGE

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SUBARACHNOID SPACE WASHOUT METHODS IN ANEURYSMAL SUBARACHNOID HEMORRHAGE (Abstract): Aneurysmal subarachnoid hemorrhage is the most dramatic form of hemorrhagic stroke. This pathology should not be regarded as a single disease, but rather as a set of events that happen in a chain-like manner, with devastating complications and requirement of proper management from the beginning. **Material and methods:** We conducted an observational and retrospective study on a group of 47 patients treated for aneurysmal HSA, with secured aneurysm, over a period of 2 years. **Results:** Patients who underwent surgery within the first 5 days of presentation by external ventricular drainage required shorter hospitalization and had faster social reintegration and better neurorehabilitation. **Conclusions:** The main cause of complications leading to coma or death in patients hospitalized for aneurysmal HSA is symptomatic vasospasm. Various surgical methods are currently used to prevent vasospasm, while others are still under development. We believe that an early approach to the subarachnoid space by opening the cerebrospinal fluid passage is mandatory for a better clinical outcome. **Keywords:** SUBARACHNOID LAVAGE, CEREBROSPINAL FLUID, SYMPTOMATIC VASOSPASM, LUMBAR DRAINAGE, VENTRICULOSTOMY, SUBARACHNOID SPACE WASHOUT.

Subarachnoid hemorrhage occurs as a consequence of a blood vessel rupture in the subarachnoid space or near it. Following this rupture, blood will invade the space between the pia mater and the arachnoid, the subarachnoid space, which normally contains just cerebrospinal fluid. About 85% of all subarachnoid hemorrhage (SAH) forms are due to the rupture of a cerebral aneurysm.

The mortality and morbidity associated with aneurysmal subarachnoid hemorrhage remain at global values of about 40% despite remarkable progress in positive diagnosis and time-efficient management, as well as their accessibility. During hospitalization for this pathology 30-70% of patients suffer a bleeding, and about 90% of them die regardless of the treatment applied.

In the development of cerebral vasospasm, the most devastating complication in terms of prognosis in SAH, hemoglobin degradation products are incriminated. This raises the issue of directly controlling them

by removing or neutralizing them from the subarachnoid space, instead of pathogenic treatment to combat their effects.

PATHOPHYSIOLOGY

The study of cerebrospinal fluid is a key element in the management of aneurysmal subarachnoid hemorrhage. Lumbar puncture is currently the diagnostic method with the highest sensitivity for obtaining a correct positive diagnosis (1, 2). A positive result for aneurysmal SAH consists of increased opening pressure, xanthochromic cerebrospinal fluid (CSF), more than 100,000 red blood cells per mm3, increased proteinuria and low or normal glycorrhea (3, 4).

In 2015 Perry *et al.* stated that the lack of xanthochromia together with erythrocytes < 2,000 x 10^6/L in CSF collected by PL exclude the diagnosis of aneurysmal HSA, the method that has sensitivity of 100% and specificity of 91.2% (4, 5).

The following table compares the characteristics of the CSF in the normal subject *vs.* the one suffering from an SAH.

TABLE I

Comparison of CSF constituents values - normal *vs.* SAH (4, 6, 7)

	Normal	SAH	Commentaries
Color	Colorless	Colorless, xanthochromic, bloody	-
pH	7.33	Increased	
Pressure	70 -180 mm H_2O	Increased in > 60% cases	High proteinorrachia correlates with a worse prognosis
Albuminorachia	< 20 mg/dL	Increased in > 60% cases	-
Proteinorrachia	35 mg/dL	Above normal limit	Increases by approximative 1 mg/dL per 1000 erythrocytes
Glycorrhachia	50 - 80 mg/dL	Normal or decreased	-
Potassium	2.8 mEq/L	Decreased	-
Erythrocytes	Absent	Present	Confusion element – traumatic LP
Bilirubin	Absent	Present	
Nucleate cells	< 3	Increased	Their presence correlates with the amount of hemorrhage

Following the degradation of hemoglobin accumulated in the subarachnoid space, heme and bilirubin are the main constituents formed, these being oxidation products that are deposited around the blood vessels, on which they exert vasoactive, cytotoxic, proteolytic and inflammatory effects. Recent experimental animal studies incriminate these substances as being responsible for the development of vasospasm by inhibiting high-conductance potassium channels of the BKCa type in the smooth muscle fibers of the vascular tunic, secondary to the induction of irreversible cerebral vasoconstriction (8, 9).

As heme is released from hemoglobin, hemoxygenase-1 degrades it into bivalent iron ions, biliverdin and carbon monoxide. Elevated levels of hemoxygenase-1 in choroid plexus cells have been identified in experimental animals with subarachnoid hemorrhage. The increased level of this proteolytic enzyme correlated with a better degree of washing of the subarachnoid

space of Hb degradation products (9, 10).

Increased pH of cerebrospinal fluid has recently been associated in controlled studies with phenomena such as late cerebral ischemia in patients with aneurysmal subarachnoid hemorrhage. This increase in pH is due to the poor clearance of Hb degradation products in the subarachnoid space (11).

The high level of proteinemia does not correlate, according to recent literature, with the risk of developing symptomatic vasospasm, but it seems to be closely linked to a more unfavorable prognosis, based on the disruption of the blood-brain barrier as the incriminating mechanism (7).

MATERIAL AND METHODS

We have analyzed data related to the length of hospitalization and the *Glasgow Outcome Scale* (GOS) score at discharge and return for follow-up control at 3-6 months, in a retrospective and purely observational manner, in patients admitted with aneurysmal subarachnoid hemorrhage for a period of 2 years (2018-2019) in Neurosurgery I Clinic from Bucharest University Emergency Hospital. Results were statistically processed with the application *Minitab.*

Patients with a confirmed diagnosis, with a Fischer grade greater than or equal to 3, who have had the incriminated aneurysm secured by endovascular method or surgical clipping and who required external ventricular drainage during hospitalization (due to intracranial hypertension syndrome or acute hydrocephalus) were included. Deceased patients, patients with complications related to the presence of the drainage device or with significant associated pathology that could act as confounders and could thus parasitize the results were excluded from the study.

Thus, a group of 47 subjects was obtained, homogeneous both in terms of age and in terms of Fischer score at admission. We followed data related to the age and sex of the subjects, the number of days of hospitalization, the time of intervention by external ventricular drainage (in the first 5 days after presentation and after 5 days), Fischer score obtained by brain CT examination at presentation, GOS score at discharge and re-evaluation (3-6 months) depending on the time of ventriculostomy. The examined data were collected from the medical charts and from the electronic archive of the department, and they were processed and included in the second table.

TABLE II

Summary of collected data

Gender	F=18; M=29
Age	Mean = 57 (55 for F, 58 for M)
Fischer score	Mean = 3.468 (3.38 for F, 3.51 for M)
GOS score at discharge	Mean = 4.42 (for 4.68 EVD in the first 5 days, 3.86 for EVD after day 5)
GOS score at 3-6 months	Mean = 4.68 (for 4.71 EVD in the first 5 days, 4.60 for EVD after day 5)
Days of hospital stay	Mean = 16.40 (min = 10, max =31)

The study started from the wish to analyze the impact of mechanical washing of the subarachnoid space in patients with aneurysmal HSA and increased risk of

cerebral vasospasm.

Ventricular drainage performs the mechanical washing of the subarachnoid space. External ventricular drainage involves cannulation of the lateral cerebral ventricle with a ventricular catheter connected to an external drainage device against an adjustable pressure, which is set according to normal intracranial pressure.

Other methods of ensuring mechanical washout of the subarachnoid space are lumbar drainage of the CSF or direct chemical washing with fibrinolytic agents and antioxidants, but these are either controversial or unapproved for current clinical use in Europe.

RESULTS

Among the group of subjects, we obtained (N = 47 subjects) the mean age was 57 years (tab. III), comparable to the data in the literature, which stipulates that SAH mainly affects people in the age range of 55-60 years (12).

TABLE III

Summary of follow-up data related to age and sex

Statistics

Variable	Gender	N	Mean	SE Mean	StDev	Minimum	Q1	Median	Maximum
Age	f	18	55.39	3.46	14.69	23.00	46.75	57.50	81.00
	m	29	58.83	2.04	10.96	31.00	51.50	59.00	79.00

The average Fischer score on admission was 3.468. A homogeneous lot was obtained regarding this score ($p < 0.005$). Data about the Fischer score for this lot of subjects is further characterized in the image below (fig. 1).

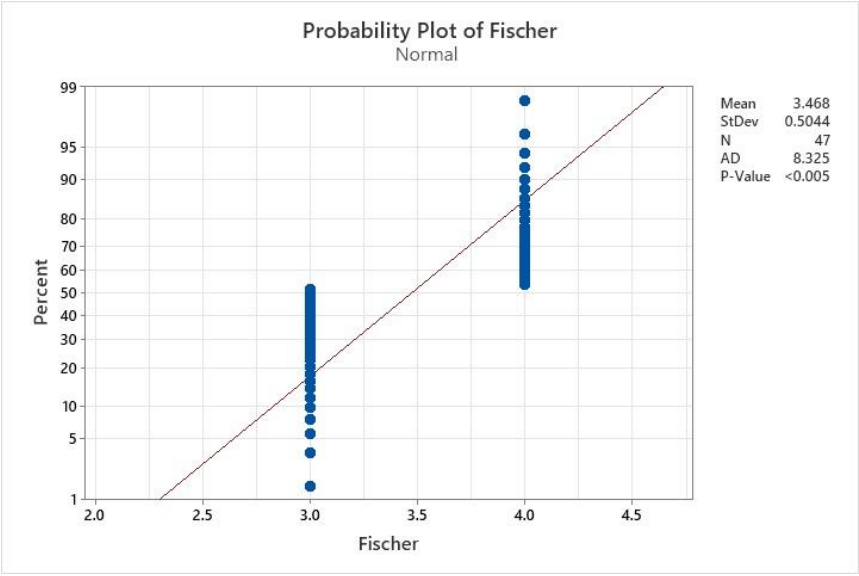

Fig. 1. The normality test for the Fischer score.

The group that underwent external ventricular drainage in the first 5 days after the onset of symptoms required an average of 7 days of hospitalization, fewer than the rest of the patients - on average 13.8 (min = 10, max = 19) *vs.* on average 21.8 (min = 12, max = 31) - and showed a better GOS discharge score on average with 0.82 (4.68 *vs.* 3.86) and 0.11 better at the time of return for follow-up (average 4.71 *vs.* 4.6). Obtaining a better GOS discharge score depending on the time of application of external ventricular drainage (EVD) was a statistically validated result using the *ANOVA* test to compare the means between 2 groups ($\alpha = 0.05$, $p<0.001$, F value > 1 – as it is shown in fig. 2 and tab. IV).

The GOS score at follow-up was 4.71 on average (min = 3, max = 5) in subjects who had DVE inserted in the first 5 days, and 4.6 (min = 3, max = 5) in the other group (fig. 3).

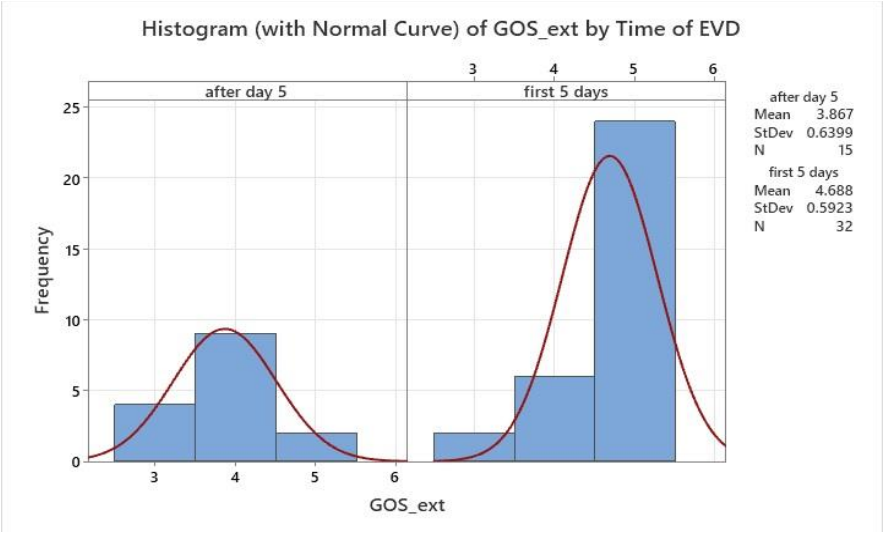

Fig. 2. GOS score at discharge depending on EVD insertion time. On the horizontal axis – GOS score at discharge. On the vertical axis - the frequency of this score in the group of subjects, depending on the time of EVD insertion.

TABLE IV

Statistical data on the number of hospitalization days according to the time of EVD insertion

Statistics

Variable	Time of EVD	N	Mean	SE Mean	StDev	Variance	Minimum	Q1	Median
DaysOfHosp	after day 5	15	21.80	1.21	4.68	21.89	12.00	20.00	21.00
	first 5 days	32	13.875	0.430	2.433	5.919	10.000	12.000	14.000

Variable	Time of EVD	Maximum
DaysOfHosp	after day 5	31.00
	first 5 days	19.000

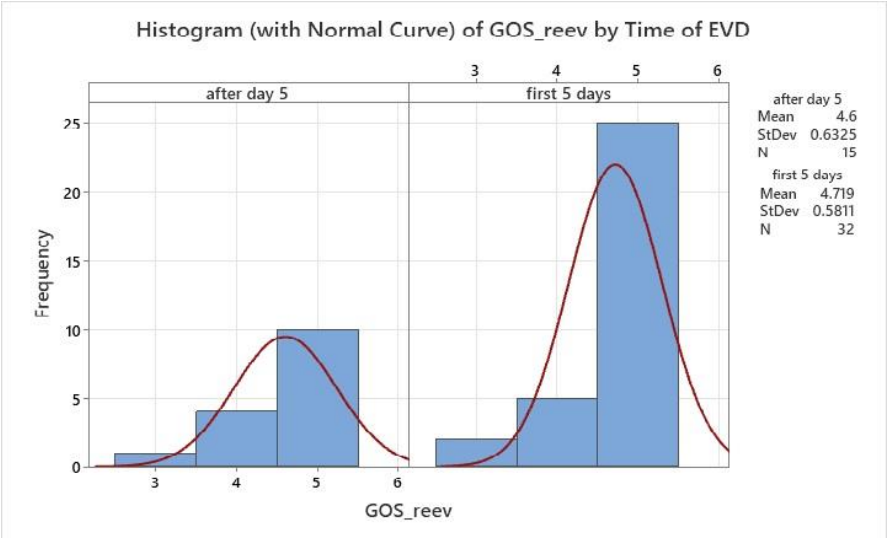

Fig. 3. GOS score at follow-up

However, regarding the differences related to GOS at discharge compared to the GOS calculated at 3-6 months, it was not possible to validate the statistical significance of the result due to the smaller number of subjects who returned for follow-up in the same service.

Following images show serial cerebral computed tomography (CT) examinations from patients in our lot of subjects.

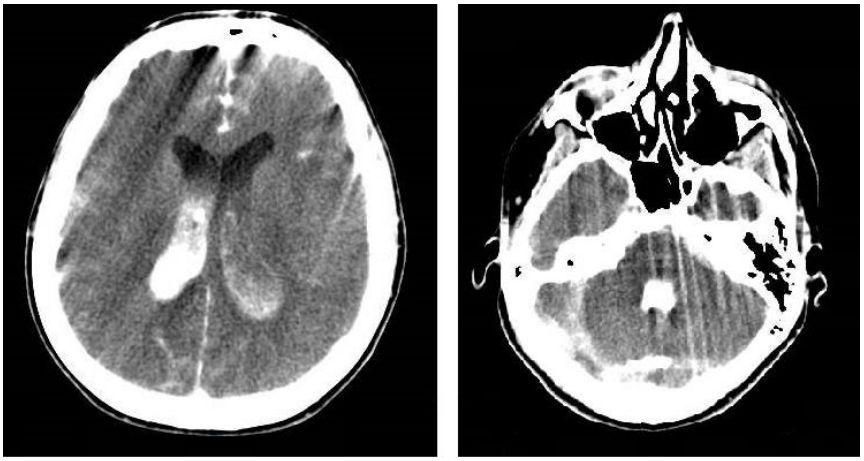

Fig. 4. Axial enhancement cerebral CT sequences from a patient with a ruptured right vertebral artery aneurysm in V4 segment, Fischer score 4: Left: thick layer of blood in the interhemispheric cisterns and in both lateral ventricles; Right: blood in the dilated fourth ventricle and surrounding the cerebellar tent. (*Collection of Neurosurgery I Clinic, Emergency University Hospital of Bucharest)*

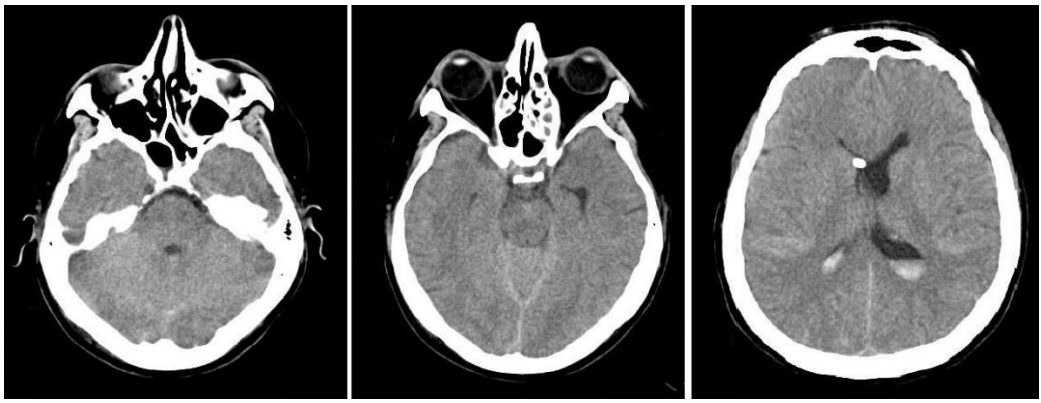

Fig. 5. Axial enhancement cerebral CT sequences from the same patient as in figure 4. Day 4 after EVD applied, almost complete remission of subarachnoid and ventricular blood and slight cerebral edema.
(*Collection of Neurosurgery I Clinic, Emergency University Hospital of Bucharest)*

DISCUSSION

Chemical washout of the subarachnoid space, by irrigating the basal cisterns with urokinase and ascorbic acid, for fibrinolytic and antioxidant effects respectively, is a method for which there is, although in small numbers, most validated date as of today's literature.

Irrigation of basal cisterns with urokinase is intended for lysis and washout of subarachnoid blood, while ascorbic acid is administered to promote the degradation of oxyhemoglobin, a highly spasmodic substance for brain vessels, in other products without spasmogenic effect. Irrigation is done from a technical point of view by installing inflow-outflow systems in the sylvian valleys (unilateral or bilateral inflow tubes) and in the opto-chiasmatic or prepontine cisterns (outflow tubes). Kodama *et al.* applied the method of chemical washing of the subarachnoid space with urokinase and ascorbic acid and obtained good results on a group of 217 patients, considering the decrease of the risk of symptomatic vasospasm in patients with Fischer III score from 37% to 2.8%. Their clinical study led to complications such as seizures, meningitis and intraparenchymal hematomas in only 4% of cases (3).

The most controversial method is given by the irrigation of the basal cisterns with Ringer's solution or urokinase and the energetic movement of the head after securing the aneurysm, this being, in fact, a mechanical method of washing the subarachnoid space.

Nguyen *et al.* (2021) obtained satisfactory results in lumbar CSF drainage in aneurysmal subarachnoid hemorrhage. In patients included in their prospective clinical study, this method reduced the incidence of clinical vasospasm, shortening hospitalization and improving the GOS score at one year (13). The mechanism of action by which this method reduced symptomatic vasospasm is probably due to the maintenance of cerebrospinal fluid circulation, thus washing the subarachnoid space of leaked blood and its products (5, 10). These findings reinforce the hypothesis of removing blood clots in the basal CSF cisterns and broaden the scope of choices for clinical practitioners to improve the

quality of treatment. However, this clinical study was performed in a relatively small group of 43 patients and the infectious risk was not negligible (10, 13).

The main disadvantages related to these methods are given by the selection of candidates, by the validation of the study results, as well as by the accessibility and acceptance of these resources by the specialists in the field.

A prospective clinical study in the United States is currently investigating whether there are significant differences in the outcome of patients with *continuous* external ventricular drainage compared to patients with *discontinuous* external ventricular drainage. The data currently available did not show arguments in favor of any of the external, continuous or discontinuous ventricular drainage methods (14).

The results of our present study suggest that early, though not immediate, intervention on the subarachnoid space in patients with high risk of developing vasospasm, in addition to complications, reduces hospitalization and in this manner also reduces the risk of nosocomial complications, offering in addition a faster social reintegration and thus reducing the costs related to neurorehabilitation. Based on these considerations, we advocate an early intervention on the subarachnoid space, by methods with washout effect, in a controlled manner, in subarachnoid hemorrhage due to vascular cause.

The risk of developing symptomatic vasospasm was estimated by placing it in a Fischer group based on the computed tomography presentation. The reduction in the risk of developing symptomatic cerebral vasospasm in these patients can be attributed to the effect of mechanical washout of the subarachnoid space by CSF drainage and by decreasing intracranial pressure.

The same result also highlights and validates the importance of mechanical intervention on subarachnoid spaces in the first 5 days after presentation.

CONCLUSIONS

Considering that a *curative treatment* of aneurysmal subarachnoid hemorrhage is out of question, this being in fact *a series of pathological phenomena* that occur causing devastating complications on brain structures, we advocate for early surgical methods that oppose the pathophysiological chain of this condition.

Adequate definition of the selection criteria of patients who can benefit from different methods of wash-out of the subarachnoid space is a key point of this type of treatment. Other key aspects are given by the moment of applying these methods in the management of SAH, ensuring the neurological safety they come with and training the specialists involved in the management of this pathology.

It is expected that ethical issues regarding the study and development of new surgical techniques on severely ill subjects will delay scientific progress in this direction.

An issue that has re-entered the surgical interest in recent years, attractive in terms of expected results, daring by reorienting from pathogenic to etiological control of cerebral vasospasm, it is expected that methods of washout the subarachnoid space in SAH of all types will become a topic of peculiar neurosurgical interest in the following decades.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE ASSOCIATION BETWEEN FACTOR V LEIDEN, MTHFR C667T/A1298C POLYMORPHISMS AND PREGNANCY OUTCOMES

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THE ASSOCIATION BETWEEN FACTOR V LEIDEN, MTHFR C667T/A1298C POLYMORPHISMS AND PREGNANCY OUTCOMES (Abstract): The present study aims to characterize the association between factor V Leiden, MTHFR C667T/A1298C polymorphisms and adverse pregnancy outcomes such as early recurrent pregnancy loss, thrombotic complications, fetal growth restriction, pre-eclamsia. **Material and methods:** We aimed to retrospectively assess the pregnancy outcomes of patients with factor V Leiden, MTHFR C667T/A1298C polymorphisms and their combination, between September 2019 and October 2020. Was determined the activity of protein C, antithrombin III and protein S activity. After isolation of blood DNA was evaluated Factor V Leiden, and MTHFR C667T/A1298C polymorphisms. **Results:** We segregated the 179 patients into 7 groups as follows: factor V Leiden mutation heterozygote and homozygote groups, patients with both factor V Leiden and MTHFR mutations, MTHFR A1298C polymorphism homozygote and heterozygote groups, and MTHFR C667T polymorphism homozygote and heterozygote groups. Although higher IVF rates were identified in the MTHFR C667T heterozygote group (23.9%) and in the FVL-homozygote group (33.3%), no statistically significant difference was calculated. Recurrent pregnancy losses were more frequently encountered in the FVL homozygote and FVL-MTHFR groups (66.6% and 66.7%), and we found a significant association between this parameter and patients with factor V Leiden and a combination of factor V Leiden - MTHFR polymorphisms ($p<0.05$). **Conclusions:** Our results suggest a strong association between factor V Leiden polymorphism, or between the combination of factor V Leiden and MTHFR polymorphism with adverse pregnancy outcomes such as thrombotic complications, early recurrent pregnancy loss and intrauterine growth restriction. **Keywords**: PREGNANCY, FACTOR V LEIDEN, MTHFR POLYMORPHISM.

Pregnancy is characterized by a hypercoagulable state, determined by increased of some coagulation factors- VII, VIII, X and von Willebrand factor activity (1), as well as a reduced fibrinolytic activity (2). These changes in the hemostatic system favor some maternal and fetal complications, which can manifest as early as con-

ception and extend during all pregnancy and also in first trimester after birth. (3). For women with acquired or inherited thrombophilia, the risks of developing pre-eclampsia (PE), placental abruption, fetal growth restriction (FGR), early and late recurrent pregnancy loss (RPL), intrauterine death and stillbirth are higher (4). However, at present the screening is routinely recommended only in presence of venous thromboembolism.

Further, the value of hereditary thrombophilia screening in complicated pregnancies is uncertain because of limitations in knowledges about its implication in development of complications and lack of evidence for effective intervention (5).

In last decades, were produced some demographic changes and thus for many women the first pregnancy is arrived after the age of 35 years and in some cases this event is obtained by an artificial reproductive technology. These women present frequently different medical complications, and the use of thrombophilia screening is needed despite the above-described limitations, especially in those that had previous pregnancy complications .

This retrospective study aims to characterize the association between factor V Leiden (FVL), MTHFR C667T/A1298C polymorphisms and adverse pregnancy outcomes such as early recurrent pregnancy loss, thrombotic complications, FGR, PE, and stillbirth.

MATERIAL AND METHODS

We aimed to retrospectively assess the pregnancy outcomes of patients with factor V Leiden, MTHFR C667T/A1298C polymorphisms, and their combination. The patients were admitted to a tertiary maternity hospital, 'Cuza Voda', Iasi, Romania, between September 2019 and October 2020. The study was reviewed and approved by the Institutional Ethics Committee of University of Medicine and Pharmacy "Grigore T. Popa" from Iasi (approval no. 17806/03.09.2019). Informed consent was obtained from all participants.

The inclusion criteria were a) pregnant patients with vascular disorders; b) positive family history for myocardial infarction, stroke or other thromboembolic complications; c) personal history of spontaneous abortions in the first trimester; d) personal history of preterm birth. We have excluded patients who used antithrombotic medication or were incapable of giving a written consent.

Blood samples were collected from 181 female patients. Blood samples were collected to the EDTA tube (five milliliters) and stored at -20°C until DNA isolation. We have determined the activity of protein C and antithrombin III using a chromogenic assay, while the protein S activity was determined using the activated partial thromboplastin time (aPTT).

Isolation of blood DNA was performed with the Qiagen kit (DNeasy Blood) according to the protocol. The concentration and quality DNA samples were evaluated by spectrophotometric method (NanoDropND1000). After analyzing the DNA samples, 179 cases were selected for evaluation. Factor V Leiden, and MTHFR C667T/A1298C polymorphisms were amplified and detected by TaqMan probes using a real-time PCR kit (Appled Biosystem). The genotyping of the obtained amplicons was performed by melting curve analysis.

Out of 181 pregnant patients, 179 were included in the study because two patients had protein C and S deficiency without any association with thrombophilic mutations. Relevant information on maternal and fetal

parameters (age, type of conception, thrombotic complications during pregnancy, PE, FGR, stillbirth, gestational age at birth, newborn weight and Apgar score) were collected in structured proforma, entered in *Microsoft Office Excel* format. Statistical analysis was performed using *SPSS software version 24.0* (IBM Corp.). Descriptive statistics and one-way *ANOVA* analysis were used for characterization of the association between thrombophilic polymorphisms and various pregnancy outcomes among study groups.

RESULTS

We segregated the 179 patients into 7 groups as follows: factor V Leiden mutation heterozygote (n=19 cases) and homozygote (n=3 cases) groups, patients with both factor V Leiden and MTHFR mutations (n= 18 cases), MTHFR A1298C polymorphism homozygote (n=13) and heterozygote (n=60) groups, and MTHFR C667T polymorphism homozygote (n=17) and heterozygote groups (n=67). The descriptive statistics for these groups and specific parameters are described in first table.

No statistically significant difference was observed between groups regarding the mother's age. Although higher IVF rates were identified in the MTHFR C667T heterozygote group (23.9%) and in the FVL-homozygote group (33.3%), no statistically significant difference was calculated.

TABLE I

Descriptive statistics of the study groups

Groups	Parameters							
	Age (years) (Mean and SD)	Conception (N/%)	Thrombotic complication (N/%)	Recurrent pregnancy loss (N/%)	Pregnancy complications (N/%)	Gestational age at birth (years) (Mean and SD)	Newborn weight (g) (Mean and SD)	Apgar score (Mean and SD)
FVL-hetero	31.4±3.84	IVF= 1 (5.3%) Natural= 18 (94.7%)	No=16 (84.2%) Yes=3 (15.8%)	No=17 (89.5%) Yes=2 (10.5%)	No=11 (57.9%) FGR=4 (21%) PE=3 (15.8%) S=1 (5.3%)	37.1±2.3	3023± 539.9	8±1.7
FVL-homo	32.4±4.82	IVF= 1 (33.3%) Natural= 2 (66.7%)	No=1 (33.3%) Yes=2 (66.6%)	No=1 (33.3.%) Yes=2 (66.6%)	No=2 (66.7%) FGR=1 (33.3%) PE=0 (0%) S=0 (0%)	38.1±2.1	3102.8± 487.4	8.3±2.1
FVL+MTHFR	29.1±4.64	IVF= 1 (5.5%) Natural= 17 (94.5%)	No=4 (22.2%) Yes=14 (77.8%)	No=6 (33.3%) Yes=12 (66.7%)	No=1 (5.5%) FGR=15 (83.4%) PE=2 (11.1%) S=0 (0%)	36.3±2.3	2853.7± 525.3	7.9±1.9

Groups	Parameters							
	Age (years) (Mean and SD)	Conception (N/%)	Thrombotic complication (N/%)	Recurrent pregnancy loss (N/%)	Pregnancy complications (N/%)	Gestational age at birth (years) (Mean and SD)	Newborn weight (g) (Mean and SD)	Apgar score (Mean and SD)
MTHFR A1298C-hetero	30±4.42	IVF= 8 (13.3%) Natural= 52` (86.7%)	No=36 (60%) Yes=24 (40%)	No=48 (80%) Yes=12 (20%)	No=50 (83.3%) FGR=3 (5%) PE=4 (6.7%) S=3 (5%)	38.4±1.6	3083.3± 466.6	8.4±0.8
MTHFR A1298C-homo	29.7±3.53	IVF= 0 (0%) Natural= 13 (100%)	No=13 (100%) Yes=0 (0%)	No=11 (84.6%) Yes=2 (15.4%)	No=12 (92.3%) FGR=1 (7.7%) PE=0 (0%) S=0 (0%)	38±1.3	3253.1± 434.1	8.6±0.7
MTHFR C667T-hetero	35.5±3.82	IVF= 16 (23.9%) Natural=51 (76.1%)	No=28 (41.8%) Yes=39 (58.2%)	No=51 (76.1%) Yes=16 (23.9%)	No=44 (65.7%) FGR=17 (25.4%) PE=6 (8.9%) S=0 (0%)	37.1±1.8	3020.8± 384	8.5±0.6
MTHFR C667T-homo	31.6±4.46	IVF= 0 (0%) Natural= 17 (100%)	No=17 (100%) Yes=0 (0%)	No=15 (88.2%) Yes=2 (11.8%)	No=11 (64.7%) FGR=3 (17.65%) PE=3 (17.65%) S=0 0%)	36.8±2.5	2955.8± 568.6	8.3±0.9

Recurrent pregnancy losses were more frequently encountered in the FVL homozygote and FVL-MTHFR groups (66.6% and 66.7%), and we found a significant association between this parameter and patients with factor V Leiden and a combination of factor V Leiden - MTHFR polymorphisms ($p<0.05$) (Table II). The same aspect of distribution was observed in case of thrombotic complications between the FVL homozygote and FVL-MTHFR groups (66.6% and 77.8%), with a p value of less than 0.05.

The highest rates of fetal growth restriction were observed among the FVL-MTHFR group (83.4%), followed by FVL homozygote group (33.3%), with a statistically significant association ($p<0.05$) (tab. II).

Although high rates of preeclampsia were noted in the MTHFR C667T homozygote (17.6%), FVL+MTHFR (11.1%), and FVL heterozygote group (15.8%), no statistically significant association could be established (tab. II). The stillbirth rates were insignificant among all groups.

TABLE II

The association between thrombophilic polymorphisms and pregnancy outcomes

Groups	Parameters				
	Age (*p* value)	Conception (*p* value)	Thrombotic complications (*p* value)	Recurrent pregnancy loss (*p* value)	Pregnancy complications (*p* value)
FVL-hetero	0.87	0.56	0.1	0.45	FGR=-.1 PE=0.3 S=0.84
FVL-homo	0.67	0.35	0.03	0.04	FGR=0.04 PE=0.48 S=0.56
FVL+MTHFR	0.32	0.40	0.02	0.01	FGR=0.02 PE=0.3 S=0.37
MTHFR A1298C-hetero	0,55	0.67	0.71	0.55	FGR=0.56 PE=0,41 S=0.44
MTHFR A1298C-homo	0,12	0.70	0.68	0.53	FGR=0.55 PE=0.88 S=0.77
MTHFR C667T-hetero	0,77	0.66	0.88	0.64	FGR=0.61 PE=0.76 S=0.56
MTHFR C667T-homo	0,78	0.87	0.67	0.52	FGR=0.23 PE=0.54 S=0.56

DISCUSSION

The purpose of our study was to evaluate the pregnancy outcomes among various groups with thrombophilic polymorphisms.

We have outlined the significant association between homozygotic mutations of factor V Leiden, or the mutant combination of factor V Leiden and MTHFR, with recurrent pregnancy loss, thrombotic complications and fetal growth restriction. Our results support other similar literature findings. The link between factor V Leiden mutation and recurrent pregnancy loss is controversial, as some showed a significant association, but other studies failed to prove this aspect (5-7). Sergi *et al*, conducted a meta-analysis on Western countries, and demonstrated a statistically significantly increased carrier frequency of FVL mutation homozygous in women with early recurrent pregnancy loss (8). Moreover, Cobzaru *et al*, demonstrated that the concomitant presence of MTHFR A1298C and Factor XIII V34L gene polymorphism had a significant association for recurrent pregnancy loss (9).

The disagreement between FVL studies may be due to the difference or heterogeneity in the patients' population so that single nucleotide polymorphisms need to be considered, along with the specific geographic

area (10-13). Our study included Caucasian women from Eastern Europe, and the results suggested a stronger bond between early recurrent pregnancy loss and the combination of mutant factor V Leiden-MTHFR carriers.

The association between factor V Leiden and thrombotic complications in pregnancy and beyond is well established in the literature (14, 15), the mutant variant increasing the risk of venous thromboembolic events with more than 20–30% (16). Our results support the literature findings and outline a strong association between factor V Leiden homozygotic mutation or heterozygotic mutation and its combination with MTHFR, and thrombotic complications in pregnancy, respectively.

As for fetal growth restriction, a recent systematic review by Howley *et al*, suggested an increased risk of giving birth to an intrauterine growth restricted fetus for women with factor V Leiden polymorphisms (17). Although we have identified a significant association between FGR and homozygotic mutation of FVL or FVL-MTHFR, we could not obtain the same results in case of preeclampsia, even though some studies suggest an important involvement of these mutation in its pathogenesis (18-20).

The major limitation of our study is the small number of cases. Further large cohort studies will allow a better statistical analysis. Moreover, multiple single nucleotide polymorphisms should be studied in order to better characterize the association between patients with thrombophilia from different regions and adverse pregnancy outcomes.

CONCLUSIONS

Our results suggest a strong association between factor V Leiden polymorphism, or between the combination of factor V Leiden and MTHFR polymorphism with adverse pregnancy outcomes such as thrombotic complications, early recurrent pregnancy loss and intrauterine growth restriction.

We failed to outline a link between these genetic polymorphisms and preeclampsia, even though the scientific literature suggests a significant association.

Further studies should focus on larger cohorts of patients, along with specific single gene polymorphisms in order to complete the picture of adverse pregnancy outcomes in the context of thrombophilia.

CONFLICT OF INTEREST AND FUNDING

Authors declare that there is no conflict of interest, and they received no funding for this study.

AVAILABILITY OF DATA AND MATERIALS

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

All authors had equal contributions. All authors gave the final approval of the version submitted for publication. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was reviewed and approved by the Institutional Ethics Committee from the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi (approval no. 17806/03.09.2019). Informed consent was

obtained from all participants and/or their legal guardians included in the study. All research was performed in accordance with relevant guidelines/regulations.

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CLINICAL EXPERIENCE IN PARTIAL THICKNESS BURNS TREATMENT USING LMW-HYALURONIC ACID TOPICS

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CLINICAL EXPERIENCE IN PARTIAL THICKNESS BURNS TREATMENT USING LMW-HYALURONIC ACID TOPICS (Abstract): **Aim:** To describe the importance of hyaluronic acid topics in partial thickness burns treatment, corresponding to each chronological stage of the burn injury, reducing the healing time, with a good control of pain management during the dressing change and improving the quality of newly formed tissue. **Material and methods:** Descriptive study developed on 20 patients treated between December 2019 - January 2021, including I, IIA, IIB burn lesions and skin graft donor zones, on a total surface area of 5-10% each, located on different zones of the body such as upper/lower limb and trunk. **Results**: For all the patients (aged 21-58 years old, 14 females), we preceded the low molecular weight hyaluronic acid (LMW-HA) application by the lesion debridement with topics based on hyaluronic acid and collagenase. In 7 cases we performed surgical excision of IIB lesion followed by reconstruction using split skin grafts, harvested from the thigh. To stimulate the detersion of the burned tissues, we combined the proteolytic effect of the collagenase with the action of the negative wound pressure therapy system (NPWT) in 7 cases. We obtained good functional and aesthetic results in all the cases, with total healing time of 14-35 days. **Conclusions:** Because of the contribution as a modulator to many of the biological processes, LMW-HA (200 kDa) became a part of therapeutic options for burns lesions and skin graft donor zones. The ease of use, biocompatibility, better esthetic and functional results were also considered as important points. **Keywords**: LOW MOLECULAR WEIGHT, HYALURONIC ACID TOPICS, BURNS, DONOR ZONES, SKIN GRAFT.

The Hyaluronic Acid (HA) is a nonsulfated glycosaminoglycan, met as a component of the connective tissues, but also of the epithelial and neural tissues. Being an important element, part of the extracellular matrix, HA is implicated in the healing process, as a modulator of the angiogenesis, inflammation and skin regeneration (1).

The hyaluronic acid can be obtained from human or animal sources (for example, human umbilical cords or bovine vitreous humors, the skin of sharks), but with the "price" of protein residuals with immunogenic effects (2). Therefore, the bacterial technology is considered as an important way to obtain high-purity HA (3). HA has different biological effects depending on its molecular weight (4). A molecular weight

of 20–200 kDa allows HA to influence important biological processes such as embryonic development and wound healing (5). High molecular weight HA (HMW-HA) has an anti-angiogenic effect; low molecular weight HA (LMW-HA) is pro-angiogenic and pro-inflammatory (6). For these properties LMW-HA is preferred for wounds medication. The topics based on LMW-HA can influence the local evolution of wounds, by reducing the healing time and controlling the qualities of the newly formed tissue (7).

MATERIAL AND METHODS

Given the good clinical evidence of efficacy and safety of HA in the management of acute and chronic wounds, we have started to treat some patients presenting first- and second-degree burns with topics based on LMW-HA, with the aim of reducing the healing time and improving the newly formed tissue.

We evaluated a group of twenty patients, treated in the Burns Unit of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, between December 2019 and January 2021.

We included in our study patients with I, IIA, IIB burn lesions and skin grafts donor zones, on a total surface area of 5-10% each, located on different anatomical regions, such as upper/lower limb and trunk.

From the whole group of patients (aged 21-58 years old, fourteen female) only 5 were smokers (one of them declaring 40 cigarettes per day). Considering the comorbidities, most of the cases (17 patients) declared at least one illness as diabetes (5 patients- 25%), leg venous insufficiency (7 patients- 35%), and urinary infection (5 patients- 25%). In ten cases (50%) we identified two preexistent comorbidities for each patient, with important effect on the healing process (as diabetes, immunodeficiency, hepatitis, ulcerative colitis, severe anemia).

Respecting the moment of the first clinical evaluation, five patients came within the first 24 hours after the injury (25%), 11 patients came at 24-72 hours distance from the accident (55%) and 4 patients came to the hospital at 5-7 days distance from the accident (20%). We consider the moment of the first evaluation of main importance, knowing that the evolution of a burn injury depends on the general and local therapy applied within the first 24-48 hours after the accident (8).

In 13 cases (65%) we identified I and II A degree burns and 7 patients (35%) had II A and II B degree injuries.

Eight patients (40%) suffered injuries covering 5-7% from the total body surface area (tbsa) and in twelve cases (60%) the burns covered 8-10% tbsa. In 12 cases the burns were situated on the lower limbs, in 4 cases on the upper and lower limbs and in 7 cases on the limbs and trunk regions.

Four patients (20%) had burns situated on the perineum region and on the buttocks, already contaminated with *Escherichia coli* at the moment of the first clinical evaluation.

All the skin grafts donor zones had negative secretions from the bacteriological point of view (7 cases). The wound's secretions were sterile (from the first clinical examination) in 8 cases (40%) and contaminated with *Staphylococcus epidermidis* in 4 cases (20%). Eight patients arrived to the first consultation with infected wounds (gram-negative bacteria).

Data were systematized and centralized in an SPSS 24.0 database and processed using the appropriate statistical functions.

A 95% confidence interval was used in data presentation.

TREATMENT PROTOCOL

In all the cases, the treatment with HA topics was preceded by the lesion debridement and cleaning with chlorhexidine and sterile saline solution.

For IIA burns, the detersion process was stimulated using local applications of the ointment containing HA and collagenase, for the first 3-5 days of evolution/once per day.

If the bacteriological examination confirmed wound bacterial contamination, we used for local applications the ointment containing HA and silver once per day, for the next 5-7 days (up to the hospital discharge), in the case of second-degree superficial burns.

For second-degree deep (II B) burns, with surgical indication, we performed early wound excision and local reconstruction using split skin grafts (7 patients, 3 patients with leg's circumferential and constrictive burns). The surgery was performed under loco-regional anesthesia in all the cases (rachianesthesia - for lower limb, perineum region and buttocks or combination between infraclavicular block and rachianesthesia, for upper limb burns and skin grafts harvested from the thigh). For two patients, with second deep burns on the trunk, we needed general anesthesia.

We used negative pressure wound therapy (NPWT) to stimulate the burn cleansing and the healing process in 5 cases-25%, (also in addition to the skin graft for 3 cases).

The burned necrosis was excised using the electric dermatome (with a thickness of 0.3 mm). The skin grafts were harvested from one thigh, using the electric dermatome, with a thickness of 0.2 mm, on a surface of maximum 3% tbsa.

To prevent the bleeding, we covered the skin grafts donor zones with Calcium-alginate dressing for the first 48 -72 hours after surgery; then, to stimulate the donor zones healing and prevent the local infection, we used daily applications with HA and silver topic for the next 5-7 days (up to the hospital discharge day). We recommended the use of LMW-HA topic for local applications, twice a day, for the first-degree burns (preceded by local cleaning with saline solution), starting with day one after injury. For second-degree superficial burns and skin grafts donor zones, we used the topic twice a day (preceded by local cleaning with liquid soap and saline solution), after the patient's discharge day.

We evaluated the progression of the healing process (we estimated the initial wound surface and thickness - using a laser Doppler blood perfusion imagery for the burned surface and the dermatome gradations, for the skin grafts donor zones). We measured the wound dimensions in the first day (the day of first clinical examination), in the eighth day of evolution (after the injury/ after surgery) or of clinical follow up (some patients arrived at the first clinical examination a few days after the injury), in the fifteenth, twenty first, twenty eighth, respectively thirty fifth day after the first clinical examination (for the cases with larger wounds). We calculated the healing rate for each period of time, and we estimated the wound healing rate per day, as a medium value.

We evaluated the blood levels of proteins and hemoglobin at the patient's admission, before and after surgery sessions, before the discharge day. We monitored the blood pressure levels, pulse rate, diuresis,

temperature and the wound's bacterial colonization for all the patients.

To estimate the local pain during the bandage removal and daily wound cleaning, we used a pain scale with ten levels, VAS - visual analogue scale (9). For pain assessment, we used paracetamol and ketoprofen.

RESULTS AND DISCUSSION

We selected for evaluation the skin grafts donor zones because they are surgical wounds with very good chances of healing. As skin graft harvesting is a surgical procedure, it takes place in sterile conditions and the wound's dimensions (surface, thickness) are mathematically established (we can precisely control the dimensions using the electric dermatome). The bleeding is controlled, and the epithelialization sources are preserved, because a split skin graft is, usually, harvested with 0.2-0.3 mm thickness (within the superficial dermis) (10, 11).

We considered that it would be interesting to compare the healing process of a "standardized wound" (the skin graft donor zone) and an accidental injury (burn) on different/symmetric anatomic regions, at the same patient. For this reason and considering that the dedicated skin grafts donor zones are the thighs, we selected for evaluation burns localized mainly on the lower limbs (16 patients, 80%).

We evaluated the daily healing progress on both, skin grafts' donor zones and burns, in area and in volume.

An essential role in quantifying the healing process is the ability to determine the size of a wound. Without determining the area and volume of a wound, we cannot assess its healing rate, appreciate the topic's efficiency, nor can we optimally use negative pressure therapy or local topics, as this is in relation to these sizes (12).

The results for the daily healing progress of the same wound, in area and in volume, were identical in the case of skin grafts donor zones evaluation, and comparable for the burned injuries (differences of 0.01 between the daily progress rate, estimated on surface and in volume) .

The dimensions of the skin grafts donor zones were between 24,000 square mm and 60,000 square mm (1.2% tbsa-3% tbsa, with a median of 2.1% tbsa) and the volumes between 4,800 and 12,000 cubic mm.

The duration of the healing process varied between 14-23 days, with an average value of 19.28 days. The healing process depended on the total burned surface, patient's age and comorbidities, the delayed period of first clinical evaluation (4 hours-7 days).

The daily rate of healing process, for skin grafts donor zones, varied between 4.34% and 5.88% from the initial wound surface or volume as well (with a medium value of 4.92%).

For the same 7 cases that needed surgery (excision and skin grafting) we followed the healing daily progression for the burned wounds. We found, for similar wound's surfaces, a lower rate of epithelialization per day.

The dimensions of the burned injuries, non-grafted zones, varied between 7,200 square mm (0.36% tbsa) and 47,200 square mm (2.36% tbsa), with a medium value of 19,667 square mm (0.98% tbsa) and the volumes between 3600 cubic mm and 10 160 cubic mm, with a median of 4,953 cubic mm.

The duration of the healing process for the burned injuries (non-grafted zones) varied between 21-35 days, with an average value of 26.5 days. The daily rate of heal-

ing progression for these injuries varied between 2.36% and 4.41% from the initial burned surface (non-grafted zones), with a medium value of 3.12% (fig. 1).

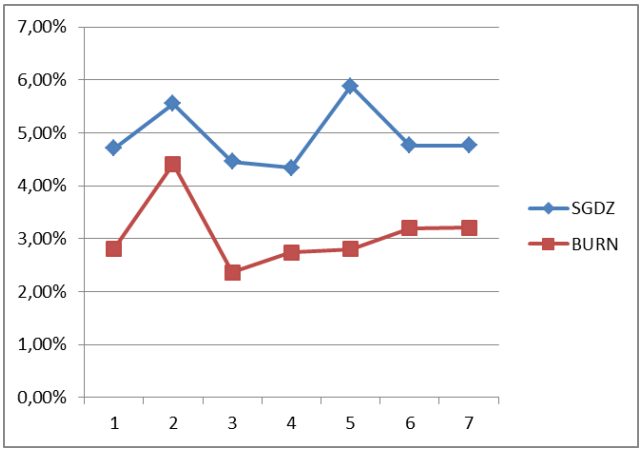

Fig. 1. SGDZ-skin graft donor zones (the daily healing rate for the 7 patients with surgical excision and skin grafts). BURN - the wounds that did not receive the excision and skin grafts (the daily healing rate for the same 7 patients, but on the burned surfaces which were not grafted)

Considering the results, we can conclude that the skin grafts donor zones will usually heal faster than the IIA burned skin, even if we use different and very efficient devices to help a burn to cure (as NWPT or HA topics). It is the difference between the etiology and pathophysiology of the two types of wounds, the surgical and the accidental one, that decides the evolution process to complete healing. We monitored the SGDZ bacteriological, as well. The results were negative secretions in all the 7 cases.

A skin graft donor zone is a surgical wound with the best chances to heal. In the absence of bacterial contamination, on a patient with good nutrition (normal proteins and hemoglobin) the skin grafts donor zones start to heal immediately after the surgery, with no delay (13-16).

A burn injury is, in fact, a skin necrosis, with different thickness levels on different areas (depending on the etiology, anatomic region, gender, age, microorganisms' density). The healing process can start only after the wound detersion, after the necrotic tissues are eliminated naturally (under enzymatic action or bacterial action). This process takes time, and the risk of wound deepening is always real.

The use of HA topics can stimulate the wound's detersion process (the ointment containing HA and collagenase) and shorten the total healing time. In 3 cases with 5-7 days delay in presenting to the hospital, we combined the efficiency of the HA and collagenase topic with the NPWT action, and so we managed to avoid surgery.

In the cases with contaminated or infected wounds, we added the ointment containing HA and silver in local wound therapy, for its efficient action against bacteria with very good results.

We used LMW-HA topics on the skin grafts too, beginning with day 7-9 after

surgery, in all the 7 cases, with very good esthetic and functional results (better local hydration, lower level of pain during the bandage changing and mobilization, faster local integration of skin grafts, more elastic scars, reduced itching, better pigmentation of newly formed skin).

Comparing these results with our experience using other topics in application on SGDZ, we concluded that the LMW-HA ointment is easier to apply on the wound. The local application determines less pain, assure better level of wound hydration (the patients feeling that the skin becomes "constrictive", the patient's need to repeat the topic application many times a day), calms the itching, enables better level of patients early mobilization. The esthetic effect is obvious, as the elasticity and pigmentation of the postoperatively scars are of good quality and the patients are satisfied by the result. For 13 patients (65%) with I, IIA and IIB burns we did not perform excision and reconstruction using skin grafts. The protocol followed in these cases combined the detersive action of NPWT or/and the HA ointment containing collagenase, with the efficient topics active against microorganisms (based on HA and silver) and the epithelial stimulation effect induced by LMW-HA topics.

Not all the patients arrived for the first clinical evaluation immediately after the accident (only 5 patients, 25%), not all the patients had negative secretions from the bacteriological point of view, at their arrival to the hospital (only 8 patients, 40%).

In twelve cases, (4 patients arrived at the first clinical evaluation immediately after the injury, 20%) the wounds were contaminated/colonized with one or two microorganisms, some of them with multiple resistance to antibiotic therapy.

Five patients (25%) had both, negative and respectively positive wounds' secretions, on different anatomic regions, on different wounds. In these cases, we compared the daily healing rate on both negative, respectively positive wounds, in area and in volume and we found significant differences (for example, case number 8: from 4.54% daily on the negative wounds to 2.85% daily on the positive wounds, for similar burn degree) (fig. 2).

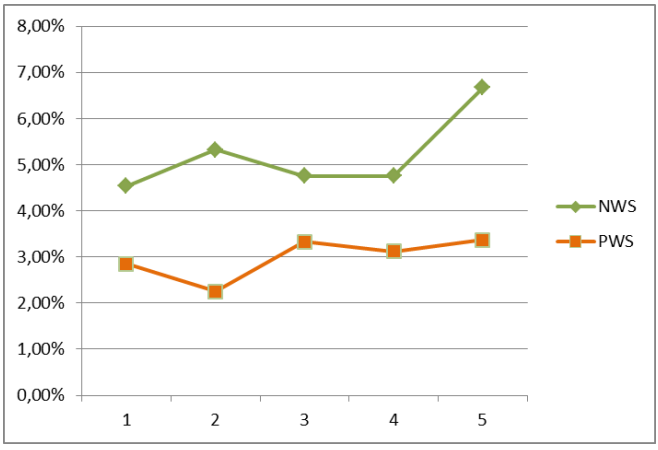

Fig. 2. NWS-negative wound' secretions. PWS-positive wound' secretions – (the daily healing rate for the 5 patients treated with HA topics, who had concomitant wounds with bacteriological negative, respectively positive secretions).

For the patients with both, negative and, respectively, positive wounds' secretions on different regions, the wounds' dimensions varied between 19,200 and 36,800 square mm (with a median of 24,420 square mm) for negative wounds, respectively from 34,800 to 50,600 square mm (with a median of 35,500 square mm) for the positive wounds. The tissue volumes varied between 3,840 and 9,120 cubic mm (with a median of 5,052 cubic mm) for negative wounds, respectively from 4,960 to 11,880 cubic mm (with a median of 7,794 cubic mm) for positive wounds.

We evaluated the daily healing progress on both types of burns, at the same patient, in area and in volume.

The results varied between 2.85% to 3.37% from the initial burned area in positive wounds, comparative with 3.57% to 6.67% from the initial burned area, for the negative wounds, for the same patient.

The duration of the healing process varied between 15 - 28 days, with an average value of 21.8 days, for negative wounds, respectively from 21 - 43 days, with an average value of 31.2 days, for positive wounds. We can conclude that the healing process is usually delayed by the wound infection, on the same patient, for lesions comparable from the dimensions and location point of view (figs. 3, 4, 5).

Bacterial colonization of the wound represents an important element for the progression of the healing process.

For example, a quantitative analysis of the distance of aggregation for bacteria from the wound surface showed that those composed of *P. aeruginosa* were located significantly deeper in the wound bed than those composed of *S. aureus* (10). This distribution of aggregates could explain the fact that *S. aureus* on the surface of the skin forms a biofilm that specifically inhibits wound healing mechanisms, thus avoiding the effects of local immunity and allowing other microorganisms to colonize and infect the lesion.

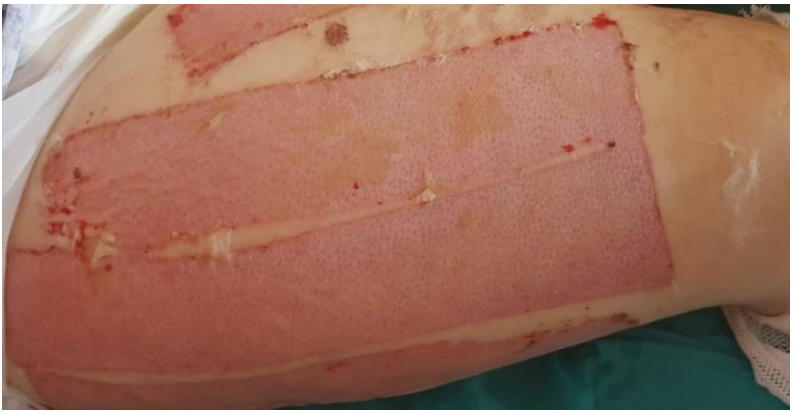

Fig. 3. Female, 47 years old, fire burn, IIA+IIB degree burn, both legs circumferential, 10% tbsa - skin grafts donor zones - day 8 after surgery, negative secretion, first day for local application of LMW-HA topic, twice a day; immediately after harvesting the graft we used alginated gauze for wound protection (Calcium alginate dressing with silver, days 1-4 and wide-meshed cotton webbing, impregnated with a non-active ingredient containing, skin-neutral ointment, days 5-8)

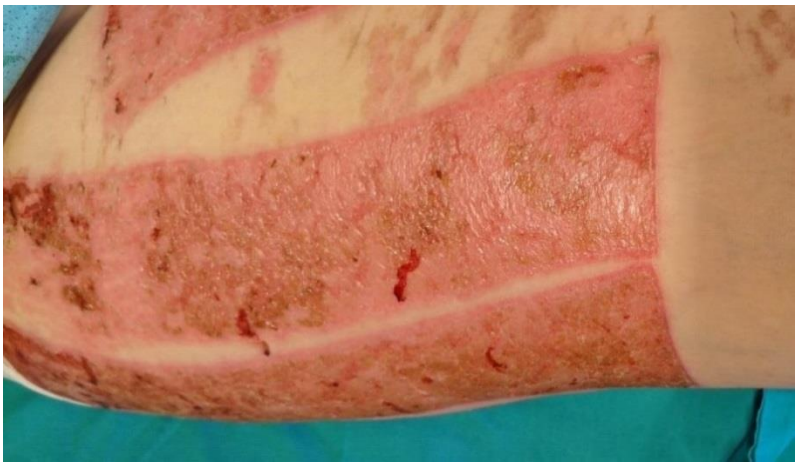

Fig. 4. Female, 47 years old, fire burn, IIA+IIB degree burn, both legs circumferential, 10% tbsa - skin grafts donor zones - day 14 after surgery, 7 days of local LMW-HA topic application, twice a day.

Fig. 5. Female, 47 years old, fire burn, IIA+IIB degree burn, both legs circumferential, 10% tbsa - skin grafts donor zones - day 21 after surgery, 14 days of local LMW-HA topic application, twice a day.

We found a correlation between the patient's late presentation to the first clinical examination (16 cases-80%) and the local bacterial colonization (12 cases with positive results of bacteriological examination-60%). There is another interesting correlation between some specific comorbidities (as diabetes, urinary infection, ulcerative colitis-50 % of cases) and the presence of Gram-negative bacteria (8 patients-40% of cases).

We used the HA ointment combined with silver in daily applications on the burned areas colonized by bacteria and we obtained a good result (good wound hydration, the persistence of a silver film on the wound surface, visible for a period of 10-12 hours after the application, smaller

quantity of secretion, lower level of local pain associated to the patient's mobilization).

CONCLUSIONS

The objectives of wound healing are to minimize blood loss, replace defects with new tissue (granulation tissue followed by scar tissue) and restore the intact epithelial barrier as soon as possible.

In these cases, healing requires tissue oxygen supply, proteins and other nutritional factors, a humid local environment, a favorable inflammatory process condition, wound debridement and correction of biological conditions. In some cases, this is easily achieved, and in others considerable effort is required.

We combined the surgical procedures with non-surgical protocols following the main goal: to promote a better and faster wound healing. The surgical protocol consisted in tangential excision of the necrotic tissue and reconstruction with skin grafts, or surgical debridement of the wound associated with local application of HA ointment containing collagenase. The non-surgical protocol used NPWT systems, in local applications stages for 5-7 days/stage and/or HA topics. The use of NPWT combined with collagenase stimulated the necrotic tissue elimination.

The use of HA topics was adapted to wounds' requirements. The local applications of HA ointment containing collagenase on recent burns stimulated the natural removal of the necrotic tissue. We used the HA ointment containing silver for the wounds with bacteria colonization, to manage the local infection and promote the faster healing.

The LMW-HA topic had a favorable outcome in all the cases, to stimulate the epithelialization and for a better scar management. The local applications of LMW-HA topics on the skin grafts donor zones, burns and skin grafts promoted a fast wound healing and reduced the pain level.

Healing is an interdependent and intricate process involving complex interactions between cells, the cellular microenvironment, biochemical mediators, and the extracellular matrix, molecules that normally lead to a restoration of affected tissue function.

The use of HA topics for the local treatment of superficial and intermediate burns was very efficient, and we observed a stimulating effect on the healing daily progress for both –skin grafts and donor zones, too. Due to the pro-angiogenic and pro-inflammatory effects, the LMW-HA topics manage to hurry the healing progression and favors the scars to become more elastic, less pigmented, with lower risk of tearing after the contact with clothes, better integrated and easier to be accepted by the patient. HA topics with LMW are painless, when applied on the wounds, promote a better wound or scar hydration, so the patient's mobility is higher.

Last, but not the least, the use of HA topics is related to the hospitalization period shortening and lower hospitalization costs, assuring a better scar quality and higher level of satisfaction for the patient.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest to disclose, and they received no specific funding regarding this scientific research.

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ELECTROHYDRAULIC *VERSUS* ELECTROMAGNETIC EXTRACORPOREAL SHOCK WAVE LITHOTRIPTER. WHAT LESSONS HAD WE LEARNED?

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ELECTROHYDRAULIC *VERSUS* ELECTROMAGNETIC EXTRACORPOREAL SHOCK WAVE LITHOTRIPTER. WHAT LESSONS HAD WE LEARNED? (Abstract): **Aim:** The present study aimed to determine if there is a difference in efficacy and safety in the use of electrohydraulic versus electromagnetic lithotripters when used for the treatment of a single kidney stone of 10-20 mm. **Material and methods:** We performed a retrospective study on a group of 106 patients with a single kidney stone which was treated by lithotripsy with devices that had different sources of shock wave generation. **Results:** The overall success of the treatment using electrohydraulic lithotripsy was 85.2% for kidney stones of 10-14 mm and 66.7% for kidney stones of 15-20 mm while for electromagnetic lithotripsy, the success rate for stones of 10-14 mm was 97% and 88.7% for kidney stones of 15-20 mm. Regarding postprocedural complications, we found 7 cases of steinstrasse, all in patients with kidney stones of 10-15 mm, of which 2 in the group of patients treated with electromagnetic lithotripsy and 5 in the case of those treated by electrohydraulic lithotripsy. We also found two post-procedural cases of perirenal hematomas, one in each group. None of the patients required additional therapeutical measures. **Conclusions:** There were no significant differences in the efficiency rate and safety profile for the treatment of kidney stones of 10-20 mm. However, the electromagnetic lithotripter has definite advantages in terms of the quality of the tracking and focusing system of the calculus compared to the bifocal system of the electrohydraulic lithotripter. **Keywords:** KIDNEY STONE, ELECTROHYDRAULIC LITHOTRIPSY, ELECTROMAGNETIC LITHOTRIPSY, EFFICIENCY OF FRAGMENTATION, SAFETY PROFILE.

Urolithiasis is a major public health problem worldwide. Although there are still no statistics for Romania, studies available show that incidence rates range from 7-13% in North America while in Europe about 4.7% of the population develop a lithiasis episode during their lifetime. For example, in Germany studies show that every year approximately 750,000 patients are diagnosed with kidney stones (1, 2).

Over the last 30 years, the treatment of urolithiasis has undergone dramatic changes, especially since Chaussy *et al.* introduced the use of extracorporeal lithotripsy (SWL) in clinical practice (3).

Currently, European Urology Association (EAU) guidelines suggest that for kidney stones smaller than 20 mm, treatment options should include, primarily, extracorporeal lithotripsy or percutaneous nephrolithotomy (PCNL), while for stones that exceed this size, gold-standard treatment is PCNL (4).

The increase of the safety profile of extracorporeal lithotripsy has been achieved through the emergence of new generations of lithotripsy, which use different shock wave generation mechanisms. Of course, the price paid to technology for the increase of the patient's comfort was a weaker disintegration of the stones and a higher rate of re-intervention (5). Extracorporeal lithotripsy using the first generations of lithotripters such as Dornier HM-3 had excellent efficacy but many disadvantages, one of the most important being the need to use general anesthesia.

Since its introduction in the medical practice, various generations of lithotripters have been developed, the difference between them consisting in the mechanism of generating shock waves. Thus, we considered it appropriate to perform a retrospective, comparative analysis between two lithotripters of a different generation, using different technologies, respectively, electrohydraulic and electromagnetic.

MATERIAL AND METHODS

To achieve this goal, we enrolled patients treated in the Extracorporeal Lithotripsy Department of Iași, "Dr. C. I. Parhon" Clinical Hospital, from January 2018-January 2019 and from February 2020-February 2022, respectively, these two intervals correspond to the use of two lithotripters with different technology. The size of the calculi was from 10-20 mm, introduced by performing paired cases. The exclusion criteria were the presence of multiple kidney stones or simultaneous ureteral stones.

Analgesia was achieved by topical application of a gel containing piroxicam, lidocaine, and cyclobenzaprine, and in very rare cases, by intramuscular administration of Tramadol. All procedures were performed in one-day hospitalization by a single urologist with significant experience in the field of extracorporeal lithotripsy and the average time between two procedures was approximately 30 ± 2 days.

The post-procedural examination was performed after 4 weeks and involved radiological examination by renal ultrasound and kidney-ureter-bladder radiography. Patients in whom radiological examination did not show certainly the presence or absence of the remaining fragments were excluded from this study. The case was considered refractory to extracorporeal lithotripsy if, after performing two procedures, no satisfactory fragmentation was found to allow the elimination of the fragments and subsequently required an endourological gesture.

SWL success was defined by the patient's stone-free status or the presence of CIRF (clinically insignificant residual fragments) below 4 mm.

RESULTS

The study includes a number of 106 patients with single kidney stones. In group A were introduced 54 patients with kidney stones treated with the second-generation electrohydraulic lithotripter, KS-88-4. Group B included patients who received extracorporeal lithotripsy using

the Dornier Sigma Compact electromagnetic source lithotripter. The characteristics of the two groups of patients are shown in first table.

TABLE I

Comparative results

	Group A	Group B	p
Total number of patients	54	52	
• Men	28	22	0.429
• Women	26	30	
Location of the calculus			
• Lower calyx calculus	9	6	
• Middle calyx calculus	3	3	0.75
• Upper calyx calculus	-	-	
• Renal pelvis calculus	42	43	
Laterality			
• Right	28	31	0.37
• Left	26	21	
Size of the calculus			
• 10-14 mm	34	34	0.95
• 15-20 mm	20	18	
Steinstrasse			
• 10-14 mm	-	-	0.437
• 15-20 mm	5	2	
Perirenal hematoma			
• 10-14 mm	-	-	0.99
• 15-20 mm	1	1	

Regarding the results obtained with the electrohydraulic lithotripter KS-88, the overall success rate was 85.2% - for kidney stones with dimensions between 10-14 mm the success rate was 88.23% (fig. 1), while for kidney stones between 15-20 mm, the calculated efficiency was 66.7% (fig. 2).

Using the Dornier Sigma Compact electromagnetic lithotripter, the overall success rate efficiency for stones of 10-14 mm was 97%, while for the ones in the category 15-20 mm the efficiency was 88.7%. Regarding the patients in the examined groups, we did not find statistically significant differences either for the 10-14 mm category (p=0,693) nor for the 15-20 mm category (p=0.202) in terms of the efficiency of the two lithotripters.

Regarding the complications (fig. 3), we report 7 cases of steinstrasse, all of which are found in patients with calculi with dimensions between 15-20 mm - 5 cases from electrohydraulic lithotripsy and 2 cases from electromagnetic lithotripsy. All steinstrasse cases were also treated by extracorporeal lithotripsy, the target being the large distal fragment. We also found two cases of perirenal hematomas, detected by post-procedural ultrasound, one case in each group. Fortunately, neither of the two

patients required complementary procedures and a 3-month reevaluation revealed complete resorption of the hematoma. In our study, there were no significant differences in the incidence of post-procedural complications, neither for steinstrasse (p=0.29) nor for perirenal hematomas (p=0.97).

The 10 patients who had calculi between 10-14 mm refractory to extracorporeal lithotripsy subsequently benefited from ureteral stenting followed by retrograde intrarenal surgery (RIRS).

From the group of the patients with stones between 15-20 mm (14 cases), in whom SWL did not give the expected results, 9 received a percutaneous procedure while 5 patients underwent RIRS.

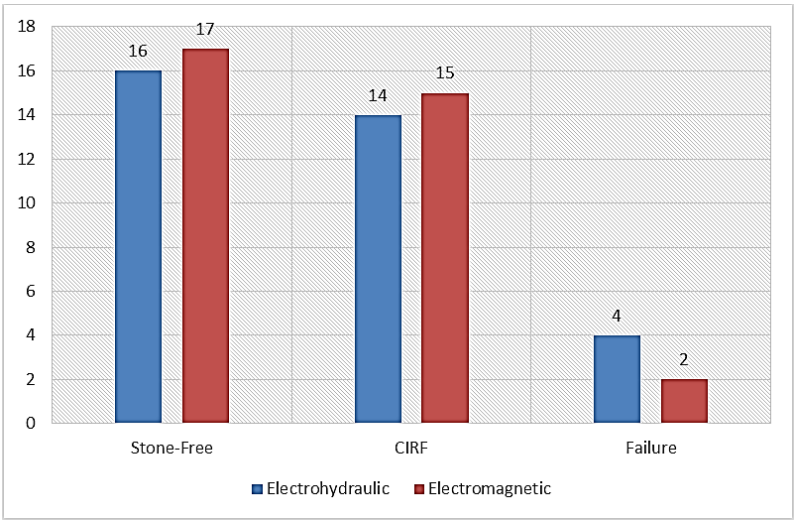

Fig. 1. Comparative results for the 10-14 mm category

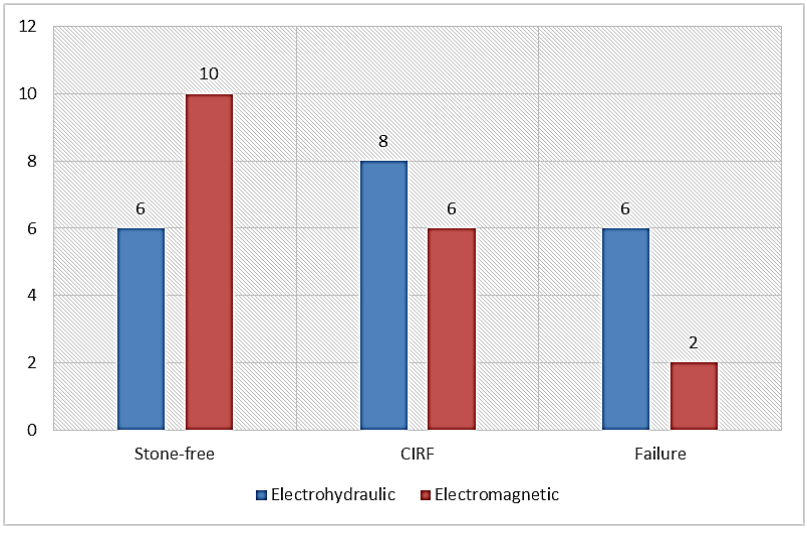

Fig. 2. Comparative results for the 15-20 mm category

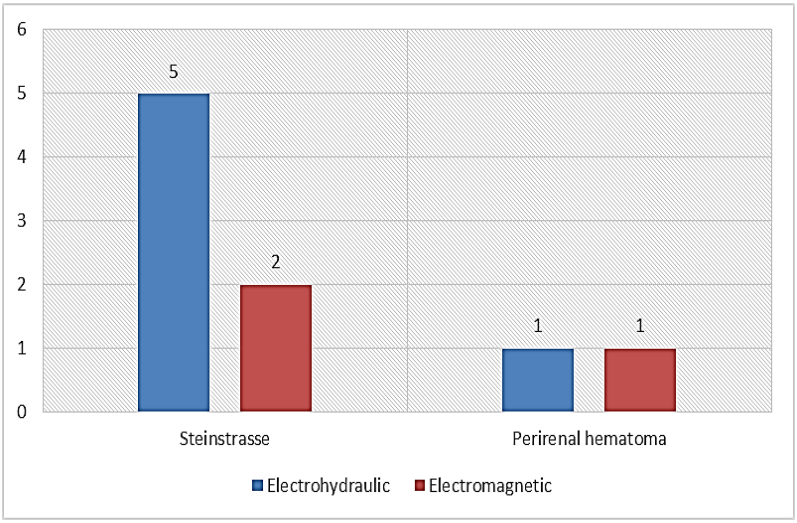

Fig. 3. Comparative results - complications

DISCUSSION

Since the introduction of lithotripsy in daily practice, various types of lithotripters have been developed, the main difference between them being the mechanism of producing shock waves. Broadly speaking, all lithotripters - regardless of the generation they belong - are composed of the power source, the wave focusing system, the coupling system, the treatment table and the control panel. The first focus (F1) is the place of wave generation, and the second focus (F2) is the place where energy converges, and fragmentation takes place. The electrohydraulic lithotripter we used, KS-88-4, made in China, uses energies between 18 and 20 kV and has a focal area of 4 cm^2 and the system of the stone's localization is bifocal, consisting of two fluoroscopy tubes located on either side of the generator in an oblique position.

In the case of electrohydraulic lithotripters, shock waves are produced by two electrodes and an ellipsoidal reflector. High voltage energy (15.000-20.000 V) is discharged by two electrodes, resulting in water vaporization and the creation of bubbles that subsequently collide and generate a high energy pressure wave that propagates spherically through the liquid, then the energy is focused and concentrated with a specially designed system. The energy at the level of the fragmentation focus (F2) in the case of electrohydraulic lithotripters is dependent on the voltage used and has variations of about 45%, which translates into a loss of energy ranging from 15-50%.

Electromagnetic lithotripters apply an electric current to an electromagnetic coil located inside a water cylinder. The discharged energy will cause a shock wave to propagate planar along with the cylinder. Electromagnetic lithotripsy does not require the replacement of electrodes, thus having the advantage that they can generate several hundred thousand pulses without requiring special maintenance and, on the other hand, the energy variation is only 3% and the fragmentation focus is a flat surface rather than a point as in the case of electro-

hydraulic lithotripters (6). The Dornier Sigma Compact lithotripter, used in our department, is equipped with a C-arm type fluoroscopic tracking system, of isocentric type, allowing rotation around a single focal point, which translates into a precise alignment between radiological detection and focusing of shock waves. For this type of lithotripter, the focal point has a size of 8.1x 105 mm and the applied pressure is 51 mPa (7).

Numerous authors have compared the results and safety profile of various types of a lithotripter, but the results are extremely varied. This variability of the data in literature seems to be generated by the lack of standardization in the evaluation of the results as well as of the numerous working protocols. These results differ not only between different lithotripsy centers whose results are published but even between two urologists using the same device within the same center (8). Precisely for this reason, the efficiency results, obtained with lithotripters of older generations, fall within a wide reference range, between 47-92%. In a similar study, conducted by Anila *et al.*, using lithotripters of different generations in patients with lithiasis characteristics similar to the patients in our study, they obtained an overall stone-free rate per month of approximately 95% for baseline calculi with dimensions between 10-20 mm (9). These results are slightly different from the results obtained by us, with the second-generation lithotripter - for calculi of 10-14 mm we obtained success rates of 81.5% and 70% for stones of 15-20 mm. In the specified study, there is no clear distinction between the two size categories of stones, the authors specifying the overall success rate of lithotripsy for stones of 10-20 mm. With the electromagnetic lithotripter, the results obtained by the mentioned authors are also slightly different, the success being 78%, compared to the results obtained by us, respectively 92.6%. The explanation for this phenomenon may arise from the fact that in the cited article, there is a significant percentage of lower calyx stones (60% in the cited article, compared to 12% of patients with lower calyx stones in our group). This is certainly very relevant and underlies the difference in the results mentioned, Weld *et al.*, pointing out in their research that pelvic lithiasis and stones at the pyeloureteral junction have a better fragmentation and elimination than calyx stones, especially lower calyx ones (10).

In a study comparable to ours, involving patients with similar lithiasis characteristics, Kavano *et al.*, obtained fragmentation rates of stones of 86%, a percentage similar to that obtained by us but stone-free rates of 54% in the first month and 68% after 3 months(11). These results are similar to those obtained by us, stating that, overall, in our study, the stone-free rate at one month, in the group of patients treated with Dornier Sigma S lithotripter, was 52% but we also considered the presence of the clinically insignificant lithiasis fragments (CIRF), which in our group of patients constituted an important percentage, respectively 32.7%. In the previous study, the percentage of patients who presented with CIFR was not specified.

As in our statistics, a slight superiority of the efficiency of electromagnetic lithotripsy versus electrohydraulic lithotripsy, Sheir and collaborators obtained rates of 72% and 68%, respectively, for kidney stones, stating that the statistical difference

was insignificant. Elbaset *et al*., using electromagnetic lithotripsy, obtained for kidney stones a fragmentation and clearance rate of lithiasis fragments of 58.6%, much lower than the results we report (12). This difference is explained by the fact that in the specified study, all kidney stones were evaluated before the procedure by computed tomography, with densities of over 1000 UH, so hard stones. In our center, there was no possibility to perform pre-procedural computed tomography.

Our results using the electromagnetic lithotripter are also confirmed by other studies conducted on large groups of patients. Thus, Tan *et al.*, on a batch of 1666 kidney stones, performed 2326 extracorporeal lithotripsy sessions - an average of 1.4 sessions for each stone, achieving an overall success rate of 81%. Of these patients, 495 required recurrences of extracorporeal lithotripsy, resulting in a retreatment rate of 29.7%. Regarding the stone-free rates obtained by Tan and his collaborators for kidney stones (53%), these are similar to the values obtained by us, respectively 52% (13).

A study with results superior to the one carried out by us is the one published by Korhmann and collaborators. They report a stone-free rate efficiency of 91% for kidney stones, the explanation for this very large difference being primarily reevaluation - respectively 5 months after the performance of lithotripsy - and the use of energies at very high levels involved the use of general anesthesia compared to our study. We used analgesia performed by applying a local anesthetic gel. Moreover, the success reported by Korhmann also comes from the fact that lithotripsy was performed in a proportion of 25% in patients who previously underwent endourological intervention (retrograde ureteroscopy, PCNL) (13, 14), while in our study, for stones in the 10-20 mm category, extracorporeal lithotripsy was the first-line treatment. In fact, in our center, almost all kidney stones larger than 20 mm are referred to as an interventional method, extracorporeal lithotripsy for this category of stones being performed exceptionally (anesthetic risk , anatomic conditions).

A well-known complication of extracorporeal lithotripsy is steinstrasse consisting in the accumulation of obstructive lithiasis fragments following the procedure. This complication is sometimes asymptomatic and transient but can cause severe problems. The data published in the literature are in the range of 2-10%, this being directly proportional to the size of the calculus (15, 16). The data obtained by us, 1.85% (EH) and 3.84% (EM), respectively, are within this range. However, for the size of the calculi analyzed by us, Soyupek and collaborators report different data, respectively 15.7% but data closer to those reported by us, were published by Javanmard *et al*., respectively 6.6% incidence of steinstrasse for kidney stones (17, 18).

The limitations of our study are primarily the relatively small number of identified paired cases, but also the impossibility of pre-procedural tomographic evaluation of patients to determine the hardness of the stones by measuring their densities and lack of analysis of the composition of fragments through infrared spectroscopy after SWL .

CONCLUSIONS

Even though the two lithotripters we considered use completely different tech-

nologies, there were no significant differences in the efficiency rate and safety profile for the treatment of kidney stones of 10-20 mm. However, the electromagnetic lithotripter has definite advantages: can provide shock waves that are equal in intensity and has a better quality of the tracking and focusing system of the calculus with a lower X-Ray exposure compared to the bifocal system of the electrohydraulic lithotripter.

Moreover, the spark gap lithotripter has the disadvantage of the need to periodically replace the electrodes compared to electromagnetic technology which is time consuming and cannot offer the same level of energy throughout the procedure.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

SERUM FERRITIN AS A DIAGNOSTIC BIOMARKER FOR KAWASAKI DISEASE

Kawasaki disease is an acute febrile systemic vasculitis of unknown etiology. Kawasaki disease diagnosis depends on the use of adjuvant diagnostic markers, such as echocardiographic findings and laboratory biomarkers, including C-reactive protein, erythrocyte sedimentation rate, albumin, alanine aminotransferase, aspartate aminotransferase, white blood cell count, hemoglobin, platelet count, and pyuria. Although the laboratory findings observed in Kawasaki disease are not specific enough to conclusively establish a diagnosis, they can be useful, particularly in patients with a high suspicion for Kawasaki disease. Diagnosis of Kawasaki disease is occasionally delayed because it is solely based on clinical symptoms. Previous studies have attempted to identify diagnostic biomarkers for Kawasaki disease. Recently, patients with Kawasaki disease were reported to have elevated serum ferritin levels. This study aims to investigate the usefulness of the serum ferritin level as a diagnostic biomarker for distinguishing Kawasaki disease from other acute febrile illnesses. Blood samples were obtained from pediatric patients with Kawasaki disease and those with other acute febrile illnesses between December 2007 and June 2011 for measuring various laboratory parameters, including serum ferritin levels. In patients with Kawasaki disease, laboratory tests were performed at diagnosis and repeated at 2, 14, and 56 days after intravenous immunoglobulin treatment. At the time of diagnosis, serum ferritin levels in patients with Kawasaki disease (188.8 μg/L) were significantly higher than those in patients with other acute febrile illnesses (106.8 μg/L, P=0.003). The serum ferritin cut-off value of 120.8 μg/L effectively distinguished patients with Kawasaki disease from those with other acute febrile illnesses, with a sensitivity and specificity of 74.5% and 83.3%, respectively. Serum ferritin may be a useful biomarker to distinguish Kawasaki disease from other acute febrile illnesses. (Kim SH, Song ES, Yoon S, Eom GH, Kang G, Cho YK. Serum Ferritin as a Diagnostic Biomarker for Kawasaki Disease. *Ann Med Lab* 2021; 41(3): 318-322).

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SENESCENT FIBROBLASTS BETA-GALACTOSIDASE INDUCED BY EXTRACELLULAR VESICLES OBTAINED FROM MSC-iPSC UNDER THE ACTION OF VARIOUS MODULATORS

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SENESCENT FIBROBLASTS BETA-GALACTOSIDASE INDUCED BY EXTRACELLULAR VESICLES OBTAINED FROM MSC-iPSC UNDER THE ACTION OF VARIOUS MODULATORS (Abstract): The absolute majority of published experimental data involve mesenchymal stem cells, although the possibilities offered by the use of pluripotent stem cells reprogrammed from mesenchymal stem cells are practically infinite. **Aim**: The goal of our study was represented by tracking the effects of stimulation and inhibition of multiple functional, intracellular pathways of senescent dermal fibroblasts in culture, in the presence of microvesicles released from pluripotent stem cells reprogrammed from mesenchymal stem cells. **Material and methods**: We used flow cytometry to measure the production of the beta-galactosidase level by dermal fibroblasts in culture after 30 passages and ultraviolet treatment. **Results**: Reduction of β-galactosidase concentrations in senescent dermal fibroblasts was evident upon administration of exosomes (approximately 20% on average) and exosomes co-administered with: LY-294002 (a highly selective PI3K kinase inhibitor, 41% on average); nutlin-3 (which stabilizes the non-genomic actions of p53, respectively the MDM2/p53 pathway, 63% on average); rapamycin (which stimulates autophagy and the mTOR pathway and related ones, 62% on average); and metformin (a pharmacologically active substance, considered a reprogrammer of energy metabolism, 73% on average). **Conclusions**: Exosomes derived from pluripotent stem cells induced from mesenchymal stem cells, administered in the culture medium of senescent dermal fibroblasts, can modify the degree and evolution of the senescence of these last cells by potentiating, activating, or stimulating some intracellular pathways of biological signal transduction, but especially through epigenetic reprogramming of energy metabolism. **Keywords:** MSC-iPSC, ENERGY METABOLISM REPROGRAMMING, EXOSOMES, FIBROBLASTS, MSC, EPIGENETICS.

The skin is the largest human organ and has a multitude of functions. It is well known that the healing of a skin wound/injury activates an impressive number of unique functionalities, which include cascaded cellular mechanisms and which, at the same time, represent a unique process in nature. While regeneration defines the specific replacement of tissue, with reference to the superficial epidermis, mucosa, or fetal skin, skin repair processes actually represent a non-specific form of healing, in which the

injury is healed by extensive fibrosis and scar formation. The clear physiological objective of wound repair in mammals ultimately equates to the formation of scars, a phenomenon that is directly proportional to the extent of the inflammatory process throughout healing (1).

The healing of injuries is thus a complex and dynamic process, at the same time supported by a multitude of cellular processes, which require strict coordination to be able to efficiently repair the damaged tissue. Alteration of cellular behaviors, which occurs for example in diabetes and the aging process, can lead to significant impairment of healing and the appearance of chronic, non-healing lesions. This chronic evolution represents a particularly important socio-economic burden due to its high prevalence and recurrence. Thus, there is a great need for a better understanding of all the complex mechanisms underlying healing (2).

Chronic lesions become superinfected with bacteria, which aggravates the inflammatory process. Keratinocytes in chronic lesions exhibit aberrant activation, resulting in hyperproliferation and impaired local migration. A very large number of cells present at the level of chronic lesions (for example, macrophages and fibroblasts) become evolutionarily senescent and transform into a senescence-associated secretory phenotype (or called SASP), which further perpetuates senescence, activates the massive release of reactive oxygen species (ROS) and further intensifies the inflammatory process. Moreover, the large amounts of final products of advanced glycation that appear contribute, at the same time, to the development of the inflammatory process and cellular senescence at the level of the lesion. Together, all these characteristics produce excessive tissue destruction and alter cellular functions, preventing normal healing (3).

Mesenchymal stem/stromal cells (MSCs), due to their competences to establish skin cells such as fibroblasts and keratinocytes, as well as their special attribute to suppress inflammation in the inflammatory focus of wounds have attracted increasing attention among researchers. Thus, exosomes transfer practical cargoes (e.g., growth factors, cytokines, miRNAs, etc.) from mesenchymal stem cells to target cells, thus affecting extraordinarily important biological functions of recipient skin cells, such as migration, the expansion and also the secretion of particularly important elements of the extracellular matrix (for example, collagen of various types) (4).

The goal of our study was represented by the tracking of the effects of stimulation and inhibition of multiple intracellular functional pathways of senescent dermal fibroblasts in culture, in the presence of microvesicles released from pluripotent stem cells reprogrammed from mesenchymal stem cells.

MATERIAL AND METHODS

The laboratory research that we carried out aimed at quantifying the evolution of the degree of senescence of dermal fibroblasts (senescent cells) by evaluating β-galactosidase through flow cytometry (5, 6), in a bi-culture, in the presence of pluripotent stem cells reprogrammed from mesenchymal stem cells (in culture) or under the action of exosomes/microvesicles released from MSC-iPSCs, in the form of concentrated supernatant, administered in the culture medium.

Storage rat dermal fibroblasts (cell line no. 1) were grown under standard laborato-

ry conditions and tested for β-galactosidase through flow cytometry after passage 1, passage 10, passage 20, and passage 30. To obtain senescent dermal fibroblasts, they were irradiated with ultraviolet, λ=315 nm, 80 mJ/cm^2, and then multiplied by means of 30 consecutive passages.

To obtain MSC-iPSCs, we used the episomal method with iPSC reprogramming vectors and the specific Stemline culture medium (Invitrogen), in full agreement with the manufacturer's specifications. We preferred this technique because the use and manipulation of potentially genetic material included in the "dangerous" chapter are totally excluded. The growth and multiplication of pluripotent stem cells reprogrammed from mesenchymal stem cells were done using an Alvetex 3D bio support -a gift from Reinnervate-, since a series of other experiments demonstrated the maximum efficiency for this type of support.

For the large-scale evaluation of possible interactions between the cell lines we used, first of all, a series of preconditioning factors for MSC-iPSCs: 1% O_2, 1 μM angiotensin II, vitamin E 0.5 μM, and low doses of lipopolysaccharide (0.1 μM) (7).

For exosome separation, the culture medium of pluripotent stem cells induced from rat aortic smooth muscle cells was centrifuged up to 100,000xg (8).

The active substances we used for the various experiments and protocols including senescent dermal fibroblasts were: 1 μM LY294002, 1 μM nutlin-3, 1 μM rapamycin, and 2 μM metformin.

All the experimental results obtained during the present research were processed entirely by statistical methodologies, using a number of well-known and optimized tests over time and for medical research, among which the One-Way ANOVA test should be mentioned, which was also completed, only occasionally, with the Student-Newman-Keuls test.

RESULTS

During the development and multiplication processes, strictly monitored, MSC-iPSCs presented a series of characteristics of embryonic morphology, constantly observed in cell culture conditions, being otherwise undifferentiated, even organizing themselves in the form of aggregates/organoids, demonstrating it therefore clearly belongs to this cell type.

To verify the viability of experimentally reprogrammed pluripotent stem cells from mesenchymal stem cells, grown on 3D Alvetex bio support, preconditioned with 1% O_2, angiotensin II, vitamin E, and low doses of lipopolysaccharide, we used 0.1 μM 5-carboxyfluorescein (succinimidyl ester), with excitation/emission maxima 498/517 nm (green fluorescence) and flow cytometry as stated. The best qualitative and quantitative development in the case of pluripotent stem cells experimentally reprogrammed from mesenchymal stem cells, starting from the 2nd passage in culture, we obtained when we multiplied them in a preconditioning medium that contained a concentration of only 1% O_2. We considered this increase to always be 100% (n=5 experiments for each set, data not shown, personal observation). Comparatively, development in the presence of angiotensin II in the preconditioning medium was 79.23±2.74% (n=5), in the presence of vitamin E it was 72.39±2.87% (n=5) and in the presence of lipopolysaccharide was 76.72±1.79% (n=5).

In addition, the most important β-galactosidase reduction effects in senescent dermal fibroblasts in monoculture, in the presence of exosomes/microvesicles at a

concentration of 0.01%, obtained in concentrated form by ultracentrifugation of the MSC-iPSC supernatant, were observed when we developed these latter cell types in a medium preconditioned with 1% O_2. Thus, under these conditions, we found amplification of β-galactosidase reduction in senescent dermal fibroblasts by approximately 20% (on average).

In a series of experiments, a number of senescent dermal fibroblasts were treated with 1 μM LY294002, considered to be a highly selective PI3K kinase inhibitor, as well as 0.01% exosomes obtained from the supernatant of MSC-iPSC. We found amplification of β-galactosidase reduction in senescent dermal fibroblasts by approximately 41% (on average) when MSC-iPSC were multiplied in a medium preconditioned with 1% O_2 (fig. 1).

In the second series of experiments, a different batch of senescent dermal fibroblasts was treated with 1 μM nutlin-3, which stabilizes with great efficiency the non-genomic actions of p53 (MDM2/p53 pathway), as well as 0.01% exosomes obtained from the supernatant of MSC-iPSC. We found amplification of β-galactosidase reduction in senescent dermal fibroblasts by approximately 63% (on average) when MSC-iPSC were multiplied in a medium preconditioned with 1% O_2 (fig. 2).

In the third series of experiments, an important number of senescent dermal fibroblasts were treated with 1 μM rapamycin, which stimulates autophagy and the mTOR pathway and related ones, as well as 0.01% exosomes obtained from the supernatant of MSC-iPSC. We found amplification of β-galactosidase reduction in senescent dermal fibroblasts by approximately 62% (on average) when MSC-iPSC were multiplied in a medium preconditioned with 1% O_2 (fig. 3).

Finally, in the fourth series of experiments, a totally different batch of senescent dermal fibroblasts was treated with 2 μM metformin, a pharmacologically active substance, considered a reprogrammer of energy metabolism, as well as 0.01% exosomes obtained from the supernatant of MSC-iPSC. We found amplification of β-galactosidase reduction in senescent dermal fibroblasts by approximately 73% (on average) when MSC-iPSC were multiplied in a medium preconditioned with 1% O_2 (fig. 4).

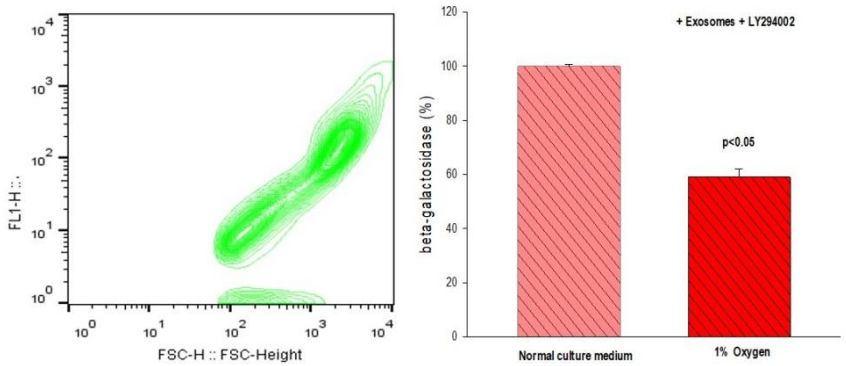

Fig. 1. Quantification by flow cytometry of β-galactosidase in senescent dermal fibroblasts in monoculture, when multiplied in the presence of LY294002 (1 μM) and MSC-iPSC-released exosomes, preconditioned in 1% O_2 medium. $p<0.05$ as compared to the normal culture medium

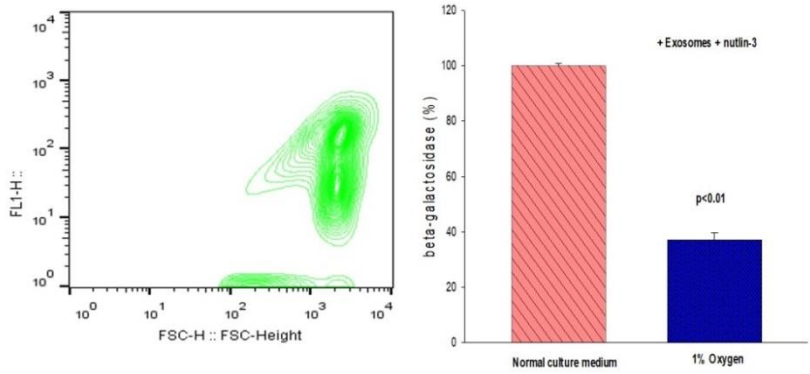

Fig. 2. Measurement of β-galactosidase in senescent dermal fibroblasts, when developed in the presence of nutlin-3 (1 μM) and MSC-iPSC-released vesicles, preconditioned in 1% O_2 medium. $p<0.01$ as compared to the normal culture medium.

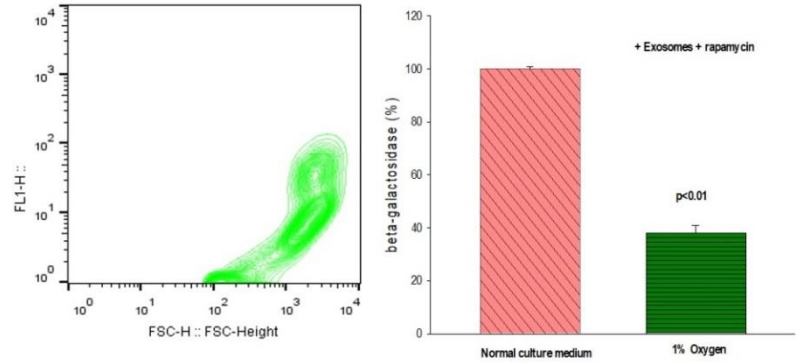

Fig. 3. Determination of β-galactosidase in senescent dermal fibroblasts, when cultured in the presence of rapamycin (1 μM) and MSC-iPSC-released extracellular vesicles, preconditioned in 1% O_2 medium. $p<0.01$ as compared to the normal culture medium.

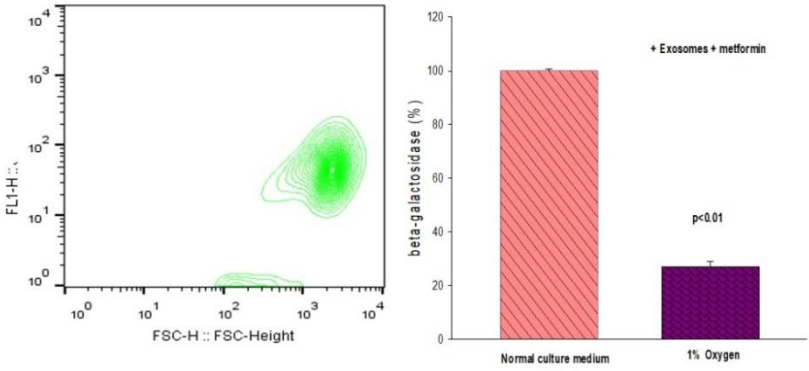

Fig. 4. Evaluation of β-galactosidase in senescent dermal fibroblasts in monoculture, when grown in the presence of metformin (2 μM) and MSC-iPSC-released extracellular microvesicles, preconditioned in 1% O_2 medium. $p<0.01$ as compared to the normal culture medium.

DISCUSSION

Mesenchymal stem cells (MSCs) have the potential to be used in cell-based regenerative therapies. Currently, hundreds of clinical trials are using MSCs for the treatment of various diseases. However, MSCs are low in number in adult tissues; they show heterogeneity depending on the cell source and show limited proliferative capacity and early senescence in vitro cultures. These elements negatively affect the regenerative capacity of MSCs and therefore limit their use for clinical applications. As a result, unique methods to generate induced MSCs (iMSCs) from induced pluripotent stem cells have actually been verified. Advancement and optimization of procedures for generating iMSCs from induced pluripotent stem cells is necessary to assess their regenerative capacity in vivo and in vitro. In addition, it is essential to compare iMSCs with primary MSCs (separated from adult tissues) regarding their safety and efficacy. Careful examination of the promising properties of iMSCs in vitro and their long-term interaction habits in animals is essential for their transfer from bench to patient (9, 10).

Human derived pluripotent stem cells (hiPSCs) are indispensable resources for the production of premium, separated cells in unrestricted quantities for both basic research studies and scientific use. They are particularly useful for studying human disease systems in vitro, making it possible to avoid the ethical problems of human embryonic stem cell research. However, there are substantial constraints when using traditional flat culture approaches, which specifically impair cell growth, differentiation performance, maintenance of stability, and facilitation of multicellular 3D structure. Embryoid bodies, which are multicellular aggregates spontaneously created from iPSCs in the suspension system, can help address these issues. Due to the unique microenvironment and cell communication within the structure of embryoid bodies, which a 2D culture system cannot achieve, they have actually been widely applied in hiPSC-derived differentiation and show considerable advantages especially in culture scale-up, performance improvement differentiation and ex vivo simulation. Embryoid bodies can also be used in early prediction of the differentiation capacity of iPSCs (11, 12).

Both mesenchymal stromal cells and fibroblasts can be isolated from almost all organs. Mesenchymal stromal cells represent the most favorable cellular healing agents with regenerative potential, and fibroblasts are among the most abundant cell types with the ability to preserve homeostasis. Due to the promising and attractive properties of MSCs, they have been well studied and thus their differentiation capacities, immunomodulatory potentials, as well as gene expression profiles are determined. Fibroblasts and mesenchymal stromal cells have been observed to have comparable morphology, gene expression patterns, surface markers, expansion, differentiation, and immunomodulatory capabilities. Therefore, it is difficult to differentiate these two types of cells. Epigenetic signatures, i.e., the methylation patterns of cells, are the only appealing functional distinction between them. Such substantial similarities show that these two cells may be associated with each other (13, 14, 15).

Cellular senescence is currently recognized as one of the nine characteristics of the aging phenomenon. Recent experimental data show, on the other hand, the major involvement of senescent cells in

tissue aging and even more so in some age-related diseases. Skin is considered to be an ideal model for the study of aging phenomena. If features of senescence are evident with skin aging, the senescent phenotype is different in different skin cell types. Moreover, the origin of cellular senescence in the skin is still far from being known and multiple origins are possible. These aspects reflect the mosaic nature of skin aging (16).

Skin aging is the most visible and obvious manifestation of the body's aging and can directly serve as a predictor of life expectancy and health status. However, it is the human desire for very long-lasting beauty that further exacerbates the interest in this subject, and therefore considerable means and efforts are devoted to studying the mechanisms of skin aging through both basic and applied research. Any type of interest, medical and non-medical, is generally welcome for the development of research in the field of the skin system, it being clear that all the results of these studies can be of great help in our attempt to deepen the understanding of complex molecular and biological processes, the type of cellular and developmental signaling, as well as the immunological type, manifested at the level of this vast organ. In fact, the skin is an ideal and relatively handy organ for observing and analyzing the impact of possible extrinsic and intrinsic driving factors of aging (17, 18).

At the skin level, aging is manifested by the appearance of photodamage and skin atrophy, with the consequent reduction of underlying tissues and impaired barrier function. To determine whether rapamycin, a drug already approved by the US authorities, which targets the mechanistic component of the mTOR complex, can reduce senescence and, at the same time, markers of aging in human skin, an exploratory, placebo-controlled interventional study was conducted, in a strictly controlled clinical dermatology setting. Topical rapamycin was observed to significantly reduce p16INK4A protein expression, consistent with a significant reduction in cellular senescence. This tissue change was accompanied by an important and relative improvement in the clinical appearance of the skin and even histological markers of aging, as well as an important increase in collagen type VII, which is an essential component of basement membrane integrity (19).

Increasing evidence suggests that chronic inflammation and senescence underlie the development of many severe age-related diseases, given that both biological processes appear to be directly proportional to the degree to which the body ages. However, until now, the existence of any specific gene related to inflammation or senescence that is common to different species or different tissues has remained unknown. Thus, these potential markers of aging could greatly help to identify possible targets for therapeutic interventions in conditions associated with aging phenomena and could also help us to deepen our understanding of the main mechanisms of aging. In addition, it was very interesting to note the experimental fact that genes involved in aging-related processes tend to be much more tightly controlled in long-lived people compared to middle-lived people. These observations could suggest the existence of a general genetic level, which would affect the lifespan of any individual. Taken as a whole, such a descriptive study has made a major contribution to a better understanding of the genetic signatures common to aging phenomena, as well as the patterns of

aging that may be tissue-specific in particular (20).

Senolytics are a relatively new class of drugs believed to selectively eliminate senescent cells. Thus, the first senolytic drugs, which include dasatinib, quercetin, fisetin, and navitoclax, were discovered using a hypothesis-only approach. The most aggressive senescent cells are highly resistant to apoptosis and show hyperstimulated anti-apoptotic pathways, which can protect them from their own secretory phenotype associated with senescence, a fact that allows them to survive, even if the neighboring cells are eliminated. On the other hand, senolytics transiently deactivate these pro-survival complexes / pathways, inducing apoptosis of those senescent cells, which have a tissue-destructive SASP. Since it takes weeks to restore senescent cell mass, senolytics can be administered intermittently in a hit-and-run approach. In a number of preclinical models, it has been shown that senolytics can delay, prevent, or significantly improve body fragility, tumorigenesis, but also cardiovascular, neuropsychiatric, hepatic, renal, and musculoskeletal disorders, pulmonary conditions, but also eye, hematological, metabolic, and skin conditions, as well as organ transplant complications, but also those of radiotherapy and, last but not least, those of cancer treatment. Early pilot tests for a number of senolytics strongly suggest that they do indeed decrease senescent cell mass, reduce inflammatory processes, and improve frailty in humans. Clinical trials involving their use in diabetes, idiopathic pulmonary fibrosis, Alzheimer's or COVID-19, osteoarthritis, osteoporosis, eye disease, bone marrow transplantation, and last but not least, in the case of the childhood cancer survivor, are in progress or have just begun (21, 22, 23).

Senescent cells, which are able to release factors in the microenvironment that cause inflammation and dysfunction, assimilating to the so-called secretory phenotype associated with senescence (also known as SASP), increase numerically with aging and, at the same time, at the level of multiple etiological sites chronic diseases. Senolytic-type pharmacological compounds, including the combination of dasatinib and quercetin, can selectively eliminate senescent cells through a mechanism of transient deactivation of pro-survival complexes, which protect them from their own apoptotic environment. Thus, in the first clinical trial in which senolytics were used, dasatinib and quercetin significantly improved physical functions in patients with idiopathic pulmonary fibrosis, a fatal disease inextricably associated with senescence. However, to date, no research has directly demonstrated that senolytics can reduce the number of senescent cells in humans. On the other hand, in the case of the combination with dasatinib and quercetin, the mass of senescent cells in humans would be significantly reduced (24).

CONCLUSIONS

The most important β-galactosidase reduction effects in senescent dermal fibroblasts in monoculture, in the presence of exosomes/microvesicles at a concentration of 0.01%, obtained by ultracentrifugation of the supernatant of pluripotent stem cells reprogrammed from mesenchymal stem cells, were obtained when we grew these latter types of cells in medium preconditioned with 1% O_2.

Reduction of β-galactosidase concentrations in senescent dermal fibroblasts was

evident upon administration of exosomes (approximately 20% on average) and exosomes co-administered with: LY-294002 (a highly selective PI3K kinase inhibitor, 41% on average); nutlin-3 (which stabilizes the non-genomic actions of p53, respectively the MDM2/p53 pathway, 63% on average); rapamycin (which stimulates autophagy and the mTOR pathway and related ones, 62% on average); and metformin (a pharmacologically active substance, considered a reprogrammer of energy metabolism, 73% on average).

All these results suggest that exosomes derived from pluripotent stem cells induced from mesenchymal stem cells, administered in the culture medium of senescent dermal fibroblasts, can modify the degree and evolution of the senescence of these last cells by potentiating, activating, or stimulating some intracellular pathways of biological signal transduction, but especially through epigenetic reprogramming of energy metabolism.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research

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NEWS

HYPERMAGNESEMIA REDUCES THE RISK OF INTRACRANIAL ANEURYSMS: A MENDELIAN RANDOMIZATION STUDY ON 23,829 INDIVIDUALS OF EUROPEAN ANCESTRY

Serum magnesium levels have been extensively studied for subarachnoid hemorrhage after intracranial aneurysm (IA) rupture as a treatment for vasospasm however its influence regarding aneurysm formation and progression has not been researched yet. The authors have used a mendelian randomization approach for 5 single nucleotide polymorphisms as variables for serum magnesium in a genome-wide association study in 23,829 individuals of European ancestry. The results proved an association between higher serum magnesium concentration and lower risk of all IAs, as well as unruptured and ruptured IAs examined separately. Also, at least partially, the results suggest that magnesium mediates the role of blood pressure in IA. Overall, this study is the first to identify a potential causal association between serum magnesium concentrations and risk of IA and it provides preliminary evidence for further research regarding the role of magnesium in IA management (Larsson SC, Gill D. Association of Serum Magnesium Levels With Risk of Intracranial Aneurysm: A Mendelian Randomization Study. *Neurology* 2021: 10.1212/WNL.0000000000012244 / doi:10.1212/wnl.0000000000012244).

Kv POTASSIUM CHANNELS AND RELAXATION OF RAT SMALL MESENTERIC ARTERIES BY HYDROGEN PEROXIDE

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Kv POTASSIUM CHANNELS AND RELAXATION OF RAT SMALL MESENTERIC ARTERIES BY HYDROGEN PEROXIDE (Abstract): H_2O_2 is an endothelium-derived hyperpolarizing factor (EDHF). The effects of exogenous H_2O_2 may vary with the vascular bed and experimental conditions. Various contributions of different K^+ channels and of cyclic GMP have been shown for the relaxing effect of H_2O_2 in small arteries from different species and vascular beds. **Aim:** Here we studied the involvement of Kv channels in H_2O_2-induced relaxation of vascular smooth muscle. **Material and methods:** We used de-endothelised rings of rat small mesenteric arteries. **Results:** H_2O_2 induced similar relaxations of rings pre-contracted by either norepinephrine (NE) or phenylephrine (PHE). Inhibition of H_2O_2 effect in the case of NE was complete with 4-aminopyridine (4-AP), a blocker of Kv channels, as shown recently by another group, using the same vascular preparation. The inhibition of H_2O_2 effect by 4-AP was weaker in the case of PHE than in the case of NE. **Conclusions:** Based on our results, and also on the recent findings from other laboratories, we may suggest that, at least in rat small mesenteric arteries, the relaxation induced by H_2O_2 is mediated to a different extent by Kv and by other mechanisms, depending on the contracting agent. Endogenous endothelium-derived H_2O_2 could activate various smooth muscle K^+ channels and thus functionally compensate for decreased NO availability. **Keywords:** HYDROGEN PEROXIDE, OXIDATIVE STRES, ENDOTHELIUM-DEPENDENT RELAXATION, RESISTANCE ARTERIES, EDHF, POTASSIUM CHANNELS.

As we have also mentioned in some of our most recent studies (1, 2, 3), we have a sustained interest in endothelial function and dysfunction (1, 2, 3, 4, 5, 6) and also in various aspects of oxidative stress (7, 8, 9, 10). Our work in such areas is placed on the background of an ongoing wide interest in: (a) endothelium-dependent relaxation (EDR) of the vascular smooth muscle (VSM), including EDH-mediated EDR, i.e., the EDR mechanisms mediated by endothelium-dependent hyperpolarization (EDH) (11, 12, 13, 14); (b) oxidative stress in relation with vascular dysfunction (15, 16). In this context, we decided to further examine now the effect of exogenous hydrogen peroxide (H_2O_2) in de-endothelised rings of rat small mesenteric arteries, based on our very early study (17); i.e., starting from our twenty years old data, which were

obtained shortly after the very first studies regarding either the mechanism of VSM relaxation by H_2O_2 (18, 19) or its role as an endothelium-derived hyperpolarizing factor (EDHF) (20, 21). Beside the general interest mentioned above, the present study has particular reasons, such as: (a) the mechanism of VSM relaxation by H_2O_2 is still incompletely explained and may vary due to many factors (13, 18, 19, 22, 23, 24), as we have also stated in our earlier paper (17); (b) the role of H_2O_2 as EDHF is now far better known and understood (better than twenty years ago or even than just five years ago), but it clearly has many sources of variability for its importance in EDH-mediated EDR and for the actual mechanisms involved (5, 6, 13, 18, 19, 20, 24).

Vascular reactivity, including EDR, is a key factor for local blood flow and for arterial blood pressure. It is very well established (11, 12, 13, 14, 24), as we have also underlined repeatedly (1, 2, 3, 5, 6), that EDR in resistance arteries is: (a) largely independent of nitric oxide (NO) synthase and cyclooxygenase; (b) mediated by EDH, which is based on endothelial Ca^{2+}- activated K^+ channels (IK1 with intermediate conductance (iK) and SK3 with small conductance (sK)). As in our previous papers (1, 2, 3, 5, 6), here the term of endothelial dysfunction denominates any endothelial malfunction that favors vasoconstriction vs. vasodilation. Again, as we cited in our recent studies (1, 2, 3), EDH-mediated EDR is important in: functionally opposing sympathetic vasoconstriction (25); flow autoregulation, myogenic response, and vasomotion (12); compensation of the endothelial dysfunction resulted from lower NO availability/effectiveness (but is also involved in pathogenesis of endothelial dysfunction and hypertension) (14, 26, 27, 28, 29, 30).

In fact, in the present study we examined the relaxing effect of exogenous H_2O_2 in de-endothelised small mesenteric arteries of the rat precisely due to the importance of endothelium-derived H_2O_2 in EDH-mediated EDR described right above, in this last paragraph of the introduction.

MATERIAL AND METHODS

Male adult Wistar rats (~200 g) were euthanized. Jejunal loops were removed and placed in physiological saline solution (PSS), containing (mM): NaCl 119; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.18; glucose 5.5. In PSS at ~5°C, 1 mm rings from first order branches of superior mesenteric artery (~0.15 mm diameter) were dissected (ocular x32, objective x1) and mounted on stainless steel wires in small vessel wire myographs connected to a PC through an AD converter. The endothelium was removed before mounting, by passing a rough hair through the lumen of the vascular fragment. The rings were then oxygenated (95% O_2 + 5% CO_2) and kept at 37°C (pH 7.2-7.4). After 30' equilibration under 1 g passive tension (PSS replaced every 15'), the rings were checked for the absence of EDR by 10^{-5} M acetylcholine upon contraction by 10^{-5} M phenylephrine. Relaxation induced by each H_2O_2 concentration was expressed as remanent active tension, % of the precontraction level. Inhibition by 4-AP (10 mM) of the relaxation induced at each H_2O_2 concentration was expressed as % of the respective relaxation in the absence of 4-AP.

All drugs were from *Sigma*. Statistics (using *GraphPad*): data expressed as mean ± SD; n=6 in each group; two-way ANOVA, multiple t-test, extra sum-of-squares F test for EC_{50} comparison; $p<0.05$ significance limit in all procedures.

This study was approved by Research Ethics Comission of the host institution.

All procedures comply with guidelines from: Act on Animal Experimentation and Animal Health and Welfare from Romania; Universities Federation for Animal Welfare; Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010.

RESULTS

Table I presents the relaxing effects of H_2O_2 in de-endothelised small mesenteric arteries of the rat, precontracted by phenylephrine 0.01 mM. H_2O_2 induced similar relaxations of rings precontracted by either norepinephrine (NE) or phenylephrine (PHE) ($p>0.05$ for all H_2O_2 concentrations tested).

Results are expressed as remanent active tension, % of the precontraction level (mean ± SD; n=6 in all cases; *$p<0.01$).

TABLE I.

Relaxing effects of H_2O_2 in de-endothelised small mesenteric arteries of the rat, precontracted by phenylephrine 0.01 mM

H_2O_2 mM	phenylephrine 0.01 mM
0.001	95.4±2.6*
0.01	86.8±3.1*
0.1	36.2±1.7*
1	12.4±2.4*

Inhibition of H_2O_2 effect in the case of NE was inhibited completely by the Kv channels blocker 4-AP (table 2; $p<0.001$ for all H_2O_2 concentrations tested). The inhibition of H_2O_2 effect by 4-AP was weaker in the case of PHE than in the case of NE (tab. II; $p<0.001$ for all H_2O_2 concentrations tested).

TABLE II.

Inhibition by 4-AP (10 mM) of the relaxation induced by H_2O_2 in de-endothelised small mesenteric arteries of the rat, precontracted by either norepinephrine 0.01 mM or by phenylephrine 0.01 mM

H_2O_2 mM	phenylephrine 0.01 mM	norepinephrine 0.01 mM
0.001	10.42 ± 1.13*	1.45 ± 2.73
0.01	11.23 ± 3.25*	5.23 ± 3.50
0.1	16.54 ± 3.21*	4.14 ± 4.26
1	25.38 ± 2.32*	5.38 ± 3.32

The inhibition by 4-AP (10 mM) of the relaxation induced at each H_2O_2 concentration was expressed as remaining % compared to the respective relaxation in the absence of 4-AP considered as 100 % (mean ± SD; n=6 in all cases); *$p<0.001$ for comparison between the effects at the same H_2O_2 concentration presented in the respective columns.

DISCUSSION

There is massive research on EDR, with sustained interest in EDH-mediated EDR, the major EDR pathway in resistance arteries (1, 2, 3, 5, 6, 11, 12, 13, 14, 20, 21, 24, 25, 26, 27, 28, 30), including the role of endothelium-derived hydrogen peroxide (H_2O_2) as EDHF (6, 13, 14, 20, 21, 24, 27). Quite many studies have tested the mechanisms for the direct relaxing effect of H_2O_2 upon VSM in various arteries and conditions and we have cited here just a few (17, 18, 19, 22, 32)

The effects of exogenous H_2O_2 may vary with the vascular bed and experimental conditions, but relaxation predomi-

nates. Various contributions of different K^+ channels and cGMP have been shown for the relaxing effect of H_2O_2 in small arteries from different species and vascular beds. We have previously shown that H_2O_2 induced similar relaxations of rings precontracted by either norepinephrine (NE) or phenylephrine (PHE), but weaker when contraction was induced by high K^+, or by NE in the presence of high K^+, or by PHE in the presence of high K^+ (17). Then we have also shown that in the case of PHE methylene blue (MEB) moderately inhibited H_2O_2 effect, high K^+ strongly inhibited it, and the combination of MEB and tetraethyl-ammonium (TEA) almost completely abolished it (17). In our earlier study TEA revealed the involvement of BKCa, in agreement with the EDHF role of H_2O_2, while MEB showed only some contribution of cGMP. Others have shown that relaxation of the similar rabbit preparation by H_2O_2 is mediated by cGMP.

In the present study, the inhibition of H_2O_2 effect in the case of NE was complete with 4-aminopyridine (4-AP), a blocker of Kv channels, as shown very recently by another group, using the same vascular preparation (22). We underline here the complexity and high quality of that study (22), which is also oriented towards the Kv implications in redox signaling, as later another group has shown that coronary microvascular Kv1 channels function as regulatory sensors of intracellular pyridine nucleotide redox potential (32). However, the relaxing effect of H_2O_2 upon VSM in other vessels, such as coronary arteries, is based on BKCa (18).

Based on our results, and also on the recent findings from other laboratories, we may conclude that in rat small mesenteric arteries the relaxation induced by H_2O_2 is mediated to a different extent by Kv and BKCa depending on the contracting agent. Ultimately, endogenous endothelium derived H_2O_2 could activate smooth muscle both K^+ channel types and thus functionally compensate for decreased NO availability.

CONCLUSIONS

It is known that EDH-mediated EDR, which is the major EDR mechanism in resistance arteries, involves endothelium-derived hydrogen peroxide as EDHF acting on BKCa in VSM. But here we confirmed that exogenous hydrogen peroxide relaxes the isolated small mesenteric arteries of the rat by involving 4-aminopyridine-sensitive Kv potassium channels in VSM.

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NEWS

ONE SMALL MOLECULE FOR A MAN, ONE BIG STEP FOR CANCER RESEARCH

Triple negative breast cancer (TNBC) is known for being the most aggressive subtype of breast cancer with limited treatment options. The goal herein is to supersede common treatment approaches with novel anti-neoplastic therapies. By using nanotechnology Saraiva and colleagues encapsulated edelfosine (a lipid member of alkyl-lysophospholipid family) in a nano-emulsion and validated its anti-tumoral effects *in vitro* and *in vivo* with promising results. Incubating MDA-MB-231 cells for 24h they showed a dose-dependent cytotoxic effect compared to the control formulation, mainly by the capacity of the drug to be highly internalized by the cancer cell lines. Furthermore, using zebrafish xenografts bearing MDA-MB-231 cells they revealed a decrease in the proliferation of neoplastic cells envisioning a promising approach to TNBC treatment (Saraiva SM, Gutiérrez-Lovera C, Martínez-Val J, *et al*. Edelfosine nano-emulsions inhibit tumor growth of triple negative breast cancer in zebrafish xenograft model. *Sci Rep* 2021; 11: 9873 / doi.org/10.1038/s41598-021-87968-4).

DICLOFENAC SODIUM ENTRAPPED IN MICROPARTICLES EXHIBIT PROLONGED ANTINOCICEPTIVE EFFECTS IN HOT PLATE TEST IN RATS

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DICLOFENAC SODIUM ENTRAPPED IN MICROPARTICLES EXHIBIT PROLONGED ANTINOCICEPTIVE EFFECTS IN HOT PLATE TEST IN RATS (Abstract): We **aimed** to evaluate the influence of microparticles containing DCF on the somatic nociceptive reactivity in rats. **Material and methods:** We used a novel procedure to entrap DCF in soft matter vesicles based on phosphatidylcholine and coated with chitosan. These original carrier systems were physicochemical characterized using a Malvern Zetasizer Nano ZS, ZEN-3500 apparatus. To investigate the effects of microparticles with DCF on the nociceptive reactivity, 3 identical groups of 5 white male Wistar rats each were used. The substances were administered orally (using an eso-gastric device) in a single dose according to the following protocol: Group 1 (Control): distilled water 0.3 ml/100 g body weight - control; Group 2 (DCF): 5 mg/kg body weight diclofenac; Group 3 (DCF-mp): chitosan-based microparticles entrapping diclofenac 5 mg/kg body weight. The somatic nociceptive reactivity was estimated using the hot plate model. The latency time reaction to paws noxious thermal stimulation was assessed before the experiment (baseline) and 15, 30, 60, 90, 120 minutes, 4, 6, 8, 10 hours, after the substances administration. Experimental protocol was approved, and the research were performed according to recommendations of the "Grigore T. Popa" University of Medicine and Pharmacy Committee for Research and Ethical Issues. The data were presented as +/- standard deviation and analyzed using *SPSS* for Windows version 17.0 and ANOVA one-way method. **Results:** We prepared original microparticles containing DCF of 562 ± 13.37 nm mean size, +45 ± 1.67 mV mean Zeta potential with a good stability in colloidal solution. The treatment with DCF resulted in substantial increase of the latency response, in the interval between 15 minutes and 90 minutes in hot plate test. In use of DCF-mp resulted in statistically significant increase of the latency time, between 2 and 10 hours in this somatic pain model in rats. **Conclusions:** The lipid vesicles stabilized with chitosan, as carrier for DCF, presented the advantage of a prolonged release of active substance, comparing with non-entrapped drug in hot plate model in rats. **Keywords:** DICLOFENAC, LIPID VESICLES, MICROPARTICLES, HOT PLATE, NOCICEPTION, RATS.

One of the most challenging research directions is the design and study of ways to entrap some nonsteroidal anti-inflammatory drugs (NSAIDs), agents frequently prescribed in medical practice, in various pathological conditions accompanied by

pain, fever or inflammation. The obtaining of new carrier systems incorporating NSAIDs is of great interest, in view of the fact that, these drugs are from various classes, with differences in terms of pharmacokinetic aspects, as well as with an increased risk of adverse effects in the body.

Diclofenac (DCF) is an active drug belonging to class II (according to the biopharmaceutical classification system) with a high degree of permeability, but with a low solubility (1). This phenylacetic acid derivative is structurally related and has pharmacological properties similar to mefenamic acid and sodium meclofenamate.

There is evidence that DCF inhibits the cyclooxygenases, the lipoxygenases, phospholipase A2 and activates the antinociceptive nitric oxide-guanzine monophosphate pathway. It is also speculated that it may also inhibit phospholipase A2 (2, 3). These additional actions may explain the high potency of DCF, which is known as one of the most powerful NSAIDs (4, 5).

The analgesic potency of DCF is 6 times higher than that of indomethacin, sulindac or codeine, 15 times higher than naproxen and 40 times higher than acetylsalicylic acid. It has a relatively long duration of action (6-8 hours), but a half-life of 1.5-2 hours, which is why it needs to be administered in 3-4 daily doses (6, 7).

DCF is a very good visceral analgesic, being indicated in acute, moderate and severe pain in acute and chronic cholecystitis, renal colic pain, and in dysmenorrhea, as well as in postoperative and post-traumatic pain, neoplastic pain, inflammatory or degenerative arthritis (8, 9, 10, 11, 12).

MATERIAL AND METHODS

Substances

DCF, chitosan, phosphatidylcholine, cholesterol, chloroform were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). The chitosan used had a degree of N-deacetylation of 79.7%, the average molecular weight was Mw = 310,000 g/mol and 3.26 polydispersity index. The 0.5% (w/w) chitosan solution was prepared in a 0.5% (v/v) acetic acid solution. The lipid used was L-α-phosphatidylcholine (egg yolk type XVI-E, 99% TLC purity). Bi-distilled water was procured from Zentiva Company, Prague, Czechia.

The preparation of lipid vesicles entrapping diclofenac

First of all, L-α-phosphatidylcholine was dissolved in chloroform and the solvent was removed by evaporation (RE-2000A Rotary Evaporator, Ya Rong Biochemical Instrument Factory, City, China). Afterward, the resulted dry lipid film was hydrated with a DCF solution in alcohol/water. The obtained solution was sonicated for 20 minutes (Sonoplus Bendeline ultrasound generator, GmbH & Co. KG Berlin, Germany). The lipid vesicles were mixed with the chitosan solution, which was thereafter dialyzed for 10 hours (dialysis tubes type D6191-25EA, with pores of 12,000 Da MWCO from Sigma Aldrich, Steinheim, Germany), to increase the pH to values close to the physiological ones.

The physico-chemical characterization of the obtained systems was assessed using a Malvern Zetasizer Nano ZS ZEN-3500 apparatus (Worchestershire, UK).

Laboratory animals

Healthy, non-genetically modified white male Wistar rats (200-250 g weight, 3 months age) were used in the research. The animals were received from the National Medical-Military Institute "Cantacuzino" for Research and Development, Băneasa Resort, Bucharest, Romania and brought to the CEMEX laboratory ("Advanced Center

for Research and Development in Experimental Medicine - CEMEX") biobase, of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Romania.

Before the experiment, the animals were positioned on a raised wire mesh, under an acrylic box and allowed 2 hours to acclimate to the testing room, in normal laboratory conditions (with 55-65% relative humidity, 23.0 ± 2.0°C temperature and 12 hours light: dark cycle).

Standard laboratory standardized food (pellets) and tap water were freely available, except during the time of the experiments. To avoid chrono-biological interferences, the investigations were performed in the interval 8-12 a.m. daily.

Hot plate test

To evaluate the effects of micro nanoparticles with DCF on the nociceptive reactivity, 3 identical groups of 5 animals each were used, as follows:

- Group 1 (Control): distilled water 0.3 ml/100 g body weight - control;
- Group 2 (DCF): 5 mg/kg body weight diclofenac;
- Group 3 (DCF-mp): chitosan-based microparticles entrapping diclofenac 5 mg/kg body weight;

The substances were administered directly in the stomach (using an eso-gastric device) in a single intake. Intragastric administration was performed using a standardized polypropylene tube for rats (size no. 16-20, length 5 cm), bought from the specialized companies for laboratory supplies.

Using the experimental somatic pain model hot plate we evaluated the nociceptive reactivity, by measuring the changes in sensitivity induced by the test substance, when thermal stimuli were applied on the paws (13, 14, 15). This test assessed the animals reaction time when was placed on a hot plate heated to 52°C (PanLab Harvard Apparatus hot-plate device). The measurement of the latency period of the response to nociceptive paw thermal stimulation was performed before (baseline), at 15 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 4, 6, 8, respectively 10 hours after the administration of the substances. The maximum exposure time to the thermal stimulus (cut-off time) was established for 12 seconds, in order to prevent the paws damage.

To quantify the intensity of antinociception, latencies are converted into percentages of the maximum possible effect (%MPE) (16, 17, 18):

$$\%\ MPE = (measured\ latency - baseline\ latency) \times 100 / (cut\ off\ time - baseline\ latency)$$

The changes in the pain threshold produced after the administration of the microsystems containing DCF, were considered relevant above 60% MPE, because the values below 60%, although they may have statistical significance, the biological one is questionable.

In this somatic pain model, the prolongation of the reaction time compared to the values obtained before the administration of the tested substances, corresponds to an antinociceptive effect of the studied substances. The shortening of the latency period of the response signifies a hyperalgesic effect of the investigated substances.

Statistical analysis

The obtained results were presented as mean values ± standard deviation of the mean (S.D.) of the latency period of the response to the painful thermal stimulus, at each time point and for each studied substance. The data were centralized, and statistically processed, using the *SPSS 17.0* software for Windows EXCEL, and the ANOVA one-way method.

Ethical aspects

The study was approved by the Ethics

Committee of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (Certificate No. 24/21.12.2020). The experimental protocol was conducted in agreement with the standards of the International Association for the Study of Pain (19), and the European regulations concerning the handling of laboratory animals (20).

RESULTS

Physico-chemical characterization of DCF-mp

Lipid microvesicles entrapping DCF, based on phosphatidylcholine and coated with chitosan were prepared. The measurements revealed that the DCF-mp have a mean size of 562 ± 13.37 nm, with a polydispersity index of 0.189.

As nanomaterials are defined as structures possessing, at minimum, one external dimension measuring 1-100 nm (21), the current lipid vesicles entrapping DCF stabilized with CHIT, having mean size 562 ± 13.37 nm, were considered to be microparticles.

The vesicles in colloidal solution have a mean Zeta potential of +45 ± 1.67 mV, which indicated their good stability.

Hot plate test

The analysis of the data obtained at different moments of time, in the dynamics of the hot plate test, reveals the following:

- the treatment with DCF was associated by a considerable increase (**p<0.01) in the response latency period, compared to the control group from 15 minutes to 90 minutes in the experiment;

- the highest latency of the response (7.58 ± 0.12 seconds) to the application of painful noxious stimulation on the paws was observed at 60 minutes in the experiment.

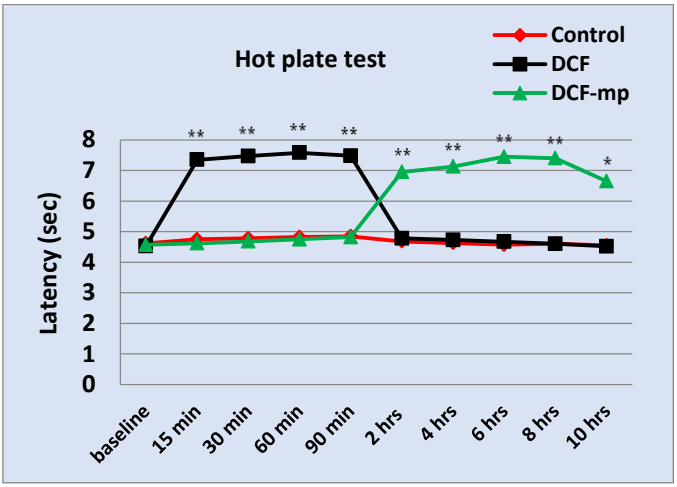

Fig. 1. The influence of DCF and DCF-mp on the nociceptive reactivity in hot-plate test in rats.

The data are presented as arithmetic mean ± S.D. of mean latency time response for five rats in a group. **p<0.01, *p<0.05 were considered statistically significant versus control.

- the administration of vesicles carrier incorporating DCF was associated with prolongation of the latency period of the

response to thermal stimulation of paws, statistically significant (**p<0.01) compared to distilled water group, in the time interval between 2 and 8 hours, with a highest value at 6 hours in the experiment (7.45 ± 0.05 seconds). The effect was maintained significant (*p<0.05) also at 10 hours in this somatic pain model in rats.

- the delay in the manifestation of the effect may be due to the particularities of the DCF release from the soft lipid vesicles stabilized with chitosan.

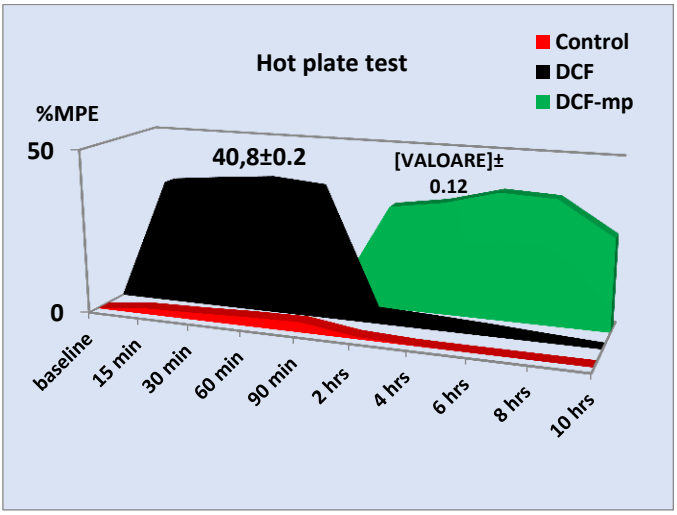

Fig. 2. Quantification of the intensity of the analgesic effect produced by DCF and DCF-mp in the hot plate test in rats**.**

The quantification of the intensity of the analgesic effect in the hot plate test highlighted that:

- the maximum analgesic effect (%EMP) produced by DCF alone or incorporated in soft lipid vesicles, was below 60% at any time moment of the determinations.

- the use of DCF resulted in an increase in % MPE statistically significant in the first 90 minutes with the peak effect noted after 60 minutes (%EMP = 40.8 ± 0.2) in the experiment.

The data are presented as arithmetic mean ± S.D. of mean %MPE for five rats in a group

- the oral administration of microparticles with DCF induced a significant increase of antinociceptive effects, in the interval between 2 hours and 10 hours.

- the treatment with DCF-mp induced a maximum possible effect of analgesia at 6 hours in the experiment (%EMP = 38.8 ± 0.12).

DISCUSSION

A modern and interesting direction of research is the design and study of ways to incorporate non-steroidal anti-inflammatory drugs (NSAIDs), agents frequently prescribed in medical practice for various diseases accompanied by pain, fever or inflammation (8, 9). The phenylacetic acid derivative diclofenac is a NSAIDs with potent anti-inflammatory activity but also with a high risk of gastric irritation (9, 22). In order to reduce the number of admin-

istrations of such a drug, with a short half-life, there is a need to obtain sustained-release systems, formulations, whose oral use could diminish the adverse gastrointestinal effects (6, 7).

Lipid-based formulations have been shown to significantly improve the bioavailability of hydrophobic drugs such as diclofenac compared to conventional dosage forms (23, 24, 25).

Lipid vesicles composed mainly of phospholipids and cholesterol. They are spherical in shape, composed of an internal aqueous compartment surrounded by a variable number of lipid bilayers. The formation of these systems is a spontaneous process that occurs when mixtures of phospholipids with cholesterol and/or other amphiphilic molecules are hydrated. Phospholipids form vesicles due to the hydrophobic effect of their acyl chains in the aqueous environment. The bonds involved are hydrogen bonds, Van der Waals forces or other electrostatic interactions (26).

Hydrophobic molecules are inserted into the bilayer membrane and hydrophilic molecules can be trapped in the aqueous center (27). As drug delivery systems, liposomes offer several benefits, including biocompatibility, self-assembly capability, capacity to carry large drug payloads, and a wide range of physicochemical and biophysical properties that can be modified to control biological characteristics (28, 29).

Liposomal formulations are characterized by properties such as particle size, charge, number of lamellae, lipid composition, and surface modification with polymers and ligands-all of them confer stability in vitro and also *in vivo* (30). The mucus covering the surface epithelium of the gastrointestinal tract possesses an adsorbent layer of phospholipids, which creates a hydrophobic zone between the epithelium and the luminal contents. At this level there are a number of lipid species reminiscent of surface-active phospholipids, of which phosphatidylcholine is suitable to incorporate diclofenac. It has been shown that in the presence of the ionic surfactant shield, the complex remains lipophilic (31).

Among all nanoparticulate systems, lipid vesicles have several advantages, represented by the low number of excipients required for their formulation, the simple preparation procedure, a high physical stability and the possibility of sustained drug release, which could be used in the treatment of various pathological conditions (25, 32).

The incorporation of drugs in organized structures is of great interest, especially in terms of absorption and targeted action in the body. It has been suggested that encapsulation with the help of phospholipids is one of the ways to improve these pharmacokinetic and pharmacodynamics aspects, but also that the drug can cause the structural modification of phospholipids, with the formation of surface-active monomers (33, 34, 35).

In our experimental research a novel micro-system entrapping DCF inside soft matter vesicles were prepared.

The use of DCF was accompanied by a rapid and progressive prolongation of the latency period of the paws withdrawal response to thermal noxious stimulation, with a statistical relevance between 15 minutes and 90 minutes, with a maximum value after one hour in the experiment, this result being in concordance with the literature information about the antinociceptive effects of this NSAID in hot plate test in rodents (36, 37).

The treatment with DCF entrapped in

microparticles was followed by significant antinociceptive effects, which have begun after 2 hours, with a maximum intensity at 6 hours, and extended around 10 hours after the administration. It is possible that the 2-hour delayed onset of the analgesic effect is due to the release after a period of latency of the active substance from the chitosan-stabilized lipid vesicles.

CONCLUSIONS

The new designed microparticles containing DCF demonstrated significant and sustained analgesic effects in hot plate test in rats.

In our experimental conditions, we demonstrated that, the use of lipid vesicles stabilized with chitosan, as carrier for DCF, presented the advantage of a prolonged release of active substance, comparing with non-entrapped drug in this cutaneous pain model in rats.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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NEWS

THE GLOBAL CHALLENGE OF COLISTIN RESISTANCE- RECENT EVIDENCE FROM ROMANIA AND ELSEWHERE

The increasing resistance of Gram-negative bacilli to a wide range of antibiotics has driven a return to colistin as the last resort in the treatment of severe infections. Given the stakes of colistin efficiency at both the individual level and generally, the manifestation and mechanisms of resistance to polymyxin E (also known as colistin) are now subject to careful monitoring and research. Recent findings from different parts of the world including Romania are reviewed and discussed in light of ensuing challenges. The increasing prevalence of multidrug-resistant strains has substantial, far-reaching negative implications for the entire world. The still excessive use of antibiotics and their diminishing effectiveness can limit treatment options to colistin, which we now know is not invulnerable. Invasive medical procedures and prolonged hospitalization can further contribute to the risk of developing resistance to this life-saving medication. In countries like Romania, where data is still scarce but sufficiently concerning, more research and communication need to be conducted with an adequate sense of urgency (Vlad MA, Ursu RG, Iancu LS, *et al*. The Global Challenge Of Colistin Resistance - Recent Evidence from Romania and Elsewhere. *Biomed J Sci&Tech Res* 2021; 40(1): 31950- 31952).

CORRELATION BETWEEN KIDNEY AND LIVER IMPAIRMENT WITH OUTCOME IN LIMB VASCULAR TRAUMA

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CORRELATION BETWEEN KIDNEY AND LIVER IMPAIRMENT WITH OUTCOME IN LIMB VASCULAR TRAUMA (Abstract): Vascular trauma represents one of the most challenging surgical emergencies. The management starts in the prehospital phase and is completed by the intervention of the vascular surgeon. Vascular trauma has a high mortality and morbidity rate, depending on the type of mechanism, localization and associated lesions and it is frequently associated with long term complications. **Material and methods**: This paper includes 146 consecutive patients with limb trauma admitted to the Department of Vascular Surgery of the "Sf. Spiridon" County Clinical Emergency Hospital, between 2011 and 2022. From the total number, 79.59% were male patients, 73.28% from rural area, mean age 45 years old. **Results:** The most frequent trauma involved the upper limb with 93 cases (brachial artery - 51 cases, axillary artery - 23 cases), followed by lower limb with 54 cases. Thirty-four cases had vascular lesions involving the nerves and 47 cases were associated with venous lesions. The most frequent mechanism was penetrating lesions via stabbing or cutting (59.59%). Intra operatively, the following procedures were performed: simple exploration and hemostasis, end-to-end anastomosis, interpositions, and bypass. Seventeen patients required amputation, and 10 had decompression fasciotomies. **Conclusions:** The prolonged time of ischemia (> 6 hours), hemorrhagic shock, reperfusion syndrome with renal or hepatic impairment are highly correlated with an increased risk of amputation and mortality. **Keywords:** VASCULAR TRAUMA, ACUTE ISCHEMIA.

Vascular trauma represents one of the most challenging surgical emergencies for the vascular surgeon because the outcome is very much time dependent. The management of trauma patients starts in the prehospital phase and is completed with the revascularization of the affected segment. There are two therapeutic management musts: first, to achieve hemostasis and secondly, to perform reconstruction of the injured artery and through that to ensure remission of the acute ischemia (1). Vascular trauma has a high mortality and morbidity rate (50-60%) (2), depending on the type of mechanism, localization and secondary lesions, and it is frequently associated with long term complications.

Vascular injury has a higher incidence

in younger patients and has a high impact on years of productive life lost (YPLL). This index is directly correlated with disability secondary to limb amputation or the loss of limb functionality contributing to increasing the number of young patients unable to work. YPLL is higher in trauma compared to deaths caused by cancer or cardio-vascular pathologies. For every traumatic death, the average YPLL is 36 years compared with 16 years for cancer and 12 years for cardio-vascular disease.

In Romania, statistic data on vascular trauma are limited and outdated.

MATERIAL AND METHODS

We included 146 consecutive patients with limb trauma admitted to the Department of Vascular Surgery from "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, between 2011 and 2022. Patient demographics, time from injury to hospital, clinical presentation aspects, biological parameters, management, intraoperative findings and evolution were analyzed in a retrospective fashion. All data were recorded and were statistical analyzed using *Microsoft Excel* and *SPSS* software. Chi square descriptive test was used to identify correlations between patient evolution and type of lesion or biological parameters at admission in the attempt to prove that patients with prolonged time of ischemia, with extensive lesions and critical biological parameters have poor outcome. End point evolution parameters were limb salvage and mortality. A p-value < 0.05 was considered significant.

RESULTS

In our study the prevalence of male patients (79.59%), majority coming from rural area (73.28%), with an age median of 45 years, and 115 patients were active workers, raising the potential impact of trauma on YPLL.

With a median of 12 patients with vascular trauma/year the Department of Vascular Surgery "Sf. Spiridon" County Clinical Emergency Hospital from Iasi can be considered an important vascular trauma center. Majority of patients (N=50; 34.24%) were from Iasi, but the addressability was from all over Moldova region (Bacău, Botoșani, Galați, Iași, Neamț, Suceava, Vrancea and Vaslui counties).

Study group general information are summarized in first table.

Trauma mechanisms were diverse, 59.59% of the vascular lesions were caused by penetrating injuries (stabbing /cuts), the rest were due to polytrauma, crush and car accidents, or following dislocations and fractures. 2 cases of iatrogenic lesion caused by catheterization were documented (fig. 1).

There is a higher percentage of the vascular trauma in the upper limb, more frequently affecting the brachial artery (51 cases), axillary artery and its branches (28 cases), comparing with lower limbs vascular trauma, with vascular lesions more frequently affecting the popliteal artery (21 cases) (fig. 2).

In 34 cases there were also major nerve lesions and in 47 cases venous lesions. In all cases, the deep vein was ligated, while neurorrhaphy was performed in 30 cases. 9 out of 10 cases with injury of the entire vasculo-nervous bundle were at the brachial level.

Patients underwent surgical artery repair via one of the following procedures: patch-plasties with autologous graft, end-to-end anastomosis, interpositions or bypass (fig. 3). Patients that required primary amputation, without an attempt of limb salvage due to mangled extremity were excluded from the study.

TABLE I
Patient general information

Patient characteristics		Number	Percent (%)
Sex			
	M	116	**79.59%**
	F	30	20.41%
Age (years)			
	median	45	-
	≤ 17	3	2.05%
	18-45	77	**52.73%**
	46-65	38	**26.02%**
	≥ 66	27	18.49%
Provenience			
	R	107	**73.28%**
	U	39	26.72%
Affected limb			
	Upper limb	92	**63.01%**
	Lower limb	54	36.99
Injured structure			
	Artery	122	**83.56%**
	Vein	47	32.19%
	Nerve	34	23.28%
Time from injury			
	First 6 hours	123	**84.25%**
	Between 6-24 hours	12	8.22%
	More than 24 hours	11	7.53%

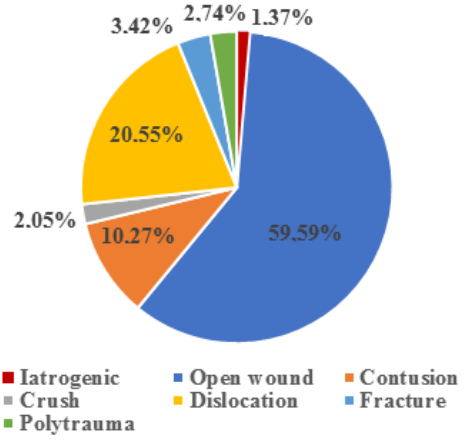

Fig. 1. Types of trauma mechanisms in the study group

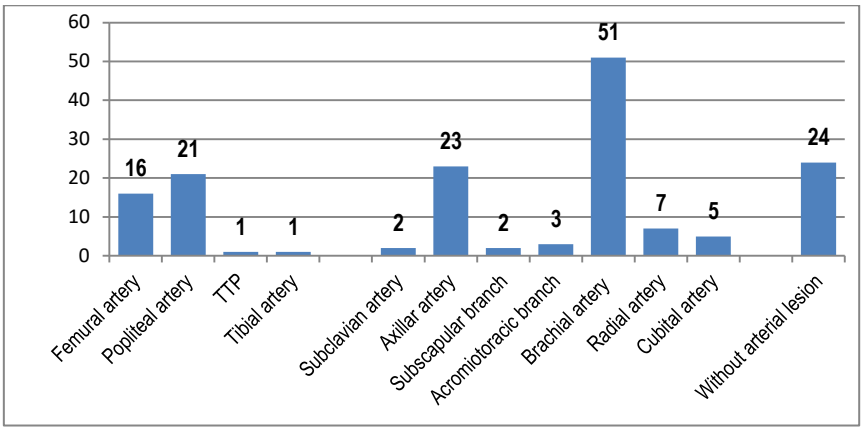

Fig. 2. Injured artery

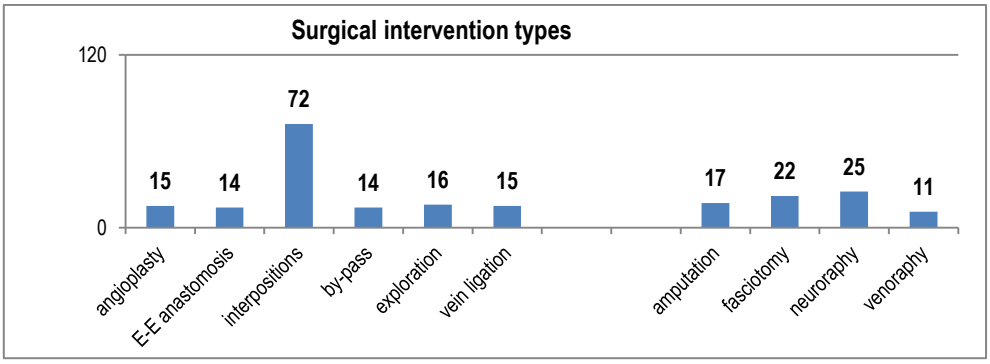

Fig. 3. Types of surgical intervention

84.83% patients had favorable evolution with peripheral pulse. Patients that underwent amputation (11.03%) or died (4.10%) were considered to have unfavorable evolution. The death causes were multiple organ failure (3 cases) and hemorrhagic shock (3 cases).

We identified correlations between patients with reperfusion syndrome, who needed fasciotomy and patients who later suffered above knee amputation ($p<0.05$). Out of 17 patients with amputation 10 had previous fasciotomies. Regarding the evolution, there was no correlation with the trauma mechanism or the affected limb (upper or lower), but the more proximal the artery was affected, the higher the risk for amputation and mortality.

The time from the traumatic event until presentation (from index symptom to surgery) and revascularization was noted. Most patients were admitted within the first 6 hours from index symptom to surgery. Higher risk of amputation and death was noted in those patients that had prolonged time of hemorrhage or ischemia ($p<0.001$) (tab. II).

We identified strong correlations and unfavorable evolution in patients with both kidney and liver impairment ($p<0.001$). Creatinine higher than 35 mg/dL and urea higher than 1.3 mg/dL are signs of kidney impairment. For the liver function the

transferases values and creatine kinases (CK) were being analyzed, the cut off values being 100 U/L, respectively 250 U/L (tab. III). CK elevated values were also a sign of poor outcome and were independently correlated with unfavorable evolution (p=0.002) (tab. III).

Organ failure as a consequence of both prolonged ischemia and reperfusion as conjunct mechanisms or independently.

TABLE II.

Correlation between time and evolution

		EVOLUTION		PATIENTS
		unfavorable	favorable	
TIME	>6 hours	8 (5.47%)	13 (8.90%)	21 (14.38%)
	</=6 hours	14 (9.58%)	111 (76.02%)	125 (85.61%)
Total		22 (15.06%)	124 (84.94%)	146
		p<0.001		

TABLE III.

Correlation between kidney and liver function with evolution

		KIDNEY PARAMETERS		TRANSFERASES LEVELS		PATIENTS
		elevated	normal	elevated	normal	
EVOLUTION	favorable	14 (9.58%)	110 (75.34%)	46 (31.50%)	78 (53.42%)	124 (84.94%)
	unfavorable	13 (8.90%)	9 (6.16%)	19 (13.01%)	3 (2.05%)	22 (15.06%)
Total		27 (18.49%)	119 (81.50%)	65 (44.52%)	81 (55.47%)	146
		p<0.001		p<0.001		

TABLE IV.

Correlation between CK levels and evolution

		CK LEVELS		PATIENTS
		elevated	normal	
EVOLUTION	favorable	65 (44.52%)	59 (40.41%)	124 (84.94%)
	unfavorable	20 (13.69%)	2 (1.36%)	22 (15.06%)
Total		85 (58.21%)	61 (41.78%)	146
		p=0.002		

DISCUSSION

In this group almost 24% of the vascular lesions were a consequence of dislocations and fractures. The real percentage is higher. The group was formed only from patients admitted in the Vascular Surgery Department leaving out a part of the patients that had type III C fractures usually admitted in the Orthopedic Department. The study group can be extended and reviewed after the inclusion of those patients.

Vascular Surgery Department from “Sf.

Spiridon" County Clinical Emergency Hospital from Iasi is the single most important trauma center in the region of Moldova and a reference center for vascular trauma. At the same time, it highlights the insufficient vascular surgeons in the region that can be a cause of delayed intervention on vascular trauma patients. The median of 12 patients/year is comparable with other notable vascular surgery centers(i.e. At a university teaching hospital in Australia, Tobin (3) reported 10 cases per year of extremity vascular injuries; in Tbilisi, Georgia, Razmadze (4) reported 10.5 cases per year; in Sweden, Kjellstrom and Risburg (5) reported 8.2 cases per year; and in Oxford, United Kingdom, Magee *et al*. (6) reported 4.7 cases per year), but the injury mechanisms are markedly different from those reported all around the world (6). Most of the traumatic lesions were due to stabbing or agricultural accidents while in other countries the main cause of trauma are car accidents or gun shots. Being mainly a consequence of agricultural accidents, this can be an explanation why the involvement of the upper limb is higher. This aspect is particular to this study, literature mostly reports lower limb trauma (7).

CONCLUSIONS

The prolonged time of ischemia (> 6 hours), hemorrhagic shock, reperfusion syndrome with renal or hepatic impairment are highly correlated with an increased risk of amputation and mortality.

The management of patients with traumatic vascular lesions is challenging, involving a multidisciplinary approach (vascular surgeon, emergency doctor, anesthesiologist, orthopedic surgeon and plastic surgeon). Reducing ischemia time by addressing the patient rapidly to the vascular surgeon is mandatory. More centers of vascular surgery have to be established all over Moldova region in order to reduce the transportation time and improve the patient survival rate and limb salvage prognostic.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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CORRELATIONS BETWEEN THE LYMPHOID INFILTRATE AND THE STEROID HORMONE RECEPTORS IN ENDOMETRIOSIS

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CORRELATIONS BETWEEN THE LYMPHOID INFILTRATE AND THE STEROID HORMONE RECEPTORS IN ENDOMETRIOSIS (Abstract): **Aim**: Endometriosis, a chronic estrogen-dependent disease, is associated with persistent pelvic pain and is classified as a neuro-inflammatory disorder. This study aimed to decipher the inflammatory milieu in endometriosis, focusing on the lymphoid infiltrate represented by the CD4+ cells, CD8+ cells, and CD68+ macrophages, and the possible correlation with steroid hormone receptor expressions. **Material and methods**: Research conducted at the "Elena Doamna" Clinical Hospital and the "Grigore T. Popa" University of Medicine and Pharmacy in Iaşi, Romania, involved retrospective analysis of 53 patients with endometriosis from 2018 to 2023. **Results:** Through immunohistochemical assays, we observed correlations between CD4 and CD8 expressions, and between CD4, CD68, and the PR score. Also, a possible causality between the macrophage infiltrate and PR expression could be suggested. **Conclusions:** The inflammatory infiltrate's analysis in endometriosis provides a deeper knowledge of its pathological development and the interplay of inflammatory cells. Understanding these receptors might guide future treatment strategies for better disease management. **Keywords:** ENDOMETRIOSIS, INFLAMMATION, LYMPHOID INFILTRATE, STEROID HORMONE RECEPTORS.

Endometriosis is a chronic, estrogen-dependent disease characterized by the ectopic growth of endometrial tissue (encompassing both glands and stroma) outside the uterine cavity. This pathology is frequently correlated with persistent pelvic pain and is contemporarily classified as a neuro-inflammatory disorder (1).

The etiopathogenesis of endometriosis has been extensively studied, yet definitive understanding remains evasive. We currently have data about the roles of genetic predisposition, estrogenic modulation, progesterone resistance, and inflammatory pathways, in conjunction with altered expression of hormonal receptors such as estrogen receptors (ER) and progesterone receptors (PR) (1, 2). Immunological components are hypothesized to be instrumental in both the inception and evolution of

endometriosis (3-5).

Within the inflammatory cellular milieu associated with endometriosis, CD4+ T cells and CD8+ T cells are discernible, albeit their precise pathological roles remain only partially delineated (6). Concurrently, macrophages are posited to be pivotal in the proliferation, angiogenesis, innervation, and nociception of endometriotic lesions (7-10).

The heterogeneity of the intralesional inflammatory infiltrate across various histological manifestations of endometriosis holds potential for profound insights into the sequential pathophysiological events and the multifaceted immune responses in endometriosis (11). Our study seeks to elucidate the composition of the inflammatory milieu through a semi-quantitative analysis of CD4+ cells, CD8+ cells, and CD68+ macrophages in ovarian and cutaneous endometriosis. Complementarily, we assessed the immunohistochemical expressions of estrogen and progesterone receptors, trying to find correlations and eventual causalities.

MATERIAL AND METHODS

The research was undertaken at both the "Elena Doamna" Clinical Hospital and the "Gr. T. Popa" University of Medicine and Pharmacy located in Iași, Romania. This study encompassed a retrospective review of clinical records combined with histopathological examinations of surgical samples (ranging from nodule removals to full hysterectomies with adnexectomy) from 53 patients diagnosed with endometriosis between the years 2018 and 2023.

Slides stained with Hematoxylin-Eosin (HE) were viewed by a pair of independent pathologists to verify the diagnosis. An immunohistochemical (IHC) test followed, utilizing specific antibodies (tab. I). Upon completion of the IHC procedure, a semi-quantitative evaluation of the immunoreactivity of the inflammatory infiltrate in the epithelium and stroma has been carried out. Also, the ERα and PR quantification used a score based on the positivity index added to the staining intensity. The analysis has been performed using their respective scores available in literature (12-16).

TABLE I.
Antibodies used in the immunohistochemical assay

Antibody	Type	Clone	Dilution
CD4	Mouse Monoclonal	4B12/Novocastra	1/100
CD8	Mouse Monoclonal	1A5/Novocastra	1/50
CD68	Mouse Monoclonal	KP1/Novocastra	1/400
ERα	Mouse Monoclonal	6711/Novocastra	RTU
PR	Mouse Monoclonal	312/Novocastra	RTU

In our statistical analysis, we employed the Hoeffding measure, a non-parametric statistical method tailored for assessing the correlation between two random variables. The Hoeffding correlation measure is adept at detecting both linear and non-linear associations, useful in situations where relationships between variables might not always adhere to a linear paradigm. The Hoeffding's D measure offers a robust quantification of association without being confined to a specific form or pattern of

relationship between the variables under investigation.

We also utilized the Pearson correlation coefficient, a widely recognized parametric statistical method that quantifies the linear relationship between two continuous variables. The Pearson test measures the strength and direction of the linear relationship, providing a coefficient value between -1 and 1. A value closer to 1 implies a strong positive linear relationship, a value closer to -1 indicates a strong negative linear relationship, and a value near 0 suggests no linear correlation. Given its sensitivity to linear associations, this test is pivotal when aiming to ascertain direct proportionality or inversely proportional relationships between variables.

Informed consent was secured from all the patients, adhering to guidelines that guarantee participants make well-informed decisions and that their rights are safeguarded (17). The study received approval from the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi (Approval No. 285/26.03.2023), in alignment with the Declaration of Helsinki's principles concerning medical research involving human participants.

RESULTS

The results were expressed as CD4 percent, CD8 percent, CD68 percent, ERα score and PR score. In order to investigate an eventual reciprocal influence of inflammation and hormonal imbalance in the endometriotic lesions, we performed a Hoeffding and also a Pearson test (figs. 1, 2). Both methods were used in order to compare their results, but also to capture both linear and potentially non-linear relationships.

	CD4 percent	CD8 percent	CD68 percent	ERα score	PR score
CD4 percent	1.000000	0.058491	0.014912	0.007265	-0.006719
CD8 percent	0.058491	1.000000	-0.009299	-0.007093	0.005025
CD68 percent	0.014912	-0.009299	1.000000	-0.003800	0.038728
ERα score	0.007265	-0.007093	-0.003800	1.000000	0.001113
PR score	-0.006719	0.005025	0.038728	0.001113	1.000000

Fig. 1. *Hoeffding correlation* coefficient regarding the association between lymphoid infiltrate and steroid hormones expressions

	CD4 percent	CD8 percent	CD68 percent	ERα score	PR score
CD4 percent	1.000000	0.533522	0.338297	0.108531	0.158922
CD8 percent	0.533522	1.000000	0.247454	0.143872	-0.190404
CD68 percent	0.338297	0.247454	1.000000	0.106933	0.300278
ERα score	0.108531	0.143872	0.106933	1.000000	0.220835
PR score	0.158922	-0.190404	0.300278	0.220835	1.000000

Fig. 2. *Pearson correlation* coefficient regarding the correlations between lymphoid infiltrate and steroid hormones expression

The non-linear correlation coefficient suggested a potential relationship between the expressions of CD4 and CD8, as well as the expressions of CD4 and CD68 and between CD68 and PR score. No correlation was found between CD68 and ERα expression, or between CD4, CD8, and hormonal receptors in the analyzed samples.

While correlation between random variables does not imply nor reveal causal directionality, this can be assessed with the help of an Additive Noise Model (ANM) (18, 19). We made use of the popular WhyPy Python packages to estimate through both Maximum Likelihood and Independence of Residuals the potential causal directionality for pairs of correlated variables in our data.

The ANM study results of CD4 (X0) and CD8 (X1) data are shown in figures 3 and 4. These results indicated that the box plots describing noise distribution in the modelling of dependencies X0~f(X1) and X1~f(X0), respectively, mostly overlap in both Maximum Likelihood (fig. 3) and Independence of Residual (fig. 4). These results did not support any clear causal directionality hypothesis.

Similarly, the ANM study of CD4 (X0) and CD68 (X1) revealed overlapping noise distributions as shown in figures 5 and 6, which is not indicative of any potential causal directionality.

Finally, the results of the ANM study of CD68(X0) and PR Score (X1) are shown in figs. 7 and 8. The Maximum Likelihood test shown in figure 7 supported a hypothesis for causal direction in which CD68 causes PR Score, while the Independence of Residuals test (fig. 8) does not result in clear support for either direction. Should a causal link exist between CD68 and PR Score, indicates that it is more likely that the first causes the second (fig. 7).

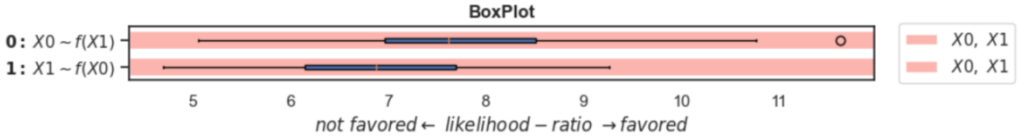

Fig. 3. ANM Maximum Likelihood for CD4 (X0) and CD8 (X1)

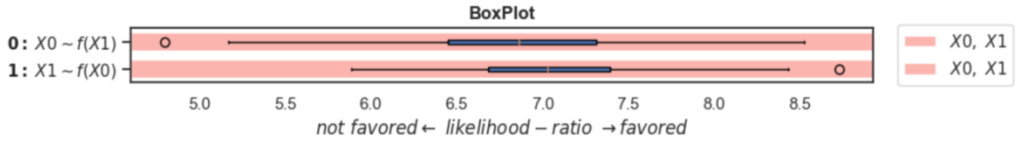

Fig. 4. ANM Independence of Residuals for CD4 (X0) and CD8 (X1)

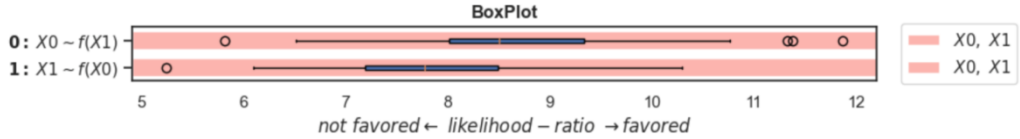

Fig. 5. ANM Maximum Likelihood for CD4 (X0) and CD68 (X1)

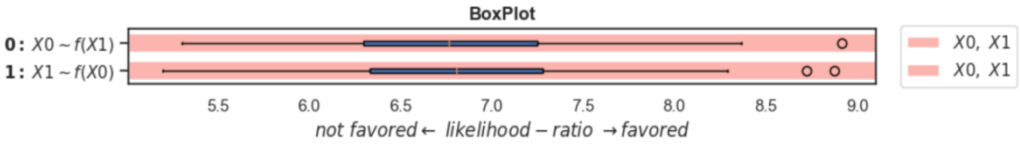

Fig. 6. ANM Independence of Residuals for CD4 (X0) and CD68 (X1)

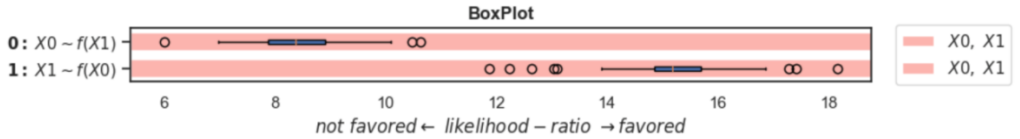

Fig. 7. ANM Maximum Likelihood for CD68 (X0) and PR (X1)

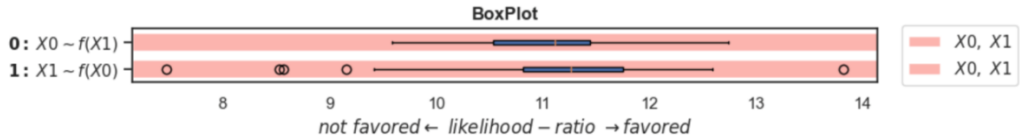

Fig. 8. ANM Independence of Residuals for CD68 (X0) and PR (X1)

DISCUSSION

A key role in the pathogenesis of endometriosis is attributed to the menstrual reflux of endometrial tissue (including stem cells) into the peritoneal cavity. Nevertheless, this phenomenon is not sufficient for the development of the disease, and the microenvironment factors that stimulate the functions of stem cells and allow the development of endometriotic implants are very important adjuncts to the mechanism of retrograde menstruation (20). Also, considering the widespread distribution of endometriosis localization in the human body, modern theories attempt to combine the effect of several factors that contribute to its development, as multifactorial, multicompartmental pathogenic phenomena such as estrogen dependence, progesterone resistance, genetic susceptibility, all under the influence of environmental factors (11). Furthermore, the pathogenic mechanisms are added to the immune system's inability to neutralize ectopic endometrial cells. Additionally, the interplay between hormonal levels, inflammation, and the progression of the disease has been extensively researched.

Since 1987, Halme *et al.* proposed an immunological aspect to endometriosis (21). They observed elevated levels of activated macrophages, belonging to the cellular component of the innate immune system, in both the peritoneal fluid and the intact endometrial tissues (referred to as eutopic endometrium) of endometriosis patients compared to women without the condition (21). Given that macrophages, which have widespread tissue presence, play a pivotal role in many inflammatory and cancer-related diseases (22), they are believed to be crucial in influencing various aspects of endometriosis. This includes the growth, development, vascularization, and nerve supply of endometriotic lesions, and even in the creation and sensation of pain (7-9).

Studies on the inflammatory cell types found in the female reproductive tract have produced varied and sometimes incon-

sistent findings (3, 5, 23). These discrepancies might be due to the changing nature of the menstrual cycle, as well as the ongoing morphological shifts in both the epithelial layer and stromal regions during different menstrual phases. However, the most commonly identified immune cells in this region include T cells, macrophages, dendritic cells, NK cells, neutrophils, and mast cells (24). Accordingly, it is considered that immune cells, such as NK cells, macrophages, dendritic cells, and neutrophils, may be involved in growth, angiogenesis, and invasion processes of endometriotic cells (24). Interestingly, B cells appear to be relatively rare in the female reproductive tract (25).

A key aspect of endometriosis pathogenesis is now considered to be linked to pelvic inflammation, but the exact pathways are yet to be determined. Several research studies have shown that cells like macrophages, lymphocytes, endometrial, and mesothelial cells can produce cytokines and inflammatory agents that further attract a variety of cells, including macrophages, lymphocytes, eosinophils, mast cells, and endometrial cells to the peritoneal cavity (25). This recruitment initiates a series of reactions that stimulate the proliferation of endometriotic cells. It appears to also promote their attachment to abnormal sites, blood vessel formation, and triggers the release of additional cytokines and chemokines, intensifying their overall impact.

Moreover, growing evidence admits that endometriosis is linked with an autoimmune status (24, 26), explained by the endometriosis-induced inflammation, leading to a deregulated immune response which may induce autoimmunity (26).

The endometriosis environment is also influenced by cytokines and defensins secreted by immune cells. In this regard, the levels of immune checkpoint inhibitors, which control the immune response, are different in endometriosis than those observed in healthy women (24).

Lymphocytes play essential roles in the process of survival, implantation, and proliferation of endometrial and endometriotic cells and numerous studies have shown aberrant functions of these cells in women with endometriosis. In this regard, recent research indicates a particular association of CD4+ T cells and CD8+ T cells with endometriosis (6). There is ongoing debate on whether changes in these cells are cause or a result of endometriosis.

CD4+ T lymphocytes are often referred as T helper cells because they aid to orchestrate and amplify the immune responses by interacting with other immune cells and releasing cytokines. CD4+ T cells can differentiate into various subsets (including Th1, Th2, Th17, and T regulatory (Treg) cells), depending on the cytokine milieu, each subset playing a unique role in immune responses.

CD8+ T lymphocytes (cytotoxic T cells) have the ability to recognize antigens presented by MHC class I molecules, which are present on almost all nucleated cells. CD8+ T lymphocytes provide immunosurveillance by constantly screening host cells for intracellular pathogens or malignancies.

Our study indicates that there is a weak correlation between the number of CD4+ T cells and CD8+ T cells in the endometriotic tissue. In contrast, other recent studies have found that the CD4/CD8 ratio in the peritoneal fluid leans towards CD8+ T cells, the preponderance of CD8+ T cells being comparable to the eutopic endometrium (28). Also, CD8+ T cells in the peritoneal fluid showed higher relative frequencies when contrasted with the peripheral blood (28).

It is considered that the endometriotic altered immune response may be correlated to damaged apoptotic processes. One study revealed in healthy women increased serum concentrations of HLA-DR- activated T cells and of CD8+ T lymphocytes throughout the luteal phase in comparison with the follicular phase (29). Contrary, in endometriosis there are no fluctuations in the peripheral blood concentrations of activated and cytotoxic lymphocytes during the menstrual cycle (26, 29).

Despite the identified correlation between the two cell populations, it is difficult to propose a hypothesis for causal direction in the endometriosis scenario. Our data suggests that the relationship between CD4+ and CD8+ T cells is multifaceted. Rather than a simple cause-and-effect manner of function, they are part of a complex immune network. Their collaboration is vital for a coordinated immune response.

Thus, T cells do not operate in isolation from the environment, their changes being able to play a role in the etiopathogenesis of endometriosis. Moreover, the immune status in endometriosis is largely regulated by epigenetic factors (26, 30).

Regarding the macrophage population, outlined in our study by the CD68 expressing cells, it is well-known that in women with endometriosis they consistently represent the majority of peritoneal immune cells throughout the menstrual cycle, accounting for 55%, with T cells following at 20% (28). The elevated counts of activated macrophages and adaptive immune cells, including T and B lymphocytes, coupled with the rise in inflammatory cytokines observed in patients with endometriosis, suggest the presence of an underlying immunological/inflammatory imbalance (31). Our results suggest that there is also weak correlation between the number of CD4+ T cells and CD68 cells in the endometriotic tissue, confirming the complex and intricate roles of the two types of cell populations and giving rise to hypothesis about their reciprocal influence. On the other hand, the ANM study of CD4 and CD68 revealed overlapping noise distributions, making it impossible to indicate any potential causal directionality at the moment.

Regarding the steroidal hormones in relation to the inflammatory microenvironment, reciprocal influences could be considered to form vicious cycle, as the presence of endometriotic implants boost the expression of ER-β while decreasing PR (32-35), triggering the production of genes responsible for various inflammatory mediators like IL-1 β, IL-6, IL-8, IL-17, TNF-α, and COX-2 (10, 11). On the other hand, the chronic inflammation accelerates the aromatase activity, causing an upsurge in estradiol levels (10).

However, hormonal imbalances play a crucial role in the endometriosis etiopathogenesis, the disease being considered as a progesterone (P4)-resistant and estrogen dependent process (26, 33).

A recent study by Hogg and associates emphasizes the relationship and the reciprocal influences between the macrophages and the steroidal hormones, describing that in the presence of estrogen, macrophages enhance their proliferative ability and become activated, transitioning to a phenotype more akin to a “wound healing” population (27, 28). Also, while endometrial macrophages don’t display the progesterone receptor (36), their gene expression markedly changes in reaction to progesterone (37), indicating an indirect mode of regulation.

In the present study, no correlation was found between CD68 and ERα expression, possibly to the limited sample size or possibly due to the polymorphism of the estro-

gen receptors. Nevertheless, our analysis of the CD68 and PR score quantifications suggest a correlation between the two. Moreover, the Maximum Likelihood test shown supported a hypothesis for causal direction in which the presence of macrophages (reflected by the CD68 percent) causes a rise in the PR score.

On the other hand, current research indicates that endometriosis commonly exhibits progesterone resistance due to diminished PR expression and functionality and there is notable dysregulation of PR expression and activity in target cells, both in eutopic and ectopic tissues, when compared to standard endometrium (38). These differences might arise from the polymorphism of PR, as PGR-B is generally considered to be the positive regulator of the effects of progesterone, and PGR-A has an inhibitory effect on PGR-B (38).

Also, some differences of opinion could be generated by the main limitation of our study, its reliance on observational analysis with a limited sample size (53 cases only). In this context, the authors were found to be in the impossibility to address potential confounders, leaving room for potential bias.

CONCLUSIONS

The analysis of the chronic inflammatory infiltrate in endometriosis offers a deeper knowledge of its pathological evolution and inflammatory cell interactions. As understanding these receptors is pivotal in ongoing research, future endometriosis treatments may focus on them for an improved disease management.

It is difficult to propose a hypothesis for causal direction regarding the CD4+ and CD8+ T cells, in endometriosis. The relationship between the two types of lymphocyte is multifaceted and cofactors that contribute to their tissular and humoral activity need to be further studied and taken into account. The present data shows that while both cell types communicate and influence each other, it is not a linear, one-to-one causation.

Our findings also indicate a weak correlation between the counts of CD4+ T cells and CD68 cells in endometriotic tissue. This underscores the intricate and multifaceted functions of these two cell types and prompts speculation regarding their mutual impact.

Also, our evaluation of the CD68 and PR score quantifications indicates a relationship between them, our evidence favoring a hypothesis where an increase in macrophages (as indicated by the CD68 percentage) leads to a heightened PR score.

Our data could lay the foundation for new treatment approaches, either in the development of macrophage-targeting medications or by developing methods to overcome resistance to progestin treatment.

There is still limited data regarding the immune landscape of endometriosis. To better understand the pathogenic mechanisms of endometriosis, a more comprehensive study is needed, with promising impact upon the treatment and randomized controlled trials might measure the effectiveness of new tailored interventions, in order to alleviate the burden of endometriosis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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TISSUE PENETRABILITY OF NANOPARTICLES LOADED WITH RHODAMINE B FLUORESCENT MARKER USING A TRIMODAL ADMINISTRATION APPROACH

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TISSUE PENETRABILITY OF NANOPARTICLES LOADED WITH RHODAMINE B FLUORESCENT MARKER USING A TRIMODAL ADMINISTRATION APPROACH (Abstract): Nanoparticles can incorporate a drug, as means of protection towards body physiological barriers, and release it in a controlled manner in a targeted tissue. This study aims to investigate the absorption of nanoparticles, loaded with Rhodamine B fluorescent marker, in different organs, by trimodal administration: endogastric, intraperitoneal and intravenous, for three days. **Materials and methods:** The extent of absorption of Rhodamine B dye in liver, gums, kidneys, ovaries and testes was determined using fluorescence microscopy in frozen sections on harvested organs from rats. The experiment required 24 adult male rats that were divided into four groups of six rats each. Group A was designated as the control group, while the other three groups, B, C and D, respectively, received Rhodamine-loaded nanoparticles for three consecutive days, at a dose of 0.05 mg/kg body weight. The methods of administration for the three study groups were as follows: endogastric for group B, intraperitoneal for group C, and injection into the tail vein for group D. On the fourth day the animals were sacrificed, and organs were removed and preserved in paraformaldehyde containers, to be studied under an immunofluorescence microscope. A four-level scale was used to evaluate organ penetrability for the given nanosystems. **Results*:*** Intraperitoneally administrated nanocarriers, marked with Rhodamine B, showed the highest degree of absorption in liver in 83.3% of the rats, compared to the endogastric route, where the same level of absorption was in only 33.3% of the animals (p=0.050). Regarding the two parenteral tested pathways, the absorption level presented statistical differences among the intraperitoneal vs. intravenous groups, in gum (p=0.011), heart (p=0.004), kidney (p=0.050) and testicle (p=0.011), whereas there was no statistical difference in liver tissue (p=0.250). **Conclusions:** The parenteral routes of administration had statistically significant higher organ penetrability, compared to the endogastric tube. Amongst the organs, liver had the most absorptive behavior due to its intrinsic metabolizing role, and there was no major difference between the intraperitoneal vs. intravenous injections. **Keywords:** RHODAMINE B, NANOPARTICLES, ENDOGASTRIC, PARENTERAL ABSORPTION.

Nanomedicine is the science of applying nanotechnology and nanosized materials in the medical field, in order to be used in the prevention and treatment of various pathologies (1). Based on the latest technological advances, nanotechnology became a frequently addressed topic in many research areas in the biological, and especially medical sciences. Within the pharmaceutical field, the primary advantages lie in the development of nanodrugs, as avant-garde medical therapies that open new horizons and challenges in targeting specific tissues (2-4).

This future drug administration was subject to skepticism at first, because nanoscale formulations have different physical and chemical properties compared to conventional drugs. In the case of noncarrier drugs, these properties are impacted by the physiological barriers of the organism, causing the destruction of the pharmacokinetic profile of drugs (2).

With the help of nanotechnology, three important directions have been approached in medicine: establishing or confirming a diagnosis following administration, control of the drug release proprieties, and influencing tissue regeneration capacity. Nanotoxicology is another field which started emerging, due to the exposure to airborne nanosized particles during manufacturing and manipulation processes (5). Nanomedicine has the ability to open new opportunities in terms of clinical applicability, by introducing new therapies for previously untreated pathologies. Drug nanosystems can integrate into their structure certain substances which, due to high toxicity, could not have been used on their own before. This decrease in toxicity, coupled with the power to control the delivery of the drug while overcoming biological barriers without degradation, prove their importance in current medicine. The therapeutic success is reflected in the in-depth research of the properties of nanodrugs to have the desired action, in the targeted tissue in the body (6, 7).

Rhodamine B as a synthetic dye has numerous uses, both in medicine, as a marker, as well as in the textile industry, food industry or cosmetology. The color obtained from the usage of Rhodamine B is green or red purple (8). This happens because exposing of the synthetic dye to light, leads to two types of reactions where reactive oxygen compounds are formed (9,10). There were some studies aiming to investigate the usage of Rhodamine B as a possible photochemotherapy for bladder cancer (11). However, the toxic profile for this compound has proved to be unsatisfactory since its administration through food in both human and animal studies can cause numerous digestive pathologies, following its accumulation in the digestive system (12).

In our study, Wistar white rats were chosen as a research tool, due to their immunological similarities with humans. Wildner demonstrated that the immunological link between rats and humans is due to major histocompatibility and the presence of activated Tregs cells (13).

The biocompatibility of nanomedicines is the starting point for treating several pathologies. Since they can overcome physiological obstacles specific to digestive tract, they offer the opportunity to reach the target organ without previously breaking down. Blais *et al*. reported the

high similarities between the metabolic pathways of humans and rats (14).

The aim of this study was to study the penetration of nanoparticles marked with Rhodamine B into several tissues using different routes of administration.

MATERIALS AND METHODS

Basic reagents

The chemicals used in this study were: Rhodamine (Octadecyl Rhodamine B Chloride (R18), Sigma), 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC, Avanti), Cholesterol (CHOL, Sigma Aldrich), Methanol (Sigma Aldrich), Chloroform (Sigma Aldrich), Milli-Q ultrapure distilled water (Merck). The substances were used without additional purification, as they were purchased at analytical grade.

Rhodamine-marked liposomes preparation

In our study we obtained liposomes (LPs) of two types, small unilamellar vesicles (SUV) and multilamellar vesicles (MLV), using a slightly modified protocol based on the thin film hydration method used by Ciobanu *et al*. (15).

The early stage consisted of dissolving DSPC (120 mg) and CHOL (80 mg) in a Chloroform/Methanol mixture (4 mL / 4 mL) (1:1 v/v). Solvent extraction was performed by evaporation (using a rotary evaporator - Heidolph Laborota 4002 - at 60 rpm, 35°C and reduced pressure) from a round-bottomed flask (100 mL), thus obtaining a thin film on the wall of the flask. Following the extractive process, the obtained lipid film was hydrated with 10 mL rhodamine solution (RHD) at 25°C. The pre-hydration step is followed by the use of the vortex leading to the formation of MLVs. To eradicate the possibility of aggregate formation and in order to obtain a homogeneous suspension, the RHD-labeled MLVs suspension was placed in a sonicator bath for 10 minutes at 25°C. Water washing by centrifugation (12 revolutions at 15,000 rpm/15 min) was used for separation of the MLVs from unabsorbed RHD. Obtaining SUVs required a supplementary step, where MLV suspension was repeatedly extruded through decreasing size polycarbonate membrane (400 nm, 200 nm and 100 nm), using a miniextruder (Avanti Polar Lipids, Alabaster, AL). In the final stage, the obtained suspension of RHD-labeled SUVs was purified by dialysis against Milli-Q water with a cellulose ester membrane bag (molecular-weight cutoff = 12400) for 5 days.

MLVs and SUVs size determination

Laser diffractometry technique (SHIMADZU SALD 7001) was used to analyze MLVs and SUVs, the measurements being made in a triplicate manner to mitigate intrinsic preparation and assay errors. The steps are as follows: a) sonication of suspensions of MLVs and SUVs (50 μL) was performed for 10 minutes at room temperature, using the sonication bath (Bandelin Sonorex); b) the sonicated suspension was put into 10mL of double-distilled water; c) the resulted solution was added to a quartz vat equipped with a stirring mechanism and analyzed.

The analyzed SUVs and MLVs distribution curves (fig. 1 and fig. 2) have a unimodal appearance and a diameter in the submicron range (75-87 nm).

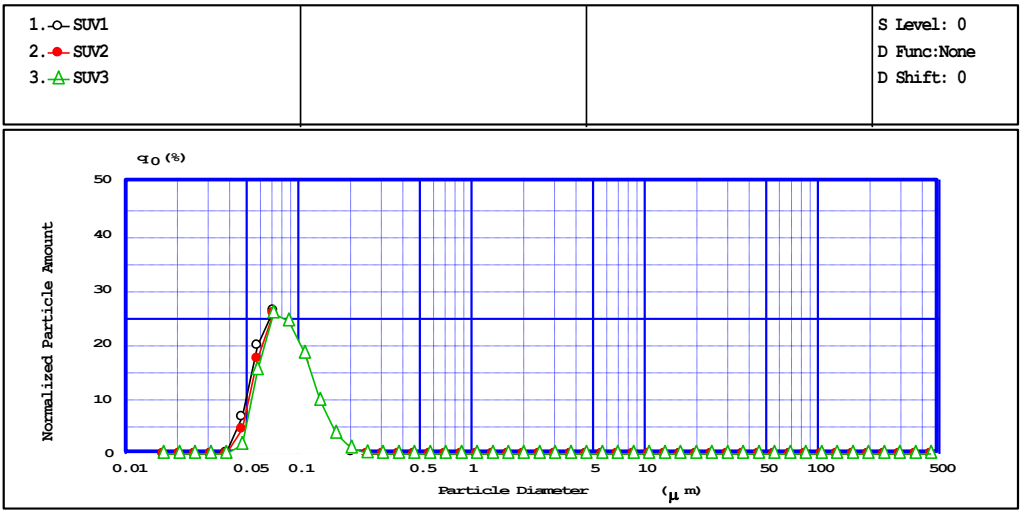


Fig. 1. Laser beam diffractometry for small unilamellar vesicles (SUVs). Distribution curves for particle diameter in the triplicate assay (SUV1=80nm; SUV2=83nm; SUV3=87nm).

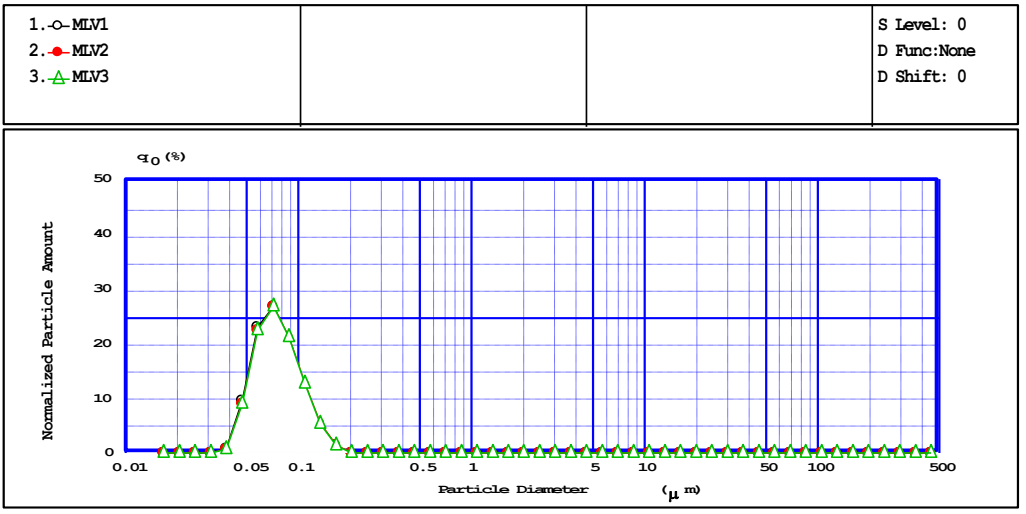


Fig. 2. Laser beam diffractometry for multilamellar vesicles (MLVs). Distribution curves for particle diameter in the triplicate assay (MLV1=75nm; MLV2=76nm; MLV3=76nm)

Phospholipid concentration assessment

The Stewart assay was used to quantify phospholipids (PLs) according to Torchilin (16). Initially we used a solution of Ammonium Ferrothiocyanate (0,1 M) and a specific calibration curve for the DSPC. Ammonium Ferrothiocyanate resulted by mixing Ferric Chloride hexahydrate (27.03 g) with Ammonium Thiocyanate (30.04 g) in double-distilled water, followed by subsequent dilution to 1000 mL.

The concentration of PLs was measured

by combining 0,5 mL suspension of SUVs (or MLVs, respectively) with 2 mL Chloroform and with 2 mL Ammonium Ferrothiocyanate solution (0,1 M) in 10 mL-tubes, protected on the exterior with aluminum foil. The previous step was done in a duplicate manner to cover for any accidental preparation errors. Each tube underwent rigorous vortexation for 30 seconds and then allowed to react 20 minutes in standard laboratory environment. After this time, the bottom layer was collected with a Pasteur pipette. The PLs-containing Chloroform phase was analyzed with a spectrophotometer at 485 nm. The concentration of PLs in the suspensions of SUVs and MLVs, respectively, was calculated by comparison of the PLs content with an anteriorly prepared standard calibration curve. PLs concentrations determined by the Stewart test were 14.5 mg/mL for the SUVs, respectively 13 mg/mL for the MLVs suspensions.

This method of calculation has the following two main advantages: a) the formation of a red color complex between the main groups of PLs with Ammonium Ferrothiocyanate, which can be quantified spectrophotometrically after extraction in an organic solution (Chloroform); b) there is no interaction between the assessment method and the inorganic phosphate.

Laboratory Animals

We used 24 white male Wistar rats, provided by the "Cantacuzino" National Medical Military Institute for Research and Development from Bucharest, Romania.

The animals were not genetically modified and presented a normal health status. Their age at the beginning of the study was 3 months, and their weight was varying between 200 and 250 grams. All animal subjects received daily appropriate nutrition and were accommodated in a controlled environment (temperature 25˚C, humidity 60%) at the Advanced Center for Research and Development in Experimental Medicine (CEMEX) of Grigore T. Popa University of Medicine and Pharmacy Iasi.

Certificate No. 93/14.06.2021 was issued by the Ethics Commission of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi.

Main experiment steps

The subjects were divided into four groups, each consisting of six rats, and were given nanoparticles loaded with the fluorescent marker Rhodamine B. Each group had a different method of administration, except for Group A, designed as the control group who did not receive any substances. The other three groups, B, C and D respectively, were made of 6 rats, and received Rhodamine B loaded nanoparticles at a dose of 0.05 mg/kg body weight for three consecutive days. The methods of administration were as follows: endogastric for Group B, intraperitoneal injection for Group C and injection into the tail vein for Group D.

On the fourth day, the animals were sacrificed, and organs (gum, liver, heart, kidney, testicle) were harvested and preserved in paraformaldehyde to be examined under an immunofluorescence microscope (Nikon's Eclipse E600). Histological sections were performed with a LEICA CM1860 UV microtome at St. Mary Children's Emergency Hospital in Iasi, Romania. Fixation was done with glycerol mixed with resins, to prevent ice crystals formation when tissue was cryogenized at -40°C. After the tissue block opacified

entirely, we sectioned it in 5.0 μm slices, at -16°C. Sections were spread with an antiroll plate on glass slides and Meyer albumin was added to support the process. Fluorescence Mounting Medium (DAKO) was applied on these tissue fragments before the cover glasses were positioned (17).

Data analysis

The data were centralized in *SPSS 18.0* databases and processed with suitable statistical functions. Both descriptive and analytical methods were used.

Indicators of the average value were used in conjunction with the ones for dispersion. In calculating the significant difference between two groups with normal distributions, t-Student Test was done at a significance threshold of 95% ($p \leq 0.05$).

The plotting of a Receiver Operator Characteristic (ROC) curve, which defines the aria under curve (AUC) and where the false-positive rate (specificity) is placed on the abscissa and the true-positive rate (sensitivity) on the ordinate, allowed the analysis of the sensitivity/specificity balance.

The Kruskal-Wallis test is a nonparametric test with which we compared three or more frequency distributions intergroupally.

RESULTS

During microscopic examination, we observed several aspects regarding the absorption of Rhodamine B marked nanoparticles. Fluorescence is identified at the level of hepatic tissue, especially due to the Kupffer cells endocytosis (figs. 3a, 3b) and in the renal tubules, with a diffuse aspect (fig. 4).

At gingival level, the gum muscle fibers and the surface epithelium are visible displaying less fluorescence than the liver tissue (fig. 5). Seminiferous tubules incorporating the fluorescent marker were observed in the testicle (fig. 6).

Intraperitoneally administrated nanocarriers, marked with Rhodamine B, showed the highest degree of absorption in liver in 83.3% of the rats in Group C, compared to the endogastric route, where the same level of absorption was recorded in only 33.3% of the animals (p=0.050).

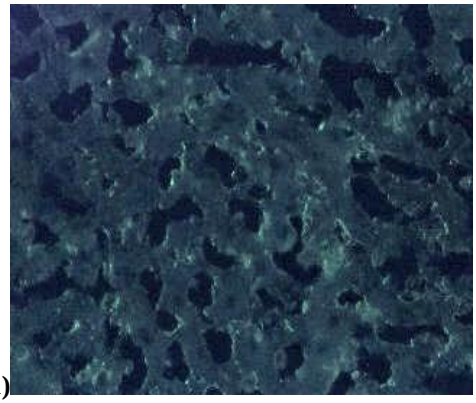
(a)  **(b)**

Fig. 3. Microscopic image of liver tissue displaying fluorescence, following administration of Rhodamine B nanoparticles: **(a)** by endogastric tube (x400); **(b)** by intraperitoneal injection (portal space, x200)

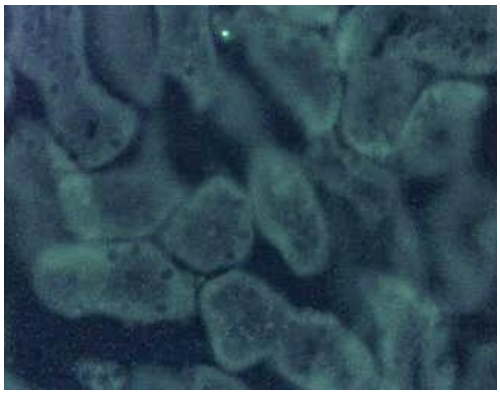


Fig. 4. Microscopic image of kidney tissue displaying diffuse fluorescence at the level of renal tubules, following administration of Rhodamine B nanoparticles by endogastric tube (x200)

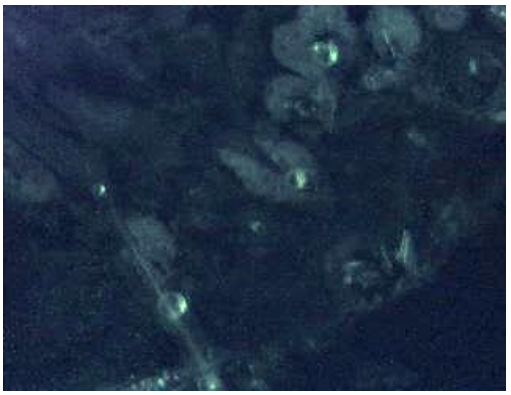


Fig. 5. Microscopic image of gingiva displaying fluorescence at the level of gingival muscle, following administration of Rhodamine B nanoparticles by intravenous injection (x200)

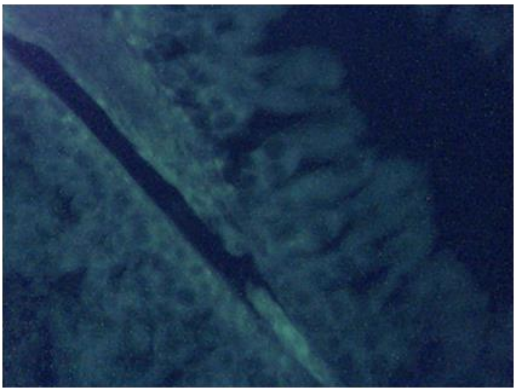


Fig. 6. Microscopic image of testicle tissue displaying fluorescence at the level of seminiferous tubules, following administration of Rhodamine B nanoparticles by intravenous injection (x200)

In what concerns the two parenteral tested pathways, the absorption level presented statistical differences among the intraperitoneal vs. intravenous groups, in gum ($p=0.011$), heart ($p=0.004$), kidney ($p=0.050$) and testicle ($p=0.011$), whereas there was no statistical difference in liver tissue ($p=0.250$). The penetrability of nanoparticles encapsulating Rhodamine B was quantified according to the observed fluorescence into four absorption levels: “very low absorption / no absorption” (-), “low absorption” (+), “moderate absorption” (++), “high absorption” (+++) (tab. I).

Kruskall-Wallis test delivers the correlation matrix between the degree of absorption of nanoparticles by type of organ, within each of the three groups. Significant differences ($p\leq0.05$) in the endogastric pathway are present between the absorption levels in the heart and kidney, heart and testicle, and kidney and testicle. In the intravenous group the only significant difference was observed between the liver and the testicle examined specimens. (tab. II).

TABLE I.

Fluorescence level following Rhodamine B marked nanoparticles absorption in the harvested organs in all study groups.

Absorption level	Number of rats / type of organs				
	Gum	**Liver**	**Heart**	**Kidney**	**Testicle**
Group A (Control)					
-	6(100%)	6(100%)	6(100%)	6(100%)	6(100%)
+					
++					
+++					
Group B (Endogastric tube)					
-	1(16.7%)	1(16.7%)	3(50.0%)	2(33.3%)	2(33.3%)
+	2(33.3%)	2(33.3%)	3(50.0%)	1(16.7%)	
++	3(50.0%)	1(16.7%)		3(50.0%)	4(66.7%)
+++		2(33.3%)			
Group C (Intraperitoneal injection)					
-	4(66.7%)		2(33.3%)	1(16.7%)	2(33.3%)
+	2(33.3%)		4(66.7%)	1(16.7%)	1(16.7%)
++		1(16.7%)		1(16.7%)	3(50.0%)
+++		5(83.3%)		3(50.0%)	
P* value for Group B *vs* Group C	**0.048**	**0.050**	0.557	0.136	**0.048**
Group D (Tail vein)					
-	2(33.3%)	1(16.7%)	2(33.3%)	5(83.3%)	2(33.3%)
+				1(16.7%)	
++	4(66.7%)		4(66.7%)		
+++		5(83.3%)			4(66.7%)
P* value for Group D *vs* Group B	0.196	0.139	**0.007**	0.075	**0.004**
P* value for Group D *vs* Group C	**0.011**	0.250	**0.004**	**0.050**	**0.011**

(-) = very low absorption / no absorption;
(+) = low absorption;
(++) = moderate absorption;
(+++) = high absorption.

TABLE II.

Kruskall-Wallis test - correlation matrix between the degree of absorption of liposomes by type of organ

Organ	Group B (Endogastric tube)				Group C (Intraperitoneal injection)				Group D (Tail vein)			
	Gum	Liver	Heart	Kidney	Gum	Liver	Heart	Kidney	Gum	Liver	Heart	Kidney
Liver	0.360	-			0.341	-			0.105	-		
Heart	0.422	0.136	-		0.759	0.105	-		0.545	0.341	-	
Kidney	0.236	0.154	**0.016**	-	0.282	0.144	0.054	-	0.148	0.341	0.148	-
Testicle	0.088	0.182	**0.050**	**0.022**	0.158	0.268	0.593	0.476	0.545	**0.015**	0.545	0.148

By drawing the ROC curves we highlighted the sensitivity and the specificity of the penetrability of Rhodamine B charged nanosystems into the examined organs, according to the route of administration (fig. 7, tab. III). In study animals receiving endogastric nanoparticles, the sensitivity/specificity balance of the degree of absorption in the liver was noted with a sensitivity of 79% and a specificity of 75% (AUC=0.792; IC95%:0.518-1.066; p=0.093), in kidneys with a sensitivity of 82% and specificity of 68% (AUC=0.722; IC95%:0.416-1.0029; p=0.200), and in the testicle with a sensitivity of 66% and specificity of 58% (AUC=0.667; IC95%: 0.331-1.002; p=0.337).

In study subjects who received Rhodamine-loaded nanoparticles by intraperitoneal injection, gum absorption degree displayed a sensitivity of 83% and specificity of 70% (AUC=0.833; IC95%:0.590-1.077; p=0.055).

In study subjects who received rhodamine-loaded nanoparticles through the tail vein, the degree of absorption in the heart was noted with a sensitivity of 75% and a specificity of 60% (AUC=0.736; IC95%:0.423-1.049; p=0.034), in the testicle with a sensitivity of 75% and specificity of 50% (AUC=0.722; IC95%:0.397-1.047; p=0.039), and in the gum with a sensitivity of 65% and a specificity of 80% (AUC=0.653; IC95%: 0.355-0.951; p=0.303).

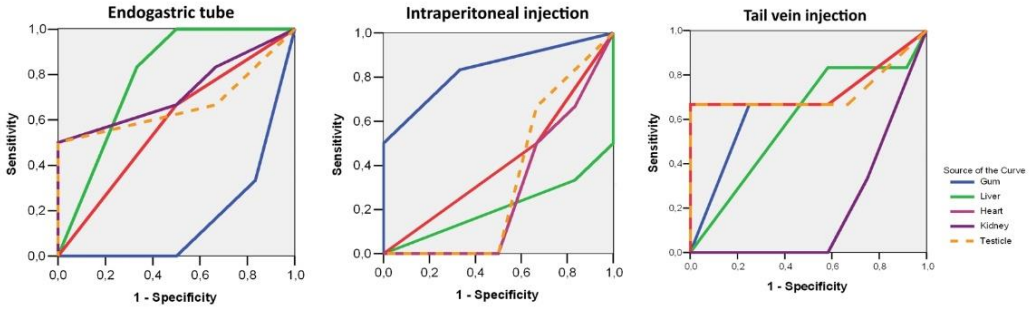


Fig. 7. Sensitivity and the specificity of the penetrability of Rhodamine B charged nanosystems into the examined organs, depending to the route of administration.

TABLE III.

Area Under the Curve

Test Result Variable(s)	Area	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Endogastric tube					
Gum	0.167	0.124	0.055	-0.077	0.410
Liver	0.792	0.140	0.093	0.518	1.066
Heart	0.583	0.171	0.631	0.248	0.919
Kidney	0.722	0.156	0.200	0.416	1.029
Testicle	0.667	0.171	0.337	0.331	1.002
Intraperitoneal injection					
Gum	0.833	0.124	0.055	0.590	1.077
Liver	0.208	0.140	0.093	-0.066	0.482
Heart	0.417	0.171	0.631	0.081	0.752
Kidney	0.278	0.156	0.200	-0.029	0.584
Testicle	0.333	0.171	0.337	-0.002	0.669
Tail Vein					
Gum	0.653	0.152	0.303	0.355	0.951
Liver	0.597	0.148	0.512	0.307	0.888
Heart	0.736	0.160	0.034	0.423	1.049
Kidney	0.194	0.102	0.111	0.006	0.395
Testicle	0.722	0.166	0.039	0.397	1.047

The test result variable(s): Gum, Liver, Heart, Kidney, Testicle has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption b. Null hypothesis: true area = 0.5

DISCUSSION

In recent times, there is growing evidence that nanosystems can be used as promising transporters towards tissues, and even certain cells from an area with specific pathology. Moreover, these carriers possess an adjustable drug release capacity, and also, they can avoid degradation due to digestion at gastrointestinal level.

Shah S *et al*. advocates for usage of correctly manufactured nanomedicines in the treatment of patients diagnosed with cardiovascular, neurodegenerative, and metabolic diseases, and in autoimmune pathologies or cancer. The numerous advantages, like avoiding first-pass metabolism or eliminating the gastrointestinal barrier in case of oral administration, coupled with efficient absorption and quick delivery in a targeted manner favors a wider-more spread implementation in the pharmaceutical industry (18). To reduce the toxicity of drug nanocarriers, their preparation uses biodegradable, biocompatible and physiological lipids (19).

The reasoning for the present paper was represented by a systematic approach to dye-marked nanoparticles absorption in several organs, by comparing three routes of administration: endogastric tube, intraperitoneal and intravenous injections. At present there is a scarcity of data in the literature regarding a comparison for absorption of these molecules in a trimodal administration.

The administration of nanoparticles can be achieved by enteral and parenteral pathways, with implications in patient quality of life. The oral route is the easiest and most common one, since has the highest patient addressability, but can be prone to variable intestinal absorption due to

digestion processes. The parenteral routes avoid intestinal barrier, but still organ absorption is correlated with blood flow distribution within the targeted tissue (20).

Intravenous injection enjoys a standard status in the hospital or out-patient clinic settings, having a rapid and entire dose delivery into the bloodstream (21). It is generally used in cases where nanosystems cannot be administered orally, patients are unconscious, or the nanodrug cannot be absorbed at the gastrointestinal level or where there are bleeding conditions in the digestive tract (22). Regarding the intraperitoneal route, it is common in the hospital practice only in limited areas like peritoneal dialysis and in the administration of radiopharmaceuticals, like 18F-fluorodeoxyglucose for the PET/CT studies (23).

The research in our preclinical study on the penetrability of Rhodamine B-loaded nanoparticles by parenteral administration and endogastric probe correlate with those conducted by Dias ML *et al*., who also investigated drug-carrying nanosystems in several delivery techniques. Dias ML has shown the effect on prostate cancer by using a chemotherapy drug (Paclitaxel) combined with a cholesterol-rich nanoemulsion given to mice. The action of this nano formula primarily targeted solid tumors and cancer cells, which increases the transcription frequency of low-density lipoprotein receptors. At the same time, the toxicity level of nanodrug structures was evaluated, together with an increase in the anticancer activity (24). Besides some major favoring elements, these carrier systems also come with some disadvantages, like a disparity between large-scale production and final selling price, presence of organic solvent residues in the final product, toxicity, and difficulty in granting licenses their usage from regulatory authorities (25).

In our study, the nanoparticles were loaded with the fluorescent marker Rhodamine B to study penetrability across several organs. Rhodamine is a synthetic dye with a stable chemical structure, easily assimilated by the body. It is widely used in immunofluorescence microscopy and flow cytometry (26), and also in biology and biotechnology as a biosensor (27, 28). Contrast dye has started being used in the last decade in formulations of nanomaterials for drug delivery with the purpose to evaluate absorption into tissues (29). Following exposure to light, Rhodamine can form reactive oxygen compounds in two ways: the first reaction is represented by the formation of reactive oxygen compounds by increasing the energy caused by Rhodamine and then transferred to biomolecules; the second reaction is characterized by the energy transfer of the immunofluorescent substance to the molecular oxygen (30).

Some studies suspect that this chemical compound is carcinogenic in rats and it can pose a genotoxic risk to mammals (31-34). However, there are currently no extensive studies proving that replacing the Rhodamine B dyes group with other fluorescent markers may mitigate these effects (35).

After administration of fluorescent marked nanoparticles, our study found that gum absorption was similar among the endogastric and intravenous administration pathways ($p=0.196$), but with a higher specificity, of 80% (AUC=0.653; IC95%:0.355-0.951; $p=0.303$), when injected into the tail vein. Gum tissue has dense vascularization, coupled with a high *in vivo* regeneration capacity (36), which is most probable to influence these findings.

Kupffer cells were highly captative of the fluorescent markers, since they are cells with important immune function due to their phagocytosis capacity, located in sinusoidal cells of the liver (37). The highest degree of absorption of nanocarriers was seen in the liver (+++) in 83,3% of the rats, in both cases of parenteral administration. We found

no statistical difference in the absorption at this level between the intraperitoneal and intravenous injections (p=0.250).

Leydig interstitial cells that secrete testosterone, along with Sertoli cells, which are support cells located in connective tissue (38), appeared fluorescent, but at a lesser extent in comparison with the liver tissue. We noted significant differences in the absorption among all three ways of administration at this level: intraperitoneal vs. endogastric (p=0.048), intravenous vs. endogastric (p=0.004), and intravenous vs. intraperitoneal (p=0.011).

Despite its toxicity evocated above (31-34), Rhodamine B itself is an optimal diagnostic method in various pathologies, being considered a fast, cheap and efficient marker (39). Incorporating Rhodamine B into nanoparticles could greatly diminish its toxicity, while still maintaining an adequate absorption profile in the target tissues. This was another reason for fulfilling the purpose of our study to make comparisons within the trimodal administration methods.

Very recent data is contradicting the genotoxicity of Rhodamine B in mammals, as it was successfully used as a biological marker, presenting low toxicity in the case of human embryonic kidney (HEK 293 cells). The absorption rate of the fluorescent marker was higher in cancer cells compared to normal cells, thus opening new opportunities in targeting tumors like skin neoplasms (B16F10 cells), breast cancer metastases (MDA-MB231 cells), lung carcinomas (A549 cells) (40).

This paper also opens up possibilities to continue the present research by implementing a more robust quantification system for the absorption of nanocarriers charged with Rhodamine B, with help of artificial intelligence processing for the fluorescence microscopy images.

Our study has involved a small number of laboratory animals, according to the Ethics Certificate issued by Grigore T. Popa University of Medicine and Pharmacy, Iași. This resulted in a limited power for our statistical analysis of the results.

CONCLUSIONS

Drug-carrying nanoparticulate systems are currently being used while also gaining increased attention, despite their high financial burden on the health systems.

Our study investigated the penetrability of nanoparticles loaded with Rhodamine B dye, following a trimodal administration method, for three consecutive days. The organ which showed the highest absorption, in both the tissular level and in the percentage of the studied subjects, was the liver, followed by kidney. The lowest absorption was seen in heart tissue.

After studying the penetrability of the fluorescent-marked nanoparticles, we have demonstrated that the studied organs had a high absorption rate, using the parenteral administration method, compared to endogastric probe.

LABORATORY ANIMALS RESEARCH ETHICS

Certificate No. 93/14.06.2021 was issued by the Ethics Commission of "Grigore T. Popa" University of Medicine and Pharmacy, Iasi.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest.

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THE PROGNOSTIC VALUE OF CEA AND CA 19-9 IN DETERMINING SURVIVAL OUTCOMES FOR PATIENT WITH LOW RECTAL CANCER

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THE PROGNOSTIC VALUE OF CEA AND CA 19-9 IN DETERMINING SURVIVAL OUTCOMES FOR PATIENT WITH LOW RECTAL CANCER (Abstract): The prognostic value of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) is a topic of interest for surgeons. The aim of this retrospective study was to evaluate the prognostic value of CEA and CA 19-9 in patients with locally advanced low rectal cancer who underwent neoadjuvant chemoradiotherapy, underlining their potential as indicators for survival outcomes**. Materials and methods:** Between November 2019 and February 2023, we evaluated the medical records of 104 patients admitted to the first Oncologic Surgical Clinic from the Regional Institute of Oncology, Iasi, Romania. Clinical and paraclinical data was recorded, and patients were segregated intro three groups: one marker elevated during follow-up (group 1), 2 markers elevated during follow-up (group 2), and no marker elevated during follow-up (group 3). The overall survival and recurrence-free survival were the evaluated outcomes, and the prognostic values of serum markers were evaluated using Cox regression model and Kaplan-Meyer survival analysis. (3) **Results:** The presence of one (hazard ratio, HR: 2.78, 95% confidence interval, CI: 1.11- 6.92, p= 0.02) or both (HR: 4.23, 95%CI: 1.44-9.32, p=0.03) elevated markers statistically decreased the recurrence-free survival time. A combination of elevated markers has a significantly higher impact on the recurrence-free survival time compared to a single elevated marker (p=0.001). Only the combination of biomarkers significantly decreased the overall survival rates (HR: 3.74, 95% CI: 0.57- 7.62, p= 0.04). (4) **Conclusions:** The combination of elevated CEA and CA 19-9 had the highest prognostic significance for the evaluated survival outcomes. **Keywords:** LOW RECTAL CANCER; CEA; CA 19-9; SURVIVAL OUTCOMES.

Low rectal cancer presents unique challenges and considerations compared to other colorectal malignancies, particularly concerning therapeutic strategies and prognostic evaluations (1). Approximately 46,000 new cases of rectal cancer are identified annually in the United States (2). The majority of them are adenocarcinomas (3).

Comparable epidemiological data was obtained from Europe, and current GLOBOCAN statistics for 2022 revealed that colon cancer ranked as the third most commonly diagnosed form of cancer in males, with 92,942 newly diagnosed cases in Eastern Europe (4). According to the data registry, colorectal cancer was identified as the second most commonly diagnosed tumor in Eastern Europe's female population, with a total of 86,575 new cases reported in 2022. In Romania, there was a close adherence to the epidemiological trend of colorectal cancer. In 2022, there were 8,056 newly diagnosed cases in males, making it the third most common kind of cancer. In females, there were 5,485 newly diagnosed cases, making it the second most common type of cancer (4).

The survival rates for rectal cancer have significantly increased in the past 30 years due to various factors, one of which is the implementation of neoadjuvant radio chemotherapy (RCT) (5). This treatment is now recommended for almost all cases of locally advanced rectal cancer (LARC) that are classified as cT3 and T4. It is evident that patients who respond well to RCT have a higher chance of achieving a better prognosis. These good responders make up approximately 15-27% of all patients treated with RCT (6).

The gold standard for evaluating the response of primary tumors after RCT is histopathological analysis of the surgical specimen. This analysis includes determining the pathological tumor regression grade (pTRG) by assessing the proportion of residual tumor cells and fibrosis within the tumor specimen (7).

Magnetic resonance imaging (MRI) is widely recognized as the most precise method for determining tumor stage, involvement of the mesorectal fascia, and invasion of blood vessels outside the wall of the rectum (8-10).

Recent investigations have shown an association between MR imaging results and pathological TRG (pTRG) (11, 12). Also, several studies suggested a scoring method called MR imaging-TRG (mrTRG), which is determined through visual evaluation of MR imaging data (13, 14).

The determination of survival outcomes is a multifaceted process, influenced by a diverse array of clinical, pathological, and molecular factors (15-17). Among these, tumor markers play a critical role in informing prognosis and guiding therapeutic decisions. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) are two such biomarkers which have been widely studied for their prognostic implications in colorectal cancer (18, 19). Their levels are often correlated with disease stage, tumor load, and patient outcomes, and they may be particularly significant in the context of low rectal cancer (20, 21).

CEA is the most prominent biomarker for colorectal cancer, and it is used preoperative staging, therapy monitoring, and postoperative follow-up [10, 11], while CA19-9 is closely associated with the biological characteristics of tumors. The rectum and colon exhibit distinct variations in embryology, architecture, and physiology.

Furthermore, there are apparent differences in the development of rectal and colon cancer. Therefore, additional research is required to investigate the significance of CA19-9 in rectal cancer, particularly in patients with locally advanced rectal cancer who are having neoadjuvant

chemoradiotherapy.

This paper examines the prognostic value of CEA and CA 19-9 in patients with locally advanced low rectal cancer who underwent neoadjuvant chemoradiotherapy, underlining their potential as indicators for survival outcomes. The pursuit is to deepen the understanding of these biomarkers beyond the usual confines of diagnostic tools, exploring how their preoperative and post-operative levels relate intricately to clinical trajectories and long-term survival rates.

MATERIALS AND METHODS

We performed a retrospective observational study at in the first Oncologic Surgical Clinic from the Regional Institute of Oncology, Iasi, Romania, between November 2019 and February 2023. Ethical approval for this study was obtained from the Institutional Ethics Committees of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi (No. 23103/23rd of October 2019) and of the Regional Institute of Oncology (No. 245/3rd of July 2019).

In this study we included patients diagnosed with low rectal tumors (less than 8 cm from the anal verge), a histopathological examination that indicated adenocarcinoma type, who had a preoperative pelvic MRI for staging, and who received neoadjuvant therapy, as well as those who offered their informed consent for participating in this study.

The exclusion criteria comprised patients with rectal cancers who needed emergency surgery, other subtypes of rectal cancer, loss of follow-up, incomplete medical data or lack of informed consent.

The following data was recorded: demographic and clinical characteristics, the levels of CEA and CA 19-9 before surgery and during follow-up, preoperative MRI parameters, histopathological examination of pre-operative biopsies and post-operative specimens, type of surgery, postsurgical evolution and survival (overall survival and recurrence-free survival). All patients underwent pelvic MRI examination the SIEMENS MAGNETOM Avanto I-class 1.5 Tesla machine (Siemens Healthcare GmbH, Erlangen, Germany).

The examination protocol included the visualization of the pelvis in all three planes:

- Sagittal – this plane was used to locate the tumor and to plan the axial and coronal sequences;
- Axial - the plane was angled perpendicular to the tumor to correctly visualize the extension of the tumor against the rectal wall, as well as the distance between the tumor and the mesorectal fascia (MRF);
- Coronal – the plane was angled parallel to the axis of the tumor, which is perpendicular to the axial series.

The sequences used are the following: multiplanar T2w and T1w that provided valuable morphological information due to the high resolution of anatomical structures. The T2w sequence was $\leq$ 3 mm thick. The preoperative parameters included: distance from anal verge, extramural venous invasion (EMVI) status, and MRI CRM (mCRM) positivity.

CA 19-9 was determined from the serum of the patients (at least 0.5 mL) using immunochemistry with electrochemiluminescence detection (ECLIA) (Roche Diagnostics GmbH, Mannheim, Germany). A cut-off value was considered <27 U/mL[3] for this biomarker. Another 0.5 mL of blood serum was used to detect CEA using the same ECLIA protocol, and the cut-offs

of this marker were considered as follows: <3.0 ng/mL (non-smokers), and <5.0 ng/mL (smokers).

The values of these biomarkers were recorded before surgery, and any increase of the serum values during follow-up were recorded. If none of these markers were increased during follow-up, the patients were included in the control group (group 3, n= 68 patients). If one of these markers were positive, the patients were included in the first group (n= 24 patients). Finally, if both of these markers were positive during monitorization after surgery, the patients were included in the second group (n= 12 patients). Our data is limited to three years after surgery, and the follow-up consisted of consultation at 3-monthly intervals for the first 2 years, and every 6 months for the third year.

Each patient underwent an extended course of radiation, receiving a total dose of 45 Gy administered in 25 fractions. This was followed with a supplementary dose of 5.4 Gy specifically targeting the primary tumor, administered over a period of 5 weeks. The preoperative concurrent chemotherapy regimens consisted of either capecitabine plus oxaliplatin (CapeOX) or fluorouracil plus oxaliplatin (FOLFOX). The surgical procedures were conducted 6-8 weeks following the preoperative neoadjuvant chemoradiotherapy.

Considering the overall survival and recurrence free survival as outcomes, we compared categorical and continuous data between groups using Pearson's $\chi 2$ test or analysis of variance (ANOVA), followed-up by Bonferroni post-hoc test. We have also used a Cox regression model with Breslow method for ties to identify the impact of the evaluated biomarkers for the prediction of overall survival and recurrence-free survival, and quantified their impact as hazard ratio (HR) and 95% confidence interval (CI).

Finally, we provided a survival analysis of our cohort of patients using Kaplan-Meier estimates, segregated depending on the presence of one or two elevated biomarkers during follow-up. A p-value less than 0.05 was considered statistically significant. These analyses were performed using STATA SE (version 17, 2023, StataCorp LLC, College Station, TX, USA).

RESULTS

This study evaluated the medical files of 104 patients with low rectal adenocarcinoma who received neoadjuvant CRT and surgical treatment. Their demographic and clinical characteristics are presented in table I. We could not find any statistically significant difference between groups regarding their baseline characteristics such as age (p= 0.72), gender distribution (p= 0.67), or body mass index (BMI) (p= 0.98).

On the other hand, patients who had one or both biomarkers increased during follow-up presented with significantly higher frequency of positive mCRM, EMVI, anterior location of the tumor, a distance from the anal verge less than 4 cm, incomplete TME, and positive pCRM (p< 0.001).

The impact of elevated serum markers on the recurrence-free survival and overall survival was tested using a Cox regression model with Breslow method for ties (table II). Our results indicated that the presence of one (HR: 2.78, 95% CI: 1.11- 6.92, p= 0.02) or both (HR: 4.23, 95%CI: 1.44-9.32, p= 0.03) elevated markers statistically decreased the recurrence-free survival time.

Also, a combination of elevated markers has a significantly higher impact on the recurrence-free survival time compared to a single elevated marker (p= 0.001).

On the other hand, only the combination of biomarkers significantly decreased the overall survival rates (HR: 3.74, 95%CI: 0.57- 7.62, p= 0.04), and the comparison between groups was statistically significant (p= 0.042).

TABLE I.

Demographic and clinical characteristics of patients included in the study

Variable	One elevated marker (group 1, n= 24 patients)	Two elevated markers (group 2, n= 12 patients)	Control group (group 3, n= 68 patients)	*p* value
Age, years (mean ± SD)	64.72± 7.41	69.16± 6.32	64.87± 7.93	0.72
Gender (n/%)	Male= 15 (62.50%) Female= 9 (37.50%)	Male= 9 (75%) Female= 3 (25%)	Male= 42 (61.76%) Female= 26 (38.24%)	0.67
BMI, kg/m^2 (mean ± SD)	25.15± 4.29	25.18± 3.88	24.86± 4.10	0.98
Positive mCRM (n/%)	Yes= 19 (79.17%)	Yes= 5 (41.67%)	Yes= 0 (0%)	< 0.001
Positive EMVI (n/%)	Yes= 14 (58.33%)	Yes= 9 (75%)	Yes= 2 (2.94%)	< 0.001
Anterior location (n/%)	Yes= 9 (37.50%)	Yes= 6 (50%)	Yes= 1 (1.47%)	< 0.001
Distance from anal verge (n/%)	< 4 cm= 19 (79.17%)	< 4 cm= 10 (83.33%)	< 4 cm= 16 (23.53%)	< 0.001
Type of surgery (n/%)	AP resection= 15 (62.50%) Hartmann procedure= 6 (25.00%) Anterior resection= 1 (4.17%) Pelvic exenteration= 2 (8.33%)	AP resection= 8 (66.67%) Hartmann procedure= 2 (16.67%) Anterior resection= 2 (16.67%) Pelvic exenteration= 0 (0%)	AP resection= 53 (77.94%) Hartmann procedure= 11 (16.18%) Anterior resection= 4 (5.88%) Pelvic exenteration= 0 (0%)	0.11
Incomplete TME (n/%)	Yes= 12 (50%)	Yes= 5 (41.66%)	Yes= 5 (7.35%)	< 0.001
Positive pCRM (n/%)	Yes= 15 (62.50%)	Yes= 4 (33.33%)	Yes= 0 (0%)	< 0.001

pCRM - pathological (positive) circumferential resection margin; mCRM - positive circumferential resection margin on MRI; mrEMVI - extramural venous invasion on MRI; SD- standard deviation; BMI - body mass index; AP - abdominoperineal resection; TME - total mesorectal excision.

TABLE II.

Group comparisons of first trimester biochemical markers and mean arterial pressure

Marker	One elevated marker (group 1, n= 24 patients)	*p* value	Two elevated markers (group 2, n= 12 patients)	*p* value	*p* value* comparison
Recurrence-free survival (HR, 95% CI)	2.78 (1.11- 6.92)	0.02	4.23 (1.44- 9.32)	0.03	0.001
Overall survival (HR, 95%CI)	2.56 (0.78- 8.40)	0.12	3.74 (0.57- 7.62)	0.04	0.042

HR- hazard ratio, CI- confidence interval, *- *p* value resulted from comparison.

In the final stage of our analysis, we outlined the impact of these elevated markers on the survival estimates using Kaplan-Meyer analysis (figures 1 and 2). The recurrence-free survival estimates for the first group were 70.83% (n= 17 patients), with a medial time until the recurrence occurred of 29 months, while the recurrence-free survival estimates for the second group were 66.6% (n= 8 patients), with a median time until recurrence of 18 months.

The overall survival Kaplan-Meyer estimates for the first group were 83.7% (n= 20 patients), with a median time until the failure event (death) of 30 months, while for the second group the overall survival rate was 75% (n= 9 patients), with a median time until death of 32 months.

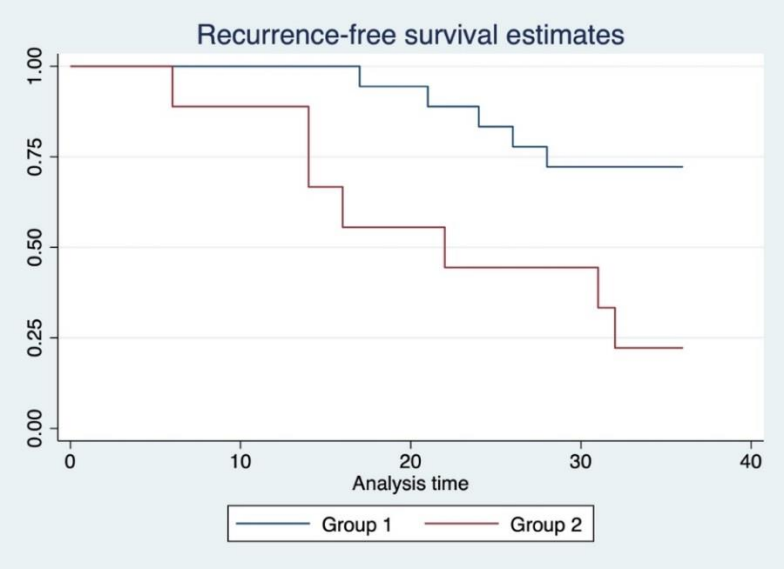

Fig. 1. Recurrence-free survival estimates corresponding to the evaluated groups. (Group 1- one elevated marker, Group 2- 2 elevated markers)

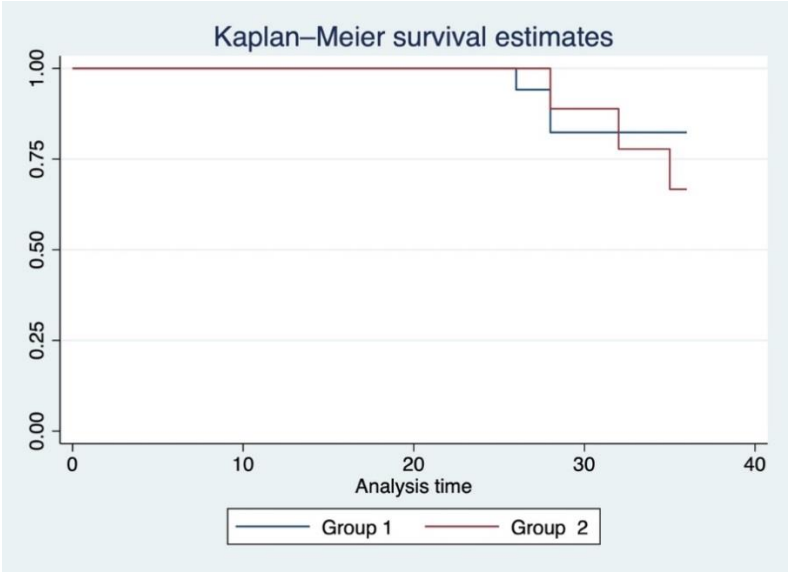

Fig. 2. Kaplan-Meier survival estimates corresponding to the evaluated groups. (Group 1- one elevated marker, Group 2- 2 elevated markers)

DISCUSSION

This observational retrospective study evaluated the prognostic value of CEA and CA 19-9 in patients with locally advanced low rectal cancer who underwent neoadjuvant chemoradiotherapy, underlining their potential as indicators for survival outcomes. Our results indicated that that the presence of one or both elevated markers statistically decreased the recurrence-free survival time. Also, a combination of elevated markers has a significantly higher impact on the recurrence-free survival time compared to a single elevated marker. On the other hand, only the combination of biomarkers significantly decreased the overall survival rates, and the comparison between groups was statistically significant.

These results are in line with previously published data. One recent study by Zheng *et al.* investigated the prognostic significance of CEA and CA 19-9 and their combination for patients with locally advanced rectal cancer (19). Their results indicated that the recurrence-free survival rates and the overall survival rated RFS and OS rates decreased as the serum levels of these markers increased during follow-up (all $p < 0.05$). The same study outlined that only the number of elevated post-neoadjuvant therapy markers was an independent prognostic factor the evaluated outcomes, and that a combination of serum markers increased the risk of recurrence and death (2.5- and 3.7-fold increased risk).

Another study included 197 cases with LARC who underwent neoadjuvant CRT and radical surgery, and the authors evaluated the prognostic significance of CEA and CA 19-9 for the following outcomes: survival rates as well as distant metastasis and local recurrence rates (22). The results from this study demonstrated that the combination of these markers achieved the best performance in terms of prognosis of disease-free survival (HR: 8.157, 95%CI: 3.232-20.591)

and distant metastasis (HR: 8.790, 95% CI: 3.324-23.248). They also found out that elevated CA19-9 was associated with poorer overall survival rates, disease-free survival rates, and local recurrence rates.

Song *et al.* investigated the prognostic significance of CEA serum levels firstly determined in six months after surgery in 100 patients with locally advanced rectal cancer treated with neoadjuvant CRT and total mesorectal excision (23). Their results indicated a median CEA value after surgery of 1.3 ng/mL, with an overall descending trend of serum values from before neoadjuvant CRT. Moreover, their multivariate analysis indicated that both a complete pathological response and an elevated postoperative CEA were significantly associated with local recurrence.

Finally, Kim *et al.* outlined the prognostic value of postoperative CEA levels over the overall survival rates and disease-free survival rates during a 5-year follow-up in a cohort of 122 patients with rectal cancer (24). Specifically, the overall survival rates were 62.3%, while the disease-free recurrence rates were 58.6% in patients with progressive decrease of CEA levels after surgery, and these rates were overall higher than those of patients with increased CEA levels after surgery. In our study, the overall survival rates and recurrence-free survival rates were higher, but the follow-up period was only of 3 years. Moreover, since the median reference time for the death occurrence in our cohort of patients was 30 and 32 months in the evaluated groups, we expect a possible decrease in the overall survival rates when evaluating data in a 5-year time-frame.

As low rectal cancer often necessitates a tailored therapeutic approach due to its anatomical and oncological characteristics, the evaluation of CEA and CA 19-9 may offer valuable insights into the aggressiveness of the disease, the likelihood of recurrence, and the chances of a favorable response to treatment. In the quest for precision medicine, dissecting the utility of these biomarkers is imperative to optimize patient management and improve prognostic accuracy, ultimately enhancing survival outcomes.

The results from this study should be interpreted considering the following limitations: the small cohort of patients, the limited time-frame for patients' follow-up. On the other hand, this study has the advantage of a homogenous cohort of patients considering the rectal tumor type and demographic data.

Artificial intelligence and risk stratification have been gaining more interest in the field of predictive medicine, and it has been frequently used in imaging data modeling in the recent years (25-31). We hypothesize that further studies, on larger cohort of patients, could include individual or combined serum markers in several machine learning or convolutional neural networks in order to better establish their prognostic value.

CONCLUSIONS

The follow-up of rectal patients who underwent neoadjuvant CRT and surgical therapy should include, among other investigation, the dynamic determination of serum values of CEA and CA 19-9.

In our study, the presence of one or both elevated markers statistically decreased the recurrence-free survival time. Also, a combination of elevated markers has a significantly higher impact on the recurrence-free survival time compared to a single elevated marker. Only the combina-

tion of biomarkers significantly decreased the overall survival rates.

Further studies should include the long-term follow-up of patients, and should incorporate more advance data modeling techniques for further prognostic stratification of these serum markers.

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CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest. No funding was received for conducting this study.

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ATHEROGENIC INDEX OF PLASMA (AIP): CAN BE A BIOMARKER FOR EARLY ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE IN EXPERIMENTAL MODELS OF OBESITY RATS?

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ATHEROGENIC INDEX OF PLASMA (AIP): CAN BE A BIOMARKER FOR EARLY ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE IN EXPERIMENTAL MODELS OF OBESITY RATS? (Abstract) **Objective:** Our experimental study evaluated the influence and correlation among the Atherogenic index of plasma and early histological endothelial dysfunction in an experimental model of obesity rats. **Materials and methods:** Twenty-one Wistar rats, male, aged 4–6 months (450-550g), were allocated into III groups. The control group consumed exclusively a standard food, while the II group was administered HFD (Hight-Fat-Diet) with 2% cholesterol and the last group received HFD and treatment with Atorvastatin 20mg/kg/day by gavages. Before and after 4 weeks of oral administration of diet and treatment, serum lipid profile and glycemia were measured. The Atherogenic Index of Plasma, Castelli risk indices (I and II) and lipoprotein combined indices (LCI) are evaluated with a formula. Also, for histological study, aortas were extracted for the histological investigation and the principal extracellular matrix components, including collagen and elastin, were examined with a microscope for indications of aortic degeneration. Endothelial dysfunction was evaluated with biochemical levels of lipid profile, abdominal ultrasonography and histological study. **Results:** Four-week diet supplementation with HFD for obesity rats caused an increase in serum lipids levels; AIP levels indicate a high risk for cardiovascular events, histological aspect we observed endothelial damage, with degenerative modification in the aortic media, indicating by the dissociation of elastic fibers and accumulation of collagen. **Conclusions:** In our study, AIP, lipoprotein combined index and Castelli's risk index I and II are correlated with endothelial injury. Obesity disturbs the lipid profile, increasing AIP in experimental models of obesity rats and can be a risk factor for endothelial dysfunction. Treatment with statin, healthy food and change in the

modifiable risk factors can improve endothelial function and early stage of atherosclerosis.
Keywords: CARDIOVASCULAR RISK FACTOR, ATHEROGENIC INDEX OF PLASMA, EXPERIMENTAL MODELS OF OBESITY, ENDOTHELIAL DYSFUNCTION.

INTRODUCTION

Cardiovascular diseases (CVD) are considered the primary cause of death in the population, affecting women and men. The World Health Organization (WHO) predicted that in 2023, 23.3 million people will die caused by cardiovascular disease (1). Also, diabetes mellitus, obesity and metabolic syndrome continue to be the main factors that limit life (2) and are a major cause of reduced quality of life and disability.

The normal equilibrium of the vascular endothelium is considered the fundamental part of vascular health. The vascular endothelium has been considered, for a long time, only a simple cellular barrier, new is defined as an active monolayer that covers the cells of the vascular lumen, thus separating the vascular wall from the circulating blood. Over the years, authors have offered multiple definitions of endothelial dysfunction – ED (3). Endothelial dysfunction is definitely like the imbalance between vasodilation and vasoconstriction with excessive reactive oxygen species (ROS) and proinflammatory agents and decreased nitric oxide (NO) bioavailability (4).

Atherosclerosis, dyslipidemia and oxidative stress (OS), at present, are considered risk factors associated with CV and are responsible for millions of deaths annually (5). A major cause of premature mortality among CVD is atherosclerosis. It is considered a chronic, systemic, complex disease, who is affecting the vascular endothelium, characterized by lipid deposition in the arterial wall. Recent scientific research supports that atherosclerosis can be slowed or stopped, by initiating specific therapeutic measures and lifestyle modification and developing new therapeutic strategies capable of improving endothelial function and cardiovascular prognosis. The pathophysiology of atherosclerosis involves inflammation, abnormalities in lipoprotein metabolism and oxidative stress, which result in the accumulation of cholesterol esters in the macrophages of the arterial wall (6).

The primary contributing cause of coronary artery disease and endothelial dysfunction is dyslipidemia. Also, dyslipidemia plays a significant role in the carcinogenesis of various tumor types (renal, prostate and ovarian cancer (7). Atherogenic dyslipidemia is characterized by an abnormal lipid profile, a low level of high-density lipoprotein cholesterol (HDL-C)and increased levels of total cholesterol (TC), also, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B and triglyceride (TG). Atherogenic dyslipidemia is the same, implicated in the pathogenesis of endothelial dysfunction, also, microvascular coronary pathology and atherosclerosis - contribute to the progression of atherosclerotic plaques (8, 9). Oxidized LDL (ox-LDL) contributes to atherosclerotic plaque development because it inhibits endothelium-dependent vasodilation by decreasing the activity of nitric oxide synthesis (10). The principal role of HDL is to protect

endothelial cells from the damaging consequences of LDL and increase their function (11). Recent studies have shown that hypertriglyceridemia inhibits endothelial function in a variety of ways (12). TG also promotes atherosclerosis by producing proinflammatory cytokines, fibrinogen and coagulation factors (13).

Furthermore, early diagnosis of endothelial dysfunction has an essential role in the prevention and detection of CVD; this could be a reversible first step in atherosclerosis. Endothelium dysfunction causes very small TG-high lipoproteins and residue molecules, recognized like ApoB which contain lipoproteins, to traverse the endothelium cell barrier, accumulating fat and atherosclerosis.

In 2001, Dobiášová and Frohlich discussed for the first time the Atherogenic Index Of Plasma (AIP) which represents the interaction between protective lipoprotein and atherogenic biochemical markers(14).A relatively recent marker of atherogenicity is the atherogenic index of plasma (AIP) and is evaluated with the classical formula *as log10 (TG/HDL-C).*It is considered a good biochemical predictor for cardiovascular pathology, which includes coronary artery physiopathology and a significant biomarker to estimate the risk of atherosclerosis.

According to previous studies, AIP was stratified into three groups: low (< 0.11), intermediate (0.11–0.21) and high-risk (> 0.21) (15). The AIP is considered a new complex lipid index that measures the equilibrium between the atherogenic and antiatherogenic components and has also been the subject of research in recent years.

Previous research has proved that the atherogenic coefficient, calculated as Non-HDL-C/HDL-C, Castelli risk indices I represent the report between [TC]/HDL-C and Castelli risk II - LDL-C/HDL-C had better predictability for CVD events and significant correlations with cardiovascular disease than simple lipid parameters.

The comprehensive lipid indexes, compared with single lipid parameters, such as non-HDL-C (TC minus HDL-C), TC/HDL-C, LDL-C/HDL-C, non-HLD-C/HDL-C (atherogenic index, AI), and TC*TG*LDL/HDL-C (lipoprotein combine index, LCI), are considered to be better predictors for cardiovascular diseases (16).

MATERIALS AND METHODS

Twenty-one Wistar male rats, 4-6 months (450-550g), come from the "Cantacuzino" Institute, Bucharest, Romania and then accommodated within the CEMEX animal research "Grigore T. Popa University of Medicine and Pharmacy", Iasi, Romania was included in an experimental study. The animals were placed in individually ventilated cages, with a specially designed room that had a temperature of 20 ± 4 °C, standard humidity (50 ± 5%) with 12 hours of cycle light/dark. They also had unlimited access to food and drink.

The experimental investigation was conducted according to the European Directive 2010/63/EU, with approval from the "Grigore T. Popa" University of Medicine and Pharmacy Iasi Ethics Committee (no. 277) and authorization from the Romanian National Sanitary Veterinary and Food Safety Authority (no. 61).

The present study was performed on three groups of 7 Wistar male rats, aged4-6 months, weighing 150 - 200 g for group control and weighing 450 - 550 g for a group with obesity. After 2 weeks of ac-

climatization, all the rats received food and treatment according to the experimental protocols.

The animals from the I group (control) have standard food and drink water ad libitum. The second and the III groups were Hight-Fat-Diet (HFD) with a 2% cholesterol-containing diet for 20 days and experimental groups (group III) were separately treated with Atorvastatin 20 mg/kg/day, every day, for the next 20 days, with gavages and water ad libitum. A recent study reported that using a dose of 20 mg/kg/day for the next Twenty days has not described any toxicity in rats and this can be a safe dose (17). Before being administered orally, Atorvastatin (Sortis, Pfizer, no NM4027) was taken in distilled water and administered once a day by gavages, using a sterile disposable for every animal feeding needle (20G × 1.1/2, Popper and Sons). Water was given to the control group via the same method as the treated groups. Every day, the quantity of water and food received was measured. Also, and on the same day, every week, body weight was measured.

Before and after 4 weeks of oral administration of Atorvastatin levels of lipid parametric (Triglyceride, Total Cholesterol, HDl-cholesterol, LDl-cholesterol) and Glycemia were analyzed. The AIP, atherogenic coefficient (AI), Lipoprotein combined index (LCI), and Castelli risk indices I and II are evaluated with the formula (results are notated in tables I and II).

Also, all the subjects were anesthetized and investigated with EKG performed in 6 leads, and abdominal ultrasonography. After 4 weeks, for histological study, the aortas were removed. The major extracellular matrix components, including collagen and elastin, have been evaluated microscopically as indicators of aortic modification.

Endothelial dysfunction has been evaluated with biochemical markers, abdominal ultrasonography and histological study.

Experimental procedures. Physiological and Biochemical markers. On the 20th day after oral administration of treatment, euthanasia was conducted on all subjects, followed by an intracardiac puncture to obtain 3-4 mL of terminal blood samples, which were then collected in 3 mL special vacutainer tubes with clot activator for biochemical assays. Following a half-hour harvesting period, the tubes were centrifuged for 15minutes at 1500x g, a temperature of 4°C. We separated serum samples for the subjects for biochemistry analysis. Following the manufacturer's explicit instructions, all reagents for the determination of biochemistry were obtained from *BioSystems S.A. (Barcelona, Spain)* and utilized with the automatic analyzer system *ACCENT-200 (PZ Cormay, Poland).*

Paraclinical Evaluation: Abdominal ultrasonography and Histopathology. The ultrasound was performed with the Vevo 2100, Visual Sonics, with a linear probe (MS 250S, 13-24 MHz). Subjects were abdominal trimmed and maintained in a supine position under inhalation anesthesia. The abdominal aorta was assessed in the longitudinal section. In B-mode the aortic wall, the aortic caliber at the mid-abdominal level and in pulsed Doppler mode the aortic flow velocity was measured.

Histopathology. Each animal had a thorough postmortem examination, which

included a rigorous examination of the bodies. During this examination, the abdominal aorta was removed and preserved in 10% formalin and let for up to 48 h for fixation, in full accordance with the updated guides for rats and mouse organ sampling and trimming for a more extensive histological study. After that, two sections of the abdominal aorta were placed on a microscope slide and stained according to the usual staining methodology for hematoxylin and eosin (H&E).

Statistical analysis. Through a comparative study between every group, all measurable parameters were evaluated. The data analysis process was carried out using *SPSS version 23.0* for Windows (SPSS, USA). Two-sided testing was used to evaluate with a statistically significant value of $p < 0.05$.

RESULTS

At the finally of the experiment study, all the animals were examined carefully. During surgery, we observed abdominal fat accumulation in group II and group III, even retroperitoneal fat was not evaluated for the classification of obesity. However, at the macroscopic evaluation, we don't observe aortic atherosclerosis in the control or obesity groups. In figure 2 shows the changes in body weights in all groups. In the tables I, II and III will see the biochemical changes in lipid profile, before and after treatment/experiment.

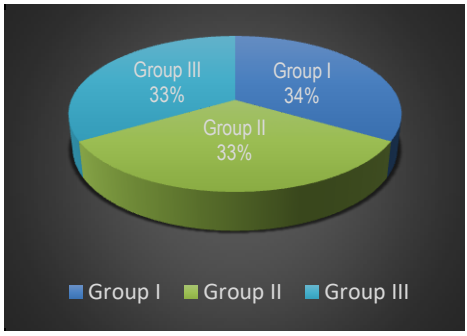


Fig. 1. Distribution of groups

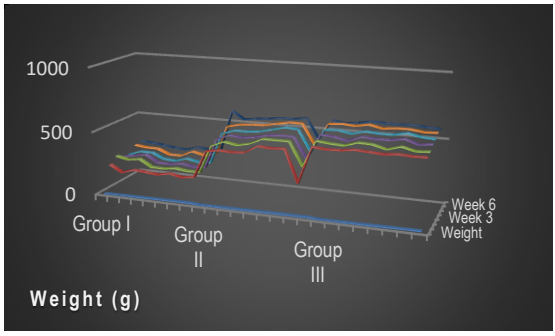


Fig. 2. Weight of the groups for six weeks

TABLE I.

Comparison of each study group before (initial) and after treatment(final) for lipidic profile and glycemia. Values as Mean ± Standard error.

Parameter / Study group	Glycemia		LDL Cholesterol		HDL Cholesterol		Total Cholesterol		Triglyceride	
	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)
Group I	84.14± 4.55	77.43± 4.51	38.43± 1.99	38.43± 0.74	52.14± 2.62	52.29± 0.68	85.71± 3.34	85.71± 3.34	96.71± 2.62	96.71± 2.62
Group II	91.29± 5.19	101.0± 2.57	51.71± 3.96	53.71± 2.42	53.29± 2.97	49.86± 2.89	178.43± 12.23	230.57± 18.19**	168.29± 9.2	182.86± 3.31
Group III	119.0± 19.34	131.29± 19.95	**49.71± 1.64**	**39.14± 2.82****	53.29± 2.15	56.14± 3.67	**270.0± 23.71**	**169.86± 3.36****	**209.57± 10.02**	**182.14± 12.61****

*OB- obesity, AT- Atorvastatin; I- Initial, Finally, **Statistically significant with $p < 0.05$;
***Statistically significant with $p < 0.001$ (paired t-test between before and after treatment values).

In the first group control, we see that there are no changes in biochemical values.

In group II- with obesity and HFD (cholesterol 2%), Total cholesterol levels increased, from an average of 178.43±12.23 mg/dL to 230.57±18.19 mg/dL, significantly ($p < 0.05$) and correlated with food received. LDL-cholesterol levels increased from 51.71±3.96 mg/dL to 53.71±2.42 mg/dL, but not statistically significant. Also, triglyceride levels increased from 168.29±9.2 to 182.86±3.31 but not statistically significant. Blood glucose levels increased, but not statistically significant during monitoring, from an average of 91.29±5.19 mg/dL to 101.0±2.57 mg/dL. In group III- with obesity and HFD (cholesterol 2%) and received Atorvastatin 20mg/kg/day, for the 20 days, it can be noted that the level of total cholesterol decreased from 270.0±23.71mg/dL to 169.86±3.36 and LDLc the same, from 49.71±1.64 mg/dL to 39.14±2.82 mg/dL (statistically significant with $p < 0.05$). The level of Glycemia and HDL_C increased but was not statistically significant. The experiment revealed that in group III, initial blood glucose values were above 119.0±19.34 mg/dL (the value considered physiological). We believe that these increased values were primarily due to stress caused by restraint, medication administration and explorations. The mean blood glucose value at the study's end was 131.29±19.95 mg/dL.

In all groups, we calculated the Atherogenic Index of Plasma- AIP with standard formula as TG/HDL_C and Atherogenic index – AI ($non\text{-}HLD_C/HDL_C$) (tab. II). In control groups, AIP increased but was not statistically modified. For group II, the obesity group with HFD (with 2% cholesterol) AI and AIP index levels increased, from an average of 2.60±0.23mg/dL to 3.42±0.46 mg/dL, also, 3.41 ± 0.18 to 3.49 ± 0.18, but not significantly and correlated with food received, age and weight and no treatment administrated. In group III**,** which received treatment with statin, we observed significantly lower levels of TC, LDLc, and TG (tab. I). Also, AIP and AI decreased statistically significantly with $p < 0.05$ in the final experiment, from levels 3.83 ± 0.29 to 2.48 ± 0.29*** and 4.04±0.73 to 2.21±0.12**** for the AI index (tab. II).

TABLE II.

Comparison of each study group before(initial) and after treatment for AIP and AI index. Values as Mean ± Standard error.

Parameter / Study group	(TG/HDL_C) ATHEROGENIC INDEX OF PLASMA (AIP)		$Non\text{-}HDL_C/HDL_C$ ATHEROGENIC INDEX (AI)	
	I (mean ± SE)	F (mean ± SE)	I (mean ± SE)	F (mean ± SE)
Group I	1.85 ± 0.04	1.85 ± 0.04	0.62±0.06	0.64±0.06
Group II	3.41 ± 0.18	3.49 ± 0.18	2.620±0.23	3.42±0.46
Group III	3.83 ± 0.29	**2.48 ± 0.29*** **	4.04±0.73	**2.21±0.12****

*OB- obesity, AT- Atorvastatin; I- Initial, Finally, **Statistically significant with $p < 0.05$;
***Statistically significant with $p < 0.001$ (paired t-test between before and after treatment values).

Table III was completed for all groups with lipid profiles and calculated Castelli risk index I and II and also, LCI. The values level of the lipid profile is evaluated with the standard logarithm of the molar ratio of the comprehensive lipid indexes, compared with single lipid parameters, such as non-HDL_C (TC minus HDL_C), TC/HDL_C-Castelli risk index I, LDL_C/HDL_C- Castelli risk index II and $TC*TG*LDL/HDL_C$ (Lipoprotein combine index, LCI).

In control groups, all the lipid index increased but was not statistically modified.

For group II, the obesity group with HFD (with 2%cholesterol) Castelli risk index I and II levels increased, not significantly and is correlated with food received, age, weight and without treatment administered. Therefore, Non-HDL_C novels were modified statistically significantly from 128.57±10.72 to 177.29±18.71. Also, the Lipoprotein Combine Index has the same, increased final levels of lipid profile from 32738.11±4776.05 to 43561.26±4996.11.

TABLE III.

Comparison for each study group before(initial) and after treatment(final) for CASTELLI RISK INDEX I and II, Lipoprotein combine index and Non-HDL_C. Values as Mean ± Standard error.

Parameter / Study group	TC/HDL_C **CASTELLI RISK INDEX I**		LDL_C/HDL_C **CASTELLI RISK INDEX II**		Non-HDL_C **(TC minus HDLc)**		TC * TG * LDL/HDL_C **LIPOPROTEIN COMBINE INDEX**	
	I	F	I	F	I	F	I	F
Group I	1.64± 0.06	1.64± 0.06	0.73± 0.03	0.74± 0.03	33.43± 3.23	33.57± 3.17	6097.69± 429.61	6111.18± 422.56
Group II	3.60± 0.23	4.42± 0.46	1.05± 0.09	1.04± 0.10	128.57± 10.72	**177.29± 18.71****	32738.11± 4776.05	**43561.26± 4996.11 ****
Group III	5.04± 0.73	**3.21± 0.12****	0.91± 0.07	**0.75± 0.08****	213.86± 25.80	**116.57± 2.69****	52008.64± 6960.58	**16262.94± 1445.30 ****

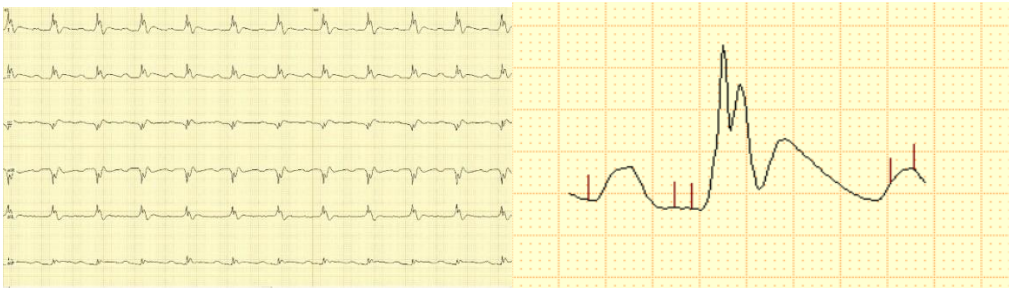
*OB- obesity, AT- Atorvastatin; **Statistically significant with p <0.05;
***Statistically significant with p < 0.001 (paired t-test between before and after treatment values).

In group III**,** which received treatment with statin, we observed significantly lower levels of TC, LDLc, and TG (tab. I). Also, AIP and lipoprotein combined index, TC/HDL_C and $TC * TG * LDL/HDL_C$ decreased statistically significantly with $p < 0.05$ in the final experiment.

As expected, the values of lipid profile - Castelli risk index I and II decreased statistically significantly from 5.04±0.73 to 3.21±0.12, 0.91±0.07 to 0.75±0.08 with statin treatment.

Finally, in the study, the recorded electrocardiograms did not reveal any pathological changes. EKG was performed on anesthetized subjects, induction with 5% isoflurane and maintenance with 2% isoflurane and 20 L/min oxygen (figs. 3, 4).

Abdominal ultrasonography performed during the present study revealed no structural changes, aortic wall thickness, atheroma plaque, or calcifications (fig. 5).



Figs. 3 and 4. Electrocardiogram performed in 6 leads, hex axial system; sinus rhythm, heart rate 400 bpm. P wave - 32 msec, 0.05 mV, PQ - 57 msec, R wave - 17 msec, 0.23 mV; QT - 88 msec, T - 0.19 mV. Calibration 200 mm/sec, 40 mm/mV.

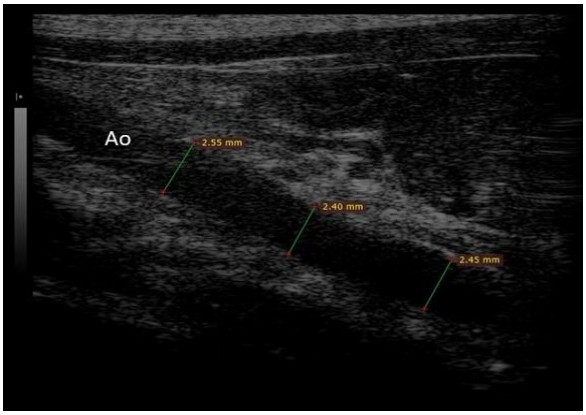


Fig. 5. Longitudinal abdominal aorta: example of aortic diameter measurement in systolic

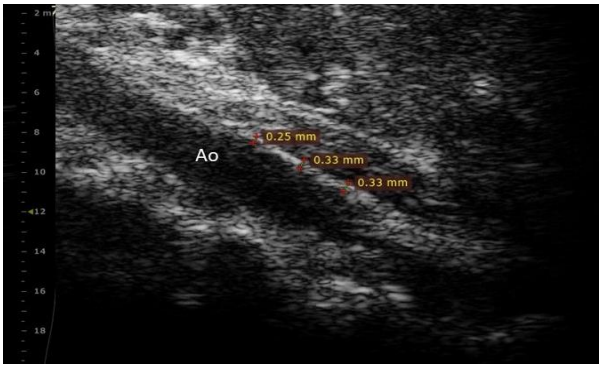


Fig. 6. Longitudinal abdominal aorta: example of aortic wall thickness measurement

Histological and structural characteristics

Slight aortic histomorphological modifications have been identified in the group with obesity *versus* the control group. All three groups have three distinct tunics: intima, media and adventitia. In all groups with obesity, the integrity of the endothelial layer was preserved but plaque for-

mation within the arteries was not seen in any group. In groups II and III, the histopathological aspect was modified for early endothelial injury (figs. 7, 8). In groups with obesity, the aorta wall with an asymmetrical thickness of tunica media with focal hypertrophy of tunica media, irregularly arranged elastic lamellae and slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis. Also, media thickening of the aortic wall, with an increase of collagen and a moderate amount of variably basophilic matrix extracellular matrix and many adipocytes in subjacent tunica adventitia in all groups (fig. 7).

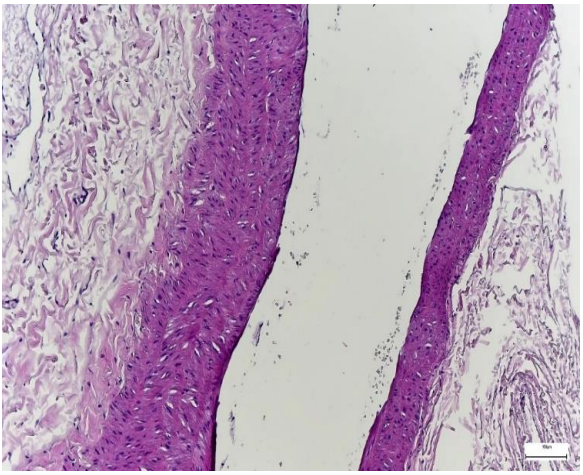


Fig. 7. Aorta wall with asymmetrical thickness of tunica media with focal hypertrophy of tunica media (on one side of the aorta in section), irregularly arranged elastic lamellae, slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis (group 2 x10 HE)

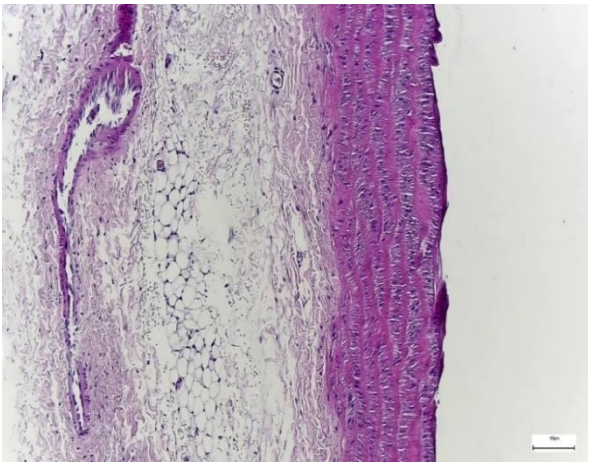


Fig. 8. Media thickening of the aortic wall, with an increase of collagen and a moderate amount of variably basophilic matrix extracellular matrix and many adipocytes in subjacent tunica adventitia (group 2 x10 HE)

DISCUSSION

Recently, AIP has been a strong marker for predicting the risk of cardiovascular diseases (18). According to previous studies, dyslipidemia is considered the most important factor for CVD. It is commonly known that standard lipid measurements, such as TC, LDL_C and TG, are associated with a significant risk for CVD events. Lipid ratios such as TC/HDL_C, LDL_C/HDL_C, LCI, AI and Castelli risk I and II are also considered to be a very important indicator of CVD. As a consequence, small-density LDL (sdLDL) is more easily oxidized to oxLDL and invades arterial deposits more easily than LDL because of the measure of particle size, it is very small. Macrophages become foam cells when oxLDL is phagocytized by macrophages, which influences arteriosclerosis and cardiovascular events.

Recent research has indicated that sdLDL is a significant predictor of arteriosclerosis and clinical application has been proposed. However, because it is a complex determination and has a high cost, the evaluation of sdLDL has limitations in clinical applications, AIP may be a good biochemical, economical and reliable indicator of coronary diseases and early stage of endothelial dysfunction.

Atherogenic Index of Plasma (AIP): In the present experiment, we observed that dyslipidemia in obesity groups, without treatment, increased the lipid profile, high AIP index, and lipoprotein combined index, then healthy controls but the summary clinical investigation is normal. In the present results, we discovered that in the obesity groups, the AIP value was more substantial than the values of controls (4.04±0.73 vs. 0.64±0.06). Also, the lipoprotein combine index decreased statistically significantly ($p < 0.05$) in group III (52008.64±6960.58 vs 16262.94±1445.30) and increased statistically significantly with ($p < 0.05$) in groups II- groups without treatment (32738.11±4776.05 *vs.* 43561.26±4996.11). To conform literature data and classification, we included Group II (with obesity) and Group III (obesity with statin treatment) in high risk for AIP. Furthermore, HDL_C contains an antioxidant enzyme and carries cholesterol from secondary tissues to the liver and is a component of the AIP. Decreased HDLc levels are associated with increased levels of pro inflammatory cytokines, TNF-alpha and interleukin-6 (19, 20) and can play an important role in the early stage of atherosclerosis. In our study level of HDL cholesterol was lower and can be improved with early treatment.

Date for literature reported the important effect of smoking, blood pressure, physical exercise and glycemia on AIP. Therefore, the authors reported a remarkable correlation between BMI, TC, and AIP in patients with cardiovascular diseases (21). In our experimental study, obesity and dyslipidemia can be the reason for high values of all parameters in the group with obesity.

In the present experimental study, will discover that all the lipid profiles were high values of the different atherogenic indices, Castelli risk and LCI. The ESC recommended, in primary prevention, to use of these lipid indexes and these parameters to evaluate and estimate the CVD risk (22).

Date from the literature confirmed several times the efficiency and benefits of treatment with a statin, in the experimental and clinical studies (23, 24, 25, 26,

27, 28, 29).

In an experimental study, animals with AIP height values and natural treatment have low-risk factors for atherosclerosis (30). Noor *et al.* show that in an experimental model of obesity rats, confirm that total cholesterol and LDL_C levels were positively correlated with the atherogenic index and treatment with methanolic extract in hyperlipidemic rats reduced the improved lipid profile and AIP (31). Lianto *et al* described that treatment with statin and high-dose Vitamin D improves levels of HDLc (32).

Previous epidemiologic studies on human subjects have shown contradictory results (5). For example, in a cross-sectional study, authors showed that the value of AIP was positively associated with waist circumference and body mass index and was inversely associated with physical activity (33). In the Turkish population-prospective study, AIP predicted CAD independently. Another study on human patients revealed that AIP was not an independent factor determining the impact of the risk of cardiovascular disease in postmenopausal women (34). In another prospective cohort study, authors evaluated the relationship between the AIP value and major adverse cardiovascular events during intensive hospitalization in patients with acute myocardial infarction (35).

Endothelial Dysfunction. Vascular endothelium has an important role in hemostasis and cardiovascular diseases. Obesity and dyslipidemia are the same, important factors for CVD because is accelerate atherosclerosis and increase cardiovascular risk. The current study used an experimental study to investigate the correlation that exists between AIP and endothelial dysfunction and compared the biochemical level of serum with histopathological aspects. In our research, we have found significantly high values of biochemical lipidic profile and AIP index, lipoprotein combines index compared with control groups. However, increased LDLc is another important marker of endothelial dysfunction since it can oxidize and generate oxidized Lipoprotein lipase cholesterol (LPL-cholesterol). This can increase the synthesis of caveolin-1, which inactivates eNOS and reduces NO production (36).

In our experimental study, non-efficient dyslipidemic profile control and high levels of LDLc can be considered the most important factors for endothelial dysfunction in obesity rats.

Lipid Profile. In practical terms, we know that dyslipidemia constitutes an important risk for CVD. Our results observed that group III who received treatment with Atorvastatin 20mg/day by gavage, profile lipid, AIP, atherogenic index, Castelli index and also lipoprotein combined index decreased significative statistically.

Abdominal and carotid ultrasonography in our results confirm that calcification and atherogenic plaque are too early to see. In daily practice, it's an investigation basically, but not relevant to endothelial dysfunction in the incipient stage.

The histopathological aspect of the abdominal aorta confirmed that dyslipidemia induced and determined incipient modified for endothelial injury, vascular endothelium is affected with asymmetrical thickness of tunica media, focal hypertrophy of tunica media, also, irregularly arranged elastic lamellae, slightly enlarged nuclei of smooth muscles, surrounded by adventitial fibrosis. Our study confirmed the associa-

tion between biochemical and histopathological aspects can be an early stage of atherosclerosis.

High AIP was the most common abnormality among the atherogenic indices (37) in practice. According to our results, AIP can be more easily applied in practice than other lipid ratios and traditional lipid indicators. It was also found that AIP can be a substantial predictor of the probability of cardiovascular disease. Prevention and early treatment for dyslipidemia can improve the lipid profile, Atherogenic Index of Plasma, lipoprotein combined index, Castelli index and endothelial function.

Our research concluded that the AIP, atherogenic coefficient (AI), Lipoprotein combined index (LCI) or Castelli's risk indices I and II can be powerful biochemical predictors of early endothelial dysfunction and cardiovascular diseases in the experimental group with obesity. Therefore, the AIP can be usually calculated in medical practice, using only a standard lipid profile, making it an easily, economical and accessible biomarker (38).

CONCLUSIONS

The prevention of cardiovascular disease remains the main objective of modern medicine and a controversial issue. Our study confirms that the AIP can be a better biochemical predictor for endothelial dysfunction and atherosclerosis, than individual cholesterol risk factors and also a good predictor of cardiovascular risk. As a result, we believe that the AIP can significantly improve the cardiovascular disease risk algorithm. Furthermore, a reduction in AIP values, which includes lowering LDLc levels, might be a crucial part of the therapy for dyslipidemia.

CONFLICTS OF INTEREST AND FUNDING

The authors declare there is no conflict of interest. This experimental study was supported by "Grigore T. Popa" University of Medicine and Pharmacy, by Andreea Clim as a Ph.D. researcher, with the supervision of Professor Ionela Lăcrămioara Şerban, M.D., Ph.D., and was effectuated in accord and supported by Advanced Research and Development Center in Experimental Medicine (CEMEX), "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania.

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All authors had equal contributions.

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WOMEN'S ANXIETY AND PERCEPTIONS OF PAP TESTS IN NORTH-EASTERN ROMANIA

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WOMEN'S ANXIETY AND PERCEPTIONS OF PAP TESTS IN NORTH-EASTERN ROMANIA (Abstract): Cervical cancer is still a substantial global public health issue among women. In the prevention and early diagnosis, cervical cancer screening plays a crucial role (1). However, acceptance of and participation in this screening are influenced by various factors. Woman's psychological status, including stress, anxiety, depression, or individual perception of health, can influence the decision to participate in cervical cancer screening. The current study **aimed** to investigate the psychological impact of Papanicolaou (Pap) testing in women in the North-eastern Romania. **Materials and methods:** This study was conducted using a questionnaire specifically developed for this study, which included structured questions aimed to assess various aspects of the psychological impact of cervical cancer screening. The statistical analysis for this study was performed using *IBM SPSS Statistics* version 26. **Results:** The study indicated a significant positive association between the perception that the Pap test was painful and anxiety related to unfavorable results and fertility concerns fertility. Younger women tend to have their Pap test more often than older women. Women with previous negative Pap test results tend to have lower levels of anxiety related to unfavorable results compared to women previously experiencing positive Pap test results. The results indicated that the regression model (i.e., age, perceptions about the Pap test - cost, pain, and embarrassment) did not significantly predict the anxiety related to unfavorable results. **Conclusions**: Our study highlights important psychosocial aspects related to cervical cancer screening and provides a foundation for improving health promotion services and risk awareness programs. **Keywords:** ANXIETY, DEPRESSION, PAP TEST, CERVICAL CANCER, CERVICAL SCREENING.

INTRODUCTION

Cervical cancer is a significant global public health problem, particularly affecting women. In the area of prevention and early diagnosis, cervical cancer screening plays a crucial role (1). In developed countries, routine screening schedules with Pap tests and/or HPV tests have led to a drastic decrease in the incidence and mortality of cervical cancer over the past five decades. In Romania, in 2020 alone, 3380 women were diagnosed with cervical cancer and 1805 died from this disease, while the 5-year prevalence was 9808 cases, according to Globocan data (2). Romania has the lowest rate of cervical cancer screening attendance

in the EU, influenced by specific social connotations. Thus, in 2019, less than 30% of Romanian women reported having had a Pap test in the last three years, which is half the European average (3). However, acceptance and participation in this screening are influenced by a variety of factors, which can be grouped into three main categories: awareness of the disease and the need for screening, structural barriers, and psychosocial beliefs about the disease and screening. Despite advances in the medical field, socio-psychological factors, particularly the psychological status of women, can significantly impact the effectiveness and participation in such screening programs. A woman's psychological status, including stress, anxiety, depression, or self-perceived health, can influence her decision to participate in cervical cancer screening. Concerns about pain, discomfort, or embarrassment associated with the procedure, or negative past experiences can discourage participation in the screening (4-7).

Discomfort with the attitude of healthcare workers, fear of being labeled, fear of being diagnosed with cancer and the implications of this diagnosis, fear of stigma, embarrassment, unacceptable touch, and negative experiences with the healthcare system can be significant psychological barriers to screening participation (8). Also, studies have highlighted that abnormal Pap smear findings evoke negative emotions ranging from anxiety to fear of developing or even having cancer (9-12).

The current study aimed at investigating the psychological impact of undergoing a Papanicolaou (Pap) test experienced by women in North-eastern Romania. This research was motivated by the need to understand how various factors influence women's experiences and perceptions related to Pap test, a critical screening tool for cervical cancer. Specifically, this study examined the interplay between age, perceptions of the Pap test (regarding cost, pain, and embarrassment), timing of the last test (within the last 2 years, between 2 and 5 years, more than 5 years ago), anxiety related to potentially unfavorable results, and fertility concerns.

OBJECTIVE AND HYPOTHESIS

The main objective of the present study was to comprehensively evaluate the psychological impact of Pap testing in women, focusing on the relationship between age, perceptions of the test (cost, pain, and embarrassment), timing of the last test, anxiety related to potentially unfavorable results, and concerns about fertility. Therefore, the current study was conducted to analyze the following hypotheses:

H1. There is a significant positive relationship between age, last test, result (positive, negative), perceptions of the test (cost, pain, and embarrassment), and anxiety related to potentially unfavorable results.

H2. There is a significant positive relationship between age, last test, result (positive, negative), perceptions of the test (cost, pain, and embarrassment), and concerns about fertility.

H3. Significant differences exist between age groups regarding the timing of the last Papanicolaou test.

H4. Women who received a positive result from a previous Pap test will experience a higher level of anxiety related to potentially unfavorable results compared to those receiving a negative result.

H5. There are significant differences in fertility-related concerns between women who received a negative result and women who received a positive result from a previous Pap test.

H6. Age and perceptions of the Pap test

(cost, pain, and embarrassment) significantly predict anxiety related to potentially unfavorable results.

H7. Age and perceptions of the Pap test (cost, pain, and embarrassment) significantly predict concerns about fertility.

MATERIALS AND METHODS

Participants and procedure

The study was conducted using a questionnaire specifically developed for this study, which included structured questions to assess various aspects of the psychological impact of cervical cancer screening. The target population were women (N = 308) aged 18-70 years old, 68.8% aged 25-45 and 27.3% aged 45-65 years old, who either participated (90.3%) or did not participate (9.7%) in cervical cancer screening programs, the selection being random. Moreover, 88.6% of participants who had previously undergone a Pap test received a negative result, while 4.9% received a positive result.

Recruitment into the study involved inviting eligible women to participate through online invitations, providing them with information about the purpose of the study, and obtaining their consent to participate. Data were collected using the Google Forms platform.

Measures

To measure the research variables, specific questions tailored to the topics of interest for the current research were used.

Anxiety related to unfavorable result

To measure anxiety related to potentially unfavorable results, we asked the participants to rate on a 4-point Likert scale, from 1 (not at all) to 4 (very much), how anxious they felt about the possibility of an unfavorable result.

Fertility concerns

To measure concerns about fertility, we used the same approach as we did to measure anxiety related to unfavorable results.

Demographic data

We asked participants to indicate their age group (18-25, 25-45, 45-65, and over 65), area of residence (rural or urban), educational level, and relationship status.

Statistical Analysis

For this study, the statistical analysis was conducted using *IBM SPSS Statistics* version 26. Our primary goal was to explore the psychological impact of undergoing a Papanicolaou (Pap) test on women, considering various influencing factors. The analyses were focused on the following variables: age, perceptions of the Pap test (regarding cost, pain, and embarrassment), timing of the last test (within the last 2 years, between 2 and 5 years, more than 5 years ago), anxiety related to potentially unfavorable results, and concerns about fertility.

First, descriptive statistics were used to summarize the demographic characteristics of the sample and the distribution of responses for each variable. Moreover, Spearman's correlation coefficients were computed to examine the relationship between age and perceptions of the Pap test (cost, pain, and embarrassment). To compare the psychological impact across different groups, we used One-Way ANOVA and Independent Samples t-Tests to analyze the differences between age groups and last Pap test (within the last 2 years, between 2 and 5 years, more than 5 years ago), and to compare differences in anxiety level and fertility concerns based on the Pap test result (in those who underwent a Pap test before). However, multiple regression analyses were performed to assess the predictive power of age, perceptions of the Pap test (regarding cost, pain, and embarrassment), and timing of the last test (within the last 2 years, between 2 and 5 years, more than 5 years ago)

on anxiety related to unfavorable results and fertility concerns.

RESULTS

The Spearman correlation analysis indicated a significant positive association between the perception that the Pap test is painful and anxiety related to unfavorable results (r = .196, p = .004). Thus, the higher the perception that the Pap test as being painful, the higher the anxiety related to unfavorable results. Moreover, the results indicated a significant positive relationship between the perception that the Pap test is being painful and fertility concerns (r = .136, p = .038). Thus, the higher the perception that the Pap test is painful, the higher the concern about fertility. Therefore, women who experience Pap smear pain had fertility concerns (tab. I).

TABLE I.

Spearman correlation analysis

	1	2	3	4	5	6	7	8
1. Age	-							
2. Last test	.239**	-						
3. Result	-.116*	-.036	-					
4. Cost	-.166**	-.057	-.018	-				
5. Pain	-.016	.117	-.012	.231**	-			
6. Embarrassment	-.014	.063	.033	.154*	.409**	-		
7. Anxiety	-.020	-.045	.117	.120	.196**	.068	-	
8. Fertility concerns	-.039	-.049	.104	.012	.136*	.054	.507**	-

Note *p< .05; **p<.01

One-Way ANOVA compared the means and revealed significant differences between age groups and last Pap test (within the last 2 years, between 2 and 5 years, more than 5 years ago) (F (3; 282) = 6.63, p = .000). Specifically, significant differences were found between the 25-45 age group (M = 1.34, SD = .58) and 45-65 age group (M = 1.69, SD = .77) regarding the last Pap test (within the last 2 years, between 2 and 5 years, more than 5 years ago). Participants in the 45-64 age group tend to have had their last Pap test longer ago compared to those in the 25-45 age group. Thus, younger women tend to have their Pap test more often than older women (tab. II).

TABLE II.

One-way ANOVA analysis

Last Pap test	Sum of Squares	df	Mean Square	F	p
Between Groups	8.19	3	2.73	6.63	.000
Within Groups	116.04	282	.412		
Total	124.23	285			

The Independent t-Test results indicated significant differences in anxiety related to unfavorable result (t (251) = -2.25, p = .025) between the participants who had previously

undergone a Pap test and received a negative result (M = 1.93, SD = .80) and those who received a positive result (M = 2.45, SD = 1.05), with higher levels of anxiety in those who received a positive result. Thus, women who previously received negative results from the Pap test tend to have lower levels of anxiety related to unfavorable results compared to women who previously received positive results from the Pap test (tab. III).

TABLE III.

Independent t-test for anxiety

Anxiety	**N**	**M**	**SD**	**t-test**	**p**
Negative result	240	1.93	.80	-2.25	.025
Positive result	13	2.46	1.05		

TABLE IV.

Independent t-test for fertility concerns

Fertility concerns	**N**	**M**	**SD**	**t-test**	**p**
Negative result	240	2.63	1.26	-1.57	.117
Positive result	15	3.20	1.26		

Regression results indicated that the regression model (i.e. age, perceptions of the Pap test - cost, pain, and embarrassment) did not significantly predict anxiety related to unfavorable results ($F (4, 205) = 1.55$, $p > .05$). Moreover, no independent variable was a significant predictor.

Also, regression results indicated that the regression model (i.e., age, perceptions of the Pap test - cost, pain, and embarrassment) did not significantly predict the fertility concerns ($F (4, 212) = .97$, $p > .05$). Moreover, no independent variable was a significant predictor.

Thus, the regression model, which relates age and perceptions of the Pap test (cost, pain, embarrassment) does not significantly predict anxiety related to an unfavorable result.

Finally, regression results indicated that the regression model (i.e., age, perceptions of the Pap test - cost, pain, and embarrassment) did not significantly predict fertility concerns ($F (4, 212) = .97$, $p > .05$). Moreover, no independent variable was a significant predictor. Thus, the regression model, which relates age and perceptions of the Pap test (cost, pain, embarrassment), does not significantly predict fertility concerns (tab. V).

TABLE V.

Regression - anxiety related to unfavorable results and concerns about fertility.

	Anxiety			**Fertility concerns**		
Variables	*B*	*SE (B)*	*β*	*B*	*SE (B)*	*β*
Age	.120	.125	.066	-.180	.189	-.066
Cost	.035	.069	.036	-.062	.106	-.041
Pain	.176	.090	.150	.226	.139	.124
Embarrassment	.000	.083	.000	-.015	.128	-.009
R^2	.029			.018		

Note: *p < .05; **p < .001

Hypothesis testing - brief results

H1. There is a significant positive relationship between age, last test, result (positive, negative), perceptions of the test (cost, pain, and embarrassment), and anxiety related to potentially unfavorable results.

Results indicated a significant positive association between the perception that the Pap test is painful and anxiety related to unfavorable results ($r = .196$, $p = .004$).

H2. There is a significant positive relationship between age, last test, result (positive, negative), perceptions of the test (cost, pain, and embarrassment), and fertility concerns.

Results indicated a significant positive relationship between the perception that the Pap test is painful and fertility concerns ($r = .136$, $p = .038$).

H3. Significant differences exist between age groups regarding the timing of the last Papanicolaou test.

There are significant differences between age groups and last Pap test (within the last 2 years, between 2 and 5 years, over 5 years) ($F (3; 282) = 6.63$, $p = .000$).

H4. Women who received a positive result from a previous Pap test will have higher anxiety related to potentially unfavorable results compared to those who received a negative result.

Results indicated significant differences ($t (251) = -2.25$, $p = .025$) between participants who had previously undergone a Pap test and received a negative result ($M = 1.93$, $SD = .80$) and participants who received a positive result ($M = 2.45$, $SD = 1.05$) regarding anxiety related to unfavorable result, with the participants who received a positive result experiencing higher anxiety compared to those who received a negative result.

H5. There are significant differences in fertility concerns between women who received a negative result and women who received a positive result from a previous Pap test.

Results indicated that there are no significant differences in fertility concerns ($t (283) = -1.57$, $p = .11$).

H6. Age and perceptions of the Pap test (cost, pain, and embarrassment) will significantly predict anxiety related to potentially unfavorable results.

Results indicated that the regression model (i.e., age, perceptions of the Pap test - cost, pain, and embarrassment) did not significantly predict anxiety related to unfavorable results ($F (4, 205) = 1.55$, $p > .05$).

H7. Age and perceptions of the Pap test (cost, pain, and embarrassment) will significantly predict fertility concerns.

Results indicated that the regression model (i.e., age, perceptions of the Pap test - cost, pain, and embarrassment) did not significantly predict fertility concerns ($F (4, 212) = .97$, $p > .05$).

DISCUSSION

This research was conducted to understand how various factors impact women's experiences and perceptions regarding Pap testing, a crucial screening tool for cervical cancer. We analyzed a sample of 307 women, exploring their distribution according to various factors. Most participants (68.95%) were in the 25-45 years age group, corresponding to the socially active population and the most likely to develop this condition. The results of previous studies show that psychological response to Pap test is a significant barrier to screening for women of all ages (11, 13), including those who had never undergone screening (14, 15). Results showed that older women more often face emotional barriers than practical barriers, while younger women more often advocate practical barriers (16). Our study

supports this hypothesis: participants in the 45-64 years age group tend to have had their last Pap test after a longer time interval compared to those in the 25-45 age group. Thus, younger women tend to have their Pap test more often than older women. Similar studies consider the same results to be influenced by public health campaigns and guideline recommendations (17). Regarding the lower rate of Pap tests in the older women group, several studies have identified a trend where older women tend to have Pap tests less frequently than younger women (18, 19).

We found significant variations in the time interval since last test. A significant percentage of the participants performed the regular test over the last 2 years, indicating continued attention to reproductive health. This group can benefit from early detection and regular health monitoring. At the same time, 24.6% of these women participated in the screening 2-5 years ago, and this may indicate a possible concern about the frequency of the tests or reflect individual circumstances such as limited access to medical services. It is also important to pay attention to the 9.5% of the participants who had the test more than 5 years ago. This group may be at increased risk, and this finding may serve as a basis for developing specific awareness and intervention strategies to stimulate participation in regular screening. A personalized and educational approach can be crucial to improving participation in screening and reducing the risk of reproductive health complications among participants.

Regarding the association between the perception that the Pap test is painful, and anxiety is related to unfavorable results, the higher the perception that the Pap test is painful, the higher the anxiety related to unfavorable results. Thus, the higher the perception that the Pap test is painful, the higher the fertility concern. Therefore, women who felt pain when they did the Pap test had fertility concerns. An explanation for this result can be that when women experience pain during the Pap test, their anxiety about potential abnormal findings (e.g. cervical cancer) increases, leading to fears about subsequent treatments that could affect their reproductive health.

Therefore, women who previously had negative Pap test results tend to have lower levels of anxiety related to unfavorable results compared to women who previously had positive Pap test results. An explanation can be that women with previous negative results may perceive themselves at a lower risk for cervical abnormalities, and this fact may contribute to a low level of anxiety while those who have previously received positive results may consider themselves likely to have unfavorable outcomes. This heightened anxiety among women with previous positive results could be due to the psychological impact of past experiences, highlighting the need for psychological support and counseling in such cases.

Our findings are consistent with previous studies showing that a positive HPV result in combination with abnormal cytology is associated with increased anxiety and stress (20-23). As reported in literature, women's experiences during a Pap test are diverse. Some women experience embarrassment, anxiety, intimidation, and fear. In particular, fear is related to the belief that knowledge of a negative diagnosis can accelerate the course of cancer (4-7). Frederiksen *et al.* have published a very detailed review (24), which summarized data from 16 studies examining psychological

out-comes in women with histologically diagnosed or treated cervical intraepithelial neoplasia or cervical cancer. One of the key findings was that the psychological outcome of women with cervical intraepithelial neoplasia was similar to those of women with abnormal cytology but not necessarily needing treatment. They interpreted this finding as a consequence of women's uncertainty about their disease status. Frederiksen *et al.* also saw that women considered a cervical intraepithelial neoplasia diagnosis to be as bad as that of cervical cancer (25).

Another study from 2020 examining short-term anxiety and distress in women receiving different results following routine human papillomavirus (HPV) primary testing at cervical screening found evidence that clinically significant levels of anxiety may be more common in women who do not understand their result or received a positive one (26, 27).

When comparing participants who had a previous Pap test and received a negative result, there were no significant differences in fertility concerns. This finding implies that factors other than test results, possibly including personal beliefs and broader reproductive health education, might influence fertility concerns.

Other variables such as age, cost, pain, and embarrassment were not individually associated with increased anxiety from a negative result or fear of infertility. This indicates that none of these variables significantly contribute to anxiety or fertility concerns when considered separately. Models including age and perceptions of the Pap test (cost, pain, embarrassment) did not significantly predict anxiety related to unfavorable results or fertility concerns. Thus, the model, which includes age and perceptions of the Pap test (cost, pain, embarrassment), does not significantly predict anxiety related to unfavorable results.

Finally, the results indicated that the model age and perceptions of the Pap test (cost, pain, and embarrassment) did not significantly predict fertility concerns. This suggests that other variables or factors not considered might play a more significant role in these aspects. All these variables can collectively generate anxiety in the context of inadequate screening or absence of appropriate informative programs. The lack of accurate information and education regarding the Pap test can amplify fears related to cost, pain, embarrassment, and negative results. In this respect, informative programs that clearly explain to the population the role of this screening, how results are interpreted, and the available treatment options are essential.

Education and awareness can reduce anxiety and fertility concerns. Clear and accessible information can help demystify the Pap test and reduce the associated shame and fears. This can lead to better acceptance and participation in the screening, thus reducing overall anxiety related to the process and its results. Studies have shown that women who had never undergone the screening are more likely to report shame and anxiety as barriers, while those who underwent the screening are more likely to quote lack of time as a barrier. Worry is a key barrier to participation in the screening among women who had largely not undergone prior screening (14). The lack of adequate knowledge is recognized as an important barrier and a significant impediment (28, 29). Lack of knowledge of the disease process or the benefits of screening not only prevents women from initiating the decision-making

process related to participation in the screening (27) but could also generate fear of the disease or screening procedure

CONCLUSIONS

In conclusion, our study highlights important psychosocial aspects of cervical cancer screening and provides a foundation for improving health promotion services and risk awareness programs. Anxiety has been shown to be a decisive factor associated with the fear of a negative result or potential fertility issues. Therefore, the non-participation in the screening is not driven by pain, embarrassment, or costs but is fundamentally rooted in the fear of a possible unfavorable result. Although individual variables such as age, cost, pain, and embarrassment do not significantly predict anxiety or fertility concerns and are undoubtly associated with a positive test result, adequate screening and well-structured informative programs are essential to reduce anxiety and improve Pap test acceptability.

The practical implications of this research could enhance the effectiveness of screening and reduce the negative psychosocial impact on patients. Psychosocial barriers to screening may most significantly interfere with initial and timely screenings; specifically, directly addressing these barriers can encourage women to undergo screening for the first time or in a timely manner, leading to early diagnosis of this condition before it progresses to cancer.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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DIAGNOSIS AND MANAGEMENT CHALLENGES OF PLACENTA ACCRETA SPECTRUM DISORDERS

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DIAGNOSIS AND MANAGEMENT CHALLENGES OF PLACENTA ACCRETA SPECTRUM DISORDERS (Abstract): Placenta accreta spectrum (PAS) disorders are associated with significant maternal and neonatal morbidity and mortality. The primary aim of this study was to determine the diagnostic accuracy of individual and combined ultrasound markers for predicting PAS disorders and to report the intraoperative complication rates for this category of patients. The secondary aim of this study was to reflect the clinical challenges of a case diagnosed with placenta accreta. **Materials and methods**: We conducted a retrospective analysis of clinical and ultrasound data from 52 pregnant patients with or without confirmed PAS disorders who gave birth in a tertiary center. Univariate analysis was used to compare differences in baseline characteristics between groups. We calculated the diagnostic accuracy of ultrasound signs for PAS disorders and the complication rates in these patients. **Results**: Placental lacunar spaces had a sensitivity of 83.87%, a specificity of 76.19%, and an accuracy of 80.77% for predicting PAS disorder. The best predictive performance for predicting PAS disorders was achieved when using at least three ultrasound markers, with a sensitivity of 90.32%, a specificity of 85.71%, and an accuracy of 88.46%. Urinary tract injuries were the most frequently encountered complications in patients with confirmed PAS. **Conclusions:** Better antenatal diagnosis of this pathology will reduce the surgical risk and improve the overall outcome for both mother and fetus. **Keywords:** PLACENTA ACCRETA, CAESAREAN SCAR, PREGNANCY, ULTRASOUND, DOPPLER.

INTRODUCTION

The term "placenta accreta" refers to abnormal placental adherence, as well as a pathology that appears on echography and resembles an abnormal placental spectrum (1). Placenta percreta, a rarer pathology, is

linked to high mortality and blood loss due to potential hematuria during pregnancy, early labor onset, and significant neonatal comorbidities (2). The incidence of abnormal placental adherence varies from 1/110 to 1/2500 of deliveries (3). With increasing rates of elective cesarean section, the risk of this pathology has increased more than fivefold (4). Studies show that by evaluating these placental anomalies with the help of the ultrasound, we can detect this pathology at around 28 weeks of gestation, giving us enough time to prepare strategically for the surgery and also to emotionally prepare the patient (5, 6).

MRI is considered mandatory after 32 weeks of gestation for the better visualization of placental abnormalities and reduction of fetal developmental exposure (7).

A full bladder is usually necessary for better visualization of the lower anterior uterine wall. The ultrasound markers such as the placental position, placental thickness, the presence/absence of the retroplacental space, the thickness of the retroplacental myometrium, the bladder line interruption, the presence/absence of the placental lacunae, the retroplacental myometrial blood flow, the presence/absence of a cervical sinus, and the cervical morphology are recorded (8).

Placental thickness and retroplacental myometrial thickness are measured by placing the ultrasound probe perpendicular to the uterine wall. The abnormal vessels have a Doppler blood flow velocity exceeding approximately 20 cm/s (9).

The ultrasound markers for PAS disorders include: loss of the clear zone, which could be described as the absence of the normal hypoechoic area between the placenta and the uterine wall; placental lacunae, which are described as irregular vascular spaces within the placenta; bladder wall interruption, which is described as a disruption of the bright echogenic line representing the bladder wall; uterovesical hypervascularity and increased vascularity in the parametrial region visible on color Doppler ultrasound (10). Accurate identification of these markers helps in the prenatal diagnosis of PAS and aids in planning the management to minimize risks during delivery (11, 12).

Choosing a stress-free time for delivery and planning cesarean section at approximately 35 weeks +/- 2 weeks are also important factors for both the maternal and fetal survival (13, 14).

The antenatal course of corticosteroid injections is helpful in promoting fetal lung development (15).

A multidisciplinary team is recommended for pre-operative planning, preordering of blood, and the availability of a urology team if necessary (16). If there is a high suspicion of a percreta, an ureteral catheter is recommended in case of further bladder dissection during surgery (17).

Associated coagulopathies and periportal hemorrhage (PPH) are often responsible for the high morbidity and mortality of “Placenta Accreta Spectrum” (PAS). The anesthesia team requires prepared blood transfusions and correction factor products in order to manage hypovolemia and coagulopathy (18, 19).

The aim of this study was to determine the diagnostic accuracy of individual and combined ultrasound markers for the prediction of PAS disorders and to report the intraoperative complication rates in this group of patients. The secondary aim of this study was to reflect the clinical challenges of a case diagnosed with placenta accreta.

MATERIALS AND METHODS

We conducted a retrospective analysis of the medical records of pregnant patients, with or without confirmed PAS disorders, who delivered at "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology of Iasi, Romania, between January 2019, and December 2023. Exclusion criteria included the following: incomplete medical records, non-viable pregnancies, multiple gestations, unavailable ultrasound data, and concurrent major health conditions that could affect pregnancy outcomes, unrelated to PAS.

Approval to access the hospital database was granted by the Institutional Ethics Committee of "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology (No. 2461/25 February 2022). Data from a total of 52 patients were included in the analysis considering the following groups: PAS positive (n= 31 patients) and PAS negative (n= 21 patients).

The type of data retrieved from the medical records included the intraoperative confirmation of PAS disorders, ultrasound evidence of PAS disorders and intraoperative or postoperative complications. We also collected data on the demographic characteristics of the study cohort, along with data regarding specific clinical risk factors (number of previous cesarean deliveries, age, BMI, smoking status, parity, and interpregnancy interval).

The chi-squared test was used to assess between-group differences in categorical variables, and the *Student t-test* was used to assess between-group differences in continuous variables. A p-value less than 0.05 was considered statistically significant.

Using this type of data, we calculated the diagnostic accuracy of ultrasound signs for PAS disorders, reporting sensitivities (Se), specificities (Sp), positive and negative likelihood ratios (LRs), and complication rates in patients with confirmed PAS disorders. Statistical analyses were performed using STATA SE (version 18.5, *Stata Corp LLC*. College Station, TX, United States of America).

RESULTS

First table shows the comparisons between the groups with respect to their demographic and clinical characteristics. Our groups were relatively homogenous regarding the following parameters: living environment (p= 0.69), mean age at diagnosis (p= 0.48), obesity rate (p= 0.45), and smoking status before and/or during pregnancy (p= 0.67).

On the other hand, our data indicated that patients with confirmed PAS disorders had significantly higher parity (2.22 ± 1.49 *vs.* 1.57 ± 0.87, p= 0.03) compared to patients with unconfirmed PAS diagnosis.

Moreover, the first group had a significantly higher number of previous cesarean deliveries (2.03 ± 0.70 *vs.* 1.57 ± 0.59, p= 0.01), and a significantly shorter interpregnancy interval compared to the second group (0.94 ± 1.44 *vs.* 1.82 ± 1.55, p= 0.02) (tab. I).

The diagnostic accuracy of ultrasound signs for the detection of PAS disorders is presented in Table II. Our results showed that placental lacunar spaces had a sensitivity (Se) of 83.87%, a specificity of 76.19% and an accuracy of 80.77% for predicting PAS disorder.

High sensitivities were also achieved by myometrial thinning of less than 1 mm (80.65%) and loss of retroplacental clear zone (80.65%), which achieved accuracies of 76.92% and 71.15%, respectively.

On the other hand, the highest specificities (Sp) were achieved by bladder wall

interruption (90.48%), placental bulging (85.71%) and hypervascularity of the uterovesical or retroplacental space (85.71%), which were reflected in high positive likelihood ratios of 6.774, 4.064 and 3.839, respectively.

TABLE I.

Comparisons of baseline characteristics between groups.

Patient's Characteristics	Pas Positive (n = 26)	Pas Negative (n = 13)	*p* Value
Living environment (n/%)	Rural= 15 (48.39%) Urban= 16 (51.61%)	Rural= 9 (42.86%) Urban= 16 (57.14%)	0.69
Age (years, mean ± SD)	30.48 ± 7.21	30.57 ± 5.06	0.48
Obesity (n/%)	12 (38.71%)	6 (28.57%)	0.45
Smoking status (n/%)	9 (29.03%)	5 (23.81%)	0.67
Parity (mean ± SD)	2.22 ± 1.49	1.57 ± 0.87	0.03
Number of previous CS (mean ± SD)	2.03 ± 0.70	1.57 ± 0.59	0.01
Interpregnancy interval (years, mean ± SD)	0.94 ± 1.44	1.82 ± 1.55	0.02

PAS - placenta accreta spectrum, SD- standard deviation, CS- cesarean section.

TABLE II.

The predictive performance of individual and combined ultrasound markers for predicting PAS disorders.

Ultrasound Signs	Se	Sp	Accuracy	+LR	-LR
Placental lacunar spaces	83.87%	76.19%	80.77%	3.5226	0.2117
Loss of retroplacental clear zone	80.65%	57.14%	71.15%	1.8817	0.3387
Bladder wall interruption	64.52%	90.48%	75%	6.774	0.3922
Myometrial thinning < 1 mm	80.65%	71.43%	76.92%	2.8226	0.2709
Placental bulging	58.06%	85.71%	69.23%	4.0645	0.4892
Bridging vessels	77.42%	66.67%	73.08%	2.3226	0.3387
Hypervascularity of the uterovesical or retroplacental space	54.84%	85.71%	67.31%	3.839	0.5269
≥3 US signs present	90.32%	85.71%	88.46%	6.323	0.1129

Legend: Se- sensitivity, Sp- specificity, +LR- positive likelihood ratio, - LR- negative likelihood ratio, US- ultrasound.

The best predictive performance for predicting PAS disorders was achieved when using at least three ultrasound markers. In this scenario, the Se was 90.32%, the Sp was 85.71%, and the accuracy was 88.46%, with a positive likelihood ratio of 6.323 and a

negative likelihood ratio of 0.112 (tab. II).

Table III shows the documented complication rates for patients with a confirmed diagnosis of PAS disorders. Our data indicated that the majority of these patients (74.19%) underwent total hysterectomy, while the subtotal hysterectomy was performed in only 22.58% of these patients.

Urological complications such as urinary bladder injury (48.38%) and ureteral injury (25.80%) were common and required multidisciplinary collaboration with urologists. In addition, significant intraoperative blood loss was documented in 32.25% of these patients, who benefited from blood transfusions (tab. III).

TABLE III.

The complication rates in patients with PAS disorders.

Complications	Rates	
	Yes (n, %)	No (n, %)
Total hysterectomy	23 (74.19%)	8 (25.80%)
Subtotal hysterectomy	7 (22.58%)	24 (77.41%)
Intraoperative urinary bladder injury	15 (48.38%)	16 (46.2%)
Intraoperative ureteral injury	8 (25.80%)	23 (73.1%)
Necessity of blood transfusion	10 (32.25%)	21 (67.74%)

Legend: number of transfusions.

DISCUSSION

Placenta accreta spectrum (PAS) refers to the abnormal adhesion or invasion of trophoblastic tissue into the myometrium. This pathology causes significant maternal morbidity such as uterine rupture, severe postpartum hemorrhage, multiple organ failure, and preterm delivery. Ultrasound is the preferred screening tool for PAS and can reduce obstetric morbidity in women at risk (20).

Our results showed that placental lacunar spaces, myometrial thinning of less than 1 mm and bladder wall interruptions achieved the highest accuracy in predicting PAS disorders. Moreover, the best predictive performance for predicting PAS disorders was achieved when using at least three ultrasound markers. These results are consistent with previously published data.

A Delphi survey and consensus published in 2023 found that only 7 ultrasound markers are relevant for PAS screening in mid-gestation in patients with a previous history of at least one cesarean delivery, myomectomy or PAS: loss of the ‘clear zone’, myometrial thinning, bladder-wall interruption, placental bulge, uterovesical hypervascularity, placental lacunae and bridging vessels (21).

A review of more than 20 studies and 3,000 pregnancies revealed that ultrasound can be considered an overall good diagnostic tool for identifying the depth of the placental invasion. The sensitivity of the uterovesical hypervascularity was low for the detection of placenta accreta. but was important for the identification of placenta increta or percreta, thus making it an important marker for the prediction of this subtype (22, 23).

In a prospective study, there were evaluated women with placenta previa and a history of at least one prior cesarean section or myomectomy. The analyzed ultra-

sound markers included the loss of the clear zone, placental lacunae, bladder wall interruption, uterovesical hypervascularity, and increased parametrial vascularity (24). When at least one sign was used, the ultrasound showed very good sensitivity. The use of three ultrasound signs has improved the detection rate of placenta percreta, highlighting the importance of considering as many markers as possible to improve surgical preparation (24, 25).

Another study analyzed the outcomes for women with placenta accreta when diagnosed antenatally or intrapartum. Women who were suspected of having PAS antenatally had a lower estimated blood loss, with a median of 4 l, and required fewer units of packed red blood cells transfusion. Coagulation testing and its results are critical not only for high-risk patients, but also for those at extreme ages, such as teenage pregnant women, who are preparing for either a vaginal delivery or a caesarean section (26).

A recent prospective cohort study conducted by Sargent *et al*., tested the predictive performance of a model based on ultrasound markers for predicting cases that will develop abnormally adherent or invasive placentation (27). The included ultrasound parameters were represented by loss of clear zone, abnormal placental lacunae, placental bulge and bladder wall interruption, and had an accuracy of 0.901.

A retrospective cohort study conducted in seven centers evaluated the performance of classic ultrasound signs for the detection of PAS using data from a total of 385 cases (28). The authors considered the classical ultrasound markers of PAS as placental lacunae, bladder-wall interruption, myometrial thinning and sub placental hypervascularity, and the following novel signs: small placental lacunae, irregular placenta-myometrium interface (PMI), vascular PMI, non-tapered placental edge and placental bulge toward the bladder.

The authors demonstrated that the model based on classical ultrasound markers achieved an AUROC (areas under the receiver operating curve) value of 0.81, while the model based on novel ultrasound markers achieved an AUROC value of 0.84 (28). Moreover, a model combining both types of ultrasound markers had an AUROC value of 0.88, suggesting that the use of multiple ultrasound markers could better predict the occurrence of PAS disorders. Thus, the imaging techniques are effective in antenatal risk stratification (29-32).

It is well known that PAS disorders are associated with complex urological complications, often requiring surgical repair (33, 34, 35). In our study, urological complications such as urinary bladder injury and ureteral injury were common and required multidisciplinary collaboration with urologists. A recent systematic review published in 2023 evaluated the rates of urological complications in patients diagnosed with PAS disorders using data collected from 6 studies (36). The authors showed that the overall rate of urological complications was 15.2%. Urological complications were encountered in 19.4% of cases undergoing hysterectomy and 12.2% of those undergoing conservative treatment.

Moreover, these types of complications were significantly more common in patients with placenta percreta (38.5%) compared to patients with placenta accreta/increta (9.4%) (36). They were also more common in cases that underwent an emer-

gency intervention (24.6%) compared to cases that underwent a planned procedure (15.4%) (36). These findings highlight the importance of prenatal diagnosis, planned surgical management and appropriate resource allocation, especially for high-risk patients (37-41).

Another retrospective study assessed the incidence of lower urinary tract injury during hysterectomy in 156 cases diagnosed with PAS disorders (42). The authors demonstrated that in the evaluated cohort 32.7% of cases were classified as PAS 1, 20.5% as PAS 2, and 46.8% as PAS 3. Surgical complications were significantly more common in cases diagnosed with PAS 3 (17.8%) compared to cases diagnosed with PAS 1 (0%) or 2 (12.5%). The incidence of lower urinary tract injury was 7.6% in the evaluated cohort and was significantly higher in the PAS 3 group.

In this study, significant intraoperative blood loss was documented in 32.25% of these patients, who benefited from blood transfusions. Shamshirsaz *et al.* conducted a retrospective study evaluating coagulopathy in 123 patients with PAS disorders (43). In the evaluated cohort, 30.1% developed coagulopathy and had an estimated blood loss was 2100cc. The overall number of units of red blood cells transfused was significantly higher in the coagulopathy group.

A recent review that included data from 10 studies evaluated the association of conservative management of PAS (placenta kept *in situ* aims) with the risk of coagulopathy and subsequent bleeding (44). This study outlined a potential risk of coagulopathy when this type of management was employed, and also the presence of a large placenta as a marker of developing coagulopathy.

Another systematic review evaluated the association between disseminated intravascular coagulopathy and conservative management of placenta percreta using data from 11 studies (45). Based on their findings, the authors suggested routine monitoring of the coagulation parameters, and signs of infection during postpartum follow-up in patients with conservatively managed placenta percreta.

This study has several limitations: its retrospective design, small sample size, lack of generalizability and limited number of ultrasound markers analyzed, reflecting our experience from a single center. Further studies, focused on developing predictive models that will include clinical risk factors.

CONCLUSIONS

Placenta accreta is considered a life-threatening condition and a dreaded pathology for obstetricians, particularly in level two hospitals with limited blood supply. It is also the leading cause of maternal mortality, postpartum hysterectomy, and ICU admission.

Ultrasound screening for PAS disorders should be performed in all patients with known risk factors. Our results showed that placental lacunar spaces, myometrial thinning of less than 1 mm and bladder wall interruptions achieved the highest accuracy in predicting PAS disorders. Moreover, the best predictive performance for predicting PAS disorders was achieved when using at least three ultrasound markers

When considering the surgical team, it should include an experienced obstetrician, a urologist, and even an available general surgeon, if needed. If hospitaliza-

tion is planned, it is important to cross-match and prepare the blood type. Suggest the elective caesarean section at 37 weeks' gestation maximum, after completion of antenatal corticosteroid treatment for lung development.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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HEPATOBILIARY MANIFESTATIONS IN INFLAMMATORY BOWEL DISEASE: RAISING CONCERN ON AN OLD DILEMMA

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HEPATOBILIARY MANIFESTATIONS IN INFLAMMATORY BOWEL DISEASE: RAISING CONCERN ON AN OLD DILEMMA (Abstract): Inflammatory bowel disease (IBD) is increasingly recognized as a multisystem disorder with significant extraintestinal manifestations, including hepatobiliary complications. This prospective study **aimed** to assess the prevalence of hepatic manifestations and associated risk factors in a real-world IBD cohort. **Materials and methods**: Between December 2023 and March 2024, 153 patients (78 with ulcerative colitis and 75 with Crohn's disease) were enrolled at a tertiary care center in northeast Romania. Comprehensive clinical evaluations, laboratory assessments, abdominal ultrasound, and vibration-controlled transient elastography with controlled attenuation parameter (CAP) were performed. **Results:** Hepatobiliary abnormalities were identified in 43.1% of patients, with steatotic liver disease (SLD) being the most common manifestation (26.8%). Notably, SLD was observed in 30.8% of ulcerative colitis and 22.7% of Crohn's disease patients. Patients with hepatic manifestations exhibited higher inflammatory markers (white blood cell count, C-reactive protein), liver enzymes, CAP values, and fibrosis, alongside longer disease duration and increased flare frequency. Multivariate analysis revealed that older age, higher body mass index, a diagnosis of Crohn's disease, and elevated CRP levels were independent predictors of hepatic involvement. **Conclusions:** These findings underscore the importance of routine liver screening and integrated hepatologic assessment in IBD management to address potential complications and improve patient outcomes. **Keywords:** INFLAMMATORY BOWEL DISEASE, HEPATIC MANIFESTATIONS, STEATOTIC LIVER DISEASE, LIVER INJURY.

INTRODUCTION

Inflammatory bowel disease (IBD) is a digestive disease with multisystemic involvement. Extraintestinal manifestations of IBD are common, occurring in both ulcerative colitis (UC) and Crohn's disease (CD), and significantly affect patient morbidity and mortality (1). Approximately 5%

of IBD patients will develop chronic liver disease, whereas up to 30% of IBD patients will have aberrant liver findings. The etiology of hepatic manifestations linked to IBD remains unclear; nevertheless, the relationship between the two conditions may be affected by immunological, genetic, and environmental variables (2). IBD progression and its complications and extraintestinal manifestations are of great interest with an important impact on the quality of life of these patients.

Fatty liver, or steatotic liver disease, represents the most prevalent hepatobiliary disorders of IBD. Its progression is directly attributable to obesity, insulin resistance, and metabolic syndrome, which is currently regarded as the hepatic manifestation of metabolic syndrome. The clinical and histological spectrum is broad, encompassing simple steatosis, steatohepatitis, nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma. According to research, people with IBD are more likely to develop NAFLD due to chronic inflammation, digestive abnormalities, prior surgery, and changes in the fecal microbiota. The prevalence appears to be at least comparable to or greater than in the general population, current data indicates a prevalence between 8% and 71% (3, 4). Moreover, lean individuals, a feature often presented in IBD patients, are mostly overlooked representing a diagnosis challenge due to their normal BMI.

In 2023, liver disease experts introduced Steatotic Liver Disease (SLD) as a new umbrella term for conditions involving fat accumulation in the liver, replacing the older “fatty liver disease” label. Within this framework, NAFLD has been renamed Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). This shift aims to reduce stigma tied to terms like “non-alcoholic” and better reflect the disease’s connection to metabolic issues such as obesity and diabetes. MASLD requires evidence of hepatic steatosis plus at least one metabolic risk factor. A notable addition is Metabolic and Alcohol-Associated Liver Disease (MetALD), a category for MASLD patients who also consume moderate alcohol. This change, driven by major liver associations, enhances classification accuracy and encourages a more inclusive, patient-focused approach to liver health (5-7).

Primary sclerosing cholangitis (PSC) is strongly associated with IBD, particularly UC. In the Caucasian population, it is estimated that 4% of UC patients may develop PSC, and three-quarters of PSC patients have UC (8). Cholelithiasis is also a frequent pathology found in patients with IBD. Individuals with Crohn's disease are twice as likely to develop gallstones. In contrast, patients with UC do not have an elevated risk compared with the general population. (9, 10). Cholelithiasis, granulomatous hepatitis, portal vein thrombosis, IgG4-related cholangiopathy, pyogenic liver abscess, hepatic amyloidosis, and PSC are occurring IBD-related hepatobiliary manifestations (11, 12).

In recent decades, therapeutic options for the management of IBD have expanded. New biological and small molecule therapies have been incorporated into the pharmacological arsenal, allowing for a more individualized approach and the pursuit of ever-stricter remission targets. Drug induced liver injury (DILI) in an IBD patient may present with elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST); alkaline phosphatase (ALP) and gamma-glutamyl transferase

(GGT) (cholestasis pattern); jaundice (hyperbilirubinemia); or a combined pattern. This complication can occur acutely with the development of acute liver failure, autoimmune hepatitis, and reactivation of hepatitis B, and some of these patients may develop chronic injury (13).

Hepatitis B virus reactivation is another issue that can be seen in patients with IBD. Anti-TNF and anti-integrin therapies have been linked to the reactivation of hepatitis B virus, especially in individuals who are positive for hepatitis B surface antigen (HBsAg), similar to other immunosuppressive treatments, such as prolonged or high-dose corticosteroid use. The frequency of hepatitis C virus (HCV) infection in patients with IBD seems to be lower than expected. The data suggests that IBD patients in Western European countries are no longer considered a risk category for HBV or HCV infection (14, 15).

The aim of this study was to assess the prevalence of hepatic manifestations and factors associated with their development in a real-world cohort of IBD patients.

MATERIALS AND METHODS

Between December 2023 and March 2024, we prospectively enrolled 153 patients with a confirmed diagnosis of IBD at a tertiary care center in northeast Romania. The study aimed to comprehensively assess these patients, capturing detailed personal medical histories that included detailed IBD features like duration or time of diagnosis, disease complications, extraintestinal manifestations, hepatic manifestations, and comorbidities. Each participant underwent a meticulous clinical examination, and blood samples were collected for extensive laboratory analysis. To evaluate alcohol consumption, we administered the Alcohol Use Disorders Identification Test (AUDIT) questionnaire, a validated tool for identifying potential alcohol abuse. Additionally, all patients received abdominal ultrasound and FibroScan examinations, conducted by an experienced physician.

Vibration-controlled transient elastography (VCTE) with controlled attenuation parameter (CAP) was performed using FibroScan® 502 Touch device (EchoSens, Paris, France). To ensure measurement accuracy, patients fasted for at least four hours prior to the procedure, as food intake can temporarily influence liver fat readings. The examination began with the standard M probe, switching to the XL probe when prompted by the device—typically for patients with greater body mass or thicker skin layers. The transducer was positioned in the 9th to 11th intercostal space, aligning with the right hepatic lobe, a standard site for liver assessment. SLD was diagnosed when CAP values exceeded 248 dB/m, a threshold supported by prior research (16), indicating significant fat accumulation in the liver.

This study was approved by the Ethics Committee of our University and was conducted according to the principles of the Declaration of Helsinki. Each participant signed a written informed consent form.

Statistical analyses were conducted using IBM *SPSS version 22.0* (IBM SPSS Inc., Chicago, IL, USA). Normality of numerical variables was evaluated using the Kolmogorov-Smirnov test. Normally distributed continuous variables were summarized as mean ± standard deviation, while non-normally distributed variables were expressed using medians and interquartile ranges. Categorical data were presented as frequencies and percentages. For group comparisons, parametric tests (e.g., Student's t-test for inde-

pendent samples) were applied to normally distributed continuous variables, whereas non-parametric alternatives (Mann-Whitney U or Kruskal-Wallis tests) were used for skewed distributions. Associations between categorical variables were examined using chi-square or Fisher's exact tests. Statistical significance was defined as a two-tailed *p*-value <0.05. Univariate and multivariate linear regression were performed to identify variables significantly associated with the presence of hepatic manifestations. Only complete datasets were included in the final analysis.

RESULTS

Study of population characteristics

The study cohort consisted of 153 patients diagnosed with IBD, including 78 patients with UC and 75 patients with CD. Detailed demographic and clinical characteristics are summarized in first table. The mean age was comparable between the UC and CD groups (47.8 ± 16.2 years *vs.* 47.1 ± 15.9 years, p=0.792), indicating no significant age difference. Gender distribution was also similar, with females comprising 44.9% of UC patients and 46.7% of CD patients (p=0.822). Rural residence was reported in 47.4% of UC patients and 46.7% of CD patients (p=0.924). Body mass index (BMI) showed no significant difference between groups, with means of 25.5 ± 4.1 kg/m² for UC and 25.3 ± 4.0 kg/m² for CD (p=0.788). Smoking status was assessed categorically: current smokers accounted for 35.9% of UC patients and 42.7% of CD patients, while former smokers comprised 14.1% and 12.0%, respectively, with no significant difference (p=0.751) (tab. I).

TABLE I.

Study of population characteristics

Characteristics	Ulcerative Colitis (n=78)	Crohn's Disease (n=75)	p-value
Demographics			
Age, years, mean (SD)	47.8 ±16.2	47.1 ± 15.9	0.792
Female, (%)	44.9% (35/78)	46.7% (35/75)	0.822
Rural residence, (%)	47.4% (37/78)	46.7% (35/75)	0.924
BMI, kg/m², mean (SD)	25.5 ±4.1	25.3± 4.0	0.788
Smoking status, (%)			
Current smoker, (%)	35.9% (28/78)	42.7% (32/75)	0.251
Former smoker, (%)	14.1% (11/78)	12.0% (9/75)	0.457
Disease Activity			
Active disease, %	7.7% (6/78)	2.7% (2/75)	0.277
Number of flare-ups, mean (SD)	1.6 (1.0)	1.7 (1.1)	0.513
New cases, (%)	11.5% (9/78)	6.7% (5/75)	0.315
Treatment Characteristics			
On any biologic therapy, (%)	24.4% (19/78)	36.0% (27/75)	0.115
On azathioprine, (%)	15.4% (12/78)	14.7% (11/75)	0.904
On mesalazine, (%)	76.9% (60/78)	36.0% (27/75)	<0.001
Current corticosteroid use, (%)	12.8% (10/78)	14.7% (11/75)	0.739
Number of corticosteroids courses in past year, mean (SD)	0.7 (1.2)	0.8 (1.3)	0.614

BMI: body mass index; SD: standard deviation.

Regarding disease activity, active disease was observed in 7.7% of UC patients and 2.7% (2/75) of CD patients (p=0.277). The mean number of flare-ups was 1.6 ± 1.0 in UC and 1.7 ± 1.1 in CD (p=0.513), and new cases were identified in 11.5% (9/78) of UC patients and 6.7% of CD patients (p=0.315), with no statistically significant differences across these parameters.

Treatment characteristics revealed notable differences in medication use. A significantly higher proportion of UC patients were receiving mesalazine compared to CD patients (76.9% *vs.* 36.0%, p<0.001). In contrast, the use of biologic therapy (24.4% *vs.* 36.0%, p=0.115), azathioprine (15.4% *vs.* 14.7%, p=0.904), current corticosteroids (12.8% *vs.* 14.7%, p=0.739), and the mean number of corticosteroid courses in the past year (0.7 ± 1.2 *vs.* 0.8 ± 1.3, p=0.614) showed no significant differences between UC and CD patients.

Disease-Specific characteristics

Disease-specific characteristics for UC and CD are presented in second table. Among UC patients, disease extent was categorized as proctitis (E1) in 25.6%, left-sided colitis (E2) in 28.2%, and pancolitis in 46.2%. For CD patients, age at diagnosis was distributed as follows: ≤16 years (A1) in 12.0%, 17-40 years (A2) in 65.3%, and >40 years (A3) in 22.7%. Disease location in CD was ileal (L1) in 34.7%, colonic (L2) in 28.0%, ileocolonic (L3) in 37.3%, and upper gastrointestinal (L4) in 6.7%. Behavioral classification revealed non-stricturing, non-penetrating (B1) disease in 70.7%, stricturing (B2) in 16.0%, and penetrating (B3) in 13.3%. Additionally, perianal disease was present in 14.7% of CD patients, with active fistulas noted in 6.7%.

TABLE II.
Disease characteristics

Ulcerative Colitis Subtypes (n=78)	
Subtype	
Proctitis (E1)	25.6% (20/78)
Left-sided colitis (E2)	28.2% (22/78)
Pancolitis (E3)	46.2% (36/78)
Crohn's Disease Classifications (n=75)	
Characteristic	
Age at Diagnosis	
A1 (≤16 years)	12.0% (9/75)
A2 (17-40 years)	65.3% (49/75)
A3 (>40 years)	22.7% (17/75)
Location	
L1 (ileal)	34.7% (26/75)
L2 (colonic)	28.0% (21/75)
L3 (ileocolonic)	37.3% (28/75)
L4 (upper GI)	6.7% (5/75)
Behavior	
B1 (non-stricturing, non-penetrating)	70.7% (53/75)
B2 (stricturing)	16.0% (12/75)
B3 (penetrating)	13.3% (10/75)
Perianal Disease	14.7% (11/75)
Active Fistulas	6.7% (5/75)

Prevalence of Hepatic Manifestations in IBD Patients

Hepatic manifestations were identified in 43.1% (66 patients) of the study cohort. The prevalence and types of hepatic manifestations are detailed in table III. Overall, SLD was the most common hepatic manifestation, affecting 26.8% of patients, followed by cholestasis in 5.88%, primary biliary cholangitis in 3.27%, occult hepatitis B virus (HBV) infection in 1.96%, hepatitis B virus infection in 1.3%, and hepatitis C virus infection in 0.65%.

When stratified by IBD type, SLD remained the predominant hepatic manifestation in both UC (30.77%) and CD (22.66%). However, differences emerged in other manifestations: cholestasis was more prevalent in CD (10.66%) than in UC (1.28%), while PSC was more frequent in UC (5.13%) than in CD (1.33%). Autoimmune hepatitis was exclusive to CD patients (4%), whereas hepatitis B virus infection was observed only in UC patients (2.56%). DILI was noted only in the personal history of 3 patients. At the time of inclusion in the study none of the patients had criteria for the diagnosis of DILI.

TABLE III.
Prevalence of hepatic manifestations

Hepatic Manifestation	Overall Cohort (N=153)	Ulcerative Colitis (N=78)	Crohn's Disease (N=75)
Primary biliary cholangitis	5 (3.27 %)	4 (5.13 %)	1 (1.33 %)
Cholestasis	9 (5.88 %)	1 (1.28 %)	8 (10.66 %)
DILI	3 (1.96 %)	2 (2.56 %)	1 (1.33 %)
Autoimmune hepatitis	3 (1.96 %)	0	3 (4 %)
Hepatitis C virus infection	1 (0.65 %)	0	1 (1.33 %)
Hepatitis B virus infection	2 (1.3 %)	2 (2.56 %)	0
Occult hepatitis B virus infection	3 (1.96 %)	2 (2.56 %)	1 (1.33 %)
SLD	41 (26.8%)	24 (30.77 %)	17 (22.66 %)

DILI: drug induced liver injury; SLD: steatotic liver disease

Comparison of Patients with and without Hepatic Manifestations

Patients with hepatic manifestations (n=66) were compared to those without (n=87) (tab. IV). Demographic and anthropometric parameters, including age, weight, height, BMI, CUN-BAE and sex distribution, showed no significant differences between the groups. However, significant differences were observed in several laboratory and clinical parameters. Patients with hepatic manifestations exhibited higher white blood cell counts (p=0.001), CRP levels (p=0.031), ALT (p=0.01), GGT (p=0.02), fecal calprotectin (p=0.01), triglycerides (p=0.012), CAP values (p=0.005) and fibrosis (p=0.001). Additionally, these patients had a longer disease duration (p=0.022), a higher number of flare-ups (p=0.023), and a greater prevalence of extraintestinal manifestations (excluding hepatic) (p=0.002). No significant differences were noted in hemoglobin, platelets, AST, ALP, total bilirubin, albumin, cholesterol, or previous surgical interventions.

TABLE IV.
Comparison of patients with and without hepatic manifestations

Parameter	Patients with Hepatic Manifestations N=66 (43.1%)	Patients Without Hepatic Manifestations N=87 (57.9%)	p-value
Age (years)	47.23±8.5	46.67±9.1	0.86
Weight (kg)	76.87±12.3	72.02±10.8	0.14
Height (cm)	171.87±6.2	170.84±5.9	0.53
BMI (kg/m²)	25.95±4.5	24.61±3.2	0.17
CUN-BAE	30.5±8.9	28.7±5.9	0.23
Sex (% Female)	43.33%	43.09%	0.98
White Blood Cells (x10³/μL)	7.82±1.5	5.82±1.2	0.001
Hemoglobin (g/dL)	13.45±1.2	13.42±1.1	0.93
Platelets (x10³/μL)	288.60±45.3	310.62±50.1	0.23
CRP (mg/L)	1.38±1.1	0.66±0.8	0.031
ALT (U/L)	34.77±12.5	22.67±8.3	0.01
AST (U/L)	26.37±9.4	22.41±7.6	0.14
GGT (U/L)	70.87±25.6	43.93±18.2	0.02
ALP (U/L)	105.27±30.2	88.33±25.7	0.07
Total Bilirubin (mg/dL)	0.82±0.3	0.62±0.2	0.06
Albumin (g/dL)	4.20±1.21	4.36±1.35	0.15
Calprotectin (μg/g)	266±58	153±41	0.01
Cholesterol (mg/dL)	224±28.3	192±18.7	0.35
Triglycerides (mg/dL)	172±15.2	112±10.5	0.012
CAP values (dB/m)	285±22	243±32	0.005
Fibrosis (kPa)	8.5±2.3	5.2±1.3	0.001
Hypertension	13.2%	10.1%	0.43
T2DM	15.5%	12.2%	0.56
Disease Duration (years)	8.37±2.5	4.02±3.1	0.022
Number of Flares	3.73±0.9	1.67±0.8	0.023
Previous Surgical Interventions	26.6%	17.6%	0.11
Extraintestinal Manifestations (excluding hepatic manifestations)	32.30%	8.13%	0.002

BMI: body mass index; CAP: controlled attenuation parameter; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; CRP, c-reactive protein; T2DM, Type 2 diabetes; CUN-BAE, Clínica Universidad de Navarra-Body Adiposity Estimator.

Risk Factors for Hepatic Manifestations

Univariate and multivariate logistic regression analyses were conducted to identify risk factors associated with hepatic manifestations (tab. V). In the univariate analysis, significant associations were found with older age (odds ratio [OR]=1.05, 95% confidence interval [CI]: 1.01–1.09, p=0.02), higher BMI (OR=1.10, 95% CI: 1.02–1.18, p=0.01), Crohn's disease diagnosis (OR=2.5, 95% CI: 1.20–5.20, p=0.01), elevated CRP (OR=1.02, 95% CI: 1.21–2.9, p=0.01), female sex (OR=1.8, 95% CI: 1.05–3.10, p=0.04), current smoking (OR=2.1, 95% CI: 1.10–4.00, p=0.03), infliximab use (OR=2.0, 95% CI: 1.05–3.80, p=0.04), and hypertension (OR=1.9, 95% CI: 1.05–3.50, p=0.03).

In the multivariate analysis, independent risk factors for hepatic manifestations included older age (OR=1.17 per year, 95% CI: 1.00–2.08, p=0.03), higher BMI (OR=1.08 per kg/m², 95% CI: 1.01–1.15, p=0.02), Crohn's disease (OR=2.2, 95% CI: 1.05–4.60, p=0.01), and elevated CRP (OR=1.11, 95% CI: 1.00–1.24, p=0.04). Female sex, CRP level, current smoking, infliximab use, and hypertension did not retain significance in the multivariate model.

TABLE V.

Univariate and multivariate analysis of factors associated with development of hepatic manifestations in IBD patients

Variable	Univariate OR (95% CI)	Univariate p-value	Multivariate OR (95% CI)	Multivariate p-value
Age (years)	1.05 (1.01-1.09)	0.02	1.17 (1.00-2.08)	0.03
BMI (kg/m²)	1.10 (1.02-1.18)	0.01	1.08 (1.01-1.15)	0.02
Crohn's Disease	2.5 (1.20-5.20)	0.01	2.2 (1.05-4.60)	0.01
CRP (mg/dL)	1.02 (1.21-2.9)	0.01	1.11 (1.00-1.24)	0.04
Female Sex	1.8 (1.05-3.10)	0.04	1.5 (0.80-2.80)	0.20
Current Smoker	2.1 (1.10-4.00)	0.03	1.7 (0.85-3.40)	0.13
Infliximab Use	2.0 (1.05-3.80)	0.04	1.6 (0.75-3.40)	0.22
Hypertension	1.9 (1.05-3.50)	0.03	1.4 (0.70-2.80)	0.34

BMI: body mass index; CRP: c-reactive protein

Lean SLD subgroup analysis

A special subgroup of patients in our data set are those with normal BMI, accounting for 73 individuals, with 26 of them having SLD. The mean age of lean IBD patients with SLD was significantly higher than that of those without SLD (54.2 ± 12.3 years *vs.* 45.6 ± 10.8 years, p = 0.002). Gender distribution also differed, with a higher proportion of males in the SLD group compared to the non-SLD group (60% *vs.* 45%, p = 0.045). Residence distribution (urban *vs.* rural) showed no significant difference between the two groups (p = 0.321). Disease duration was longer in the SLD group (12.5 ± 6.7 years *vs.* 8.3 ± 5.2 years, p = 0.004), and the mean number of flare-ups was greater (3.2 ± 1.5 *vs.* 2.1 ± 1.2, p = 0.001). Disease activity scores were also elevated in the

SLD group, with ulcerative colitis (UC) patients showing a higher mean Mayo score (6.8 ± 2.1 *vs.* 4.5 ± 1.9, p = 0.003) and CD patients demonstrating a higher mean Crohn's Disease Activity Index (CDAI) (220 ± 50 *vs.* 180 ± 45, p = 0.007). Regarding treatment options, a greater percentage of patients with SLD were treated with biologics (55% *vs.* 40%, p = 0.028) and immunomodulators (45% *vs.* 30%, p = 0.041) compared to those without SLD. Corticosteroid use was more common in the SLD group, though the difference was not statistically significant (35% *vs.* 25%, p = 0.112).

Significant differences were observed in laboratory parameters between the two groups. Patients with SLD had higher mean C-reactive protein (CRP) levels (15.4 ± 7.2 mg/L *vs.* 10.2 ± 5.6 mg/L, p = 0.001) and elevated liver enzymes, including alanine aminotransferase (ALT) (45 ± 15 U/L *vs.* 25 ± 10 U/L, p < 0.001), aspartate aminotransferase (AST) (40 ± 12 U/L *vs.* 22 ± 8 U/L, p < 0.001), and gamma-glutamyl transferase (GGT) (55 ± 20 U/L *vs.* 30 ± 12 U/L, p < 0.001). Lipid profiles revealed higher mean total cholesterol (210 ± 35 mg/dL *vs.* 180 ± 30 mg/dL, p = 0.004) and triglycerides (150 ± 40 mg/dL *vs.* 120 ± 35 mg/dL, p = 0.012) in the SLD group, alongside lower high-density lipoprotein (HDL) levels (40 ± 10 mg/dL *vs.* 50 ± 12 mg/dL, p = 0.008). Moreover, metabolic abnormalities, such as abnormal glucose levels, were also more prevalent in the SLD group (25% *vs.* 15%, p = 0.048).

DISCUSSION

Our study highlights that nearly half (43.1%) of patients with IBD exhibit some form of hepatic manifestation, with SLD being the most common finding. This high prevalence reinforces the notion that hepatic involvement is a frequent extraintestinal feature of IBD, as previously documented (17,18). The prevalence of hepatic manifestations in our cohort is notably higher than the pooled prevalence of extraintestinal manifestations reported by Kilic *et al.*, which stood at 31.3% across IBD patients globally (1). Hepatobiliary manifestations constitute a significant subset of extraintestinal manifestations, and our elevated prevalence may reflect the meticulous diagnostic approach employed, including the use of FibroScan. This advanced tool likely enhanced the detection of subclinical liver abnormalities, such as early steatosis or fibrosis, which might be missed by conventional methods like ultrasound alone. This aligns with findings by Veltkamp *et al.*, who emphasized the utility of non-invasive tools in identifying hepatic steatosis and fibrosis in IBD patients (2).

The prevalence of SLD is 26.8% in our cohort (30.77% in UC and 22.66% in CD), and is the dominant hepatic manifestation, consistent with the growing recognition of MASLD as a common comorbidity in IBD. The prevalence aligns with findings by Sartini *et al.*, who reported a 28% prevalence of NAFLD in IBD patients, emphasizing the role of metabolic risk factors (19). Another recent study reported that 25% of IBD patients had MASLD, with higher rates in those with obesity and insulin resistance, supporting our observation of BMI as a risk factor (20).

An intriguing finding is the association of hepatic manifestations with CD rather than UC, with CD identified as an independent risk factor (OR=2.2, 95% CI: 1.05–4.60, p=0.01). Results from current

literature are heterogenous. A study that compared the two conditions reported that CD patients had a higher prevalence of hepatic steatosis compared to UC patients, possibly due to metabolic factors like BMI (21). One more recent study found that CD patients had a higher risk of gallstones and hepatic steatosis, linked to ileal dysfunction and chronic inflammation (22). Of note is the fact that the prominence of CD as a risk factor may reflect its association with metabolic factors, which are more pronounced in CD patients due to malabsorption or chronic inflammation altering metabolic profiles, results similar with other studies (23).

Patients with hepatic manifestations exhibited elevated white blood cell counts, CRP, ALT, GGT, fecal calprotectin, triglycerides, longer disease duration, and more flare-ups. These markers indicate active inflammation and metabolic dysfunction, reinforcing the interplay between IBD activity and liver health. The association between longer disease duration and hepatic manifestations is consistent with the findings from literature in which IBD patients with longer disease duration are more likely to develop MASLD (24).

Multivariate analysis identified older age (OR=1.17 per year, p=0.03), higher BMI (OR=1.08 per kg/m², p=0.02), CD (OR=2.2, p=0.01), and elevated CRP (OR=1.11, p=0.04) as independent predictors of hepatic manifestations. These findings are supported by results from a recent meta-analysis showing that MASLD significantly impacts clinical outcomes in patients with metabolic risk factors (25). Elevated CRP, a marker of systemic inflammation, supports the hypothesis that chronic inflammation in IBD contributes to liver pathology, being involved in the progression of NAFLD (26).

The interplay between immunosuppressive therapies and liver injury also warrants careful consideration. Although DILI was documented only in a small number of patients in our study, its occurrence is clinically significant given the expanding therapeutic armamentarium for IBD management (12).

Our cohort revealed that lean IBD patients with SLD were significantly older than those without SLD (p = 0.002). This observation aligns with findings from the general MASLD population, where age is a recognized risk factor for disease progression. For example, Unger *et al*. (2020) reported that older age was associated with a higher prevalence of hepatic steatosis in lean patients, irrespective of underlying conditions (27). Moreover, a higher prevalence of Crohn's disease (CD) among lean IBD patients with SLD (65% *vs.* 50%, p = 0.031) was observed, suggesting a specific association between CD and liver steatosis, results that are in line with data from current literature (28). Additionally, our SLD group exhibited longer IBD duration and greater disease activity. These results parallel those of Sartini *et al.*, who demonstrated that prolonged IBD activity increases the risk of liver steatosis, emphasizing chronic inflammation's role in MASLD pathogenesis (19). The consistency with prior studies underscores the interplay between IBD severity and liver involvement in lean patients.

Although this study provides significant insights into the epidemiology and risk variables associated with hepatic symptoms in IBD, it also possesses limitations. The single-center design and limited sample

size may constrain the generalizability of the results. Future multicenter studies with bigger cohorts and longitudinal follow-up are essential to clarify the natural history of hepatic involvement in IBD and to enhance therapy methods.

CONCLUSIONS

In conclusion, the high prevalence of hepatic manifestations, particularly SLD, among IBD patients calls for routine liver screening and a proactive management strategy. Our findings advocate for the integration of hepatologic assessment into the standard care of IBD patients, which may ultimately improve clinical outcomes by mitigating the impact of these extraintestinal complications.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflicts of interests. The research did not receive external funding.

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THE IMPACT OF BARIATRIC SURGERYON CORTISOL AND OXYTOCIN IN OBESE PATIENTS

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THE IMPACT OF BARIATRIC SURGERY ON CORTISOL AND OXYTOCIN IN OBESE PATIENTS (Abstract): Morbid obesity is linked to widespread endocrine dysregulation, including heightened stress and responses and altered social-bunding signals. Cortisol, the primary effector of the hypothalamic-pituitary-adrenal (HPA) axis, drives insulin resistance and systemic inflammation, while oxytocin, a hypothalamic neuropeptide, influences appetite control, emotional coping and social behaviors. Although bariatric surgery is stablished as the most effective long-term treatment for severe obesity, its impact on these two pivotal hormones remains incompletely defined. **Aim:** To evaluate and compare plasma samples of cortisol and oxytocin concentrations in adults with morbid obesity before and three months after bariatric surgery, relative to healthy non-obese controls. **Materials and methods:** In this prospective longitudinal study, we enrolled 21 patients (age 25-60 years; Body Mass Index (BMI) $\geq$ 40 kg/m^2) scheduled for either sleeve gastrectomy or Roux-en-Y gastric bypass, alongside 10 healthy volunteers (BMI 22 $\pm$ 1 kg/m^2). Fasting blood samples were drawn on the morning surgery (T0) and at a three-month follow-up visit (T2). Plasma cortisol and oxytocin levels were quantified via high-sensitivity competitive immunoassays. Repeated-measures analyses compared hormonal shifts within the surgical cohort and against controls. **Results:** At baseline, patients exhibited markedly elevated cortisol (86.6 $\pm$ 50.2 ng/mL) and oxytocin (553 $\pm$ 115 pg/mL) compared with controls (54.3 $\pm$ 40.1 ng/mL and 380 $\pm$ 100 pg/mL, respectively). Three months following surgery, mean cortisol declined by 66% to 29.5 $\pm$ 22.4 ng/mL ($p < 0.001$), approximating control values, whereas oxytocin decreased modestly to 483 $\pm$ 120 pg/mL ($p = 0.02$), indicating partial normalization. The magnitude of cortisol reduction correlated strongly with the degree of weight loss and oxytocin adjustment aligned with patient-reported improvements in psychological well-being. **Conclusions:** Bariatric surgery elicits rapid and significant recalibration of both stress-related and social-bonding hormonal axes. The pronounced cortisol decreases and the trend toward normalized oxytocin suggest dual benefits, metabolic and psychological, in the early postoperative period. These findings support integrating targeted nutritional and psychological interventions to

reinforce endocrine recovery and enhance long-term surgical outcomes. **Keywords:** BARIATRIC SURGERY, CORTISOL, OXYTOCIN, MORBID OBESITY, STRESS HORMONES, SOCIAL BONDING, IMMUNOASSAY.

INTRODUCTION

Obesity is a complex, chronic disease characterized by excess adiposity and associated with elevated risk of cardiovascular disease, type 2 diabetes and decreased life expectancy (Rutters *et al*., 2012). Bariatric surgery has emerged as the most effective intervention for achieving sustained weight loss and metabolic improvement in individuals with severe obesity, with procedures such as sleeve gastrectomy and Roux-en-y gastric bypass demonstrating durable benefits over medical therapy alone (Bando *et al*., 2023). Beyond reductions in body mass index, these surgical approaches elicit profound alterations in glucose homeostasis, gut hormone secretion and energy expenditure, suggesting mechanisms that extend beyond simple caloric restrictions (Cornejo-Pareja *et al*., 2019).

Except adipose tissue promotes dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated cortisol secretion that contributes to visceral fat accumulation and insulin resistance (Bose *et al*., 2009). Chronically increased cortisol levels exacerbate systemic inflammation and impair lipid metabolism, thereby reinforcing the cycle of metabolic dysfunction in obesity (Incollingo Rodriguez *et al*., 2015). Several studies have documented normalization of both basal and postprandial cortisol concentrations following bariatric surgery, reflecting restoration of HPA axis homeostasis; however, the magnitude and time course of these changes vary according to the type of surgical procedure and the postoperative interval examined (Casteràs *et al*., 2024; Cornejo-Pareja *et al*., 2019).

Oxytocin, a neuropeptide synthetized in the hypothalamus, plays key roles in social bonding, stress modulation and the regulation of appetite and energy balance (Lawson, 2017). Circulating oxytocin levels have been reported to be elevated in individuals with morbid obesity compared to lean controls, though these levels may decline following substantial weight-loss interventions (Pataky *et al*., 2019). Preclinical and early clinical trials indicate that oxytocin administration enhance lipolysis, increases fatty acids oxidation and supports lean mass preservation, independent of reductions in food intake (Kerem *et al*., 2021). Nevertheless, the literature on post-bariatric changes in endogenous oxytocin is mixed with some cohorts showing significant decreases at 12 months after sleeve gastrectomy and others reporting stable levels despite marked weight-loss (Becetti *et al*., 2023; Pataky *et al*., 2019).

Given the central roles of cortisol and oxytocin in coordinating metabolic and psychosocial adaptations, elucidating their joint response to bariatric surgery may uncover insights into both the psychological and behavioral drivers of postoperative recovery. In this longitudinal comparative study, we evaluate plasma samples of cortisol and oxytocin before and three months after bariatric intervention in patients with morbid obesity, benchmarking these changes against healthy controls to advance understanding of endocrine remodeling in the early post-surgical period.

MATERIALS AND METHODS

We conducted a prospective study with 21 patients diagnosed with morbid obesity (BMI ≥ 40 kg/m^2), all undergoing bariatric surgery in the 3rd Department of Surgery of the "St. Spiridon" Hospital in Iasi, Romania. Of these, seven patients underwent laparoscopic sleeve gastrectomy and the remaining fourteen underwent Roux-en-Y gastric bypass. As a reference group, we included ten normal-weight adults (mean BMI 22 ± 1 kg/m^2), aged between 30 and 35 years, without metabolic comorbidities.

For enrollment in the obesity cohort, the following were required> BMI at least 40 kg/m^2, recommendation for bariatric surgery, age between 21 and 65 years and written informed consent. The volunteers in the control group were not chronic alcohol users, did not have active infectious disease and were not taking vitamin supplements, maintaining their usual medication regimen until the day of collection.

Excluded from both groups were people using antioxidants or anti-inflammatory treatments, those with a recent history of trauma or surgery (last six months), pregnant or lactating women and those engaged in restrictive diets (ketogenic, vegan, etc.) that could have affected hormonal levels. All blood samples were collected in the morning, before launch, both for the initial assessment and for the three-month control. Plasma separated by centrifugation was stored at -80 ° and analyzed in duplicate for cortisol and oxytocin, and the values were averaged for interpretation.

Cortisol Determination

Plasma cortisol was analyzed using a competitive ELISA kit (Fine Test, China) at 1:20 dilution. Optical density was read at 450 nm, with a standard curve spanning 0.0391-25 ng/mL (S1-S7) and a zero-concentration control (S8). Intra and inter-assay variability were < 8% (tab. I)

TABLE I.
Competitive ELISA Standard Curve for Cortisol: Optical density (450 nm) vs. Concentration (0-25 ng/mL)

Standard	OD at 450 nm	Conc. (ng/mL)
S1	0.518	25
S2	0.947	12.5
S3	1.042	6.25
S4	1.243	3.125
S5	1.65	1.562
S6	1.954	0.781
S7	2.115	0.391
S8	2.54	0

Figure 1 depicts a competitive ELISA standard curve for cortisol, demonstrating an inverse correlation between optical density (OD = 450 nm) and cortisol concentration (0.0391-25 ng/mL), with OD value rising as hormone levels decrease. Assay precision is evidenced by low variability (<8%) and a zero-concentration control (S8) showing maximal OD (2.54), confirming analytical reliability.

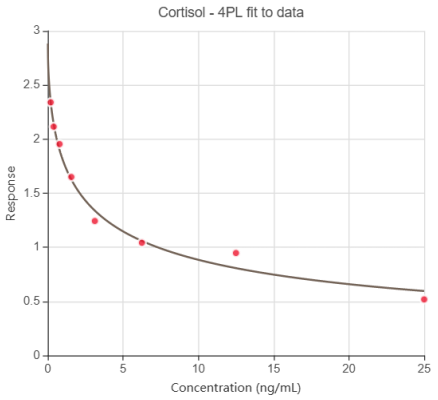

Fig. 1. Cortisol ELISA Standard Curve - Relationship between Optical Density (OD) and Concentration

Oxytocin Determination

Plasma oxytocin levels were quantified using a competitive ELISA kit (Abcam Oxytocin ELISA Kit). Serum samples underwent solid-phase extraction on C18 cartridges minimize matrix interference, followed by 1:8 dilution in assay buffer. Absorbance was measured at 405 nm with concentrations interpolated from a seven-point standard curve (15.6-1000 pg/mL; two-fold dilutions). The assay's sensitivity was 15 pg/mL with intra- and inter-assay coefficients of variation < 10% (tab. II)

TABLE II.

Competitive ELISA Standard Curve for Oxytocin (Dilution 1:8): Optical Density (OD = 405 nm) *vs.* Concentration

Standard	OD at 405 nm	Conc. (pg/mL)
S1	0.165	1000
S2	0.254	500
S3	0.373	250
S4	0.502	125
S5	0.628	62.5
S6	0.71	31.2
S7	0.758	15.6

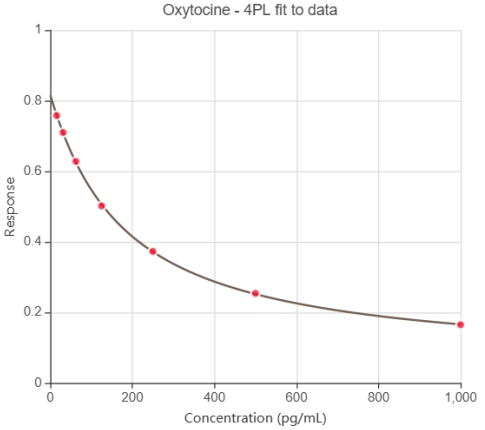

Fig. 2. Competitive ELISA Standard Curve for Oxytocin (Dilution 1:8)-Relationship between Optical Density (OD) and Concentration

Table II and figure 2 illustrates the standard ELISA curve for oxytocin (1:8 dilution), demonstrating an inverse correlation between OD at 405 nm and hormone concentration (15.6-1000 pg/mL). At maximum concentrations (1000 pg/mL), the OD is minimal (0.165), while at low concentrations (15.6 pg/mL) the OD reaches 0.758. The curve covers a wide dynamic range, suitable for the quantification oxytocin in physiological and pathological samples. The logarithmic trend line confirms the reliability of the method, with coefficients of variation below 10%.

Data Analysis. Normality of hormone distributions was assessed by the Shapiro-Wilk test. Repeated-measures analyses of variance (ANOVA) evaluated within-subject changes from T0 to T2, with post-hoc Bonferroni corrections for multiple comparisons. Independent-sample t-tests compared baseline patient values to controls. Pearson correlation coefficient quantified relationship between hormone changes and clinical parameters, including percent excess weight loss and self-reported quality of life scores. Statistical significance was defined as $p< 0.05$. Analyses were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY).

Quality Control and Ethical Considerations

All reagents were lot-verified and assay runs included quality control samples covering the analytical range. Laboratory personnel were blinded to participant group and time point. The study adhered to the Declaration of Helsinki and complied with institutional guidelines for human subjects research.

RESULTS

The investigation recruited 31 participants stratified in three cohorts> a reference group of 10 metabolically healthy

individuals (mean body mass index: 22 ± 1 kg/m^2), a clinical population of 21 adults meeting criteria for severe obesity (BMI ≥ 40 kg/m^2) and an intervention subset comprising 10 surgically managed patients reevaluated 90 days post-bariatric procedure. Demographic analysis revealed pronounced gender disparity in the obese cohort, with 95.2% female representation (20 females and 1 male) and an age distribution spanning young adulthood to senior years (21-65 years). These obese group exhibited a high burden of chronic metabolic and cardiovascular comorbidities, notably insulin resistant diabetes, persistent hypertension and reflux pathology. Pharmacotherapeutic patterns indicated prevalent use of glucose-modulating agents, gastric acid suppressants, thyroid hormone replacements and cardio-selective β-adrenergic antagonists. Postprocedural monitoring documented clinically significant anthropometric improvements, with mean BMI declining from 50.5 kg/m^2 (± 6.3) preoperatively to 39.0 kg/m^2 (± 7.6), reflecting pronounced early-phase weight reduction following surgical intervention.

Hormonal Dynamics:

Cortisol and Oxytocin

Cortisol Levels Across Groups

At baseline, patients with morbid obesity exhibited elevated fasting serum cortisol levels (86.6 ± 50.2 ng/mL) compared to the control group (54.3 ± 40.1 pg/mL). Three months post-surgery, a significant reduction in cortisol levels was observed (29.5 ± 22.4 ng/mL), aligning closely with control values. Statistical analysis confirmed Baseline vs. Control: p = 0.02 and Postoperative vs. Baseline: p < 0.001. This substantial decrease suggests a normalization of HPA axis activity following weight loss induced by bariatric surgery (fig. 3).

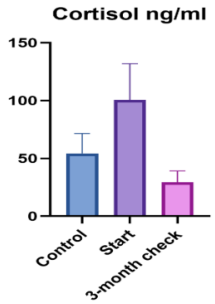

Fig. 3. Cortisol levels in control, before and after surgery

Oxytocin Levels Across Groups

Baseline oxytocin concentrations were higher in obese patients (553 ± 115 pg/mL) compared to controls (380 ± 100 pg/mL). After three months, oxytocin levels decreased to 483 ± 120 pg/mL. Comparative analyses revealed statistically significant differences between cohorts. The baseline obese group demonstrated marked divergence from healthy cohorts (p = 0.01). Postoperative evaluations showed significant anthropometric improvement relative to preoperative measures (p = 0.02), though comparison between surgically managed patients and the control group did not attain statistical significance (p = 0.15). These results indicate a partial normalization of oxytocin level post-surgery, though not fully reaching control values (fig. 4.)

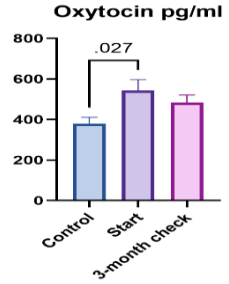

Fig. 4. Oxytocin levels in control, before and after surgery

Correlation Analyses

Age-Related Hormonal Variations

A moderate positive correlation between age and baseline cortisol levels was identified (r = 0.31) (fig. 5) suggesting increased HPA axis activity with advancing age. Postoperative reductions in cortisol were more pronounced in patients under 40 years, indicating age-related differences in hormonal adaptability (fig. 6).

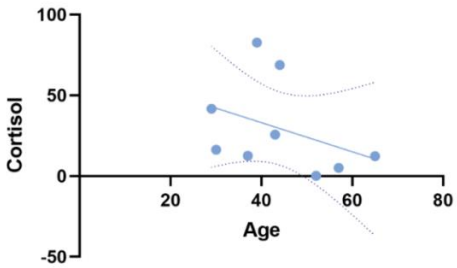

Fig. 5. Correlation between age and cortisol levels recorded preoperatively

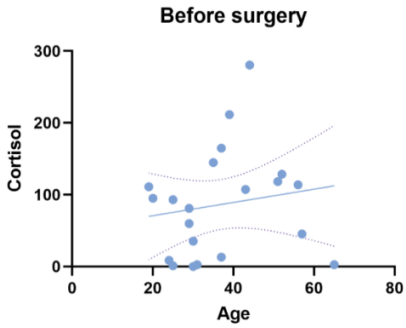

Fig. 6. Correlation between age and cortisol levels recorded postoperative

No significant correlation between age and oxytocin levels was observed at baseline (r = -0.05) (fig. 7) or postoperatively (fig. 8), implying that oxytocin dynamics are relatively age-independent in this cohort.

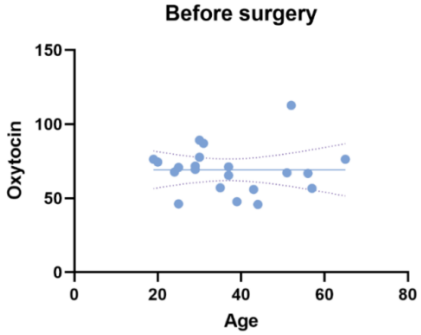

Fig. 7. Correlation between age and oxytocin levels recorded preoperatively

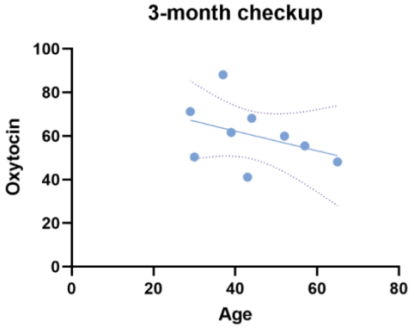

Fig. 8. Correlation between age and oxytocin levels recorded postoperatively

Weight-Related Hormonal variations

A significant positive correlation was observed between baseline body weight and serum cortisol levels (r = 0.57), indicating that individuals with higher body weight tended to have elevated cortisol concentrations. Following bariatric surgery, a notable inverse correlation emerged between the extent of weight loss and the reduction in cortisol levels (r = -0.68), suggesting that greater weight loss was associated with more substantial decreases

in cortisol. These relationships are visually depicted in figure 9 and 10, which illustrates the linear associations between body weight and cortisol levels both before and after surgical interventions.

A moderate positive correlation was observed between baseline weight and serum oxytocin levels (r = 0.49), suggesting that higher body mass may be associated with increased oxytocin secretion in the preoperative state. This association is visually illustrated in figure 11, where a trend line indicates a gradual elevation in oxytocin levels with increasing weight. The dispersion of values suggests moderate interindividual variability, yet the overall directionality supports a consistent physiological link.

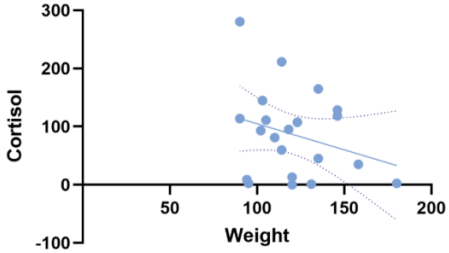

Fig. 9. Correlation between body weight and cortisol levels recorded preoperatively

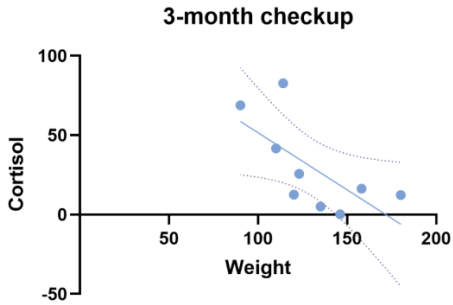

Fig. 10. Correlation between body weight and cortisol levels recorded postoperatively

Three months post-surgery, oxytocin levels decreased in most participants, and this reduction demonstrated an inverse correlation with the amount of weight lost (r = -0.42). This trend suggest that oxytocin secretion may adapt to changes in energy balance or adipose tissue mass. The postoperative association is depicted in figure 12, where a downward trend can be noticed in oxytocin values relative to reduced weight, reinforcing the hypothesis of hormonally mediated metabolic recalibration following bariatric surgery.

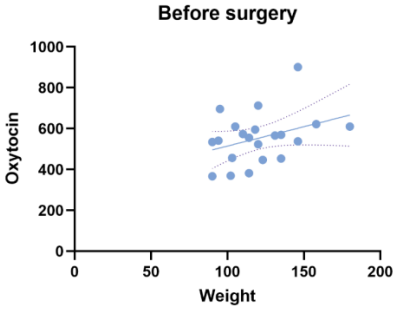

Figure 11. Correlation between body weight and oxytocin levels recorded preoperatively

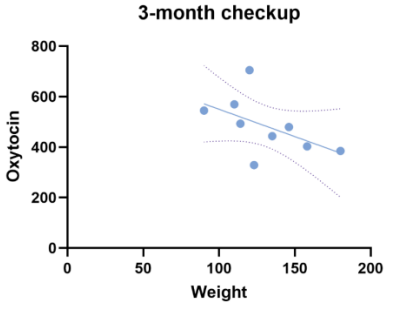

Figure 12. Correlation between body weight and oxytocin levels recorded postoperatively

BMI-Related Hormonal Variations

At baseline, BMI was positively correlated with serum cortisol levels (r = 0.51), supporting the hypothesis that adiposity contributes to elevated stress hormone secretion. Figure 13 illustrates this association where a clear upward trend can be observed: as BMI increases, cortisol levels tend to rise. This reinforces the role of excessive adipose tissue as a chronic physiological stressor.

Three months after bariatric surgery, a significant reduction in BMI was observed and this was strongly associated with a decrease in cortisol levels (r = -0.65). The inverse relationship is presented in figure 14, where lower BMI values post-surgery align with diminished cortisol secretion. This suggest a restoration of HPA axis homeostasis following weight loss and potentially a reduced allostatic load in post-bariatric patients.

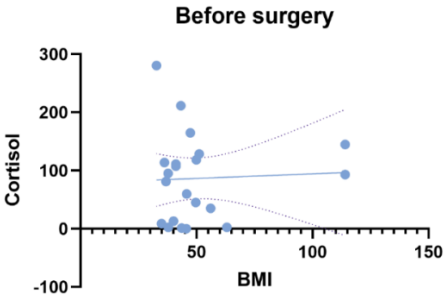

Fig. 13. Correlation between BMI and cortisol levels recorder preoperatively

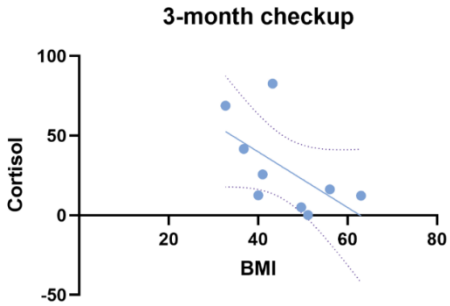

Fig. 14. Correlation between BMI and cortisol levels recorder postoperatively

Similarly, a moderate positive correlation existed between BMI and oxytocin levels prior to surgery (r = 0.46) (fig. 15). Participants with higher BMI tended to present with increased oxytocin concentrations. This could reflect a compensatory neuroendocrine mechanism, possibly modulating appetite, reward sensitivity or metabolic regulation in obesity.

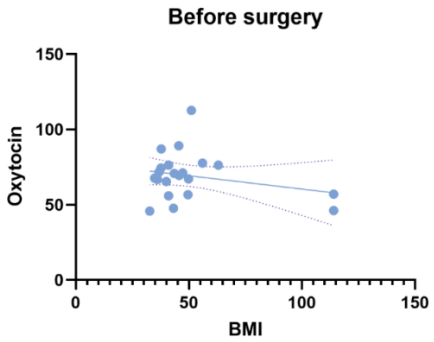

Fig. 15. Correlation between BMI and oxytocin levels recorder preoperatively

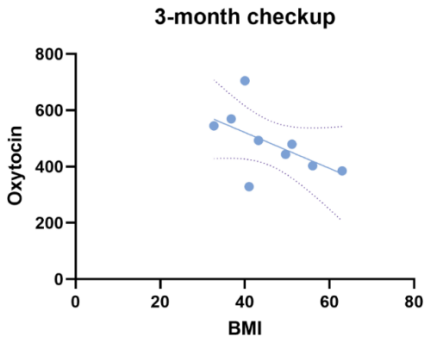

Fig. 16. Correlation between BMI and oxytocin levels recorder postoperatively

Following surgical intervention and BMI normalization, oxytocin levels shoved an inverse correlation with BMI (r= -0.39). This inverse trend suggests a potential normalization of oxytocinergic tone in relation to reduced fat mass (fig. 16). These findings contribute to the emerging understanding of oxytocin as more than a social neuropeptide, implying roles in energy metabolism and post-obesity hormonal adjustment.

Pearson correlation coefficients quantified relationship between hormonal levels and clinical parameters (weight, BMI). All analyses were two-tailed with significance set at $p < 0.05$. Data were processed using *SPSS v 26.0.*

DISCUSSION

Analysis of hormonal evolution following bariatric surgery highlights a rapid normalization of the HPA axis and a partial adjustment of the oxytocinergic system, in strict accordance with the results reported by numerous clinical studies and reference reviews.

Regarding cortisol, the increased preoperative levels in morbid obesity (86.6 ± 50.2 ng/mL) align with data from the international literature, where obese patients present, on average, morning values between 80 and 90 ng/mL (Cornejo-Pareja *et al*., 2019). The significant 66% reduction at three months postoperatively to 29.5 ± 22.4 ng/mL confirms the normalization trend described by (Cornejo-Pareja *et al*., 2019) where rapid weight loss restores HPA homeostasis by 30-70% in the first year after surgery.

The statistically significant inverse correlation between percentage weight loss and cortisol decrease (r = -0.68) reflects similar observations from a Japanese study of patients undergoing sleeve gastrectomy where higher preoperative cortisol levels were correlated with lower weight loss (Bando *et al*., 2023). The role of cortisol as a prognostic biomarker is also supported by analyses that identified significant relationship between pre-surgical cortisol levels and the risk of long-term weight regain (Casteràs *et al*., 2024). Thus, our data complement the vast literature by highlighting an early, pronounced decline correlated with clinical parameters, suggesting the need to integrate cortisol monitoring into postoperative protocols to optimize long-term outcomes.

Regarding oxytocin, the values recorded before surgery (553 ± 115 pg/mL) fall within the range described by variable studies on plasma oxytocin in obesity, where levels can be up to 50% higher than in normal weight conditions (Pataky *et al*., 2019). The approximately 13% decrease to 483 ± 120 pg/mL, although partial, is consistent with reports indicating a reduction in oxytocin in the first 6-12 months after bariatric surgery, mediated by decreased fat mass (Becetti *et al*., 2023). The inverse correlation between weight loss and oxytocin adjustment (r = -0.42) supports the hypothesis of compensatory mechanisms by which excess adipose tissue stimulates the release of oxytocin, a roe also emphasized by preclinical experiments that attest to the lipolytic effects of the hormone (Ding *et al*., 2019). Furthermore, the connection between oxytocin adjustment and improved postoperative quality of life has been highlighted in several studies, where partial normalization of this neuropeptide was associated with reduced anxiety symptoms and improved social support (Lawson, 2017).

Beyond the effects of bariatric surgery, both age and adiposity exert independent influences on cortisol and oxytocin dynamics. Multiple clinical investigations have demonstrated that advancing age is associated with higher basal cortisol secretion, even after adjusting for BMI, whereas the relationship between oxytocin and age appears more variable.

Philips *et al.* (2011) reported that in men aged 50-70 years, plasma cortisol concentrations rose with increasing age and fell with higher BMI, highlighting a dual influence of chronological and metabolic factors on HPA axis tone. A meta-analysis by Tenk *et al.* (2016) further confirmed that the peripheral cortisol levels actually decline with healthy aging but may remain elevated in the presence of obesity, suggesting that adiposity can counteract the age-related decrease in cortisol secretion.

In our own cohort, we observed a modest positive correlation between age and baseline cortisol ($r = 0.31$), and a more pronounced cortisol reduction after surgery in younger patients, findings that align with these earlier reports and underscore the complex interplay between age, BMI and stress hormone regulation. The preoperative increase in cortisol reflects a neuroendocrine response to chronic stress, similar to that observed in populations subjected to intense psychosocial stress, such as nursing students, where academic stress is a significant predictor of depressive symptoms (Iorga *et al*., 2017).

Regarding adiposity, cortisol exhibits a U-shaped relationship with BMI, with nadirs in the overweight-class I range and rising levels at both lower and higher extremes of BMI (Schorr *et al*., 2015). Our positive baseline correlation ($r = 0.51$) and strong inverse link between BMI reduction and cortisol decrease ($r = -0.65$) mirrors these observations, reinforcing the concept that excessive fat mass constitutes a persistent activator of the HPA axis. Similarly, a large Korean cohort demonstrated that higher cortisol excretion was associated with favorable metabolic profiles despite greater adiposity, illustrating that cortisol adaptations may also reflect compensatory metabolic responses rather than simple stress markers (Park *et al*., 2011).

Oxytocin's association with age is less consistently reported. Some studies suggest stable oxytocin across the adult lifespan while others note subtle declines post menopause (Kerem, Lawson, 2021). In our data, age bore no significant relationship to oxytocin levels either pre- or post-surgery, implying that obesity-related factors overshadow age effects for this neuropeptide in the 21-65-year range.

With respect to BMI, oxytocin correlates positively with adiposity indices. Variable Oxytocin Levels in Humans revealed significant correlations between oxytocin and weight, waist circumference and BMI ($R^2 = 0.15$-0.16), reflecting higher oxytocin in more obese individuals (Pataky *et al*., 2019). A smaller pilot in women corroborated a positive BMI-oxytocin association ($r \approx 0.45$) (Skinner *et al*., 2019). Our finding of a baseline correlation ($r = 0.46$) and an inverse relationship between BMI loss and oxytocin reduction ($r = -0.39$) fits within this framework, suggesting that as fat mass decreases the compensatory elevation of oxytocin likewise diminishes.

Postoperative biomarker monitoring (e.g., cortisol, oxytocin) should be complemented with periodic psychological assessment, similar to recommendations for

nursing students, where screening is essential for early interventions and approaches that help patients' quality of life (Iorga *et al*., 2017).

CONCLUSIONS

In this study, it was highlighted that bariatric surgery causes significant changes in the hormone profile, especially in terms of cortisol and oxytocin, in the first three months postoperatively. These hormonal changes reflect complex physiological adaptations associated with weight loss and adiposity reduction.

Plasma cortisol levels decreased significantly, indicating a reduction in the activity of the HPA axis. This decrease in cortisol suggest an improvement in the stress response and a possible reduction in systemic inflammation associated with obesity.

Regarding oxytocin, a moderate decrease in plasma levels was observed postoperatively. This reduction may reflect an adaptation of the neuroendocrine system to the new post-surgical physiological conditions and a possible recalibration of appetite and eating behavior regulatory mechanisms.

The correlations identified between weight loss and hormonal changes suggest that body mass loss directly influences hormonal regulation. These findings highlight the importance of monitoring endocrine parameters in the postoperative period to better understand physiological adaptations and optimize intervention strategies.

In conclusion, the results obtained highlight the significant impact of bariatric surgery on hormonal regulation, highlighting the need for an integrated approach that includes endocrine monitoring in the postoperative management of severely obese patients.

CONFLICTS OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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IMPACT OF SIMULATED BRUSHING WITH DENTIFRICES ON ACRYLIC DENTURE BASE RESINS

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IMPACT OF SIMULATED BRUSHING WITH DENTIFRICES ON ACRYLIC DENTURE BASE RESINS (Abstract): Aim of the study: The study aimed to evaluate the impact of simulated brushing with denture cleaning dentifrices on the surface roughness and weight loss of three types of PMMA denture base materials: heat-cured, CAD-CAM milled, and DLP printed. **Materials and Methods:** Ninety standardized samples were fabricated and subjected to brushing simulations using two commercial dentifrices (*Corega PurFrisch, Glaxo Smith Kline and Protefix Higiena, Queisser Pharma*) and distilled water as control. Surface roughness (Ra) and weight were measured at baseline, after 20,000 and 40,000 cycles, simulating up to four years of clinical use. **Results:** showed a progressive increase in surface roughness and a corresponding decrease in weight across all materials. Heat-cured PMMA exhibited the highest resistance to surface changes, with Ra values remaining below 0.4 µm and minor weight loss. DLP printed PMMA demonstrated the greatest susceptibility, with roughness values exceeding 0.56 µm and the most significant mass reduction. CAD-CAM milled samples showed intermediate behavior. The choice of dentifrice had minimal influence compared to the manufacturing method and mechanical action of brushing. **Conclusions:** The findings highlight the role of material processing techniques in determining long-term durability and suggest that brushing remains the primary factor in surface degradation. Clinicians should consider these differences when recommending cleaning methods and selecting denture materials. **Keywords:** POLYMETHYL METHACRYLATE RESIN, DENTURE HYGIENE, DENTURE CLEANING DENTIFRICE, SURFACE ROUGHNESS, WEIGHT LOSS.

INTRODUCTION

Partial or complete edentulism significantly affects aesthetics, speech, chewing, diet and quality of life. Dental prostheses are the standard treatment, with polymethyl methacrylate (PMMA) being the most commonly used denture base resin for over 70 years due to its aesthetics, strength, affordability, and biocompatibility (1-7). Heat-cured PMMA remains widely used, but it has drawbacks such as leaching of residual monomers, which may cause hypersensitivity and compromise material stability through water sorption and shrink-

age (6, 8). These issues have led to the development of improved materials and techniques.

Modern approaches like CAD/CAM and injection molding have enhanced denture fabrication. CAD/CAM dentures, made from pre-polymerized resins, offer better fit, reduced porosity, improved hygiene, good mechanical properties and color stability (9-14). However, challenges include difficulties in recording occlusal relationships, higher material and laboratory costs, and the absence of a trial denture stage (15-17).

Maintaining proper hygiene of PMMA-based dentures is essential for oral health and the long-term performance of the prosthesis. Mechanical cleaning methods, such as manual brushing, ultrasonic baths, and the use of effervescent denture cleansers, are widely recommended due to their effectiveness in reducing plaque and biofilm (18, 19). Additionally, common household agents such as bleach and vinegar offer practical and accessible alternatives for denture cleaning (20, 21). For individuals with limited manual dexterity, particularly elderly patients, denture cleaning dentifrices and antiseptic soaps, present a convenient and effective solution (22-24). However, long-term use of these agents may compromise the material's properties, leading to surface roughness, increased monomer release, and reduced flexural strength.

The aim of this study was to evaluate the effect of brushing with denture cleaning dentifrices on the surface properties of PMMA denture base material. The null hypothesis of the study stated that brushing PMMA denture base material with two different dentifrices does not produce a statistically significant change in surface roughness or weight loss, under simulated conditions equivalent to two years of use in a healthy individual.

MATERIALS AND METHODS

The experimental study was conducted at the Faculty of Dental Medicine, Grigore T. Popa University of Medicine and Pharmacy Iași, in partnership with the Faculty of Materials Science and Engineering, Gheorghe Asachi Technical University of Iași.

A total of 90 resin samples were fabricated using materials suitable for conventional, CAD-CAM subtractive, and additive denture manufacturing (fig. 1).

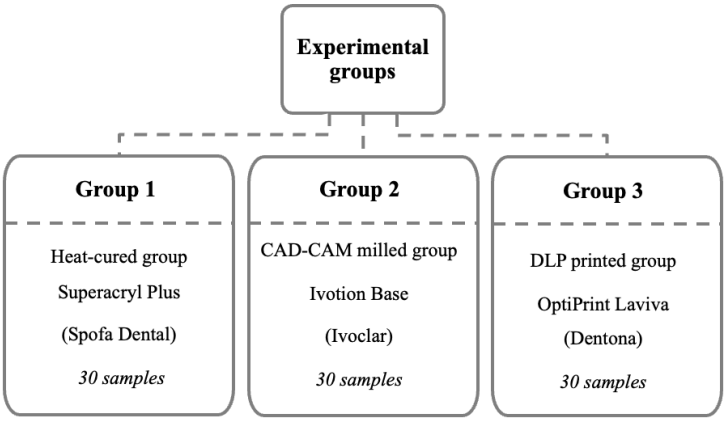

Fig. 1. Experimental groups

The samples were tested using a soft bristle brush (Tribest), and two commercially available denture cleaning dentifrices, Corega PurFrisch (GlaxoSmithKline), Protefix Higiena (Queisser Pharma).

The sample design was created using Autodesk MeshMixer (Autodesk Inc., USA) and featured dimensions of 65 mm in length, 10 mm in width, and 2 mm in thickness (fig. 2).

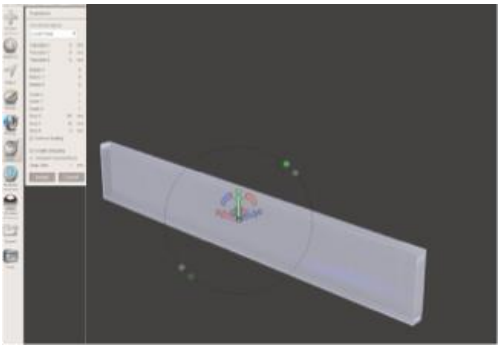

Fig. 2. Digital design of the PMMA samples created in Autodesk MeshMixer

Heat-cured samples were fabricated using Superacryl Plus resin (Spofa Dental), following the manufacturer's guidelines. For the subtractive manufacturing group, a 5-axis dental milling machine (VHF K5 Plus, VHF, Germany) was employed to mill the experimental samples from CAD/CAM denture base resin discs (Ivotion Base, Ivoclar). For the additive manufacturing method the STL files were transferred to a digital light processing (DLP) printer (Asiga MAX, ASIGA, Australia) and printed using OptiPrint Laviva resin (Dentona).

All samples were finished and polished using 1000-grit abrasive paper under continuous water cooling to obtain a smooth, mirror-like surface. After polishing, the samples were immersed in distilled water and maintained at 37 °C for 24 hours.

The brushing simulation in this study was conducted using the ZM-3 Toothbrush Simulator (SD-Mechatronik), which is equipped with three heads designed to accommodate toothbrushes. Three samples were tested simultaneously. The toothbrushes were secured in the simulator using screws located on the sides and top of each head. These screws were adjusted to ensure accurate alignment and leveling of each toothbrush during the simulation.

The brushing simulator was programmed to operate at a frequency of 75 strokes per minute, with each stroke covering a distance of 3.8 cm and applying a vertical load of 0.2 N (200-g vertical load). The testing device provided horizontal and vertical movements. The brushing test was carried out for 20,000 and 40,000 cycles, simulating up to two to four years of prosthesis use under conditions representative of a healthy person.

For each sample, three roughness measurements were recorded after the finishing and polishing process using a contact-type roughness tester, Form Talysurf® (Taylor Hobson, Leicester, England). The arithmetic mean roughness parameter (R_a) was used.

After the brushing procedure, the samples were carefully removed from the simulator and subjected to ultrasonic cleaning with pure water for 10 min twice before the measurements, and their surfaces were dried with warm air.

Each sample was subsequently weighed using an analytical balance with an accuracy of 0.1 mg (Adam, Adam Equipment, UK) in order to determine the material weight.

Data analysis was carried out using IBM *SPSS Statistics* software (version

26.0; IBM Corp., Armonk, NY, USA). Comparisons between groups were made using the Kruskal-Wallis test, with statistical significance determined at a threshold of $p < 0.05$.

RESULTS

Across all materials, surface roughness increased with the number of brushing cycles, confirming that prolonged mechanical action impacts denture base surfaces. However, the extent of this increase was clearly material-dependent. Heat-cured PMMA showed the highest surface stability, with surface roughness rising only slightly from a baseline of 0.366 ± 0.069 µm (distilled water) to 0.398 ± 0.067 µm after 40,000 brushing cycles. Similarly small changes were observed with Corega PurFrisch (from 0.371 ± 0.065 µm to 0.378 ± 0.066 µm) and Protefix Higiena (from 0.369 ± 0.073 µm to 0.376 ± 0.072 µm), suggesting excellent resistance to wear over time. In contrast, DLP printed PMMA exhibited the greatest surface changes, with roughness values increasing substantially across all conditions. The Protefix subgroup showed the highest increase, from 0.495 ± 0.066 µm at baseline to 0.565 ± 0.063 µm at 40,000 cycles—a change of +0.070 µm. Even in distilled water, the DLP group experienced an increase from 0.494 ± 0.067 µm to 0.561 ± 0.063 µm, further emphasizing the material's susceptibility to surface degradation. CAD-CAM milled PMMA demonstrated intermediate behavior. The surface roughness increased from 0.455 ± 0.109 µm (distilled water) to 0.488 ± 0.104 µm, and from 0.462 ± 0.096 µm to 0.470 ± 0.092 µm in the Corega group. While this group was more stable than the DLP-printed samples, it was still more affected than the heat-cured group (tab. I).

TABLE I.

Surface roughness (Ra) of the PMMA study groups immersed in different cleansing agents before and after 20,000 and 40,000 brushing cycle

Study groups	Cleansing agents immersion	Surface roughness (Ra) (µm) Mean ± Std. Deviation		
		Baseline	20,000 Cycles	40,000 Cycles
Heat-cured PMMA	Distilled water N=10	0.366±0.069	0.381±0.068	0.398±0.067
	Corega PurFrisch N=10	0.371±0.065	0.373±0.065	0.378±0.066
	Protefix Higiena N=10	0.3690±0.073	0.3710±0.074	0.376±0.072
	p-value	0.987	0.885	0.701
CAD-CAM milled PMMA	Distilled water N=10	0.455±0.109	0.465±0.108	0.488±0.104
	Corega PurFrisch N=10	0.462±0.096	0.465±0.095	0.470±0.092
	Protefix Higiena N=10	0.459±0.112	0.463±0.101	0.467±0.111
	p-value	0.983	0.999	0.754

Study groups	Cleansing agents immersion	Surface roughness (Ra) (μm) Mean ± Std. Deviation		
		Baseline	20,000 Cycles	40,000 Cycles
DLP printed PMMA	Distilled water N=10	0.494±0.067	0.525±0.063	0.561±0.063
	Corega PurFrisch N=10	0.489±0.061	0.493±0.063	0.523±0.061
	Protefix Higiena N=10	0.495±0.066	0.529±0.062	0.565±0.063
	p-value	0.898	0.485	0.270

Independent-Samples Kruskal-Wallis Test; significant level $p < 0.05$

Over the course of the simulated brushing cycles, all PMMA denture base materials showed a gradual decrease in weight, indicating material loss as a result of mechanical wear. However, the extent of this weight loss varied depending on the type of material and, to a smaller degree, the cleansing agent used. The heat-cured PMMA group started with the highest initial weight, averaging around 8.69 mg across all subgroups. After 40,000 brushing cycles, the samples immersed in distilled water dropped to 8.49 ± 0.276 mg, while those treated with Corega and Protefix fell to 8.395 ± 0.261 mg and 8.359 ± 0.2938 mg, respectively. In comparison, the CAD-CAM milled PMMA group began with a slightly lower initial weight of approximately 8.46 mg. While the weight loss was still moderate, it was more noticeable than in the heat-cured samples. After 40,000 cycles, samples treated with distilled water weighed 8.311 ± 0.038 mg, and those brushed with Corega or Protefix ended at 8.231 ± 0.039 mg and 8.210 ± 0.037 mg, respectively. The most substantial changes were observed in the DLP printed PMMA group. These samples began with the lowest baseline weight (around 8.355 mg) and showed the greatest material loss after brushing. By the end of the 40,000 cycles, weights had decreased to 8.176 ± 0.072 mg in distilled water, 8.125 ± 0.067 mg with Corega, and 8.105 ± 0.064 mg with Protefix (tab. II).

TABLE II.

Weight of PMMA study groups immersed in different cleansing agents before and after 20,000 and 40,000 brushing cycle

Study groups	Cleansing agents immersion	Weight (mg) Mean ± Std. Deviation		
		Baseline	20,000 Cycles	40,000 Cycles
Heat-cured PMMA	Distilled water N=10	8.691±0.280	8.601±0.281	8.491±0.276
	Corega PurFrisch N=10	8.685±0.284	8.565±0.252	8.395±0.261
	Protefix Higiena N=10	8.689±0.269	8.539±0.2896	8.359±0.2938
	p-value	0.988	0.818	0.513

Study groups	Cleansing agents immersion	Weight (mg) Mean ± Std. Deviation		
		Baseline	20,000 Cycles	40,000 Cycles
CAD-CAM milled PMMA	Distilled water N=10	8.462±0.035	8.391±0.032	8.311±0.038
	Corega PurFrisch N=10	8.461±0.033	8.361±0.031	8.231±0.039
	Protefix Higiena N=10	8.460±0.035	8.350±0.036	8.210±0.037
	p-value	0.513	0.024*	0.000*
DLP printed PMMA	Distilled water N=10	8.356±0.017	8.256±0.012	8.176±0.072
	Corega PurFrisch N=10	8.355±0.016	8.235±0.046	8.125±0.067
	Protefix Higiena N=10	8.355±0.013	8.225±0.037	8.105±0.064
	p-value	0.963	0.005*	0.000*

Independent-Samples Kruskal-Wallis Test; significant level $p < 0.05$

DISCUSSION

Maintaining a smooth and clean denture surface is essential for minimizing microbial colonization and biofilm formation, particularly from organisms like *Candida albicans*, which is associated with inflammation, halitosis, and systemic infections (4,25). Denture surfaces can become contaminated during fabrication and clinical use, increasing the risk of cross-infection between patients and clinicians (26-29).

For surface roughness (Ra), the null hypothesis was retained across all material groups-heat-cured, CAD-CAM milled, and DLP printed PMMA-regardless of the cleansing agent used (Corega PurFrisch, Protefix Higiena, or distilled water). Although the type of cleansing agent did influence surface roughness to some extent, its impact was less significant than the manufacturing method or the number of brushing cycles. In most cases, Corega and Protefix produced smaller increases in roughness compared to brushing in distilled water, suggesting that properly formulated denture cleansers may offer a slight protective effect against surface wear. These findings agree with Paranhos *et al*. (30), who emphasized that mechanical action plays a more critical role than cleanser composition in influencing surface degradation of denture materials.

While a slight increase in surface roughness was observed after 20,000 and 40,000 brushing cycles, this effect was consistent across all cleansing agents and within the expected threshold of clinical tolerance. Even after extended brushing, surface roughness values remained below 0.6 µm, which is near the threshold for microbial adhesion but still within clinically acceptable limits (31,32).

Regarding weight loss, the null hypothesis was retained for the heat-cured PMMA group at all time points ($p > 0.05$), indicating no significant influence of the denti-

frice type on material mass loss. However, for CAD-CAM milled and DLP printed PMMA, the null hypothesis was rejected after 20,000 and 40,000 brushing cycles, with p-values < 0.05. This implies that the type of cleansing agent had a statistically significant effect on weight loss in these two material groups, especially after prolonged mechanical wear. The findings align with and expand upon existing literature, emphasizing that both the manufacturing method and the mechanical stresses induced by brushing play pivotal roles in determining material performance. Alfouzan *et al*. (33) conducted a detailed investigation into the effects of brushing and thermal aging on 3D-printed resins, reporting significant increases in surface roughness confirmed through profilometry and scanning electron microscopy (SEM). Similarly, Chang *et al*. (34) reported that while brushing increased roughness and weight loss in both conventional and printed denture base materials, the milled resins showed greater mechanical stability, with material hardness identified as a key factor in abrasion resistance.

The observed differences in material performance can largely be attributed to variations in material composition and polymerization processes. Heat-cured PMMA, which undergoes conventional thermal polymerization, demonstrated the highest stability and resistance to degradation. CAD-CAM milled resins, although pre-polymerized, may be more prone to surface loss due to their relatively brittle structure. DLP printed resins, fabricated using photo-initiated polymerization, typically exhibit lower crosslink density and higher water sorption, making them more vulnerable to wear (6).

In general, mechanical brushing remains an effective method for maintaining denture hygiene; however, it also contributes to the gradual wear of PMMA materials. Over time, the combination of mechanical action and abrasive components in dentifrices—such as silica or calcium carbonate—can lead to deterioration in surface polish and a measurable reduction in denture mass (6). Moreover, surface abrasion is influenced by multiple variables: the abrasiveness of the dentifrice, brush stiffness, brushing force, technique, frequency, and the denture material's mechanical properties (32, 35).

Despite the contributions of this study, several limitations must be noted. First, the in vitro design, while enabling control and reproducibility, does not replicate the complex conditions of the oral cavity, such as temperature changes, salivary enzymes, pH fluctuations, and microbial activity. Secondly, only two denture cleaning dentifrices (Corega PurFrisch and Protefix Higiena) were tested, limiting the applicability of results across the wide range of products available. The brushing brushing parameters were standardized, though in real-life conditions, patient habits vary greatly in technique, force, and frequency, potentially affecting material wear outcomes. Moreover, the study focused solely on surface roughness and weight loss. Important properties such as color stability, hardness, and microbial adhesion were not evaluated.

Future studies should aim to incorporate in vivo elements or simulate the oral environment more realistically through artificial saliva, thermocycling, and microbial challenges. Additionally, expanding the range of dentifrices, incorporating more

diverse material types, and examining biofilm accumulation and patient-centered outcomes would enhance the clinical relevance of the findings.

CONCLUSIONS

The findings of this study demonstrate that brushing with denture cleaning dentifrices leads to a progressive yet controlled increase in surface roughness and a gradual loss of material weight across all PMMA denture base materials tested. Importantly, the differences observed between cleansing agents were minimal, indicating that brushing itself, rather than the chemical composition of the dentifrices, was the primary factor influencing surface degradation.

Among the three types of PMMA materials evaluated, DLP-printed PMMA was the most susceptible to surface wear and weight loss, while heat-cured and CAD-CAM milled PMMA exhibited superior resistance to these changes under identical experimental conditions. This highlights the manufacturing method as a key determinant of the material's mechanical durability and overall performance.

At all-time points, the choice of denture cleanser did not significantly impact surface roughness or weight within each material group, further emphasizing that the mechanical action of brushing was the dominant contributor to wear. Clinically, this suggests that all tested cleansing agents are similarly effective in preserving surface integrity, particularly for heat-cured PMMA.

These results underscore the importance of selecting appropriate materials for denture fabrication and educating patients on gentle but effective cleaning techniques. While regular brushing is essential for maintaining oral hygiene, it can accelerate wear in less durable materials. Therefore, proper hygiene practices combined with the selection of more wear-resistant materials can help reduce microbial colonization, minimize the risk of denture-related infections, and ultimately extend the functional lifespan of prosthetic devices.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests. The research did not receive external funding.

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THE ASSESSMENT OF THE PROTECTIVE EFFECT OF THEOBROMINE, CAFFEINE AND THEOPHYLLINE ON INDUCED-OXIDATIVE STRESS IN ZEBRAFISH INTESTINE

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THE ASSESSMENT OF THE PROTECTIVE EFFECT OF THEOBROMINE, CAFFEINE AND THEOPHYLLINE ON INDUCED-OXIDATIVE STRESS IN ZEBRAFISH INTESTINE (Abstract): Oxidative stress, through the disbalance between oxidant and antioxidant mechanisms, plays an important role in intestinal disorders and dysfunctions. Evidence reported that methylxanthines possess antioxidant and anti-inflammatory properties, but their role in reducing oxidative stress-induced intestinal damage remains poorly understood. **Aim**: In this study, adult zebrafish (*Danio rerio)* were exposed to zinc oxide nanoparticles (ZnO-NPs) to induce oxidative intestinal injury and to evaluate the potential protective effects of methylxanthines (caffeine, theophylline, and theobromine). **Materials and methods:** Adult zebrafish were randomly divided into eight groups: control, oxidative stress-induced (treated with ZnO nanoparticles), oxidative stress and theobromine, oxidative stress and caffeine, oxidative stress and theophylline, theobromine treated group, caffeine treated group, theophylline treated group. After 15 days of exposure, intestinal samples were processed for histopathological evaluation; microscopic images were analyzed for villus, epithelial integrity and goblet cells density. Representative biochemical markers of antioxidant defense-superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx1), and reduced glutathione (GSH) were quantified to assess redox status. **Results:** Histological examination revealed that ZnO-NPs induced intestinal damage, including epithelial disruption, blunt villus shortening and a discrete increase in the number of goblet cells, which were attenuated in oxidative stress methylxanthine-treated groups. The biochemical data supported these findings, showing enhanced antioxidant enzyme activities and restored GSH levels in protected groups. Correlation analysis demonstrated associations between enzymatic antioxidant capacity and histopathological integrity. **Conclusions:** Methylxanthines can differentially modulate the intestinal oxidative stress, providing a basis for future studies on their potential therapeutic or dietary application. Theobromine appears to confer greater preservation of intestinal structure and mucosal balance. **Keywords:** OXIDATIVE STRESS, INTESTINE**,** THEOBROMINE, CAFFEINE, THEOPHYLLINE, ZEBRAFISH.

INTRODUCTION

Evidence shows that due to their high prevalence and impact, gastrointestinal diseases represent a consistent problem for modern medicine. The oxidative stress, generating a high level of reactive oxygen species (ROS), which cannot be countered by antioxidant mechanisms, is deeply in-

volved in the pathogenesis of a large number of intestinal diseases, including colorectal cancers, inflammatory bowel disease, diverse metabolic disorders and enteric infections (1, 2). To date, current therapies to these conditions remain ineffective in restoring normal intestinal integrity and physiological functions. Understanding the correlation between oxidative stress and antioxidant defense mechanisms requires a complex process, based on the selection of an appropriate biological model, a distinct target tissue, and specific bioactive compounds (3). Based on this reasoning, our study was designed to explore how and if methylxanthines influence oxidative balance in zebrafish intestinal tissue, reflected in the histopathological analysis of intestinal morphology (4). The motivation for our investigation started from the limited existing research about the potential of methylxanthines to modulate intestinal oxidative stress. Our previous biochemical investigation demonstrated that ZnO-NP exposure in adult zebrafish disrupts antioxidant defenses and elevates oxidative stress markers, while methylxanthines such as theobromine, caffeine, and theophylline exhibit notable antioxidant activity. Building on these findings, the present study evaluates the histopathological changes in zebrafish intestine following ZnO-NP exposure and assesses the protective effects of these methylxanthines. In this context, we intended to find an answer for three main questions related to oxidative stress: why focus on intestine, why use zebrafish and why investigate methylxanthines (5). This approach integrates three key perspectives already mentioned: the intestine as a primary site of absorption for most bioactive compounds and presenting oxidative vulnerability, the zebrafish as an extremely relevant model for redox biology, and methylxanthines as widely consumed bioactive molecules with both beneficial and adverse gastrointestinal effects (6). Together, these elements provide complex information to assess the potential protective role of methylxanthines against intestinal oxidative stress.

MATERIAL AND METHODS

Study design. The experimental design and exposure protocol were identical to those used in a companion study focusing on biochemical antioxidant parameters, submitted separately for publication. In brief, adult zebrafish (*Danio rerio)* (6 months old, both sexes) were acclimated for two weeks before being randomly distributed into eight groups (n = 15 per group, in triplicate): Experimental groups were exposed for 15 days to zinc oxide nanoparticles (ZnO-NPs; 0.69 mg/L) alone or in combination with methylxanthines (caffeine, theophylline, or theobromine; 50 mg/L each). Additional groups received methylxanthines only under the same conditions.

Histological processing. At the end of the exposure period, zebrafish were euthanized by gradual cooling in ice-chilled water, following AVMA guidelines (2020). Intestinal samples were fixed in 10% neutral buffered formalin for 24 h, dehydrated, cleared in xylene and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E) for morphological examination. The slides were examined using a light microscope (Zeiss Axiolab 5), and representative images were captured with a digital camera system (Axiocam 208 color camera). Histopathological evaluation included qualitative assessment of structural alterations including epithelial degeneration, adipocyte distribution, and villus disorganization, and quantitative,

based on goblet cells count.

Biochemical Data. The activities of SOD, CAT, and GPx1, along with reduced GSH levels, were performed as part of the companion biochemical study submitted separately. The resulting data were used here to establish correlations with intestinal histopathological changes.

Statistical Analysis. Data were collected and subjected to analysis using statistical analysis as Spearman's rank correlation, Principal Component Analysis (PCA) and multiple linear regressions. The normality of distributions within the groups was verified for each variable across groups using the Shapiro-Wilk test ($p > 0.05$ for all parameters).

Ethics. The study design and protocol were approved by the Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy Iasi, Romania (No. 325/15.06.2023).

RESULTS

The intestinal morphology of zebrafish from the control (fig. 1A), theobromine (fig. 1F), caffeine (fig. 1G), and theophylline groups (fig. 1H) presented a normal morphology, with a mucosal lining presenting well-organized villi and intact epithelial linings, in which goblet cells are normally distributed, indicating that the tested methylxanthine doses did not exert any structural alterations. In contrast, the histological evaluation of zebrafish intestines revealed that oxidative stress induced marked structural alterations, including epithelial degeneration, villi appeared blunt, fused or eroded, without the normal regular disposal, lamina propria is enlarged, with inflammatory cell infiltration, and there is a relatively moderate increase number of goblet cells, consistent with their hyperplasia and hypertrophy (fig. 1B).

These lesions are characteristic of oxidative damage in intestinal tissues, where excessive production of ROS compromises mucosal defense, fact that will affect mucosal morphology, disrupting the intestinal barrier.

Among the oxidative stress-treated groups, co-treatment with theobromine resulted in substantially milder histopathological alterations; (fig. 1C). Although, the villi remain irregularly distributed, the intestinal epithelium in the OS + theobromine group exhibited improved structural preservation and a more balanced goblet cell morphology, compared with the OS + caffeine (fig. 1D) and OS + theophylline groups (fig. 1E).

ZnO-NP exposure induced oxidative stress, reflected by increased GPX1, SOD and CAT levels with concurrent GSH depletion, this biochemical imbalance being followed by a moderate rise in goblet cell number, suggesting an acute mucosal defense reaction (tab. I).

However, Spearman correlation analysis (fig. 2) revealed no significant associations between the number of intestinal goblet cells and the biochemical oxidative stress markers (GPX1, CAT, SOD, and GSH).

Although weak positive trends were observed with and CAT ($r_s = 0.57$), these did not reach statistical significance ($p > 0.05$), indicating that the epithelial response was not directly coupled with the magnitude of enzymatic antioxidant modulation.

Principal Component Analysis (PCA) was applied to the normalized group means to explore covariation patterns among oxidative stress biomarkers and the histological parameter (number of goblet cells). The first two components (F1 and F2) explained 72.18% of the total variance (42.84% and 29.33%, respectively), indicating a good model fit for biological interpretation (fig. 3).

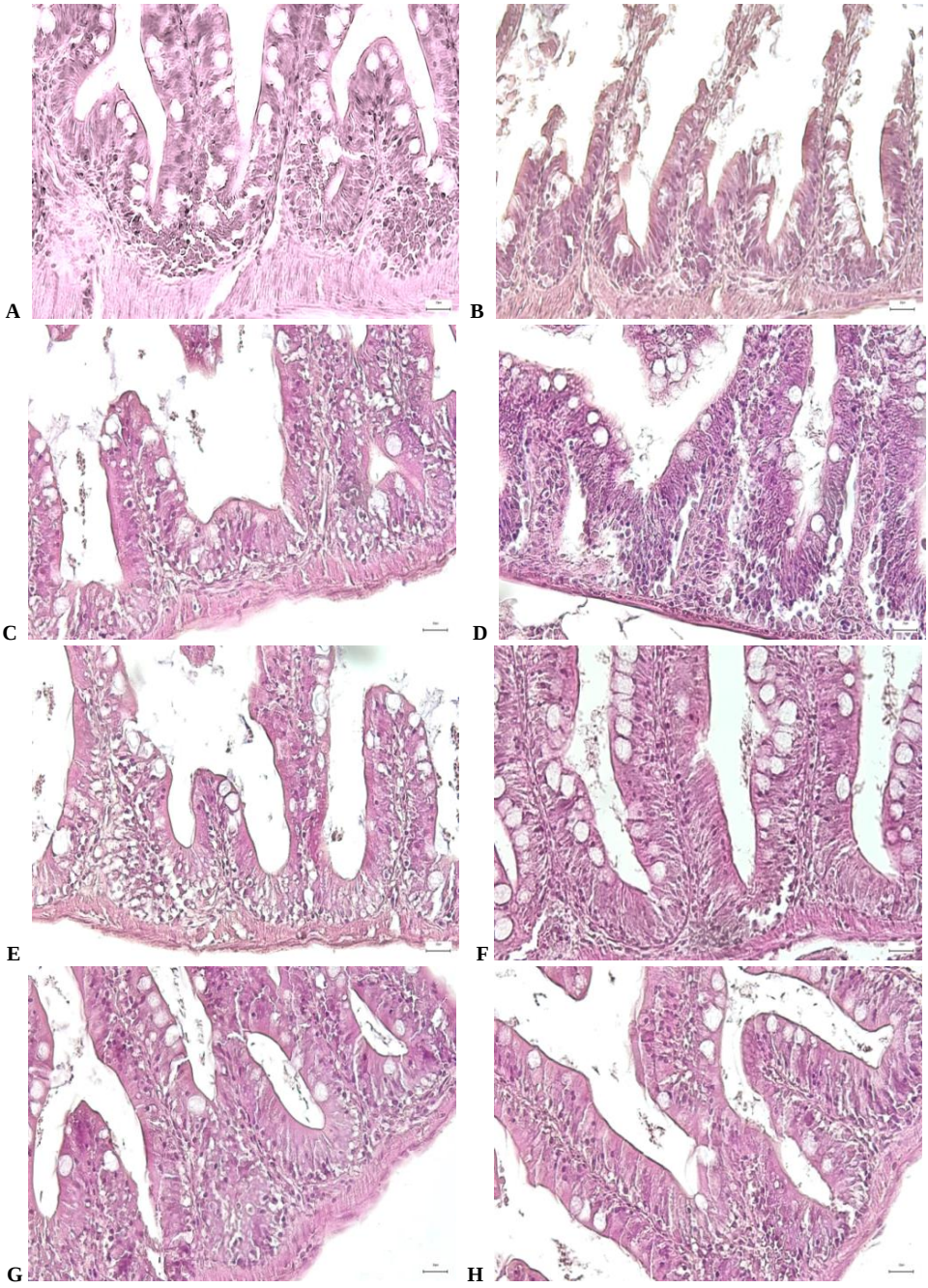

Fig. 1. Zebrafish intestine. A. Control; B. Oxidative stress; C. Oxidative stress and theobromine; D. Oxidative stress and caffeine; E. Oxidative stress and theophylline; F. Theobromine; G. Caffeine; H. Theophylline (Col. H&E, scale bar=20 μm)

TABLE I.

Mean ± SD or median [IQR] values of oxidative stress biomarkers and intestinal goblet cell counts in zebrafish

Group	GPX1 pg/g	CAT pg/g	SOD ng/g	GSH µg/g	No. of goblet cells
L1 control	84.270 (79.45-86.19)	38.255±10.43	0.122 (0.10-0.13)	2.900 (2.80-2.98)	8.63±1.91
L2 (ZnO-NPs)	142.24 (112.64-163.67)	100.756±19.43	1.090 (0.93-1.24)	1.200 (0.96-1.43)	11.08±1.69
L3 (ZnO-NPs+ caffeine)	65.240 (59.28-73.54)	71.173±19.06	0.490 (0.38-0.61)	1.670 (1.11-1.80)	8.38±1.95
L4 (ZnO-NPs+ theophylline)	79.420 (69.01-88.57)	82.082±21.46	0.650 (0.47-0.76)	2.000 (1.70-2.43)	8.29±2.05
L5 (ZnO-NPs+ theobromine)	25.030 (23.14-37.96)	76.012±20.37	0.568 (0.31-0.95)	3.040 (2.47-4.70)	8.33±2.01
L6 (caffeine)	67.435 (54.20-80.72)	90.760±19.27	0.820 (0.41-1.07)	2.118 (1.99-2.98)	9.00±2.00
L7 (theophylline)	51.070 (41.40-59.52)	78.551±20.52	0.570 (0.17-0.61)	3.070 (2.39-3.95)	9.33±1.79
L8 (theobromine)	52.340 (47.89-65.05)	76.455±14.55	0.470 (0.27-0.54)	3.170 (2.57-4.08)	9.50±1.69

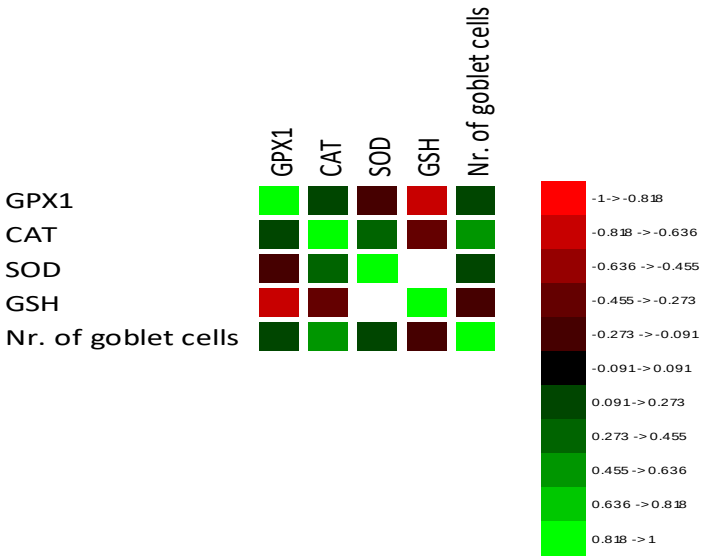

Fig. 2. Correlation matrix.

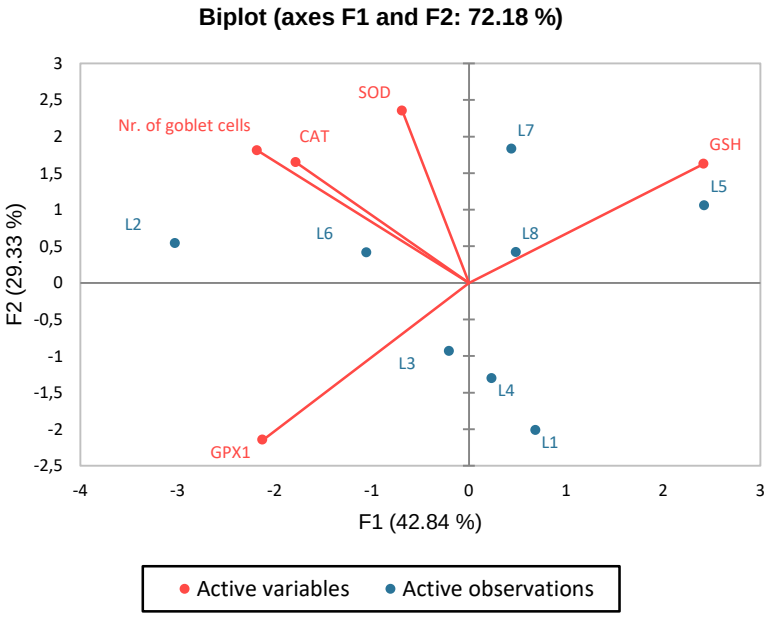

Fig. 3. PCA analysis for biochemical parameters and number of goblet cells

Axis F1 primarily opposed GPX1 (negative projection) to GSH (positive projection), describing a redox gradient between intense oxidative stress characterized by peroxidase hyperactivity and depleted glutathione, and a restored redox state defined by elevated GSH and reduced GPX1 activity. Axis F2 was defined mainly by positive loadings of CAT, SOD, and the number of goblet cells, indicating an adaptive enzymatic response associated with epithelial protection. Among experimental groups, the ZnO-NPs group was positioned in the negative region of F1, close to GPX1, confirming a strong oxidative stress profile with limited epithelial adaptation. The co-exposure groups occupied an intermediate position, due to a partial restoration of redox balance under the influence of methylxanthines. Notably, ZnO-NPs + theobromine group was projected near GSH and goblet cells, consistent with improved antioxidant status and epithelial recovery.

The methylxanthine-only groups were located in the upper-right quadrant, close to CAT, SOD, and goblet cells, reflecting a moderate enzymatic activation and maintenance of GSH levels, typical of adaptive, non-stressful antioxidant stimulation.

PCA revealed that the number of intestinal goblet cells clustered with the antioxidant enzymes CAT and SOD, indicating a coordinated adaptive response involving both enzymatic defense and epithelial protection. Conversely, the number of goblet cells were positioned opposite to GPX1, reflecting the harmful impact of severe oxidative stress on mucosal integrity. Their proximity to GSH show that restoration of intracellular thiol balance parallels epithelial regeneration. Thus, methylxanthine exposure, particularly with theobromine, shifted the epithelial profile toward a protective and secretory phenotype, mitigating

the histopathological effects of ZnO-NPs.

A multiple linear regression model was constructed using the number of intestinal goblet cells as the dependent variable and GPX1, CAT, SOD, and GSH as explanatory variables. The model showed a high coefficient of determination (R^2 = 0.872; adjusted R^2 = 0.701), indicating that approximately 70% of the variability in goblet cell number could be explained by the biochemical parameters. The residuals followed a normal distribution (Shapiro-Wilk p = 0.783), confirming the validity of the model assumptions (fig. 4).

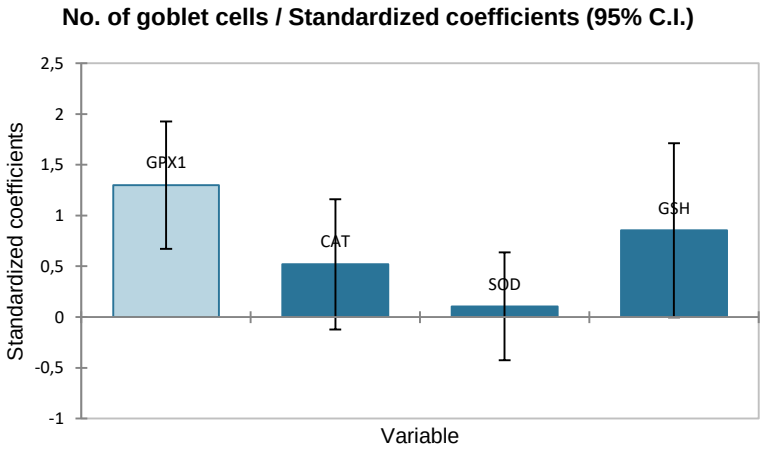

Fig. 4. Multiple linear regression model showing standardized coefficients (β ± 95% CI) for oxidative stress biomarkers (GPX1, CAT, SOD, GSH) as predictors of intestinal goblet cell number in zebrafish.

The standardized coefficients revealed differentiated contributions of oxidative markers to epithelial alterations. GPX1 (β=1.299, p=0.007) and GSH (β=0.854, p=0.051) showed positive associations with goblet cell number, whereas CAT and SOD displayed weaker, non-significant effects (p>0.05). Among the oxidative biomarkers included in the model, GPX1 emerged as the strongest predictor of goblet cell number, followed by GSH. These findings are consistent with PCA, which positioned goblet cells near CAT/SOD and apart from GPX1, indicating that mucosal hypersecretion occurs both as an irritative response under strong oxidative load and as an adaptive response when redox balance is restored.

DISCUSSION

The particular susceptibility of the intestine to oxidative stress presents major challenges, as it contributes to the development of numerous complex diseases with poorly and only partially understood mechanisms, limiting the effectiveness of current therapeutic approaches. Prolonged exposure to oxidative stress has been linked to alterations of intestinal mucosa, affecting the heterogenous cell population of intestine, by directly damaging the enterocytes and the goblet cells, changes that

are often reflected by a reduction in villus height and loss of epithelial integrity (1).

Zebrafish models are increasingly used to decipher the implications of oxidative stress in intestine, providing an advantage over other experimental models, by facilitating an efficient translation of results in humans (7). Because zebrafish oxidative qualitative mechanisms - such as ROS production, lipid peroxidation or apoptotic signaling - are largely comparable with those in humans, zebrafish models represent a potential translational system, generating reproducible data that enhance our understanding of oxidative stress mechanisms, with potential implications for human health and therapeutic development.

Exposure to ZnO Nps triggers a sequence of mechanisms that interact and influence the morphology of the intestine. The excess ROS, through a specific oxidative induction, subjects enterocytes to a specific apoptotic process, which can be able to generate changes in villus length and morphology (8). On the one hand, ZnO will promote inflammation and the presence of activated immune cells will increase tissue injury. On the other hand, studies reported another major impediment in restoring the integrity of intestinal barrier, namely a strong inhibition of intestinal stem cells proliferation, who will no longer be able to replace the nonfunctional epithelial cells (9). Each time it should be noted that these effects are strongly dependent on dose, exposure route and exposure time.

Methylxanthines, a class of naturally occurring alkaloids that include caffeine, theobromine, and theophylline, are widely consumed through beverages and foods such as coffee, tea, and cocoa (10). These compounds exhibit various bioactive properties, including antioxidant, anti-inflammatory, and neuroprotective effects (11). While numerous studies have explored the impact of methylxanthines on oxidative stress in respiratory or nervous or cardiovascular systems (12), limited data exist regarding their influence on intestinal oxidative damage in zebrafish (*Danio rerio*), a rapidly emerging experimental model in gastrointestinal research.

One may question why research on methylxanthines has not advanced further in the field of gastrointestinal pathology. In this context, we have to consider that their primary molecular targets are more functionally relevant to the nervous and respiratory systems, (where they are already extensively applied), whereas their gastrointestinal effects are broad, nonspecific, and difficult to interpret or translate into therapeutic applications (13).

Although methylxanthines have traditionally been explored for their effects on the nervous and respiratory systems, we consider that their potential relevance to intestinal oxidative stress deserves renewed attention. With both direct and indirect antioxidant properties, acting as free radical scavengers while also modulating endogenous defense mechanisms such as SOD, CAT, and GPx activity. In addition, methylxanthines influence adenosine receptor signaling, a key pathway in the regulation of intestinal inflammation and oxidative injury, particularly under hypoxic or ischemic conditions (14).

Their phosphodiesterase-inhibiting activity has a significant role to maintain epithelial protection by maintaining intracellular cAMP levels, stimulating barrier integrity, and decreasing ROS generation. Because they have well-established pharmacological profiles, accessibility, and systemic antioxidant effects, methylxan-

thines may be regarded as promising but insufficiently investigated agents against oxidative stress-mediated intestinal injury. Further research is needed to define their molecular interactions in the intestinal microenvironment and to explore their possible role as adjuvant therapies, in oxidative or inflammatory gastrointestinal disorders. (15)

The histological evaluation of zebrafish intestine in our study revealed that oxidative stress induced specific structural alterations, including epithelial degeneration, villus shortening, and inflammatory cell infiltration (16, 17). These lesions are characteristic of oxidative damage in intestinal tissues, where excessive production of reactive oxygen species (ROS) disrupts epithelial integrity and compromises mucosal defense. In contrast, the intestinal morphology of zebrafish from the control, theobromine, caffeine, and theophylline groups remained normal, with well-organized villi and intact epithelial linings, indicating that the tested methylxanthine doses did not exert injuries of the intestinal wall, which is very important in determining their precise role under normal conditions (18).

When ZnO-NPs are added to tank water, zebrafish constantly ingest water, so the intestinal mucosa receives a direct dose of ZnO-NPs, which accumulate in the intestine. Although some studies report a decrease in goblet cell density following ZnO-NP exposure, our findings revealed a relatively discrete increase after 15 days of treatment. We consider that this fact suggests a compensatory mechanism that enables the intestinal mucosa to adapt the oxidative stress resulting from NPs accumulation. Goblet cell differentiation, with mucus hypersecretion could be considered as transient response to ZnO-Np induced alterations, which helps preserve the integrity of intestinal barrier, supported by the protective mucus coating (18, 19). With higher doses or prolonged exposures, depletion of secretory cells become evident, impairing intestinal epithelium integrity and is followed by the goblet cell loss, as in severe chronic exposure, reported in other studies (20, 21).

Co-treatment with theobromine attenuated these histological changes, resulting in improved epithelial preservation and a more balanced goblet cell distribution compared with the oxidative stress + caffeine and oxidative stress + theophylline groups. These findings suggest that theobromine provides a stronger protective influence against oxidative stress-induced mucosal injury, possibly through its antioxidant and anti-inflammatory actions, including ROS scavenging and effect on cell membranes (22).

The statistical analyses provided additional insight into the relationship between oxidative stress biomarkers and intestinal epithelial alterations. Although ZnO-NP exposure produced a pronounced biochemical imbalance - characterized by elevated GPX1, CAT, and SOD levels with concurrent GSH depletion - Spearman's rank correlation revealed no significant linear associations between oxidative stress markers and goblet cell counts. The weak positive trend observed with CAT (r_s = 0.57, $p > 0.05$) indicates that epithelial secretory adaptation may not be directly proportional to enzymatic modulation but rather reflects a complex, nonlinear tissue response to oxidative injury.

Principal Component Analysis (PCA) provided additional insight, explaining 72.18% of the total variance and distinguishing clear redox and epithelial patterns.

The first component (F1) opposed GPX1 and GSH, describing a transition from oxidative overload toward restored redox balance. The second component (F2), defined mainly by CAT, SOD, and goblet cells, indicated an adaptive enzymatic-epithelial response. The ZnO-NP group clustered with GPX1, confirming a strong oxidative profile, while theobromine co-treatment was projected near GSH and goblet cells, reflecting partial restoration of thiol balance and epithelial recovery. Also is consistent with histological observations of reduced epithelial damage. The methylxanthine-only groups were associated with moderate antioxidant activation and preserved mucosal integrity.

The multiple linear regression model confirmed these multivariate patterns, showing that oxidative biomarkers collectively explained approximately 70% of the variability in goblet cell number (R^2 = 0.872; adjusted R^2 = 0.701). GPX1 emerged as the strongest independent predictor (β = 1.299, p = 0.007), followed by GSH (β = 0.854, p = 0.051), while CAT and SOD displayed weaker, non-significant effects. These relationships indicate that the goblet cell hyperplasia may occur as a result of two factors: an irritative reaction under intense oxidative condition and as an adaptive process, accompanying the restoration of redox homeostasis (23). Together, these multivariate analyses support the hypothesis that theobromine modulates oxidative-epithelial interactions more efficiently than caffeine or theophylline, promoting epithelial recovery through a balanced antioxidant response.

CONCLUSIONS

Collectively, the histological and statistical data demonstrate that although all three methylxanthines share a similar chemical structure, their biological effects under oxidative stress are different. Theobromine exhibits the most effective modulation of oxidative-epithelial interactions, promoting intestinal protection through balanced antioxidant responses and maintenance of epithelial integrity. When interpreted alongside the biochemical data (SOD, CAT, and GPx), these histological findings support the potential of theobromine as a milder, but biologically active methylxanthine, providing a better protection against oxidative damages, compared with caffeine and theophylline, with significant implications for managing oxidative stress-related intestinal injuries.

CONFLICT OF INTEREST AND FUNDING

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MOTIVATION AND DECISION-MAKING FACTORS IN CHOOSING BARIATRIC SURGERY. A PROSPECTIVE STUDY

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MOTIVATION AND DECISION-MAKING FACTORS IN CHOOSING BARIATRIC SURGERY. A PROSPECTIVE STUDY (Abstract): Understanding patients' reasons for undergoing bariatric surgery and their expectations regarding their next surgery it is important to provide best clinical practice and reduce unrealistic expectations. **Materials and methods**: The present study represents a prospective cohort, which included 65 patients undergoing bariatric surgery at the Iași Bariatric Surgery Center, Romania, between April 2023 and April 2024. The preoperative evaluation included the analysis of the reasons why the patients opted for surgery, as well as the collection of anthropometric, socio-professional data and associated comorbidities. The postoperative evaluation aimed at weight loss and the evolution of the results obtained following the surgery. **Results**: The mean change in body mass index (BMI) before and after surgery was 14.42 kg/m². At 1 year after surgery, the mean percentage of excess weight loss (%EWL) was 66.04%. Before surgery, the most frequently reported reason for requesting surgery was weight loss (47.9%), and this was maintained postoperatively, followed by improved self-esteem and reduced comorbidities. **Conclusions:** The main reason for seeking bariatric surgery was weight loss. However, despite significant weight loss, self-esteem was the element that generated the greatest satisfaction after surgery. Our findings may be useful in the clinical context, when patients are informed before surgery about what to expect, as well as when meeting with a patient after surgery to discuss aftercare. **Keywords:** OBESITY, BARIATRIC SURGERY, EXPECTATION, WEIGHT LOSS.

INTRODUCTION

Obesity has become one of the greatest global public health challenges of the 21st century. Obesity has reached epidemic proportions worldwide, with over 1 billion people classified as obese in 2022, representing 13% of the global population (1,2,3).

Bariatric surgery is currently considered the most effective long-term treatment for morbid obesity when lifestyle interventions fail to achieve healthy and sustained weight loss (4).

Bariatric surgery is divided into two classes: restrictive procedures (e.g., sleeve gastrectomy) and gastrointestinal bypass procedures (e.g., Roux-en-Y gastric bypass). Both restrictive and gastrointestinal bypass procedures produce significantly greater weight loss and more profound improvements in glucose tolerance than medical treatments (5).

Bariatric surgery improves morbidities such as diabetes and cardiovascular disease

and reduces the risk of developing cancer (6, 7).

It is associated with a low risk of complications and has been shown to be more effective than conservative treatment for long-term weight loss and control of type 2 diabetes (8).

Although bariatric surgery is typically associated with significant overall weight loss (e.g., 30%–50% of excess weight within the first 6 months and 71% of excess weight at 1 year after surgery), a reduction in medical and psychological comorbidities, and an improved quality of life. Insufficient weight loss (<50% excess weight loss) is also the most common reason for performing additional bariatric surgery (9).

Although bariatric surgery is recognized as a safe and successful treatment for obesity despite good results in terms of weight loss after surgery, patients are not always satisfied due to unrealistic expectations (10, 11).

Patients undergoing nonsurgical weight loss treatments have similar unrealistic expectations, but some studies indicate that high expectations lead to greater weight loss (12, 13).

Previous studies exploring patients' expectations of bariatric surgery have focused primarily on weight loss and, to some extent, on quality of life. Preoperative reasons for seeking bariatric surgery have been explored to some extent, but no prospective follow-up has been conducted after surgery (14, 15).

This study aims to explore the main reasons for seeking bariatric surgery and to further investigate which aspects patients report as most satisfying after surgery. In addition, we aimed to study patients' perceived satisfaction with the surgical outcome and the association with postoperative weight loss and quality of life. Our hypotheses are that, regardless of patient-related factors, weight loss remains the most important reason for seeking surgery, and the degree of satisfaction with the postoperative outcome depends on the amount of weight loss.

MATERIALS AND METHODS

We performed a prospective analysis of data collected in a dedicated, anonymized bariatric database of patients who underwent bariatric surgery between April 2023 and April 2024, at our Reference Center for Bariatric and Metabolic Surgery, Sf. Spiridon County Emergency Hospital Iași, Romania.

Thus, 83 obese patients were accepted for laparoscopic sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB) procedures and were invited to participate in the study. Preoperative patient evaluation included laboratory tests, upper gastrointestinal endoscopy, abdominal ultrasound, chest radiography, as well as nutritional, cardiological, endocrinological, psychological, anesthesiologic, and pneumological (sleep apnea test) assessments. All patients in whom *Helicobacter pylori* infection was detected endoscopically underwent bacterial eradication treatment before surgery.

The inclusion criteria were to qualify for bariatric surgery, according to national guidelines (a BMI > 40 kg/m^2 or a BMI > 35 kg/m^2 and comorbidities, e.g. diabetes). The exclusion criteria were failure to attend the 12-month follow-up, therefore 65 patients remained in the study.

Other exclusion criteria were age under 18 years, inability to read or understand Romanian language, severe decompensated hepatic and renal disease, severe decom-

pensated cardiovascular pathology, uncontrolled psychiatric disease. Informed consent was obtained from all participants before the start of the study. The study was approved by the local ethics committee.

A questionnaire designed specifically for this study was distributed to patients before surgery and at the 12-month follow-up after surgery. A questionnaire designed specifically for this study was distributed to patients before surgery and at 12-month follow-up. It included questions in the form of reasons for seeking bariatric surgery. Participants in the study were asked to rate 14 items on a Likert scale from 1 (not important) to 5 (very important). The items were weight loss, taking less medication, improvement in comorbidities, having less pain, chances of being employed, improvements in social life, expectations to live longer, improved intimacy, improved self-esteem, improved fertility, improved ability to perform sports, having smaller cloth size, improved psychiatric health, and improved work performance. Participants were also asked to rate their top three reasons for seeking surgery, or their top three satisfaction items.

It was initially developed in English and subsequently translated and in Romanian by the two independent certified translators and the questionnaire were finalized and distributed to the patients in printed form.

In the questionnaire, participants were asked to self-report their age, height, weight, marital status, children, smoking, current goal, level of education, comorbidities and medication use. BMI (kg/m^2) was calculated based on reported height and weight.

Statistical Analysis. Categorical baseline characteristics and comorbidities were compared between surgery types using Pearson's chi-square test; Fisher's exact test was used when expected cell counts were <5. For the occupation variable (3-category distribution), a permutation-based chi-square test was applied due to sparse expected counts. Changes in binary questionnaire items between baseline and 12-month follow-up were assessed using McNemar's test (exact for small numbers of discordant pairs; otherwise asymptotic with continuity correction).

RESULTS

Statistical analysis. The study included a total of 65 patients who completed the preoperative questionnaire and had complete data at all preoperative and postoperative assessments. Of the total participants, 53 (81.5%) were female and 12 (18.5%) were male (fig. 1), indicating a clear predominance of female patients in the analyzed cohort (tab. I). Demographic characteristics

The mean age at the time of the preoperative assessment was 40.5 years. A total of 27 patients (41.5%) were under 40 years of age, while the majority of participants were over 40 years of age. Regarding family status, 36 patients (55.4%) had children and 29 (44.6%) did not have children. Regarding smoking, 23 patients (35.4%) were smokers, while 42 (64.6%) declared that they did not smoke.

Socio-professional characteristics. The analysis of occupational status showed that 30 patients (46.2%) were employed, 30 (46.2%) were unemployed, and 5 patients (7.7%) were retired. The educational level was relatively balanced: 33 patients (50.8%) had university education, and 32 (49.3%) had no university education. A total of 27 patients (41.5%) were under chronic drug treatment at the time of the

preoperative evaluation. Anthropometric characteristics

The preoperative body mass index (BMI) had a mean value of 43.39 kg/m², indicating severe obesity. The majority of patients, 42 (64.6%), had a BMI > 40 kg/m², while 23 (35.4%) had a BMI < 40 kg/m² at baseline. At 12 months after surgery, the mean BMI decreased significantly to 28.9 kg/m², reflecting a substantial improvement in weight status. The mean decrease in BMI between preoperative and postoperative periods was 14.42 kg/m². Also, the mean percentage of excess weight lost (%EWL) at 12 months was 66.04%, indicating a favorable surgical outcome.

Associated comorbidities. The main comorbidities reported preoperatively were: hepatic steatosis, dyslipidemia, hepatomegaly, arterial hypertension, obstructive sleep apnea syndrome (fig. 2). These results confirm the high prevalence of metabolic and cardiovascular diseases associated with severe obesity in the studied population.

Type of surgery. The types of bariatric surgery performed are shown in figure 3. (SG) was the most frequently used procedure, performed in 64.6% of patients, followed by (RYGB) in 35.4% of patients.

TABLE I.

Characteristics of the study population

Characteristic	Total (n=65)	RYGB (n=23)	SG (n=42)	p value
Age (years): <40	27 (41.5%)	7 (30.4%)	20 (47.6%)	0.179
Age (years): ≥40	38 (58.5%)	16 (69.6%)	22 (52.4%)	
BMI (kg/m²): <40	23 (35.4%)	6 (26.1%)	17 (40.5%)	0.246
BMI (kg/m²): ≥40	42 (64.6%)	17 (73.9%)	25 (59.5%)	
Children: Yes	36 (55.4%)	15 (65.2%)	21 (50.0%)	0.238
Children: No	29 (44.6%)	8 (34.8%)	21 (50.0%)	
Smoking: Yes	23 (35.4%)	5 (21.7%)	18 (42.9%)	0.089
Smoking: No	42 (64.6%)	18 (78.3%)	24 (57.1%)	
Occupation: Working	30 (46.2%)	14 (60.9%)	16 (38.1%)	0.044
Occupation: Retired	5 (7.7%)	3 (13.0%)	2 (4.8%)	
Occupation: Unemployed	30 (46.2%)	6 (26.1%)	24 (57.1%)	
Education: University degree	33 (50.8%)	15 (65.2%)	18 (42.9%)	0.085
Education: No university degree	32 (49.2%)	8 (34.8%)	24 (57.1%)	

Baseline characteristics were broadly comparable between surgery groups. No statistically significant differences were observed for age category, BMI category, parental status, smoking status, or education level (all p>0.05). Occupational status differed between groups (p=0.044), with a higher proportion of employed patients among the RYGB group and a higher proportion of unemployed patients among the SG group.

Overall, the most prevalent comorbidities at baseline were hepatic steatosis (83.1%) and dyslipidemia (70.8%), followed by

hepatomegaly (49.2%), sleep apnea (40.0%), and arterial hypertension (26.2%) (tab II). Sleep apnea was more frequent in the RYGB group compared with the sleeve gastrectomy group (56.5% *vs.* 31.0%, p=0.044), and hepatomegaly was also more frequent in the RYGB group (73.9% *vs.* 35.7%, p=0.003). Hepatic steatosis showed a non-significant trend toward higher prevalence in the RYGB group (95.7% *vs.* 76.2%, p=0.080), while dyslipidemia and arterial hypertension did not differ significantly between surgery types (p=0.680 and p=0.241, respectively).

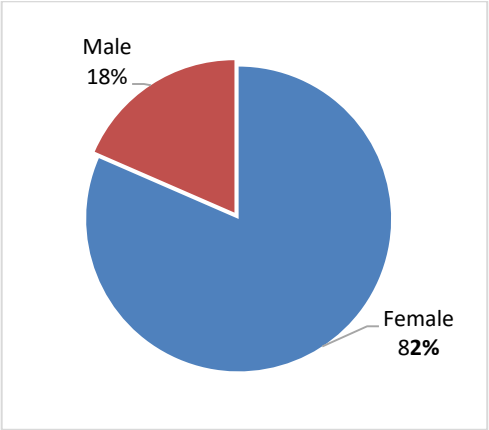

Fig. 1. Sex distribution of sample patients

TABLE II.
Distribution of comorbidities

Comorbidity	RYGB (n=23)	SG (n=42)	p value
Dyslipidemia	17 (73.9%)	29 (69.0%)	0.68
Sleep apnea	13 (56.5%)	13 (31.0%)	0.044
Arterial hypertension	8 (34.8%)	9 (21.4%)	0.241
Hepatic steatosis	22 (95.7%)	32 (76.2%)	0.08
Hepatomegaly	17 (73.9%)	15 (35.7%)	0.003

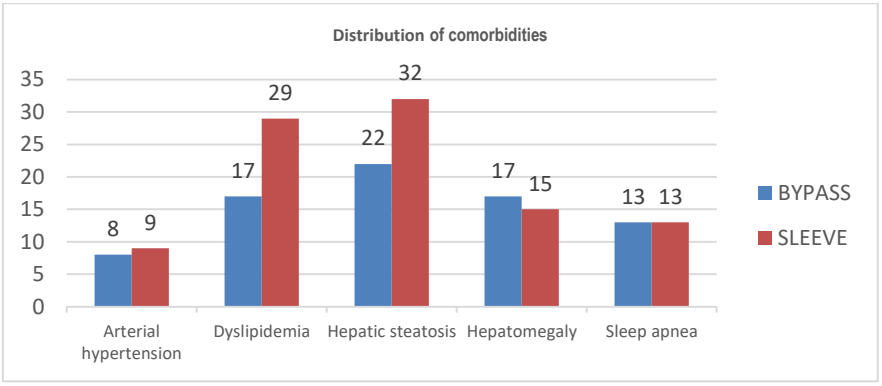

Fig. 2. Distribution of most common comorbidities in sample patients

The three most common reasons (fig. 3) for choosing bariatric surgery were weight loss (73.8%), followed by improved social life (50.8%) and improved self-esteem (43.1%). At 12 months, the most frequently endorsed items were weight loss (87.7%), improved self-esteem (63.1%), and improved co-morbidity (55.4%).

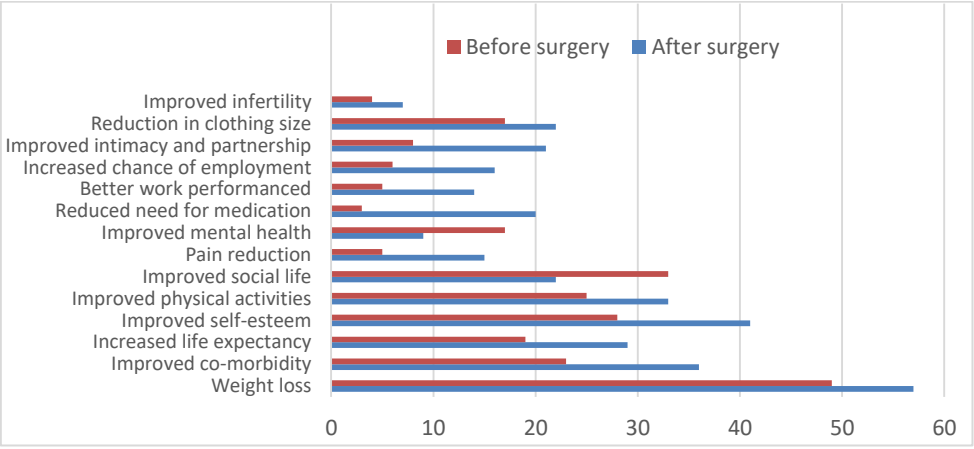

Fig. 3. The most important reason to seek bariatric surgery and items of greatest importance for satisfaction 12-month follow-up

TABLE III.

The most important reason to seek bariatric surgery

Reason	Before surgery	After surgery	p value
Weight loss	48 (73.8%)	57 (87.7%)	0.004
Improved co-morbidity	23 (35.4%)	36 (55.4%)	<0.001
Increased life expectancy	19 (29.2%)	29 (44.6%)	0.021
Improved self-esteem	28 (43.1%)	41 (63.1%)	0.031
Improved physical activity	25 (38.5%)	33 (50.8%)	0.077
Improved social life	33 (50.8%)	22 (33.8%)	<0.001
Pain reduction	5 (7.7%)	13 (20.0%)	0.021
Improved mental health	17 (26.2%)	9 (13.8%)	0.134
Reduced need for medication	2 (3.1%)	20 (30.8%)	<0.001
Better work performance	5 (7.7%)	12 (18.5%)	0.118
Increased chance of employment	6 (9.2%)	16 (24.6%)	0.021
Improved intimacy and partnership	8 (12.3%)	21 (32.3%)	0.002
Reduction in clothing size	14 (21.5%)	23 (35.4%)	0.110
Improved fertility	5 (7.7%)	7 (10.8%)	0.688

From baseline to 12-month follow-up, several motivations / satisfaction items changed significantly (tab. III). The proportion endorsing weight loss increased (73.8% to 87.7%, p=0.004), as did improved co-morbidity (35.4% to 55.4%, p<0.001), increased life expectancy (29.2% to 44.6%, p=0.021), improved self-esteem (43.1% to 63.1%, p=0.031), pain reduction (7.7% to 20.0%, p=0.021), increased chance of employment (9.2% to 24.6%, p=0.021), and improved intimacy/partnership (12.3% to 32.3%, p=0.002). Reduced need for medication also increased (3.1% to 30.8%, p<0.001). In contrast, endorsement of improved social life decreased from baseline to 12 months (50.8% to 33.8%, p<0.001). No statistically

significant changes were observed for improved physical activity, improved mental health, better work performance, reduction in clothing size, or improved fertility (all $p>0.05$).

DISCUSSION

The results of the present study show that weight loss was one of the most important reasons for patients to choose bariatric surgery, as well as one of the main factors of satisfaction at 12 months postoperatively. This result is expected and is consistent with data previously reported in the literature, which indicate weight loss as the main determinant of the decision to resort to bariatric surgery (10). In our study, improved social life and, increased self-esteem occupied the second and third places in the hierarchy of reasons why patients resorted to bariatric surgery. Regarding postoperative satisfaction, weight loss is important for patients and improved self-esteem was a frequently mentioned factor. This finding supports the results of other studies that have shown that self-esteem plays an essential role in the subjective assessment of the success of bariatric surgery (16). The increase in self-esteem is likely the result of both significant weight loss and improved perceptions of body image and social integration. However, existing data suggest that improvements in self-esteem may occur early after surgery, even before significant weight loss is achieved. Previous studies have shown a significant increase in self-esteem just a few months after surgery, suggesting the existence of additional psychological mechanisms independent of weight loss (17). In addition, weight loss has been shown to be associated with significant increases in social and professional relationships, which may indirectly contribute to increased self-esteem (16). Obesity-related comorbidities were an important reason for resorting to bariatric surgery, and they were among the top three criteria for postoperative satisfaction. This observation can be explained by the fact that the population included in the present study presented important co-morbidities that improved or healed after weight loss. Improvements in quality of life after surgery have been reported as early as 3 months postoperatively in some studies (18). However, studies with longer follow-up periods suggest that these improvements may decline over time (19).

CONCLUSIONS

It is important for patients to understand the importance of lifestyle changes and maintaining a healthy lifestyle after surgery to achieve successful weight loss and avoid weight regain. While some patients are well informed, others may need additional counseling, as weight regain after bariatric surgery has been shown to be unexpected, and patients have reported being unprepared for, and having unrealistic expectations of weight loss (20). A future challenge will be to effectively identify patients who need additional support before, as well as after surgery. To further address the effect of expectations on outcomes such as weight loss and weight maintenance after bariatric surgery, prospective studies with repeated and long-term follow-up of patients, including men, who have undergone different types of bariatric surgical procedures, are needed.

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DIAGNOSTIC AND PROGNOSTIC UTILITY OF COMPLETE BLOOD COUNT-DERIVED INFLAMMATORY MARKERS AND C-REACTIVE PROTEIN IN PENILE CANCER

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DIAGNOSTIC AND PROGNOSTIC UTILITY OF COMPLETE BLOOD COUNT-DERIVED INFLAMMATORY MARKERS AND C-REACTIVE PROTEIN IN PENILE CANCER (Abstract): Systemic inflammatory indicators obtained from standard blood tests have garnered heightened interest as prospective diagnostic and prognostic instruments in oncological urology. **Materials and methods:** This study assessed the diagnostic efficacy of complete blood count-derived inflammatory indices and C-reactive protein (CRP) in individuals with penile cancer. A total of 38 individuals with penile cancer were compared to 38 control participants receiving surgery for benign scrotal conditions. **Results**: Notable disparities were identified between the two groups for baseline characteristics. Patients with penile cancer were much older and had reduced blood hemoglobin levels and elevated serum creatinine values in comparison to controls. All assessed inflammatory markers were markedly enhanced in the cancer cohort, suggesting an increased systemic inflammatory load. Mean CRP levels were significantly elevated in patients with malignancy, accompanied by markedly higher neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), and aggregate index of systemic inflammation (AISI). An examination of the ROC curves for inflammatory markers revealed statistically significant differences between groups. The NLR exhibited strong discriminating capacity for penile cancer, with an area under the curve (AUC) of 0.829. CRP had significant diagnostic efficacy (AUC 0.700). Conversely, composite indices such as SIRI and AISI showed relatively little discriminatory ability, with AUC values of 0.694 and 0.697, respectively. **Conclusions:** Individuals with penile cancer exhibit markedly increased systemic inflammatory markers in comparison to controls. Among the assessed indices, NLR and CRP demonstrated the greatest diagnostic efficacy, whereas more complex composite indices provided minimal additional differentiation. **Keywords:** PENILE CANCER, INFLAMMATORY MARKERS, NEUTROPHIL-TO-LYMPHOCYTE RATIO, C-REACTIVE PROTEIN, SII, AISI, SIRI.

INTRODUCTION

The early identification of reliable diagnostic and prognostic biomarkers remains a priority for improving cancer patient stratification, optimizing treatment decisions, and enhancing long-term survival outcomes. Recent studies have shifted attention to blood-based biomarkers derived from routine laboratory tests. These markers possess the advantage of being readily accessible,

minimally invasive, and cost-efficient compared to tissue-based molecular assays. According to Czajkowski *et al*., the neutrophil-to-lymphocyte ratio (NLR) has attracted substantial attention for its potential to reflect systemic inflammation and the interplay between tumor biology and immune response (1). In penile squamous cell carcinoma (PSCC), elevated NLR has been repeatedly associated with poorer overall survival (OS), recurrence-free survival (RFS), and cancer-specific survival (CSS) (2). Systemic inflammation markers extend beyond NLR. C-reactive protein (CRP), for example, is synthesized by hepatocytes in response to interleukin-mediated pathways such as IL-6 and IL-1β, which trigger transcriptional activation mechanisms involving C/EBP proteins (1). While CRP is classically associated with infection or trauma, elevated levels have been observed across multiple malignancies. In penile cancer, Qin *et al*. propose that high CRP values might be linked to worse prognosis in advanced disease stages (3). Altogether, this framing underscores why a systematic synthesis examining complete blood count-derived inflammatory markers alongside CRP can provide tangible benefits: clarifying contradictory findings in the literature; quantifying predictive value across diverse patient groups; and exploring how these readily available metrics interface with disease management algorithms currently applied to penile cancer patients (4). We aimed to compare complete blood count (CBC)-derived inflammatory markers and CRP levels between patients with penile cancer and a control group.

MATERIALS AND METHODS

Data of patients who were diagnosed with PSCC who underwent penile amputation at “Dr. C.I. Parhon” Clinical Hospital, Iasi, and Neamt County Hospital between January 2021 and December 2025 were collected retrospectively.

Penile amputation was performed after confirmation of neoplasia by a previous biopsy in accordance with the recommended guidelines. The blood sample was taken before surgery. Patients with disorders that could influence inflammatory markers, including additional malignancies, active or chronic infections, immunosuppressive diseases, systemic inflammatory conditions, the use of immunosuppressive medications, and renal or hepatic dysfunction, were excluded from the study. The control group was formed by patients who presented for benign scrotal pathology, namely hydrocele, in a number equal to that of the study group, in the order in which they presented for surgical treatment in the last period of the study. From both groups, we collected data including age at the time of surgery, complete blood count, C-reactive protein, and serum creatinine. The CBC-derived inflammatory markers have been calculated as follows: NLR = neutrophil count / lymphocyte count, MLR = monocyte count / lymphocyte count, Systemic Immune-Inflammation Index (SII) = platelet / × neutrophil-to-lymphocyte ratio, Systemic Inflammation Response Index (SIRI) = (neutrophil count × monocyte count) / lymphocyte count; Aggregate Index of Systemic Inflammation (AISI) = (Neutrophil count × Monocyte count × Platelet count) / Lymphocyte count (5-7).

The Mann-Whitney U test was employed to compare each parameter between groups. Data were presented as mean ± standard deviation and median; $p < 0.05$ was accepted as statistically significant. The area under the receiver operating char-

acteristic (ROC) curves was assessed for the markers that were significantly different between the two groups.

Approval was obtained from the hospital Ethics Committee (Approval No.1372/09-FEB-2026). Statistical analysis was performed using the *Statistical Package for the Social Sciences version 26.0* (SPSS Inc., Chicago, IL, USA).

RESULTS

We included a total of 38 patients diagnosed with penile cancer and 38 subjects in the control group. Comparative analysis demonstrated statistically significant differences between the two groups for several baseline clinical and laboratory parameters, including mean age, serum hemoglobin levels, and serum creatinine levels. Patients in the cancer group were significantly older and exhibited lower hemoglobin concentrations and higher serum creatinine levels compared with controls.

Regarding systemic inflammatory status, marked differences were observed between the two groups. All evaluated inflammatory markers showed statistically significant elevations in the cancer group compared with the control group (tab. I). These findings suggest a substantially higher inflammatory burden among patients with malignancy.

TABLE I.

Comparison of characteristics between the two groups

Parameter	Penile cancer (n=42)	Control (n=98)	p
Mean age (years), SD	65.71 (±10.79)	42.65(±13.92)	**0.001**
Serum Hemoglobin (g/dl)	13.12 (±2.61)	14.77 (±1.02)	**0.0013**
Serum creatinine mg/dl	1.58 (±0.13)	0.92 (±0.16)	**0.05**
CRP (mg/L)	13.64 (±12.80)	2.98 (±2.84)	**0.0069**
NLR	3.64 (±2.18)	1.84 (±0.62)	**0.001**
MLR	0.41 (±0.23)	0.29 (±0.12)	**0.007**
SII	1001.88 (±766.08)	468.10 (±184.64)	**0.001**
SIRI	2.42 (±1.94)	1.39 (±0.6)	**0.003**
AISI	718.39 (±736.23)	313.15 (±188.85)	**0.003**

To further evaluate the discriminatory performance of the inflammatory markers that differed significantly between the two groups, we performed receiver operating characteristic (ROC) curve analyses, focusing particularly on CRP and NLR. The ROC analysis revealed that NLR demonstrated good discriminatory ability, with an area under the curve (AUC) of 0.829. CRP showed a moderate discriminatory capacity, with an AUC of 0.700.

In contrast, the composite indices SIRI and AISI exhibited relatively poor discriminative performance, with AUC values of 0.694 and 0.697, respectively. These results are illustrated in Figure 1. During the study, we did not record any patient deaths. Pathological examination revealed that 60.52% (n=23) have been classified as T1, 31.57% (n=12) as T2, and 7.89% (n=3) as T3.

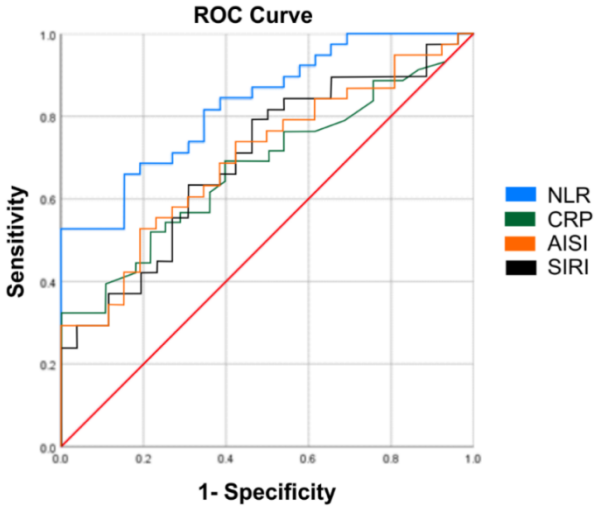

Fig. 1. ROC analysis of the inflammatory markers

DISCUSSION

To investigate oncological diseases, markers are used that, although effective, are often too expensive for current practice (8). On the other hand, CBC-derived inflammatory markers are cheap and have proven useful in certain types of cancer (9, 10). CBC profiles, including absolute counts and subtype proportions, provide a window into the systemic immune status of patients with penile squamous cell carcinoma. Distinct white blood cell subtypes, neutrophils, lymphocytes, monocytes, and platelets, reflect different facets of inflammation and immunity, and when these parameters shift in characteristic ways, they can mark progression or prognosis in PSCC. Neutrophils, as primary components of the innate immune system, play a dual role in cancer. Tumor-associated neutrophils can be recruited via chemokine gradients established within the microenvironment and may contribute to tumor initiation and growth by activating cancer cells or suppressing cytotoxic lymphocyte function. Such suppression could manifest as elevated peripheral neutrophil counts relative to lymphocytes, leading to an elevated NLR. The elevation of NLR itself can arise from several physiological scenarios: increased neutrophil counts coupled with reduced lymphocyte numbers; increased neutrophils without lymphocytic changes; or diminished lymphocytes without altered neutrophils (11).

Elevated NLR has been linked not only to disease-specific mortality but also correlates with nodal stage severity, offering a potential bridge between routine hematology results and staging models traditionally constructed from imaging or intraoperative findings (12). Lymphocytes are central to immune surveillance against malignancy. Their depletion reduces the capacity to mount adaptive immune responses and is linked to enhanced tumor progression due to weakened control over aberrant cells. A reduction in circulating lymphocytes relative to platelets, the platelet-to-lymphocyte ratio (PLR), has been suggested as a marker for poor prognosis in diverse cancers

because it reflects both diminished immunity and increased platelet-mediated tumor support functions (13). According to Steffens *et al.*, combining NLR with CRP levels has shown potential in improving predictive ability for poor outcomes, especially in non-metastatic patients who nonetheless harbor aggressive local or regional disease patterns (14).

Elevated monocyte-to-lymphocyte ratio (MLR), a CBC-derived value, is another CBC-derived value proposed as an independent prognostic factor across cancers (13). In PSCC research cohorts, reduced LMR may indicate a skewing toward a microenvironment dominated by macrophages, which typically favors tumor facilitation over elimination. Platelets themselves transport proangiogenic and antiangiogenic factors within their granules and microparticles (15).

CRP belongs to the pentraxin family of proteins and is categorized as an acute-phase reactant, with production predominantly occurring in hepatocytes under stimulation by proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α. Under conditions of infection, trauma, or malignancy, circulating CRP levels can rise profoundly, sometimes exceeding baseline by over 1000-fold, in a relatively short timeframe (16, 17). In PSCC studies investigating prognostic markers, threshold values above 15 mg/L have been associated with increased nodal disease incidence and reduced cancer-specific survival (18). Lower but persistently elevated ranges, for example above 5 mg/L, have been incorporated into multi-factor prognostic scoring models alongside surgical margin status and pathological stage to stratify patient mortality risk (16).

Tumor-associated macrophages, malignant keratinocytes, and reactive stromal fibroblasts secrete IL-6 continuously, maintaining hepatic CRP synthesis without reverting to basal levels (1). Unlike infection-mediated acute peaks that resolve as pathogens are cleared, malignancy-associated CRP elevation can remain stable or fluctuate at moderately high levels depending on ongoing tumor activity and metastatic spread. This chronicity imbues CRP readings with prognostic relevance: persistent concentrations above modest thresholds have correlated with reduced disease-specific survival and advanced pathological stages in genitourinary cancers including PSCC (19). In patients with PSCC, correlations have been observed between elevated pre-treatment CRP and adverse clinical indicators such as extranodal extension or bilateral pelvic lymph node metastasis (20).

Elevated neutrophil-to-lymphocyte ratio (NLR) values have been repeatedly associated with adverse outcomes, such as reduced overall survival (OS) or cancer-specific survival (CSS), though the strength of these associations may diminish after adjustment for established pathological factors, such as tumor grade, lymph vascular invasion, and clinical nodal status (21). The platelet-to-lymphocyte ratio (PLR) shows similar trends, with higher values generally observed among individuals with extranodal extension or advanced TNM staging. In smaller PSCC cohorts, PLR elevation is associated with worse OS, complementing the picture presented by NLR but with a slightly different biological emphasis, capturing platelet-driven microenvironment changes coupled with adaptive immune suppression. While precise cut-off thresholds vary across reports due

to population and assay differences, values exceeding 133.5 have been proposed via receiver operating characteristic curve analysis for stratifying risk before surgical intervention (13).

While the systemic indices discussed earlier capture host inflammatory status in a relatively accessible way, they remain indirect proxies for intratumorally events. Recent advances in molecular pathology have reclassified penile neoplasms into discrete biological subtypes based on HPV status, gene mutations, and protein expression profiles (12). By overlaying CBC- or CRP-derived inflammatory metrics onto these molecular categories, it becomes possible to examine whether specific genomic contexts consistently produce certain systemic inflammatory phenotypes. For example, an elevated neutrophil-to-lymphocyte ratio (NLR) might align more with TP53-mutated, HPV-negative tumors characterized by high PD-L1 expression, reflecting immunosuppressive microenvironments that weaken adaptive immune surveillance (22).

CONCLUSIONS

Penile squamous cell carcinoma presents unique challenges due to its multifactorial etiology and the intricate interplay between tumor biology and host immune responses. The integration of complete blood count-derived inflammatory markers such as NLR, platelet-to-lymphocyte ratio, and MLR, alongside C-reactive protein measurements, offers a promising avenue for enhancing prognostic assessment and clinical management. These biomarkers provide accessible, minimally invasive, and cost-effective tools that reflect systemic inflammation and immune dysregulation associated with tumor progression, nodal metastasis, and survival outcomes.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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GIANT UTERINE MYOMA AND OVARIAN MYOMA - ASSOCIATION OF TWO RARE ENTITIES. CASE REPORT AND LITERATURE REVIEW

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GIANT UTERINE MYOMA AND OVARIAN MYOMA- ASSOCIATION OF TWO RARE ENTITIES - CASE REPORT AND LITERATURE REVIEW (Abstract): Uterine myomas are the most common benign tumors of female reproductive system. They affect 25% of women of reproductive age. Giant uterine myomas are quite rare and are a challenge both for a complete diagnosis and for surgical management. Extra-uterine myomas are rare and they usually appear in the genitourinary tract (ovaries, vulva, broad ligament, round ligament, urethra). Primary ovarian leiomyoma is one of the rarest solid tumors of the ovary accounting for 0.5%–1% of all benign ovarian neoplasms. We report a 45-years-old nulliparous woman who came with abdominal distension and fatigue for several months. Abdominal-pelvic ultrasound and computer tomography showed a large myoma-like mass that filled the pelvis and abdomen. The diagnosis of giant uterine myoma was established and after careful discussion with the patient total hysterectomy was proposed. Surgery confirmed the diagnosis of a giant uterine myoma with several parasitic omental blood supplies. An additional ovarian myoma was discovered and total hysterectomy with right adnexectomy was performed. Our case stresses several clinical points that should be kept in mind before planning a surgery for a giant myoma - the possible presence of parasitic blood vessels and adherences which can pose some difficulties for the surgical team and the coexistence of different pathologies masked under the size of the tumor. It is important before planning a surgery to discuss with the patient the possibility to adapt the surgery according to associated pathology. **Keywords:** UTERINE LEIOMYOMA, GIANT LEIOMYOMA, GIANT MYOMA, LARGE MYOMA, OVARIAN MYOMA.

Uterine myoma is the most common benign tumors of female reproductive system. It affects 25% of women of reproductive age (1). Giant uterine myomas are quite rare and are a challenge both for a complete diagnosis and for surgical management. The challenge in diagnosis comes from their size, as they can hide other associated conditions, which sometimes are discovered during surgery and require changing the initial surgical plan. We present a case with two rare associated entities: a large uterine myoma associated with an ovarian fibroma.

CASE REPORT

A 45-year-old nulliparous woman comes to gynecological exam for a progressive increase of the abdomen and lower abdominal pain. Her medical history was normal, and she has no pregnancies. Her vital signs were all within normal limits. Clinical examination reveals the distension of the abdomen and the presence of a firm not tender lumpy abdominal and pelvic mass (fig. 1). A rectal examination was performed (the patient being a virgin) and it revealed a very large firm central mass filling the pelvis and abdomen. It was difficult to specify the origin of tumor. The results of routine laboratory testing were normal. Transabdominal ultrasound confirmed the presence of a large tumor highly suggestive of a uterine leiomyoma localized intramural and subserous. Bilateral ovaries could not be detected. An abdominal and pelvic computed tomography (CT) showed a large mass, approximately 25/20/13 cm in size, occupying the abdomen from pelvis to the upper abdominal cavity.

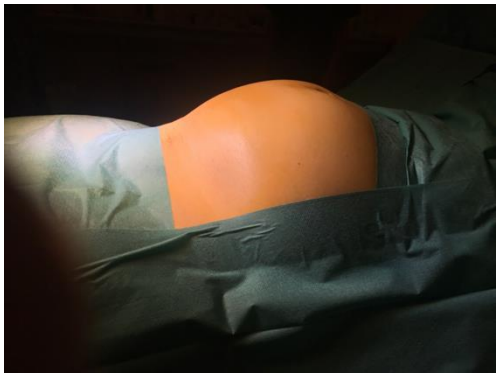

Fig.1. Clinical picture of the abdomen shows an important distension of the whole abdomen.

In the light of the clinical examination, the routine laboratory findings and US and CT results, the preoperative diagnosis was giant uterine myoma and after the informed consent of the patient was obtained, total abdominal hysterectomy was proposed.

Laparotomy revealed a large multilobular tumor originating from the uterus occupying the whole abdomen. Adhesions and parasitic vessels between the mass, omentum and descending colon were present. After careful dissection of the adhesions the examination of the ovaries revealed normal left ovary and a 3-cm myoma-like tumor at the right ovary. Because this tumor and giant uterine myoma total hysterectomy and right adnexectomy were performed. The postoperative evolution was uneventful, and the patient was discharged three days after the operation in excellent condition. The removed specimen was 4.5 kg in weight and size was 25/20/12 cm (fig. 2). Microscopic examination revealed a multilobulated leiomyoma, endometrial polyp and ovarian myoma.

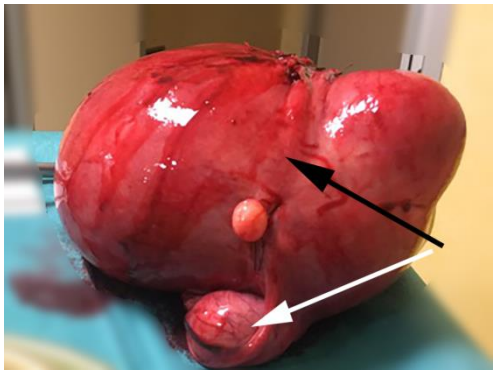

Fig.2. Surgical specimen- black arrow - the whole uterus, white arrow the ovarian myoma.

DISCUSSION

Uterine leiomyomas are common benign tumors affecting 25% of women of reproductive age (1). Uterine myoma can be asymptomatic or can cause a variety of symptoms, including abnormal bleeding,

pain, infertility, miscarriage and compression on neighboring organs. Although the size of leiomyoma varies from microscopic to large, giant uterine myomas are uncommon. Giant uterine myomas are almost always symptomatic because of the tumor bulk-related symptoms.

Extra-uterine myomas are rare and present a great diagnostic challenge. They usually appear in the genito-urinary tract (ovaries, vulva, broad ligament, round ligament, urethra) (2). Ovarian fibromas are benign ovarian tumors of sex cord stromal origin. Primary ovarian leiomyoma is one of the rare solid tumors of the ovary, accounting for 0.5%–1% of benign ovarian neoplasms (3). Ovarian fibroma is generally asymptomatic and seen in women >50 years of age. Most ovarian leiomyomas are small, measuring only a few millimeters to centimeters in diameter but sometimes they can get larger and the ovary may become completely absorbed by the tumor (4). The differential diagnosis includes ovarian fibroma-thecoma, cellular fibroma, and sclerosing stromal tumor leiomyosarcoma. Ovarian fibroma is benign and complete surgical resection is the treatment.

A useful tool for diagnosis of uterine or ovarian myoma is the ultrasound examination where typically a myoma has whorled pattern appearance with variable echogenicity, depending on the presence of degeneration and calcification. In some cases, cystic degeneration with extensive edema forming cystic fluid-filled spaces can occur and the diagnosis can be more difficult to establish. Computed tomography and magnetic resonance imaging can also be used to diagnose fibromas.

The association of ovarian myoma and uterine myoma was reported by Doss et al. (5). According to our knowledge this is the first case that associates a giant uterine myoma with ovarian myoma and endometrial polyp. Our case stresses several clinical points that should be kept in mind in cases with giant uterine myoma. First, as leiomyomas enlarge, they can outgrow their blood supply and several parasitic blood vessels can appear from the structures it is adherent to. Such structures include the omentum, common iliac artery and inferior mesenteric artery (6, 7). Both parasitic vessels and adhesion can pose some difficulties to surgical team during surgery. Various types of degeneration such as hyaline, cystic, myxoid or red degeneration may appear in cases of giant myoma (8,9). These degenerations give atypical appearances and can cause confusion in diagnosis with imaging techniques like USG, CT, and MRI. The most frequent confusion that could appear is with an ovarian tumor (10,11). The final diagnosis can be made either intraoperative or histologically. Also imaging technique can in some cases miss some associated pathologies due to the size of the giant myoma. The patients should be informed about this possibility and about the need for adapting or changing the planned surgery to attempt a complete recovery.

CONCLUSIONS

Giant uterine myoma needs a careful preoperative exploration, as its size can sometimes hide other associated pathologies. To prevent discovering these pathologies during the surgery and to avoid surgical management without patient's consent, it is appropriate to carefully explore all the cases. Even so it is important to discuss with the patient and to inform him that due to the size of the tumor it is possible to change the surgical plan.

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NEWS

WHEN AND HOW TO CORRECT VITAMIN D DEFICIENCY?

In the last teen years, more than 30,000 articles regarding the importance of vitamin D in some physiological process, have been published worldwide. Now, we know the importance of vitamin D in immunoregulation, insulin production, cellular growth and cardiovascular protection. What we don't really know is: what is the optimal level of vitamin D to cover all needs of the tissues that have vitamin D receptor (VDR)? For human well-being we need at least 20 ng/mL (the cut-off for treatment) and Endocrine Society it recommended achieving more than 30ng/mL with the preferred range of 40-60 ng/mL. Recommendations are: infants up to 1 year, 400-1,000 IU/day, for children over one year 600-1,000 IU/day, and for adults 1,500-2,000 IU/day. There are no reports regarding the intoxication with vitamin D in adults (Pludowski P, Holick MF, Grant WB, et al. Vitamin D supplementation guidelines. *Journal of Steroid Biochemistry and Molecular Biology*. doi: 10.1016/j.jsbmb.2017.01.021. Epub 2017 Feb 12).

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THE MEDITERRANEAN DIET IMPACT ON PREVENTION AND TREATMENT OF COGNITIVE DECLINE

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THE MEDITERRANEAN DIET IMPACT ON PREVENTION AND TREATMENT OF COGNITIVE DECLINE (Abstract): The world's population aged over 60 years is predicted to double in the next 50 years. Increased age makes us vulnerable to various neurodegenerative pathologies among them cognitive impairment and dementia. The economic and social burden of caring for persons with cognitive decline is increasing, and to date there are no effective pharmacologic agents to prevent or treat the disease or even its precursor stages. Identifying simple and effective interventions, like modifiable risk factors to prevent cognitive decline and dementia is likely to be an important strategy for delaying the onset, and reducing the number of patients is a major public health priority. There is growing evidence that diet -a modifiable risk factor- could be one component of an effective prevention strategy against cognitive decline, although no firm conclusion can be drawn at this point. A healthy Mediterranean diet (MeDi), rich in fruit, vegetables, whole grains, nuts, olive oil, and fish may be neuroprotective. Greater adherence to the MeDi is associated with slower rate of cognitive decline and lower risk of cognitive impairment and dementia, but findings are conflicting, mainly due to significant heterogeneity between studies in terms of populations studied and methods used to assess diet and cognition. All these reasons highlight the need for randomized clinical trials and for further research. **Keywords**: COGNITIVE IMPAIRMENT, NUTRITION, MEDITERRANEAN DIET.

Aging is a normal and physiological process. Genetic studies claim that we are born with a genetic determined cognitive reserve which is affected as we get older by environmental and external factors resulting in a decline. Epidemiological studies predict that the number of persons affected by cognitive decline will double every 20 years and reach 106.8 million, affecting 1 out of 85 persons by 2050. Therefore, is a growing public health concern with significant socioeconomic burden, being one major cause of death and disability worldwide (1).

Cognitive impairment can be influenced by several factors and the potential effect of nutrition has become a topic of increasing scientific and public interest (2). In

recent years, there has been increasing evidence supporting the role of nutrition in and that to achieve a benefit, an intervention would likely need to be started in the preclinical stage. Emerging evidence suggests an association between dietary habits and cognitive performance (3). Nutrients and diets, together with other aspects of daily living (such as exercise), can affect multiple brain processes by regulating neurotransmitter pathways, synaptic transmission, membrane fluidity and upregulating genes for maintaining synaptic function and plasticity (4).

A common approach to explore the impact of nutrition is studying dietary patterns comprising combinations of nutrients and foods. A frequently studied dietary pattern is the Mediterranean diet, which is rich in fruits and vegetables and unsaturated fatty acids and has been proven to have vascular and anti-inflammatory benefits and may be neuroprotective (5). It was first described by dr. Walter Willet from Harvard University in 1990 and recognized as UN cultural patrimony in 2013. Its definition consists in recommendations regarding the number of portions per day of different classes of nutrients. It favors the consumption of whole grains, fruits and vegetables, olive oil, fish, nuts and dairy over red or processed meat products and refined sweets. It also recommends increased water consumption and 1 or 2 glasses of red wine per day. The Mediterranean dietary pattern refers not only to the type of food consumed, but also to lifestyle and the social customs associated with the way of eating. It therefore seems reasonable to assume that this pattern of healthy eating can modify cognitive status and reduce the prevalence of cognitive impairment, and mood disturbances in the elderly population (6).

THE ADHERENCE TO THE MEDITERRANEAN DIET

The Mediterranean Diet was largely studied due to its effect in many pathologies. Cognitive decline means brain aging which involves neurodegeneration with loss of synapses and neurons, increased oxidative stress, inflammation and vascular impairment. The primary foods of the Mediterranean diet contain bioactive nutrients and phytochemicals like polyphenols, flavonoids, vitamins, minerals and fiber, arginine and antioxidants with anti-inflammatory proprieties and neuroprotective effect (7). The fish and olive oil contain a lot of omega 3 acids (polyunsaturated fatty acids), part of the neural membrane functioning, increase the activity of antioxidation enzymes, protecting against oxidative stress, neuronal death and the formation and aggregation of amyloid in the brain (8).

Prospective studies have shown that populations with higher adherence to MeDi exhibit lower risk of cardiovascular diseases (9). Reduced inflammation and improved endothelial function produce vasodilatation and increased cerebral blood flow, reducing oxidative stress and neuronal cell death. Interventional studies have reported improvements to blood pressure, lipid profiles, insulin sensitivity, CRP levels and oxidative stress. (10).

Greater adherence to the Mediterranean diet has been associated with a lower rate of cognitive decline in many observational and interventional studies (11). The international recommendations for globally applicable dietary guidelines were translated into the Healthy Diet Indicator (HDI) by the World Health Organization (WHO) in 1990 (12). The HDI based on initial WHO recommendations has been associated with a lower prevalence of cognitive impairment. There-

fore, the Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES) which included 3 population-based cohorts: Survey in Europe on Nutrition and the Elderly, a (SENECA), Rotterdam Study (RS), Nurses' Health Study (NHS) totaling 21,837 men and women from Europe and US concluded that a higher HDI score was not related to reduced rates of cognitive decline in European and American older adults (13).

Beside many studies regarding the association between Mediterranean style of eating and cognitive decline, there are a few meta-analyses which gather and select the most important data about this subject. Researchers from Mayo Clinic, USA, screened 664 studies through November 2012, aiming to determine whether there is an association between the Mediterranean diet (MeDi) and cognitive impairment. Only five studies met the eligibility criteria and showed that higher adherence to the MeDi reduced the risk of mild cognitive impairment with 33%. Results also suggested that a higher adherence to the MeDi is associated with a significant reduced risk of developing Alzheimer disease, and a reduced risk of progressing from mild cognitive impairment to dementia (14).

Living on a Mediterranean diet also reduces the risk of stroke, depression, cognitive impairment and Parkinson disease. Higher adherence to MeDi has protective action and is consistently associated with reduced risk for stroke, mainly ischemic and in the male population. Moderate adherence was linked to reduced risk for depression (15). Not living in the Mediterranean area has not been an impediment for the benefit of MeDi on cognition. Lower adherence to a Mediterranean diet was associated with higher AD and mild cognitive impairment compared to healthy subjects in an Australian population (16). An analysis conducted in a group of healthy elderly Swedish men and women, showed that adherence to MeDi was positively associated to healthier cognitive aging, even though no association was found between Mediterranean score and cognitive function or brain volume measured by magnetic resonance (17).

Not all studies favor the benefit of MeDi. For example, results from the cognitive sub-study of the Women's Health Study reported no association of the Mediterranean diet with cognitive decline. Differences in methodologies used to assess adherence, different sample size, the heterogeneity of groups or populations tested and poor reliability of food questionnaires, among other factors may be the reason why some studies have found no correlation between adherence to a Mediterranean diet and delayed or reduced cognitive decline (18).

PREVENTION WITH THE MEDITERRANEAN DIET

Successful aging depends on the homeostatic reserves of different physiological systems. Recent literature states that MeDi has also effects on cognitive frailty. This novel entity represents a critical intermediate status of the aging process that is at increased risk for negative health-related events and it is starting to be investigated also in association with nutrition (19). There is not a clear understanding of the pathways converging into frailty, but it seems that the losses imposed by ageing, the impact of acute or chronic disease, and the own genetic endowment of the individual may have important roles (20). The potential for reversibility of frailty and its

different phenotypes suggests that these clinical features may be important for secondary prevention in neurodegenerative diseases. Studies have reported an inverse association between adherence to a Mediterranean diet and lifestyle and prevalence of frailty (21).

Preventing cognitive decline is the subject of recent research. One of them investigated the association between MeDi and the prevention of mild cognitive impairment (MCI) and the progression from MCI to Alzheimer disease (AD) in a multiethnic community in New York. 1,393 cognitively normal participants were included from who 275 developed MCI and 106 were diagnosed with AD for 4.5 years. Subjects that consumed MeDi had 28% less risk of developing MCI and 48% less risk of developing AD compared to control. In the end, there was a significant protective effect of MeDi against MCI and AD, and even a reduced risk of MCI conversion to AD. Studies looked deeper into the effects of MeDi on the domains of cognitive function of healthy older adults, respectively on memory and executive function. In a met analysis with 41,492 participants where the primary outcome was cognitive function, but divided into domains of memory and executive function results revealed a significant association between MeDi and older adults' episodic memory and global cognition, but not working or semantic memory. Also, compared with controls, the MeDi improved delayed recall, working memory and global cognition, but not episodic memory, immediate recall, attention, processing speed or verbal (22).

A study published this year investigated the associations of MeDi and its components with magnetic resonance imaging measures of cortical thickness for the four lobes separately and averaged. Higher MeDi score was associated with larger frontal, parietal, occipital, and average lobar cortical thickness and higher adherence to MeDi was associated with larger cortical thickness. While higher legume and fish intakes were associated with larger cortical thickness, especially parietal and precuneus, higher carbohydrate and sugar intakes were associated with lower entorhinal cortical thickness. This study opens new paths for research, but these novel findings need cross-sectional and prospective studies for validation (23).

Another proposed neuroprotective dietary pattern, the Mediterranean-DASH diet Intervention for Neurodegeneration Delay (MIND diet), has been recently described. The MIND diet is a modified version of MeDi but incorporates additional foods based on current evidence in the study of the effect of diet on dementia. In some population-based studies MIND score was more predictive of cognitive decline than MeDi score, and higher MIND scores were associated with lower risk of Alzheimer disease. These results show that greater adherence to MeDi and MIND diet patterns are associated with better overall cognitive function in older adults and lower odds of cognitive impairment, which could have important public health implications for preservation of cognition during aging (24).

THE THERAPEUTIC ROLE OF THE MEDITERRANEAN DIET

The literature available today is also focused on nutritional therapy or intervention, in delaying the neurodegeneration processes with various nutrients and diets. Regarding this new goal, a recent parallel-group randomized clinical study investigated 447 participants on a Mediterranean diet

if it influences cognitive status compared with a control diet for 6 years. The *Prevención con Dieta Mediterránea* (PREDIMED) study used neurocognitive testing at inclusion and at the end of the study, among which the Mini-Mental State Examination, Rey Auditory Verbal Learning Test (RAVLT), Wechsler Scale and the Color Trail Test (CTT). The participants were randomly assigned to MeDi supplemented with extra virgin olive oil (1L/week), MeDi supplemented with mixed nuts (30 g/d), or a control low fat diet. The follow-up was available only to 334 participants after intervention and data showed that the group allocated to MeDi plus olive oil scored better on the RAVLT, MMSE and Color Trail Test compared with controls; no between-group differences were observed for the other cognitive tests. The MMSE and CTT scores were also higher for participants allocated to MeDi and nuts versus control. In the end, the investigators concluded that, although not in all cognitive tests, a Mediterranean diet supplemented with olive oil or nuts is associated with improved cognitive function per se, but also compared with a low-fat diet (25).

Furthermore, actions are taken in the medical world to use diets as interventional strategies in treating different diseases. This year, the BRIDGE (Building Research in Diet and CoGnition) randomized controlled trial started and aims to investigate the effect of MeDi, with and without weight loss, on cognitive functioning in obese older adults (BMI ≥ 30 kg/m^2 and ≤ 50 kg/m^2, age ≥ 55 years). They will be randomized in a 2:2:1 allocation scheme to: Typical Diet Control; MeDi alone, without weight loss; or MeDi lifestyle intervention to promote weight loss and weight loss maintenance. Both MeDi intervention groups will meet for one individual session and 27 group sessions over an 8-month period. Individuals in the control group will not receive instruction on changing lifestyle habits. Outcomes will be assessed at baseline, 8 and 14 months. The primary outcome is cognitive functioning; secondary outcomes will include changes in body weight, diet, cardiovascular, metabolic, and inflammatory biomarkers (26).

Regarding the MeDi role in treating cognitive decline, the group led by Samieri showed that MeDi postpones cognitive aging with 1 year and reduces the risk of MCI and the conversion rate from MCI to dementia. Before that, Feart et al stated that there is a therapeutic window of 5 years before the clinical diagnosis of Alzheimer's disease in which MeDi can be used as a therapeutic strategy with very good effects on the cognitive function (27). The cognitive decline may be also reduced by lowering the cardiovascular risk, mission in which MeDi has already proved efficient, by lowering blood pressure, cholesterol, lipids, body weight and supporting the pharmacological treatment in many metabolic diseases (28).

CONCLUSIONS

In our opinion nutrition plays a decisive role in the maintenance of cognition, thus successful brain aging is an achievable goal for dietary therapies. We reviewed studies that highlight the implication of epigenetic nutritional events in modulating the cognitive abilities and that raises the possibility of dietary manipulations as a viable strategy for treating brain related pathology. In our review evidence is suggestive of a neuroprotective role for the Mediterranean Diet, but variation between studies makes it difficult to draw firm conclusions. Further

investigation is needed to determine whether the MeDi represents an optimal dietary pattern for protection against neurodegeneration in representative populations.

Our conclusion is that higher adherence to Mediterranean Diet is associated with slower cognitive decline and lower risk of developing Alzheimer disease. Long-term randomized controlled trials promoting a Mediterranean Diet and life style may help establish whether improved adherence helps to prevent or delay the onset of Alzheimer disease and other types of dementia. What we are sure and has been proved is that in brain aging and preventing cognition decline a multidomain intervention is required including proper nutrition, supported physical exercise and updated pharmacological treatment.

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NEWS

PREDICTING GESTATIONAL DIABETES MELLITUS THROUGH SERUM SEX HORMONE BINDING GLOBULIN LEVEL

A group of researchers conducted a study that assessed the sex hormone binding globulin(SHBG) as a valuable biochemical marker in predicting gestational diabetes mellitus(GDM). Circulating SHBG levels are inversely associated with insulin resistance. In this case controlled study were included 90 pregnant women, 45 women with GDM and 45 matched controls. They measured the serum SHBG levels by Enzyme-linked immunosorbent assay(ELISA) method between 24 and 28 weeks of gestation. Compared with the control group, SHBG concentrations were significantly lower in the GDM group; 23nmol/L vs. 78 nmol/L. The autors concluded that patients with GDM have lower circulating levels of SHBG than normal glucose tolerance pregnant women, so circulating concentrations of SHBG represent a potentially useful new biomarker for prediction of risk of GDM.(Tawfeek MA, Alfadhli EM, Alayoubi AM, El-Beshbishy HA, Habib FA. Sex hormone binding glubulin as a valuable biochemical marker in predicting gestational diabetes mellitus. *BMC Women's Health* 2017; 17: 18 DOI**:** 10.1186/s12905-017-0373-3)

Delia Nicolaiciuc

A NEW REALITY: GUT MICROBIOTA AND INFLAMMATORY SYSTEMIC DISEASES

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A NEW REALITY: GUT MICROBIOTA AND INFLAMMATORY SYSTEMIC DISEASES (Abstract): Microbiota refers to the total microorganisms of the microbial community, while the term microbiome refers to a group of microbes that includes bacteria, bacteriophages, fungi, protozoa and viruses. Microbiota is an organ with its own functions that can modulate the expression of genes involved in the defense of mucosal barrier, angiogenesis and postnatal intestinal maturation. Changes in the composition of gut microbiota -dysbiosis- may be associated with nosocomial infections, necrotizing enterocolitis in premature infants, inflammatory bowel disease (IBD), obesity, rheumatologic autoimmune diseases and allergies. Dysbiosis increases intestinal permeability and the microbial translocation through the mucosa, thus resulting inflammation and metabolic endotoxemia. Therefore, are generated many proinflammatory cytokines and oxygen free radicals, all of which are considered triggers for the development of immuno-inflammatory systemic disease. Many clinical studies have examined the link between autoimmune diseases and dysbiosis using 16S rRNA genetic analysis. Clear evidences of association with intestinal dysbiosis have been described in patients diagnosed with IBD, spondylarthropathies, rheumatoid arthritis or systemic lupus erythematosus. **Keywords**: MICROBIOATA, INTESTINAL DYSBIOSIS, INFLAMMATORY BOWEL DISEASE, SPONDYLARTHROPATHIES, RHEUMATOID ARTHRITIS, SYSTEMIC LUPUS ERYTHEMATOSUS.

Bacterial intestinal flora includes about 10^{14} bacteria which represents 10 times more than the number of cells in the human body (1, 2). Since birth, the normal intestinal microbiota contributes to the development of intestinal function, modulates the immune system, helps to regulate and maintain intestinal function, provides protection against infection and tolerance to food intake. We can talk about a symbiosis of the bacteria-host interaction. Intestinal microbiota diversity includes more than 1,500 microbial species, dominant bacteria group being phyla species: *Firmicutes* and *Bacteroidetes*. The phyla group also includes: *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* (3).

Microbiota has multiple roles in the development of the intestinal immune system by: the modulation of intestinal mucous layer and lymphoid structures, differentiation of immune cells and immune mediators'

production, positive stimulatory effect on the innate and adaptive immune system (4).

Changes in the composition of gut microbiota - dysbiosis - may be associated with nosocomial infections, necrotizing enterocolitis in premature babies, inflammatory bowel diseases (IBD), obesity, rheumatologic autoimmune diseases and allergies. Dysbiosis increases intestinal permeability and the microbial translocation through the mucosa, thus resulting inflammation and metabolic endotoxemia. Therefore, many proinflammatory cytokines and free radicals are generated (5).

Dysbiosis influence the intestinal mucosa by interacting with epithelial cells and by the enteric nervous system, leading to changes in intestinal motility, sensory function and the perception of pain (6). Also, dysbiosis is associated with the development of gastrointestinal and extra intestinal disorders, as well as with an impaired liver function (5,7).

MICROBIOTA AND INFLAMMATORY BOWEL DISEASE

Genetics and environmental factors and the host immunity forms a triad that it has been shown to regulate the TCR function (Toll like receptor). When this relationship is disturbed, it can develop aberrant TCR signals which contribute to the formation of inflammasomes that will cause intestinal inflammation (8).

Risk factors for the occurrence of IBD include factors that influence the composition of intestinal microbiota - maternal exposure, breastfeeding, diet, antibiotics, infections and factors affecting mucosal immune system - smoking, Nonsteroidal anti-inflammatory drug (NSAIDs), oral contraceptives, vaccination, intestinal permeability, appendectomy, stress (9). A model of colitis on laboratory mice showed that, after the administration of 4 antibiotics for 4 weeks, the deletion of commensal gut microbiota can cause severe intestinal mucosal impairment (10). Extended use of antibiotics is associated with increased risk of Crohn's disease (CD), but not with ulcerative colitis (UC) (11).

One study attempted to investigate the impact of IBD on intestinal microbiota being analyzed 89 sigmoid mucosal biopsies in healthy individuals and in patients with CD and UC who achieved remission. On these biopsy samples 16sDNA and rARN genetic analysis were performed. Results showed an abundance of *Bacteroides* in the control group and in patients with UC, an increase of *Firmicutes* in both IBD, a reduced activity of *Faecalibacteria* in patients with CD, an increase in the prevalence and activity of *Papillibacter* in healthy persons; only *Prevotella* was positively associated with CD (12).

Dysbiosis in CD is characterized by: a greater number of mucosal bacteria compared to healthy individuals, alteration of the balance between beneficial and aggressive bacteria and by the reducing of the phyla diversity group *Firmicutes* and *Bacteroides* (13). Increased prevalence of intracellular pathogens in CD may be due to the innate immune system's inability to control persistent infections caused by intracellular bacteria.

Patients with ileal and colonic CD have a low concentration of commensal bacteria concerning the *Clostridiales* group as *Faecalibacterium prausnitzii* and *Roseburia* (14), these being regarded as predictive marker for postoperative ileal CD (15). An increased number of bacterial species found in CD include *Escherichia coli* especially B2 and D groups and those with adherent / invasive strains associated with a severe ileal disease (16).

MICROBIOTA AND INFLAMMATORY RHEUMATIC DISEASES

Except reactive arthritis in which there are clear evidences that bacterial infections can cause articular manifestations, currently studies show only hypotheses regarding the role of gut microbiota in immune-mediated arthritis. However, a study conducted on mice having ankylosing spondylitis (AS) and positive antigen HLA-B27 and which were maintained in germ-free conditions highlighted that they didn't developed articular inflammation (17).

Recent studies (18) argue that, intestinal dysbiosis in patients with SA correlates with the presence of the antigen HLA-B27. To detect bacteria associated to HLA-B27, 16S rRNA sequencing had been used and elevated populations of *Paraprevotella* and *Bacteroides vulgatus* have been highlighted. Altered intestinal bacterial composition characterized by: increasing populations of *Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Porphyromonadaceae, Bacteroidaceae*, alongside the decreasing of *Veillonellaceae* and *Prevotellaceae* constitutes a risk factor for developing SA (19).

A recently published study (20) supports the important role of intestinal dysbiosis (especially Dialister gender) in spondylarthropathies. There have been included 27 patients of which 14 showed microscopic intestinal inflammation and 13 without gut inflammation. Ileal and colonic biopsies were performed. To compare the intestinal microbial composition 16SrARN sequences were used. The results showed that the microbiota of patients with spondylarthropathies was associated with intestinal inflammation and *Dialister* gender was positively correlated with the ASDAS score (Ankylosing Spondylitis Disease Activity Score).

Other diseases such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) may have as pathogenic mechanism intestinal dysbiosis which contributes to the development of arthritis through activation of autoreactive T cells in the gut (21). Dysbiosis in RA is characterized by a depletion of *Haemophilus spp*. - negatively correlated with the level of autoantibodies - and an increasing of *Lactobacillus salivarius* - especially in cases of very high RA activity (22). Regarding SLE patients, trials evidenced a significant decrease in *Firmicutes* and *Firmicutes/Bacteroidetes* ratio and a significant increase in *Clostridiaceae* and *Lachnospiraceae* groups that correlated with disease progression (23).

MICROBIOTA AND INFLAMMATORY BOWEL DISEASE ASSOCIATED WITH ARTICULAR MANIFESTATIONS

Regarding the association between IBD and rheumatic disorders, namely AS, it must not forget that these conditions have common genetic background and are considered distinct phenotypes of an immune-mediated inflammatory disorder. The strongest genetic association is represented by the antigen HLA-B27.

Intestinal epithelial cells produce mucins and antimicrobial peptides such as lysozyme, defensins and lectins that have a critical role in intestinal homeostasis. Patients with active CD show a marked decrease of DEFA5 and DEFA6 α-defensins, leading to an impaired mucosal commensal microbial flora. Controversially, the studies showed an increased expression of α-defensins in patients with SA having also subclinical ileal inflammation (24, 25).

Regarding IL-17 / IL-22 cytokines relevant to IBD and spondylarthropathies, clear evidences indicate that the interaction between gut microbiota and host causes the activation of immune cells with subsequent production of these types of proinflamma-

tory cytokines (26).

CONCLUSIONS

We can conclude with confidence that the gut microbiota plays an important role in the development of immuno-inflammatory diseases, namely in intestinal and articular disorders. Nevertheless, the specifically pathogenic mechanism remains a challenge for the practitioner physician. The analysis of gut microbiota opens new perspectives in research and for understanding of these systemic diseases.

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NEWS

A NEW BLOOD TEST USEFUL FOR DIFFERENTIAL DIAGNOSIS OF CHRONIC DEGENERATIVE NEUROLOGICAL DISORDERS

Neurofilament light chain protein (NLCP) is a biomarker of degeneration of large myelinated axons. This biomarker is elevated in the cerebrospinal fluid (CSF) in patients with chronic degenerative neurological disorders such as atypical parkinsonian disorders (APD), amyotrophic lateral sclerosis, and frontotemporal dementia but is not increased in Parkinson disease (PD). The use of this biomarker to differentiate between PD and APD is influenced by the patient's decision because lumbar puncture is an invasive maneuver that lowers its compliance and delay treatment decisions. Because prognosis differ dramatically between PD and APD, early differentiation between these diseases is very important. A recent cohort study evaluated the usefulness of NLCP levels determination in the blood in order to differential diagnosis of PD and APD i.e., multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD), with same accuracy as CSF NLCP. The study included 504 people grouped in three cohorts. Two cohorts included healthy controls and patients with PD, MSA, PSP and CBD with average disease duration of four to six years. The third cohort was comprised of patients with APD with disease duration ≤3 years. The results showed that blood levels of NLCP were elevated in patients with APD compared to healthy individuals and those with PD in all cohorts ($p < 0.001$). Determination of blood levels of NLCP can distinguish between PD and APD, with high diagnostic accuracy (0.81 – 0.91). This accuracy was similar to that obtained when used NLC determination in CSF (0.85–0.90). In researchers' opinion, these findings can improve the diagnostic algorithm of parkinsonian disorders (Hansson O, Janelidze S, Hall S, Magdalinou N, Lees AJ, Andreasson U, Norgren N, Linder J, Forsgren L, Constantinescu R, Zetterberg H, Blennow K. Swedish BioFINDER study. Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. *Neurology*. 2017 Feb 8. pii: 10.1212/WNL.0000000000003680).

Cătălina Luncă

A RARE CAUSE OF HIGH INTESTINAL OBSTRUCTION

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A RARE CAUSE OF HIGH INTESTINAL OBSTRUCTION–CASE REPORT (Abstract): Portal vein thrombosis is a frequent thrombotic complication of cirrhosis. Thrombosis can extend to the mesenteric veins, causing acute intestinal ischemia. We report the case of a 61-year-old female who developed high intestinal obstruction caused by distal jejunal infarction one month after an episode of spontaneous bacterial peritonitis. The patient underwent segmental enterectomy with end-to-end anastomosis, with favorable clinical outcome, which allowed us to initiate anticoagulation treatment with low-molecular-weight heparin. Intestinal ischemia due to by mesenteric vein thrombosis can be tardily diagnosed, since it causes nonspecific clinical symptoms. The treatment of mesenteric vein thrombosis requires an interdisciplinary team and includes resection of non-viable intestinal segments, anticoagulation, thrombolysis or trans catheter thrombectomy. However, the use of anticoagulation in cirrhotic patients increases both the risk of esophageal varices rupture and the extent of the bleeding. **Keywords**: PORTAL VEIN THROMBOSIS, CIRRHOSIS, MESENTERIC VEIN THROMBOSIS, INTESTINAL INFARCTION.

Portal vein thrombosis (PVT) is one of the most common thrombotic events in patients with liver cirrhosis (LC). PVT is less common in patients with compensated LC (1%) but has a prevalence of 8-25% in subjects with advanced liver failure, awaiting transplantation (1,2). If in non-cirrhotic patients PVT is often related to prothrombotic states (myeloproliferative neoplasms, inherited coagulation disorders) (3), in cirrhotic subjects the pathophysiology of PVT is more complex. The risk of developing PVT is independently correlated with the severity of the liver damage, since such patients present the three constituents of the Virchow's triad (4, 5). A recent report demonstrated that the activity of von Willebrand factor-cleaving protease is independently correlated with PVT in cirrhotic subjects (6). Modified platelet indices, such as mean platelet volume and platelet distribution width, have been associated with prothrombotic state in cirrhotic patients (7), but a recent study proved that platelet hyperactivity is not correlated with a higher incidence of PVT (8).

While the obstruction of the portal and splenic veins leads to portal hypertension-

related complications, the occlusion of the mesenteric vein is associated with high mortality rates precipitated by intestinal infarction (3, 9). The clinical presentation of intestinal infarction includes persistent abdominal pain, gastrointestinal bleeding, abdominal guarding, contracture and multi-organ dysfunction syndrome (10).

CASE REPORT

ZM, a 61-year-old female, presented at hospital with complaints of vague abdominal pain, nausea, fever and chills. The onset of symptoms was 7 days prior to admission. Her past medical history included LC caused by chronic hepatitis C, for which she had completed the interferon-free therapy in the previous month, with sustained virologic response (SVR).

On admission, she exhibited diffuse abdominal tenderness, tachycardia (110 beats per minute) and normal blood pressure. Routine laboratory tests showed inflammation, slightly elevated creatinine levels, mild thrombocytopenia, anemia and elevated bilirubin levels.

Abdominal ultrasound revealed a small amount of free abdominal fluid (not suitable for paracentesis), enlarged spleen and coarsened liver echotexture. The clinical context, along with the newly developed ascites was suggestive of spontaneous bacterial peritonitis. Antibiotics, antalgics and spasmolytics led to the remission of the symptoms.

The patient was readmitted with nausea and recurrent abdominal pain a month after her initial presentation. On the third day of hospital stay, during the bowel cleansing protocol for colonoscopy, the patient complained of worsening abdominal pain, incoercible vomiting and absence of bowel movements. Laboratory tests revealed worsening renal function and progressive inflammatory syndrome. Abdominal ultrasound detected dilated intestinal loops. Upper endoscopy revealed grade II esophageal varices and gastric distension. Abdominal radiography (X-ray) (fig. 1) showed multiple air-fluid levels in the topography of the small intestine.

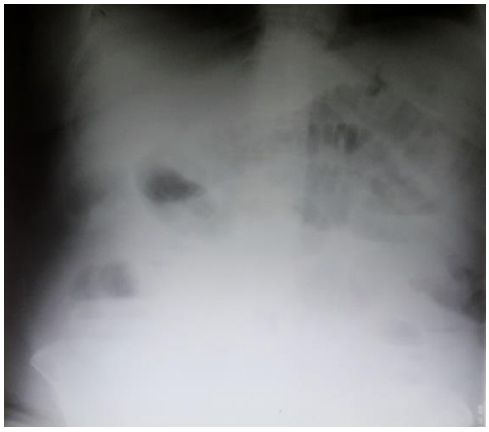

Fig. 1. Plain Abdominal X-ray. Distended intestinal loops, air-fluid levels

Fig. 2. Abdominal CT. The arrow points towards portal vein thrombosis

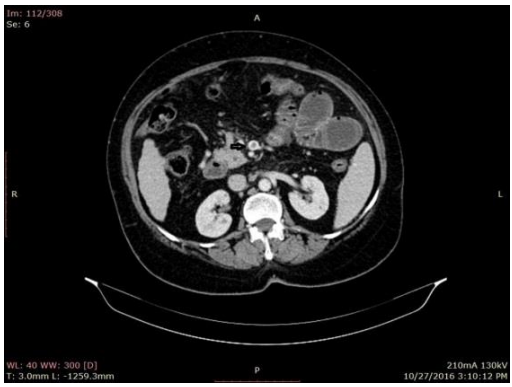

Fig. 3. Abdominal CT. The arrow points towards superior mesenteric vein thrombosis

Fig. 4. Abdominal CT. Bowel wall thickening (up to 12 mm), distended loops

Emergency contrast enhanced computed tomography (CT) described an extensive thrombosis of the portal vein and superior mesenteric vein. The distal part of the jejunum presented radiographic signs of ischemia while the proximal loops appeared dilated and with multiple air-fluid levels (fig. 2, 3, 4). The patient underwent segmental enterectomy (intraoperative aspect-venous jejunal infarction) with end-to-end anastomosis. The clinical outcome was favorable and we initiated low-molecular-weight heparin. Abdominal Doppler ultrasonography performed two months after surgery revealed permeable portal and superior mesenteric vein, with hepatopetal portal flow and no residual venous thrombosis.

DISCUSSION

The development of PVT in a cirrhotic patient is asymptomatic in 43% of cases. However, 39% of patients develop gastrointestinal bleeding and 18% present acute abdominal pain, mostly due to intestinal infarction (5). Diagnosis relies on Doppler ultrasonography. CT and magnetic resonance imaging are indicated to evaluate the extent of the thrombus and to screen for local malignancies (11).

Only 5-15% of cases of intestinal ischemia are attributed to mesenteric venous thrombosis (MVT). MVT is generally associated with malignancies, prothrombotic states, surgery, inflammatory bowel disease, intra-abdominal infections and trauma (12). Infarction due to MVT occurs when the thrombosis fully occludes the venous return. Associated vasospasm, which can persist even after the remission of venous drainage obstruction, has also been reported (13). Venous mesenteric infarction is associated with a mortality rate of 20-60% and frequently requires extended resections, with high risk of postsurgical complications (9).

MVT is rarely localized in the inferior mesenteric vein. The affected bowel segment is usually the ileum (64-83%) and the jejunum (50-80%). The colon is involved only in 14% of cases, due to its numerous collaterals (13).

Subjects usually seek medical care after 5-14 days and the intensity of pain is disproportionate to the clinical exam (13). Our patient presented fluctuating abdominal discomfort, which worsened during bowel cleansing protocol. Fever and signs of peri-

toneal irritation suggest the progression of intestinal infarction (12) but our subject was afebrile during hospitalization. Ascites formation and hypotension are correlated with poor outcome (12).

Laboratory tests are not specific for the diagnosis of MVT and include elevated serum lactate, leukocytosis and hemoconcentration (12). Plain abdominal X-ray is usually negative but this case presented as a high intestinal obstruction, with visible air-fluid levels.

Contrast-enhanced CT is the preferred diagnostic test for MVT. Intestinal ischemia is suggested by wall thickening, bowel distention, modified enhancement patterns, abnormal mesentery and newly developed ascites (13). Although splanchnic angiography is still the gold standard imaging test for MVT, it should be employed only in patients who could undergo intra-arterial treatment. Doppler ultrasound is specific but has low sensitivity as it cannot detect small mesenteric branches (13).

Baveno VI consensus recommends that in patients with acute PVT, non-candidates for liver transplant, anticoagulation with low-molecular-weight heparin or vitamin K antagonists could be considered when the thrombosis extends to the superior mesenteric vein (14). Thrombolysis and trans catheter thrombectomy have proven ineffective in patients with LC. Enterectomy of non-viable bowel segments is recommended (14).

However, early diagnosis of MVT and prompt initiation of anticoagulation can prevent intestinal infarction and the need for surgery. Enterectomy could not be avoided in this case because the patient developed high intestinal obstruction. After the procedure, we considered low-molecular-weight heparin to be the only therapeutically option. Although anticoagulation could increase the risk of esophageal variceal bleeding, we used non-selective beta-blocker for primary prevention of this complication (11, 14). The clinical outcome was positive with complete recanalization of portal and superior mesenteric veins.

Interferon-based therapy for hepatitis C has previously been associated with MVT and ischemic colitis (15), but our patient had undergone the interferon-free regimen. To our knowledge, this is the first published case of acute PVT and MVT after interferon-free treatment in a patient with SVR. Even after viral clearance, advanced hepatic fibrosis and portal hypertension persist and increase the risk of PVT in cirrhotic patients.

CONCLUSIONS

Portal vein thrombosis with extension to the mesenteric vein can complicate the natural course of cirrhosis, independent of viral clearance. The diagnosis of this complication requires a high index of clinical suspicion in cirrhotic patients presenting with new onset ascites and abdominal pain.

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NEWS

FATAL FOLIC ACID TOXICITY IN HUMANS

Folic acid is B-9 vitamin. Folic acid is prescribed commonly for pregnant women to prevent neural tube defects in the fetus, patients under chemotherapy, pernicious anemia and to reduce the risk of stroke and cardiovascular disease. Acute or chronic ingestion of a large dose of folic acid generally manifests as neurological complications, which are reversible. A 23-year-old pregnant woman committed suicide by consuming folic acid tablets and succumbed to death within 36 h. Postmortem toxicological analysis detected folic acid in viscera. Death following acute consumption of folic acid is rare and has been not reported in the literature (Devnath GP, Kumaran S, Rajiv R, Shaha KK, Nagaraj A. Fatal Folic Acid Toxicity in Humans *J Forensic Sci.* 2017 Mar 6).

A. Knieling, Diana Bulgaru Iliescu

MAGNESIUM IN PEDIATRIC NEPHROTIC SYNDROME

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MAGNESIUM IN PEDIATRIC NEPHROTIC SYNDROME (Abstract) **Aim**: In the present study, we aimed to evaluate serum Mg concentration in children with nephrotic syndrome and highlight the relationship between Mg concentration and acute nephropathy. **Material and methods**: We conducted a clinical study in 27 patients, aged 2 to 17 years, admitted to the Nephrology clinic of the Iasi "Sf. Maria" Children′s Hospital between 2011-2015, with the diagnosis of idiopathic nephrotic syndrome (NS), first episode or relapse. In each patient, we investigated serum urea and creatinine, total cholesterol, total serum proteins, serum magnesium, urinary proteins, creatinine clearance and renal histopathology. We also used a control group of 14 children with normal renal function. **Results**: 12 patients had NS in the acute phase, 6 steroid responsive and 6 steroid resistant. The remaining 15 patients were in the remission phase of NS, 7 steroid responsive and 8 steroid resistant. Significantly decreased serum Mg levels were found in NS active group compared to control group. Renal histopathological analysis in steroid responsive patients revealed the following pathological aspects: optically normal glomeruli (1 case), minimal mesangial changes (5 cases), IgM nephropathy (4 cases), mesangioproliferative glomerulonephritis (4 cases). **Conclusions**: The variations of serum and urinary Mg levels in children with acute nephropathy may be useful for early diagnosis and improving therapy. **Keywords:** MAGNESIUM, NEPHROTIC SYNDROME, CHILDREN.

Nephrotic syndrome (NS) is one of the most frequently encountered glomerulopathy in children, being characterized by altered permeability of the glomerular capillaries and the inability to control urinary protein loss, leading to hypoproteinemia, hyperlipidemia and edema (1). Of all NS cases, about 90% are idiopathic, with favorable response to corticosteroids, and good prognosis. Ten percent of cases are steroid resistant, with little or no response to usual corticosteroid treatment (2, 3).

In terms of histopathology, NS can have the following aspects: minimal change disease (76.4%), mesangioproliferative glomerulonephritis (2.3%), membranoproliferative glomerulonephritis (7.5%), focal segmental glomerulosclerosis (6.9%), and membranous nephropathy (1.5%). Of the patients with minimal change disease, 95% respond favorably to corticoids (4). In NS, impaired glomerular function causes tubulointerstitial alterations through different mechanisms: glomerular hemodynamic and

selectivity disturbances, immunologic mechanisms, inflammatory mediators, leukocyte migration. All these lead to destruction of the glomeruli and renal tubules, and finally to tubulointerstitial lesions (5). About one third of steroid-resistant cases evolve towards end stage renal disease within 5 years of disease onset. Histopathology with minimal glomerular damage and mild mesangioproliferative glomerulonephritis has a benign clinical and pathological course without major tubulointerstitial lesions. Instead, focal segmental glomerulosclerosis and diffuse mesangioproliferative glomerulonephritis associate significant tubulointerstitial alterations and an increased risk of developing chronic kidney disease (6). Thus, early recognition of the histopathologic type and of the degree of the tubulointerstitial damage could be useful in establishing a targeted and effective therapy.

In the present study, we aimed to evaluate serum Mg concentration in children with nephrotic syndrome and highlight the relationship between Mg concentration and acute nephropathy.

MATERIAL AND METHODS

We conducted a retrospective study of 27 patients, aged 2 to 17 years, admitted to the Nephrology clinic of the Iasi "Sf. Maria" Children′s Hospital between 2011-2015 with the diagnosis of idiopathic nephrotic syndrome, first episode or relapse. Also, included in the study were patients diagnosed before the year 2011, but who during the study interval had at least one hospitalization for treatment and reassessment. Of the 27 patients, 12 had acute nephrotic syndrome, 6 steroid-responsive and 6 steroid-resistant. The remaining 15 patients were in the remission phase of NS, 7 favorably responding to corticosteroid therapy and 8 being steroid-resistant.

Inclusion criteria were: age 0 - 18 years; diagnosis of idiopathic nephrotic syndrome. Exclusion criteria were: diagnosis of congenital nephrotic syndrome or secondary nephrotic syndrome; simultaneous presence of other diseases such as systemic lupus erythematosus, Henoch-Schonlein purpura, conditions that could cause magnesium loss (gastrointestinal illnesses, burns), administration of nephrotoxic substances (loop diuretics, gentamicin, contrast agents).

The following data were collected from each patient: socio-demographic characteristics, biochemical parameters: urea, creatinine, total cholesterol, total serum proteins, serum magnesium, urinary proteins, creatinine clearance, and renal histopathologic appearance. Measurement of all investigated parameters was performed using the standard methods of biochemistry and histopathology laboratories of the "Sf. Maria" Children's Hospital. Creatinine clearance was calculated using the formula: Cl Creat (ml/min/1,73m^2) = ((0,035 x Age (years)) + 0,236) x 100) / serum creatinine (mg/dL) (7) and renal histopathological analysis.

We used a control group of 14 children, aged 2-17 years, with normal renal function, hospitalized in the General Pediatrics Clinic with functional abdominal pain syndrome, in which we followed-up the serum Mg levels for comparing them with the levels reported in the study group. The study was conducted with the approval of the Ethics Committee of the institution for the use of medical data and informed consent was obtained from all patients. Statistical analysis was performed using unpaired t-test and Fisher's test. The results were expressed as mean ± standard devia-

tion. Coefficient p values <0.05 were considered statistically significant.

RESULTS

The study was conducted on a sample of 27 patients (16 males and 11 females), aged 2 to 17 years, diagnosed with idiopathic nephrotic syndrome, which had at least one hospitalization in the Nephrology Clinic in the interval 2011-2015. The diagnosis of nephrotic syndrome was based on the presence of the following biological changes: hypoproteinemia, hypercholesterolemia, proteinuria. For NS in remission phase, the values of these parameters were within normal ranges.

In the study group, 12 patients had acute NS and 15 patients NS in remission phase. Depending on the response to treatment, steroid resistance was detected in 6 patients with active SN and 8 patients in remission (fig. 1).

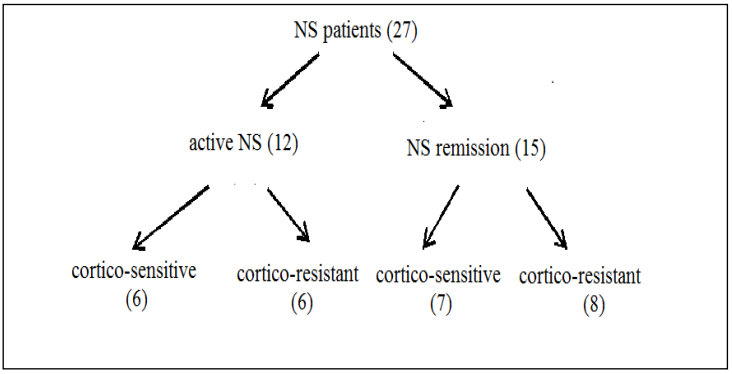

Fig. 1. Distribution of nephrotic syndrome in the study group

The mean age was 6.58 ± 4.14 years for active NS group, and 9.66 ± 4.65 years for remission NS group.

TABLE I

Biochemical parameters in study patients

Parameter	active NS (n=12)	NS remission (n=15)
total serum proteins (g/l)	44.62 ± 7.18*	68.21 ± 5.40
total cholesterol (mg/dl)	381.16 ± 134*	169.06 ± 31.49
urinary proteins (mg/dl)	300 ± 0*	0
urea (mg/dl)	32.91 ± 11.62	27.33 ± 6.14
creatinine (mg/dl)	0.71 ± 0.58	0.59 ± 0.12
creatinine clearance (ml/min/1,73m^2)	82.23 ± 31.38	96.90 ± 23.63
Serum Mg (mg/dl)	1.96 ± 0.30*	2.31 ± 0.77

* p<0.0001

The investigated parameters, namely total serum proteins, total cholesterol and urinary proteins, showed mean levels significantly different in patients with active NS compared to children with NS in remission. Thus, in the active NS group compared to remission NS group, serum proteins levels were low (p<0.0001), serum

total cholesterol levels were higher ($p<0.0001$) and proteinuria was present in all children with active NS, while patients in remission showed no urinary protein loss ($p<0.0001$) (tab. I). All these data confirmed the acute nature of the disease in the active NS group, and the favorable response to treatment with occurrence of remission and normalization of parameters that are typically affected in acute nephropathy for the group with NS in remission. Regarding renal function parameters, serum urea and creatinine, one case of azotemia was reported in active NS group (serum creatinine 2.47 mg/dl), while in NS remission group there was no case of nitrogen retention. Also, creatinine clearance values did not vary significantly between acute NS patients and NS remission group (tab. I).

Renal histopathological analysis of steroid-resistant patients in the 2 groups revealed the following pathological aspects: active NS group: optically normal glomeruli (1 case), minimal mesangial changes (2 cases), IgM nephropathy (3 cases); remission NS group: minimal mesangial changes (3 cases), mesangioproliferative glomerulonephritis (4 cases), IgM nephropathy (1 cases). The most common histological aspect was minimal mesangial changes (36%), followed by the mesangioproliferative aspects (29%) and IgM deposits (28%).

TABLE II.

Serum Mg (mg/dl) in the groups active NS, remission NS and control. Statistical significance – unpaired t test

Group	n	Mean	Standard Deviation	Standard Error	Min	Max	p
Active NS	12	1.96	0.30	0.08	1.53	2.30	<0.05*
Remission NS	15	2.31	0.77	0.19	1.61	4.81	0,71
Control	14	2.23	0.10	0.02	2.10	2.43	

*vs. control

The results showed decreased serum Mg levels in active NS group (1.96 ± 0.30 mg/dl) compared to control group (2.23 ± 0.10 mg/dl), the difference being statistically significant ($p<0.05$) (tab. II). However, although serum Mg was significantly lower in patients with acute nephropathy compared to controls ($p<0.05$), divalent cation concentration showed no significant variation between active and remission groups.

DISCUSSION

Literature data (8, 9) have suggested a link between serum and urinary Mg and tubulointerstitial function, given that both reabsorption of filtered Mg and retention of serum Mg occur in renal tubules. Any structural or functional alteration of the renal epithelium interferes with the tubular loss and reabsorption of Mg, leading to increased Mg urinary excretion. Hypomagnesemia is a common electrolyte disorder which occurs in approximately 12% of hospitalized patients. This incidence may increase to 60-65% in patients in Intensive Care Units due to nutritional deficiencies, hypoalbuminemia, hypotension, sepsis, use of such medications as diuretics or amino-

glycosides (10).

The results of our study show that serum Mg levels were significantly lower in patients with active nephropathy compared to those in remission ($p<0.5$). Since Mg is a divalent cation and its homeostasis depends on kidney filtration, low Mg levels in children with active NS reflect an alteration of renal excretion and reabsorption of Mg (11, 12).

In the literature, there are few studies regarding the implications of Mg deficiency on renal function after an episode of acute kidney injury (AKI). Santos et al. (13) showed that hypomagnesemia is a risk factor for irreversible renal function recovery in patients with AIDS (acquired immunodeficiency syndrome) and AKI. Similarly, Alves et al. (14) investigated Mg variation in a group of 232 patients admitted in the Intensive Care Unit and who experienced impaired renal function. It has been demonstrated that the prevalence of hypomagnesemia was 63% in the study group, the patients who showed no recovery of renal function having a significantly higher prevalence of hypomagnesemia (70%) compared with those with recovered renal function (31%).

Cheungpasitporn et al. (15) investigated the link between Mg variation and the occurrence of renal impairment in a group of 9241 patients, of which 124 developed AKI. Their results showed that the lowest incidence of AKI was in patients with serum Mg levels within the normal range, between 1.7 and 2.1 mg/dl. Instead, both hypomagnesemia (< 1.5 mg/dl) and hypermagnesemia (> 2.3 mg/dl) were associated with a higher incidence of developing kidney damage.

There are several theories that may explain the association between magnesium deficiency and renal impairment in AKI. Mg competes with calcium (Ca) transport in the cell membranes, leading to decreased intracellular calcium concentration, and secondary to smooth muscle relaxation (16). Administration of Mg produces peripheral vasodilatation, predominantly arteriolar, not only by stimulating the production of nitric oxide, but also by its ability to antagonize directly with Ca (17).

In addition to vasodilatory effects, Mg is associated with effects on microcirculation, such as: increased deformability of red blood cells, reduced platelet aggregation, anti-inflammatory effects, maintaining the endothelial integrity (18). Other similar studies (8, 19) which have investigated the homeostasis of Mg in nephrotic syndrome, showed the existence of increased Mg urinary excretion, in focal and segmental glomerulosclerosis and mesangioproliferative glomerulonephritis. Also, Gheissari et al. (20) revealed elevated renal Mg excretion, suggesting the possible use of Mg concentration as an early marker for detecting the tubular malfunction and as an indicator of residual tubular lesions.

Our results showed, in patients with steroid resistant NS, histopathological lesions which are consistent with literature data, indicating the presence of minimal change glomerulonephritis in steroid resistant NS in proportions ranging from 25% (21) to 45.5% (4).

CONCLUSIONS

The present clinical trial is particularly useful in medical practice, suggesting the importance of investigating the variations of serum and urinary Mg in pediatric acute nephropathy, along with standard measures of other ions such as Na, K, Cl, with the possible early detection of patients with unfavorable response to therapy.

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ADVANCED SARCOIDOSIS WITH APPARENT ONSET BY RESPIRATORY FAILURE

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ADVANCED SARCOIDOSIS WITH APPARENT ONSET BY RESPIRATORY FAILURE (Abstract). We present the case of a 14 years-old male (with poor living condition, non-smoker) hospitalized for a sudden onset of a rest dyspnea, mucopurulent cough. We found bronchial sibilant rales, wheezing, cyanosis, peripheral adenopathies, hepatosplenomegaly, purulent tonsilitis. The chest-x-ray revealed bilateral mediastinal lymphadenopathy and bilateral lung infiltrations. Spirometry: severe mixed ventilatory dysfunction without reversibility. Sputum: negative microscopy and culture for Koch bacillus. Stool examination: cysts of Lamblia giardia. The patient refused bronchoscopy and mediastinoscopy, so it was performed axillary lymph node biopsy which confirmed sarcoidosis (non-caseating epithelioid granulomas). The treatment included antibiotics, antiparasites, oral and inhaled corticosteroids (CS), bronchodilators, oxygen, with clinical/functional improvement after 3 months. CS was followed 1.5 years with poor compliance. A relapse occurred after 3 years and the CS were reinserted. The computerized tomography (CT) scan revealed a diffuse interstitial fibrosis with bronchiectasis. The case particularity relies on the atypical early onset of the sarcoidosis, with respiratory failure and progression to lung fibrosis despite CS treatment. The association of proinflammatory risk factors such as multiple infections needs to be noted.
Keywords: SARCOIDOSIS, LUNG FIBROSIS, RESPIRATORY FAILURE

Sarcoidosis (S) is a multiple organ granulomatous disorder of unknown etiology. It usually affects young and middle aged adults and initially involves target organs – lung, lymph nodes, skin, eye, joint and liver but it can overtake all organ systems. Organ like salivary glands, heart, nervous system, muscles, bones, kidney are also frequently affected in extra pulmonary S (1). The S diagnosis is established upon clinical and radiological aspects supported by the histopathological evidence of the noncaseating epithelioid granulomas. Commonly seen immunological aspects are depression of the delayed cellular immune response and B lymphocytes over response (1, 2). The treatment depends upon staging, locations, respiratory functional tests (RFTs) and symptoms. For stage II or III pulmonary sarcoidosis (PS) and impaired

RFTs, oral steroids (OS) are recommended. The proper length of therapy for patients who respond to treatment is unknown (6 months - 1 year) (3). Every 3 months, clinical features, chest-x-ray and RFTs will be monitored. Angiotensin-converting enzyme activity (ACE) seems to be sensitive for the diagnosis and evolution assessment and the OS therapy monitoring (4).

CASE REPORT

A 14-year-old male (with poor living condition, from rural area, non-smoker) was admitted in the Pulmonology Clinic with an apparently acute onset of a rest dyspnea, high fever, asthenia, weight loss (7 kg/2 weeks), nasal obstruction, productive cough. The patient's medical history noticed frequent adenoiditis and repeated acquired pneumonia. We found cyanosis, axillaries enlarged lymph nodes, diffuse bilateral sibilants, hepatosplenomegaly, purulent tonsillitis. The chest-x-ray revealed bilateral mediastinal/hilar adenopathies, diffuse interstitial opacities and bilateral basal infiltrative condensation (fig.1). Spirometry: severe mixed ventilator dysfunction (the Vital Capacity - VC 50%, Forced Expiratory Volume in one second - FEV1 52%) and oxygen saturation: 90% in ambient air.

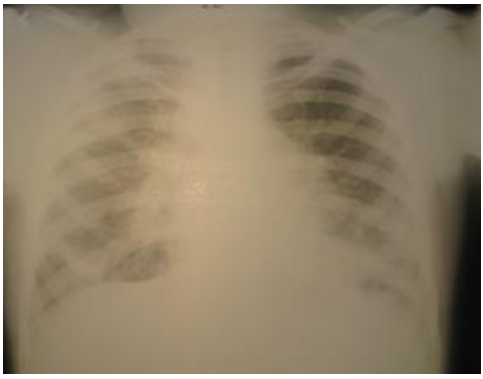

Fig. 1. Chest radiograph: stage II sarcoidosis

The blood tests: leukocytosis 21.000/µL with neutrophilia, increased hepatic enzymes (57.103 U/L), mild anemia, dysproteinemia (α2 - 16%; β - 21%; γ - 26,7%; albumins - 29%) and pathological antibodies: IgG - 1100mg%; ASLO - 600 UI. Blood cultures and tuberculin skin tests were negative and Koch bacilli were negative in sputum (microscopy and culture). Pharyngeal exudate reveals 2 germs: *Staphylococcus aureus* and *Streptococcus beta-hemolyticus*. Stool examination: cysts of *Giardia lamblia.* The patient refused the mediastinoscopy and bronchoscopy however the axillaries lymph node biopsy confirmed the S by highlighting of non-caseating epithelioid granulomas.

The final diagnostic was: evolutive stage II pulmonary sarcoidosis with extra pulmonary lymph nodes and liver/spleen involvement; bronchopneumonia; severe mixed ventilatory disorder and respiratory failure; digestive lambliasis.

A complex treatment was started: oxygen therapy, oral corticosteroids (OCS) 0.7 mg/kg (initially for 3 months, then with gradually decrease with 10 mg/month till 20 mg/kg). The patient had 10 days with antibiotics, antiparasitic treatment, inhaled corticoids and long term bronchodilators. Clinical status improved slowly and the patient was discharged after 14 days with OCS. After 2 months we noticed a favorable evolution with weight gain, return in appetite and RFTs improvement with 33%. The OCS lasted 1.5 years but the patient had a modest compliance to medical controls and treatment.

A relapse of the disease occurs after 3 years. RFTs dropped toward a very severe ventilatory disorder (VC 39%, FEV1 42%) and ACE raised to 120 U/l announcing an evolutive phase of S. The computerized

tomography (CT) scan revealed a diffuse interstitial fibrosis with bronchiectasis and right upper honeycomb image (fig. 2, 3).

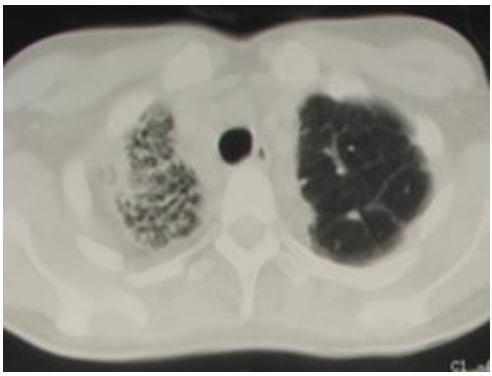

Fig. 2. Thoracic CT: stage III/IV sarcoidosis with extended fibrosis and bronchiectasis in the right upper lobe and emphysema in the left upper lobe

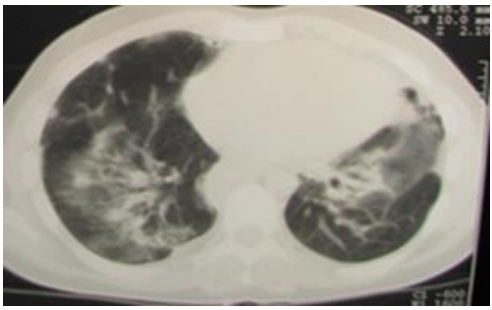

Fig. 3. Thoracic CT: stage III/IV sarcoidosis with diffuse interstitial fibrosis and bronchiectasis

We reinserted OCS, inhaled corticoids/bronchodilators and antibiotics in short cure. We also recommended dental medicine treatment and respiratory rehabilitation. 1-year treatment was conducted with favorable outcome. The patient's reassessment was difficult because of the lack of cooperation to medical investigation and controls. The patient is now in a stable condition but with advanced ventilatory mixed disorder (VC 45%, FEV1 48%). He is following combined inhaled corticoids/bronchodilators.

DISCUSSION

Sarcoidosis can occur at any age but it is relatively rare for children. Our case had an apparently acute onset at the age of 14, but in reality the patient was already in the second stage of the disease with extra pulmonary involvement and a previous slow onset. It was found that an acute onset with erythema nodosum and bilateral hilar lymphadenopathy usually denotes a self-limiting development, while the insidious onset and extra pulmonary lesions predict an evolution toward progressive lung fibrosis (5).

Our patient had cumulated several known risk factors which rushed the disease progression: late diagnosis in a secondary stage of S, with respiratory insufficiency, coexistence of repeated bacterial infection and parasites which made a pressing on immunological host response (1, 6).

We used ACE as a biologic marker to help diagnosis and disease evolution monitoring. It is recognized that elevations greater than two times the upper limits of normal are frequently met in S and much less common in other diseases (tuberculosis, hypertiroidism, Gaucher's disease, lymphoma) (1).

The need for differential diagnostic with lymphoma and tuberculosis was strong and this shows the diagnostic difficulties of the disease, settled here by the histological typical aspect in the axillaries lymph node biopsy. The repeated respiratory infections and parasites may contribute to inflammation, to trigger the disease and its evolution (7).

The inhaled corticosteroids (CS) had a

good tolerance even though he presented bronchial obstruction and diffuse interstitial peri bronchiolar lesions. Several studies have demonstrated some benefit of CS inhaled therapy (8, 9).

CONCLUSIONS

Our case is a useful opportunity for clinical remarks firstly given for the atypical onset - early in the puberty, in an acute presentation with respiratory failure (determined both by the diffuse sarcoid infiltrations and by the over imposed bacterial bronchopneumonia).

We can also note the lack of common symptoms (Löfgren syndrome) and the first presentation in an advanced stage of sarcoidosis (stage II), with extra pulmonary determinations and the rapid evolution towards diffuse interstitial fibrosis despite the corticoid treatment. The patient's lack of compliance to investigations and treatment with frequent OCS abandonment may also contribute to the rapid progression.

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CORTICOSTEROID TREATMENT IN THE SETTING OF DECOMPENSATED LIVER CIRRHOSIS WITH RELATIVE ADRENAL INSUFFICIENCY: A CASE REPORT AND A BRIEF REVIEW OF THE LITERATURE

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CORTICOSTEROID TREATMENT IN THE SETTING OF DECOMPENSATED LIVER CIRRHOSIS WITH RELATIVE ADRENAL INSUFFICIENCY: A CASE REPORT AND A BRIEF REVIEW OF THE LITERATURE (Abstract): Relative adrenal insufficiency (RAI) is the term used to describe inadequate production or action of glucocorticoids with respect to the severity of the illness. RAI is frequently found in critically ill patients particularly with septic complications and it is also present in both critically ill and stable patients with liver cirrhosis. In the following study a case report of a patient with decompensated cirrhosis and RAI is presented followed by a brief review of the literature. A 65-year-old male with liver cirrhosis of alcoholic etiology was admitted to hospital with bilateral leg edema, ascites, and marked weakness. At admission, his blood pressure was 82/52 mmHg and he had sinus tachycardia of 130/min. Laboratory analysis revealed hyponatremia (122 mmol/L), while ascites fluid analysis showed no infection. During the first 48 hours of hospitalization the patient remained persistently hypotensive despite adequate vascular filling and the addition of noradrenaline. A standard-dose short synacthen test was performed which revealed a poor cortisol response, which is a compatible criterion for the diagnosis of RAI. Intravenous hydrocortisone therapy was initiated, which resulted in a rapid improvement in patient's general condition, and increase in blood pressure. As the patient became hemodynamically stable without the need of noradrenaline, the hydrocortisone dose was weaned progressively, and he was discharged after 18 days of hospitalization in a stable condition. **Keywords:** RELATIVE ADRENAL INSUFFICIENCY; LIVER CIRRHOSIS; HEPATO-ADRENAL SYNDROME; CORTISOL.

Relative adrenal insufficiency (RAI) is the term given to inadequate production or action of glucocorticoids with respect to the severity of illness, and it is frequent in critically ill septic patients (1) as well as in both stable and decompensated cirrhosis with or without sepsis (2-5). The term "hepato-adrenal syndrome" defines an adrenal dysfunction in critically ill cirrhotic patients, and suggests that adrenocortical insufficiency may be a feature of liver disease *per se*, with a different pathogenesis from that occurring in septic shock (4). Only a few studies assessed corticosteroid therapy in critically ill cirrhotic patients with RAI (4,6-8), and the results were

mostly controversial. Some studies reported favorable effects (4,6,7), while a randomized control study (8) has shown no benefit. In this paper, we report a case of a patient with decompensated cirrhosis and RAI, with a brief review of the literature.

CASE REPORT

A 65-year old male with decompensated liver cirrhosis of alcoholic etiology was referred to our hospital with large ascites (grade 3), bilateral leg edema and marked weakness. He had a long history (25 years) of alcohol abuse, although he denied any alcohol intake during the last year. On examination, the patient presented cutaneous arterial "spider" angiomas, palmar erythema, Dupuytren's contractures, grade 3 ascites, peripheral edema, gynecomastia, parotid enlargement, peripheral muscle wasting, mild jaundice, hepatomegaly and splenomegaly. His blood pressure was 78/48 mmHg, he had sinus tachycardia (130/min), and an increased respiratory rate (22/min). The digital rectal examination was normal. Laboratory analysis at admission revealed mild macrocytic anemia, leukocytosis with mild eosinophilia, and thrombocytopenia, elevations of AST, ALT and GGT, hyperbilirubinemia, hypocholesterolemia with low HDL- and LDL-cholesterol values, prolonged INR, hypoalbuminemia, electrolyte disturbance (sodium 122 mmol/L, potassium 2.9 mmol/L). The urine culture was negative and a chest X-ray showed no pathological elements. Abdominal ultrasound showed hepatomegaly with cirrhotic characteristics (nodular structure, heterogeneous echotexture, irregular margins), and signs of portal hypertension (enlarged portal vein of 14 mm, slow portal venous flow, enlarged splenic vein of 11mm, grade 1 splenomegaly) as well as grade 3 ascites. Large-volume paracentesis was performed associated with albumin infusion (8g for every liter of ascitic fluid removed). Ascites fluid analysis showed no infection (< 75 polymorphonuclear cells/mmc). Upper gastrointestinal endoscopy found large esophageal varices. During the first 24 hours of hospitalization, his blood pressure remained low despite vascular filling, and the patient was referred to the intensive care unit where intravenous norepinephrine was added to fluid replacement therapy. However, the blood pressure remained low (80/50 mm Hg), the patient's general condition deteriorated and he complained of marked muscle weakness and fatigue; consequently, an adrenocortical insufficiency was suspected and in the third day of hospitalization a standard-dose short synacthen test (SD-SST) was performed which showed a poor cortisol response, compatible with the criteria of RAI (baseline serum total cortisol < 15 μg/dL, delta cortisol < 9 μg/dL). Thus, in our patient serum total cortisol levels at baseline and 60 minutes after the corticotropin stimulation were 9.6 and 12.5 μg/dL, respectively, with a delta cortisol value of 2.9 μg/dL.

The patient received hydrocortisone intravenously 50 mg every 6 hours, beginning with the third day of hospitalization, and the blood pressure values as well as the patient's general condition greatly improved during the following 24 hours. The norepinephrine dose was gradually reduced and in the fifth day of hospitalization the vasoactive treatment was stopped with no significant change on the blood pressure. The hydrocortisone was weaned progressively (50 mg/day) and the patient was discharged after 18 days of hospitalization

in stable condition on spironolactone and propranolol.

DISCUSSION

Over the last two decades, several studies have reported that in critically ill patients with sepsis the adrenal glands respond inappropriately to stimulation, a condition named initially "relative adrenal insufficiency" (RAI). The name was replaced more recently by the term "critical illness related corticosteroid insufficiency" (CIRCI), defined as an "inadequate cellular corticosteroid activity for the severity of the patient's illness" (9). Both cirrhosis and septic shock share many hemodynamic abnormalities (e.g. hyperdynamic circulatory failure, peripheral vasodilatation, low blood pressure, increased production of proinflammatory cytokines) (10,11) and, consequently, several studies reported that RAI was found in critically ill cirrhotics with or without sepsis (2-4,6,7,12), in patients with stable cirrhosis (3,5,13), and in liver transplant recipients (4,14). The term "hepatoadrenal syndrome", used for the first time by Marik et al (4) defines adrenal insufficiency in critically ill advanced liver disease with sepsis and/or other complications, suggesting that adrenocortical insufficiency may be a feature of liver disease *per se*, with a different pathogenesis from that occurring in septic shock.

Mechanisms of RAI in cirrhotic patients remain largely unknown; however, they may include endotoxemia, impaired synthesis in total cholesterol, HDL and LDL, increased levels of proinflammatory cytokines, and "exhaustion" of the adrenal cortex due to the low levels of steroidogenic substrate (total cholesterol, HDL, LDL) (15).

The diagnosis of RAI in cirrhotic patients is based on the laboratory tests. SD-SST measures total serum cortisol at baseline and 60 minutes after intravenous or intramuscular injection of 250 μg of synthetic ACTH [tetracosactrin (synacthen Novartis Pharma AG, Basel, Switzerland) or cosyntropin (cortrosyn, Amphastar Pharmaceuticals, Rancho Cucamonga,CA, United States)]. A basal serum total cortisol level < 5 μg/dL indicates RAI, while a level > 15 μg/dL will rule out RAI (11). In critically ill patients with cirrhosis RAI is defined either by basal serum total cortisol < 15 μg/dL (2,6) or a delta cortisol (difference between basal serum total cortisol and serum total cortisol after stimulation) < 9 μg/dL (2,6,8,12). Other studies used a peak cortisol level (highest serum total cortisol concentration after stimulation) < 18 μg/dL (7) for the definition of RAI. Most of the studies that included critically ill cirrhotic patients used SD-SST for the diagnosis of RAI (2,6,7,12), while only a few performed a low dose-short synacthen test (LD-SST) (3,4) which uses 1 μg of synacthen given intravenously and serum cortisol measured after 30 minutes. Due to the limitations of all available diagnostic tests, SD-SST remains the most used test for the diagnosis of RAI in critically ill cirrhotic patients, while LD-SST seems to be more appropriate in non-critically ill patients with cirrhosis (11).

The data on corticosteroid therapy in critically ill cirrhotic patients and RAI is controversial, some studies reporting favorable results (4,6,7), while others showing no benefits (8). Harry et al (7) retrospectively assessed the effect of hydrocortisone treatment in a case-control study involving two groups of cirrhotic patients with hemodynamic instability and vasoactive treatment. The group that received hydrocortisone treatment (300 mg/daily)

had a decrease in noradrenaline requirement, but no benefit in survival. Marik et al (4) evaluated the effect of hydrocortisone given intravenously (300 mg/daily) in a group of cirrhotic patients with hemodynamic instability and found that in patients with RAI hydrocortisone therapy was associated with a significantly lower mortality rate and with a significant reduction of the norepinephrine dosage compared with those patients without RAI. Fernandez et al (6) using SD-SST, reported RAI in 17 of 25 cirrhotics with septic shock, and found that patients who received hydrocortisone therapy had higher rates of shock resolution and hospital survival. In a randomized controlled trial, Arabi et al (8) reported that hydrocortisone therapy (50 mg intravenously every six hours until shock resolution) in cirrhotic patients with septic shock was associated with higher rates of shock reversal and significant reduction in vasopressor doses; however, such therapy did not reduce hospital mortality and had higher frequency of shock relapse and gastrointestinal bleeding.

CONCLUSIONS

We presented a case of a cirrhotic patient in which RAI was associated with hemodynamic instability and hyponatremia. In our case, treatment with corticosteroids was beneficial and allowed reduction of vasopressor dosage. We therefore conclude that RAI should be suspected and SD-SST performed in any patient with decompensated cirrhosis, hypotension refractory to vasopressors and fluid resuscitation, marked muscle weakness and fatigue, hyponatremia and eosinophilia. Once the diagnosis of RAI is established, treatment with intravenous glucocorticoids can be considered for hemodynamically unstable cirrhotic patients with adrenal insufficiency as it is associated with reduction of vasopressor dosage and may improve survival.

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NEWS

TEA DRINKING AND TUBERCULOSIS

Numerous studies showed that tea drinking helps to prevent obesity, cardiovascular diseases, autoimmune diseases, neurodegenerative diseases and tumors. Chinese researchers discovered the negative association between tea drinking and tuberculosis (TB). Tea leaves contain epigallocatechin-3-gallate, a flavonoid which inhibit the bacterial growth and the bacillus survival in macrophages. The increasing tea consumption is associated with a decreased risk of TB. Subjects who consumed 151-300 g tea / month had a lower risk to develop TB than those with a lower intake of tea. The green tea led to the most evident protection against TB, compared to oolong and black tea. Future studies are needed to determine whether drinking tea can be used in the prevention and control of TB (Mengshi Chen; Jing Deng; Wufei Li; Dan Lin; Congxu Su; Mian Wang; Xun Li; Benjamin Kwaku Abuaku; Hongzhuan Tan; Shi Wu Wen. Impact of tea drinking upon tuberculosis: a neglected issue. *BMC Public Health* 2015;15(515)).

Cătălina Luncă

CORRELATIONS BETWEEN ARTERIAL HYPERTENSION INDUCED BY ANGIOTENSIN II AND SYSTEMIC INFLAMMATION IN RAT

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CORRELATIONS BETWEEN ARTERIAL HYPERTENSION INDUCED BY ANGIOTENSIN II AND SYSTEMIC INFLAMMATION IN RAT (Abstract): Angiotensin II has particularly shown to play a key role in the regulation of inflammatory processes in hypertension. **Aim:** The present study aims to correlate the angiotensin II – induced hypertension with systemic inflammation. **Material and methods:** We conducted an experimental study on Wistar male rats who received Ang II via subcutaneous miniosmotic pumps for 2 weeks. Rats were exposed to a 12h light /12h dark cycle. Sham rats were used as control. Systolic blood pressure measurements and a flow cytometric analysis of lymphocyte surface markers were performed. After 14 days, the animals were euthanized under anesthesia with xylazine/ketamine. **Results:** Systolic BP progressively and significantly increased in rats with Ang II chronic infusion. We observed a statistical significant difference ($p = 0.00001$), in terms of T lymphocytes percentage between control rats plasma and Ang II treated rats plasma, in 14 days. **Conclusions:** Angiotensin II is an important mediator of hypertension and directly promotes inflammation by noticeably increasing the quantity of T cells in kidney tissue. **Keywords:** ANGIOTENSIN II, HYPERTENSION, INFLAMMATION.

Hypertension is prevalent in 25% of the adult population and contributes to the development of coronary heart disease, stroke and chronic kidney disease (1). In 2003, the World Health Organization - International Society of Hypertension (WHO-ISH) provided a definition of hypertension as arterial pressure measurements of 140/90 mm Hg or greater (2). In only 5%-10% of patients, the causes of high blood pressure are identified. For the rest of cases the etiology remains unknown, and the hypertension is defined as "essential".

Numerous studies showed that renin angiotensin aldosteron system (RAAS) contributes to cardiovascular and renal homeostasis by generating angiotensin peptides, mainly angiotensin II (Ang II). It has been shown that increased levels of Ang II cause hypertension, cardiovascular hypertrophy (1), increased vascular reactivity to different vasoconstrictor agents (1,3), inflammation (4) and endothelial dysfunction (5). Ang II causes a mild increase in blood pressure having as results T cell activation and inflammation, these factors having as result a

sustained hypertension (6). The present study intends to highlight some correlations between arterial hypertension induced by angiotensin II and systemic inflammation.

MATERIAL AND METHODS

Male Wistar rats (250–260 g) (n=9) received Ang II (300ng/kgc/min) in NaCl 0.9% via subcutaneous miniosmotic pumps, for 2 weeks as previously reported (7). Rats were exposed to a 12h light /12h dark cycle. Sham rats were used as controls (n=9). Care, use, and treatment of all animals in this study were in strict agreement with the guidelines of the animal care from the University of Medicine and Pharmacy "Grigore.T. Popa" Iasi. Basal systolic blood pressure (SBP) measurements were performed for every rat by tail-cuff plethysmography, twice/week at a basal period. After 14 days the animals were euthanized under anesthesia with xylazine/ketamine and we collected blood samples. Flow cytometric analysis was used to study lymphocyte surface markers. Comparisons within groups were performed using paired Student´s t-test.

RESULTS

Systolic blood pressure. Before the treatments, systolic blood pressure was comparable in the two groups (~120 mmHg). Though, systolic BP gradually and significantly increased in animals with Ang II chronic infusion (208±2 mmHg for Ang II versus 120±5mmHg for control group, in the 14th day).

Cellular viability in plasma. In the Ang II treated rats plasma we observed a statistical significant increasing number of PI-positive cells (non-viable) compared to cells derived from control rat plasma (fig. 1).

The cellular viability in plasma from control rats, respectively Ang II treated rats, is shown in fig. 2 and 3.

T and B lymphocytes into plasma. In figures 4 and 5 we revealed the presence of T and B lymphocytes in rat plasma (control lot vs. Ang II lot). We observed a statistical significant difference ($p = 0.00001$), in terms of T lymphocytes percentage between control rats plasma and Ang II treated rats plasma, for 14 days (fig. 6). As shown in figure 7, the percentage of B lymphocytes plasma, was stastistically significant higher on rats treated with Ang II for 14 days ($p<<<0.05$) compared to control lot.

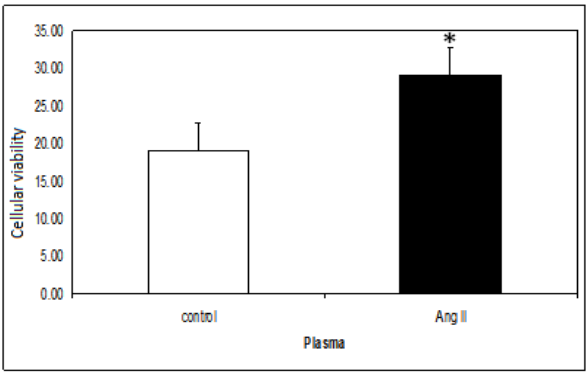

Fig. 1. Cellular viability percentage in rats plasma (control; Ang II) (*p=0.002)

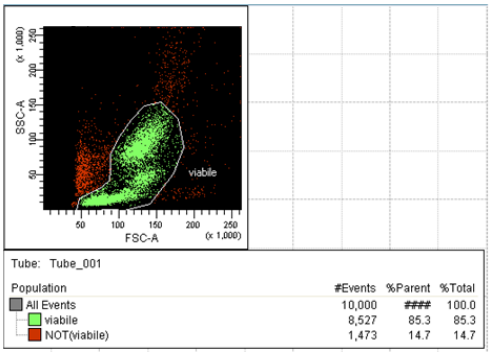

Fig. 2. Cellular viability in plasma (control lot)

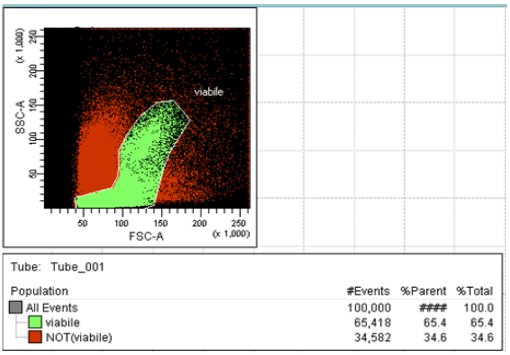

Fig. 3. Cellular viability in plasma (Ang II lot)

T and B lymphocytes into plasma. In figures 4 and 5 we revealed the presence of T and B lymphocytes in rat plasma (control lot vs. Ang II lot). We observed a statistical significant difference ($p = 0.00001$), in terms of T lymphocytes percentage between control rats plasma and Ang II treated rats plasma, for 14 days (fig. 6). As shown in figure 7, the percentage of B lymphocytes plasma, was stastistically significant higher on rats treated with Ang II for 14 days ($p<<<0.05$) compared to control lot.

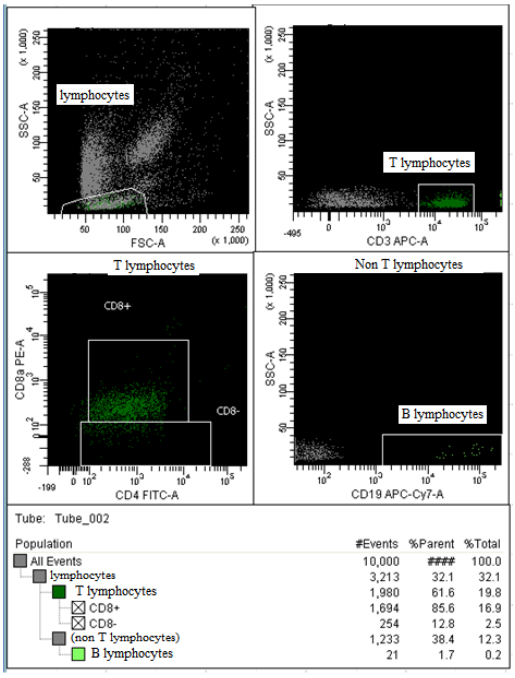

Fig. 4. T and B lymphocytes in plasma (control lot)

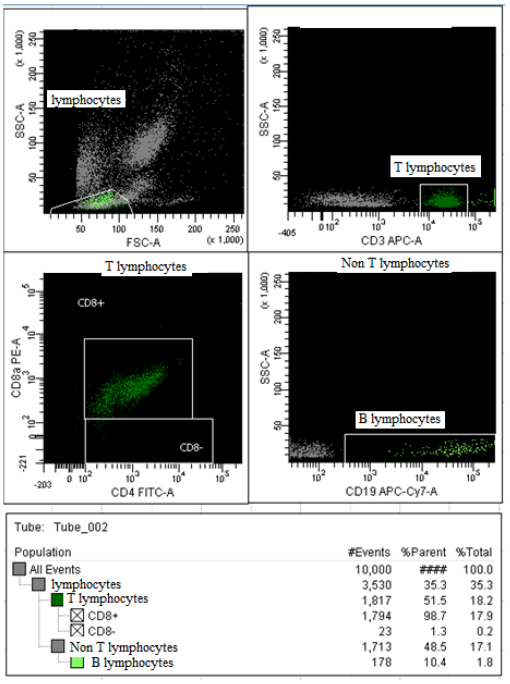

Fig. 5. T and B lymphocytes in plasma (Ang II lot)

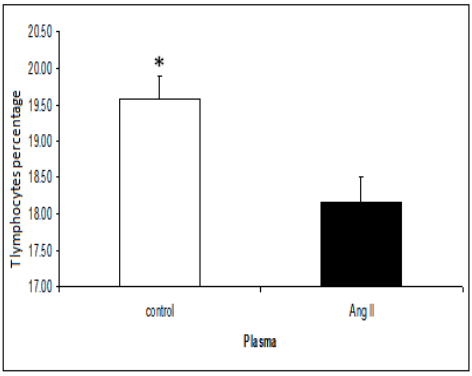

Fig. 6. Percentage of T lymphocytes in plasma (*p = 0.00001)

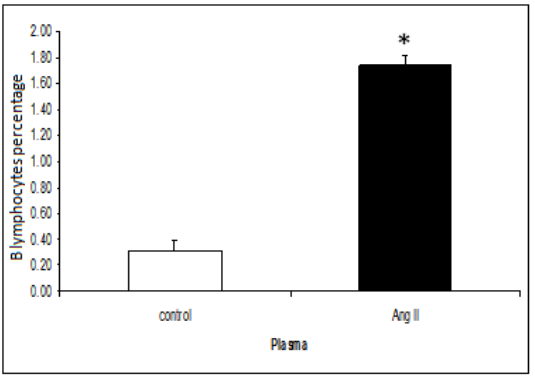

Fig. 7. Percentage of B lymphocytes in plasma (*p <<<0.05)

DISCUSSION

Hypertension should be considered as a potential multifactorial inflammatory disease. Morphological abnormalities in the renal parenchyma and arteries seem to determine hypertension. Inflammatory processes might induce renal vasoconstriction, ischemia and injury that could sustain systemic hypertension. Arterial and tubulointerstitial inflammatory cells infiltration in response to renal damage might further increase renal and vascular alterations through the production of oxidants and other soluble inflammatory mediators (7,8). Increased levels of blood pressure may induce proinflammatory and procoagulant responses.

Up-regulation of local and systemic inflammatory mediators, such as components of the renin-angiotensin system (RAS), endothelial adhesion molecules, chemokines, cytokines and tissue factor (TF), was demonstrated in essential hypertension (9,10,11). The renin angiotensin aldosteron system (RAAS) contributes to cardiovascular and renal homeostasis by generating angiotensin peptides, mainly angiotensin II (Ang II). The effector of the RAAS, angiotensin II, was known mainly as a humoral factor formed by the action of kidney-derived rate limiting enzyme *renin* (12).

Angiotensin II directly modulates cytokine release (13), endothelial adhesion molecule expression and the production of plasminogen activator inhibitor-1 (PAI-1) (14), thus favoring inflammatory vascular and renal damage (15, 16). These studies indicate that angiotensin II, in addition to its well-known cardiovascular effects, could also favor leukocyte recruitment within renal parenchyma in essential hypertension.

The flow cytometry results of the present study comparing control and Ang II treated animals noted that there is a statistically significant difference (p = 0.00001) in the percentage of T lymphocytes in plasma derived from control group rats compared to the percentage of T lymphocytes derived from the plasma of Ang II treated group for 14 days. Also, the percentage of B cells was significantly higher in plasma of rats that were treated with Ang II for 14 days (p <<< 0.05) compared to the percentage of B cells derived from the plasma of controls, confirming hypotheses from the literature regarding the involvement of angiotensin II in the development of inflammatory reactions.

CONCLUSIONS

Our paper reports that T cells play an essential role in the genesis of hypertension and support the involvement of inflammation in the occurrence of this disease. In our study, angiotensin II increased T cell markers activation. Angiotensin II markedly increased the number of T cells in plasma. Thus T cells might represent a novel therapeutic target for the treatment of high blood pressure.

Acknowledgments

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NEWS

RELATIONSHIP OF CAFFEINE WITH ADIPONECTIN AND BLOOD SUGAR LEVELS IN SUBJECTS WITH AND WITHOUT DIABETES

Insulin resistance or abnormal insulin secretion, are the characteristics of type2 diabetes mellitus (DM2), resulting in a diminished body glucose disposal. Those with chronic hyperglycemia, insulin resistance, or DM2 are at greater threat for its associated risk factors like hypertension, dyslipidemia, and cardiovascular disease. Genetic background and diet are considered to be one of the risk factors for developing type2 diabetes. Interestingly, among the several factors present in diet, coffee is considered as a potent dietary-component associated with reduced risk of diabetes and its complications. Most generally consumed beverage is coffee in the world, and its beneficial effects on health have been attracting substantial consideration. The common protective effect of coffee against diseases like cancer, cardiovascular diseases and type 2diabetes mellitus has been considered. Better glucose tolerance and a substantially lower risk of type 2 diabetes have been connected with high coffee consumption in diverse populations. The components responsible for the obvious beneficial effect of coffee on glucose metabolism remains blurred. Animal studies have shown that intake of the coffee components like chlorogenic acid, quinic acid and trigonelline, has enhanced glucose metabolism. Intervening metabolic studies on short term in humans have shown that caffeine can intensely lower insulin sensitivity. However, a regular high caffeine intake on long term has been linked with improved insulin sensitivity. An important secretory product of adipocytes is adiponectin, a marker and perhaps a mediator of metabolic and cardiovascular disease risk acts as a hormone with anti-inflammatory and insulin sensitizing properties. Further adiponectin levels are low in insulin-resistant subjects regardless of their obesity. A growing body of evidence has shown that high adiponectin levels confirm a protective effect against glucose intolerance budding in patients who are at high risk for diabetes. Further, it might be anticipated that the adiponectin level be affected with coffee consumption. In conclusion, the study shows the long term use of caffeine is more efficient on blood sugar and adiponectin levels, which needed in the prevention of complications in diabetic subjects (Bhaktha G, Nayak BS, Mayya S et al.Relationship of Caffeine with Adiponectin and Blood Sugar Levels in Subjects with and without Diabetes. Journal of Clinical and Diagnostic Research. 2015, Vol-9(1):1-3).

Doina Butcovan

THE AUTOIMMUNE CONSTELLATION IN LICHEN AMYLOIDOSIS

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THE AUTOIMMUNE CONSTELLATION IN LICHEN AMYLOIDOSIS (Abstract): Localized cutaneous amyloidosis is a rare disease among white people, being more common in South-Asia, China and South America. The disease is characterized by deposition of amyloid material in the papillary dermis without visceral involvement. Nevertheless, there is a growing list of immune-mediated disorders that have been linked to cutaneous amyloidosis. We present two cases of concomitant occurrence of lichen amyloidosis and autoimmune thyroiditis/atopic dermatitis in two Caucasian women. **Keywords:** AMYLOIDOSIS, LICHEN AMYLOIDOSIS, HISTOLOGIC STAINS.

Lichen amyloidosis (LA) is defined as a chronic itchy skin condition, characterized clinically by the presence of hyperkeratotic papules and histologically by the deposition of amyloid in the papillary dermis. It occurs more frequently in males aged 50-60 years and is primarily located on the lower limbs, but the trunk or forearms can also be involved. The condition is the most common subtype of localized cutaneous amyloidosis (LCA), in which the protein is deposited only in the skin, without involving other organs (1).

Amyloidosis refers to the deposition of amyloid in various tissues and all clinical types of amyloidosis share a common amyloid P component: a nonfibrillar protein derived from a precursor known as serum amyloid P. Clinically, Shimizu classified amyloidosis into two major classes (2007):

- Systemic amyloidosis with 4 subtypes - systemic AL amyloidosis (AL stands for amyloid light chain), systemic AA amyloidosis, amyloid A being derived form α- globulin called serum amyloid A, familial systemic amyloidosis and hemodialysis-related amyloidosis.
- Localized cutaneous amyloidosis (LCA) with 2 major forms:
  - Primary localized cutaneous amyloidosis: lichen amyloidosis, macular amyloidosis, nodular localized cutaneous amyloidosis (amyloidosis cutis nodularis atrophicans), poikiloderma-like cutaneous amyloidosis, anosacral cutaneous amyloidosis
  - Secondary localized cutaneous amyloidosis - the deposition of amyloids is associated with another skin disorders (lichen simplex chronicus, verruca vulgaris, basal cell carcinoma, seborrheic keratosis, actinic keratosis etc).

What does the term *amyloid m*ean*?* The amyloid was defined in 1854 by Rudolph Virchow as the iodine-stainable starchlike which shares some common features such as eosinophilic amorphous appearance with hematoxylin – eosin stain (2).

LA is a rare, often misdiagnosed disease, which accounts for approximately 10% of cutaneous amyloidoses. In the past, the amyloid was considered to be a single substance regardless of the variation in clinical presentation. Nowadays, using the criteria of Congophilia and fibrillar morphology, more than 20 biochemically different forms of amyloid have been identified.

There are some special stains for the amyloid deposits, in addition to the classic Congo red: apple green birefringence, thioflavin T, crystal violet metachromasia, methyl violet, Pagoda red no.9, amyloid-P component antibody (3).

Although histologically LA involves amyloid deposits only in the papillary dermis, the epidermis is also involved, having as result the appearance of hyperkeratosis and acanthosis. The pathological examination usually shows massive deposits of amyloid in the dermis, highlighted by Congo red staining revealing amorphous appearance (4).

The etiology of LA is still a mystery, viral and genetic factors, feminine gender, atopy or local friction have been listed as possible triggering factors. The most affected areas are the shins or the extensor surfaces of the extremities, but involvement of the abdominal wall, anosacral region, scalp, ears, interscapular region or sites of varicose veins has also been reported (5-7).

CASE REPORTS

We present two cases diagnosed and treated at the Dermatology and Pathology Departments of the "St. Spiridon" University Hospital, Iasi, Romania in the interval January- April 2014.

Case 1 was a 50-year-old woman presenting with a history of intermittent pruritic, brownish papules, 4-5 mm in diameter, distributed bilaterally and symmetrically on the extensor area of the pretibial surfaces, (fig.1). Similar but smaller and flatter papules were also noted on the extensor surfaces of the arms. She had no previous skin disease and family history was negative. Case 2 was a 62-year-old female presenting similar papular and pruritic lesions but confined to the trunk. She had no family history of any similar dermatological disease. Thyroid function tests showed high TSH (thyroid-stimulating hormone) level

Fig. 1. Hyperkeratotic papules distributed on the pretibial areas

and normal T3 (triiodothyronine), T4 (thyroxine) levels. Anti-thyroid microsomal antibody was positive whereas antithyroglobulin antibody was negative. Routine complete blood cell count, blood chemistry, urinalysis and serum electrophoresis were within normal limits in both patients.

Initial differential diagnosis included: dyschromatosis pigmentosa reticularis, pretibial pruritic popular dermatitis, hyperkeratotic lichen planus, and pretibial thyroid dermopathy (also known as pretibial myxedema).

No evidence of systemic amyloidosis deposition was found.

A punch biopsy specimen of a papule was taken from the pretibial area (case 1) and from the trunk (case 2) for routine microscopic examination.

Results of histological examination showed epidermal acanthosis with hyperkeratosis associated pigment incontinence. Both cases had hematoxylin & eosin (H&E) stains available for review and paraffin blocks for immunohistochemical staining. The H&E slides were reviewed and the diagnoses were made using well-established histopathologic criteria. Special stains were performed: PAS, Congo red, Fontana, methyl violet and van Gieson. The Congo red stain was analyzed in polarizing microscopy.

Immunohistochemistry was performed on formalin-fixed, paraffin–embedded (4 - µm thick) sections using a standard technique (streptavidin– biotin – peroxidase technique) with appropriate positive and negative controls. Novocastra Multi Cytokeratin (clone AE1/AE3 cocktail ready to use) with Dako's En Vision system was used for detection.

In papillary and reticular areas an amorphous eosinophilic material was found. The deposits expanded the papillae and the elongated rete ridges were displaced laterally, without involving the vascular or adnexal structure (fig. 2). The amorphous deposits stained positive light red with PAS, Congo red, and methyl–violet (fig. 3), light yellow with van Gieson stain (fig. 4), and appear green to fluorescent yellow in polarizing microscopy (fig. 5). Fontana stain showed melanin pigment associated with the amorphous deposits and the increase pigmentation of the basal layer (fig. 6). Immunohistochemical stain with anti-keratin antibody demonstrated focal reactivity in amyloid deposits (fig. 7).

Based on the clinicopathological and laboratory findings, a final diagnosis of LA was made.

The treatment of LA is often difficult and the condition recurs frequently. Potent topical corticosteroids (with or without occlusive dressings), intralesional corticosteroids, topical calcineurin inhibitors, dermabrasion, PUVA and UVB phototherapy, systemic retinoids, cyclosporine or topical 10% of dimethylsulfoxide have been used for the medical management of LA (8,9).

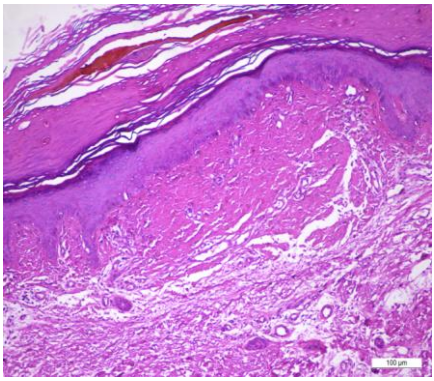

Fig. 2. Hyperkeratosis, elongation of the rete ridges and dermal papillary deposits of amorphous eosinophilic material, H & E, original magnification, x 200.

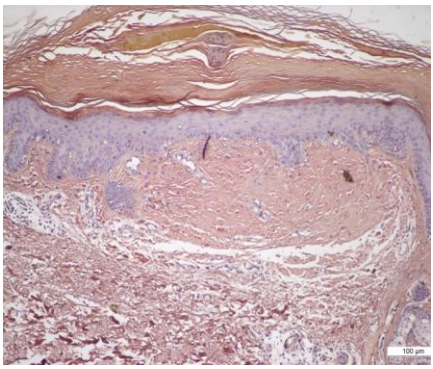

Fig. 3. Amyloids satin reddish brown in Congo red, original magnification, x 100

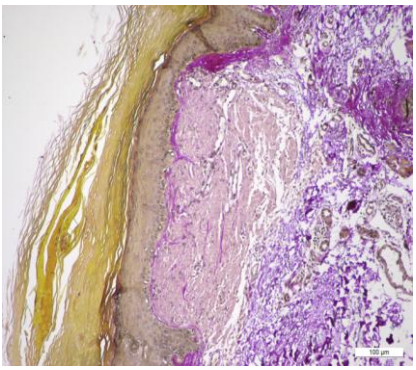

Fig. 4. Amorphous yellowish material, characteristic of amyloid in papillary and reticular dermis, van Gieson, original magnification, x 100.

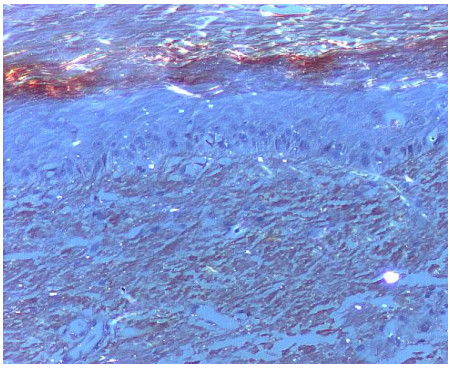

Fig. 5. Amyloid green to fluorescent yellow in polarizing microscopy, original magnification, x100.

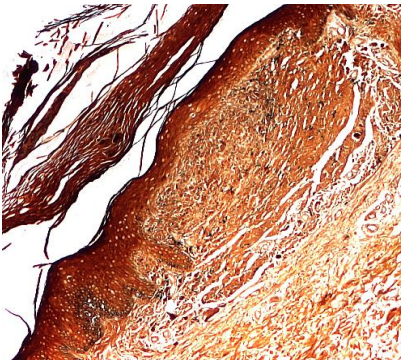

Fig. 6. Melanin pigment in amyloid deposit and basal layer, Fontana, original magnification, x40

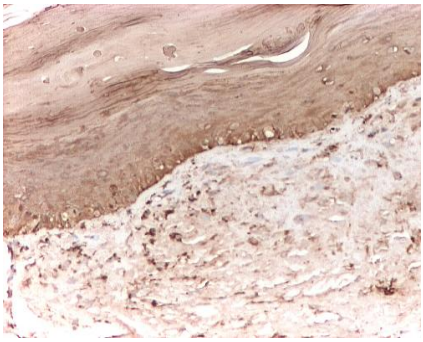

Fig. 7. Focal positivity in amyloid deposits for cytokeratins AE1/AE3, original magnification, x 200.

In our patients the treatment with oral antihistamine in association with topical applications of corticosteroids had a poor response.

DISCUSSION

In literature LA has been associated with a wide range of conditions: atopic dermatitis, dermatomyositis, systemic lupus erythematosus, autoimmune thyroiditis, hyperthyroidism and ankylosing spondylitis; however no association has been supported by statistically relevant data (10-13).

The differential diagnosis is often a challenge to many dermatologists, and we must always look for lichen simplex chronicus (affects predominantly females aged 30 to 50 years and clinically appears like chronically excoriated plaques), pretibial pruritic papular dermatitis (with a striking clinical resemblance to lichen amyloidosis but without amyloid deposition), pretibial thyroid dermopathy (a latter manifestation of thyroid disease), prurigo nodularis (hyperkeratotic nodules that vary in size from 0.5 to 3 cm), dyschromatosis pigmentosa reticularis (a rare genodermatosis characterized by generalized hyperpigmentation).

In lichen amyloidosis nerve fibers are localized at the dermoepidermal junction but not in the papillary dermis, suggesting that the pruritus may result from hypersensitivity of the nerve fibers that remain following the unexplained loss of nerves at the dermoepidermal junction. It is also possible that hyperthyroidism, by itching, may induce the occurrence or spread of LA (14).

Pigment incontinence, amorphous material and hyperkeratosis support the diagnosis of pruritic lesion, although non-pruritic variants of lichen amyloidosis have been described (15).

CONCLUSIONS

The histopathological findings in these two cases were similar to those reported in the literature, with amyloid depositing in the papillary and reticular areas and immunohistochemical staining positive for these deposits with antikeratin antibody AE1/AE3.

Lichen amyloidosis, the most common form of primary cutaneous amyloidosis, it is a rare disease among white people, with male predominance.

In our opinion, the occurrence of LA in two women, during a short period of time, in association with two autoimmune diseases is a strong indication that the etiopathogenic process is still unclear and that the genetic profile may be the strongest link to understanding the whole process.

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NEWS

THE IMPORTANCE OF CYTOKERATIN 19 EXPRESSION IN THE DIFFERENTIATION OF BASAL CELL CARCINOMA AND TRICHOEPITHELIOMA

Basal cell-carcinoma (BCC) is the most common skin tumour constituting approximately 70% of all skin malignancies. It occurs mostly in the elderly especially in the head and neck regions in sun- exposed areas. It is a locally aggressive tumour with very rare metastatic rates. With immunohistochemical studies, it has been shown that BCC originates from follicular stem cells and basaloid epithelia of follicular projections of the hair buds. On the other hand, trichoepithelioma (TE) is a rare, benign tumour of skin adnexa originating from follicular germinative cells. They are commonly located on the face and hairy skin. There are three subtypes of TE: desmoplastic, solitary and multiple. Lesions are generally solitary and sporadic papules or nodules in skin color. They show similarities with BCC since they are formed from basaloid islands and cordons with peripheral palisading in fibrous stroma. In small skin biopsies, if morphological findings of BCC and TE are overlapping, differential diagnosis may be especially difficult. In previous studies, for the differentiation of these two tumours, some immunohistochemical markers such as CD10, bcl2, CK15, and Ber-EP4 have been used. In several studies, cytokeratin 19 (CK19) has been determined to have a high specificity for undifferentiated basaloid cells. CK19 is a small (40 kDa) acidic keratin that is expressed in germinative basaloid cells. In the non-neoplastic skin tissue samples, while positive staining with cytokeratin 19 in the outer root sheath of hair follicles and sweat glands were observed, there was no staining in basal layers. In conclusion, CK19 expression may be helpful in the differential diagnosis of BCC and TE especially in small skin biopsy samples in which morphologic differentiation is difficult (Bedir R, Sehitoglu I, Yurdakul C, et al. The Importance of Cytokeratin 19 Expression in the Differentiation of Basal Cell Carcinoma and Trichoepithelioma.Journal of Clinical and Diagnostic Research. 2015, Vol-9(1):1-4).

Doina Butcovan

SEVERE HYPOTHYROIDISM IN OLDER PEOPLE: SPECIFIC FEATURES OF DIAGNOSIS AND TREATMENT. CASE REPORT AND LITERATURE REVIEW

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SEVERE HYPOTHYROIDISM IN OLDER PEOPLE: SPECIFIC FEATURES OF DIAGNOSIS AND TREATMENT. CASE REPORT AND LITERATURE REVIEW (Abstract): Prevalent causes of overt hypothyroidism are autoimmune destruction of the thyroid gland and previous thyroid surgery or radiation therapy. However, due to the side effects of amiodarone or lithium treatment thyroid dysfunction is increasingly frequent, especially in elderly patients who are anyway prone to a certain degree of hypothyroidism. This paper presents the case of a 66 years old patient who was admitted for severe physical asthenia and weight loss occurring during the past year. On admission we found anemia, which was considered secondary to a possible digestive tumor. The Comprehensive Geriatric Assessment (CGA) showed malnutrition, depression and a minor cognitive deficit. The absence of any obvious digestive cancer determined us to expand the investigation area. Cardiac ultrasound showed fluid in the pericardium which made us reconsider the diagnosis and take into account the presence of hypothyroidism, diagnosis that was subsequently confirmed by hormonal investigations and thyroid ultrasound. Hypothyroidism (either subclinical or overt forms) is a diagnosis that is easily overlooked in elderly patients because of its non-specific manifestations - symptoms may be wrongly attributed to normal aging or consequences of such diseases as depression, Parkinson or Alzheimer disease. Therefore, the measurement of serum thyroid-stimulating hormone (TSH) levels should be part of the biochemical work-up in all elderly subjects. **Keywords**: SEVERE HYPOTHYROIDISM, ELDERLY.

Hypothyroidism denotes deficient production of thyroid hormone by the thyroid gland. There are two clinical forms: subclinical and overt hypothyroidism (myxedema). Subclinical hypothyroidism is characterized by an elevated thyroid-stimulating hormone (TSH) level in the presence of normal serum free thyroxine (T4) and triiodothyronine (T3) concentrations. Subclinical hypothyroidism may progress to overt hypothyroidism in approximately 2-5% cases annually (1). The common causes of hypothyroidism are autoimmune destruction of the thyroid gland and previous thyroid surgery or radioiodine therapy, as well as various types of medication, including amiodarone, cytokines and lithium (2).

A mild degree of hypothyroidism is common in the elderly. It affects 5-20% of women and 3-8% of men. In elderly popu-

lation symptoms may be atypical: physical asthenia, lethargy, depression, cold intolerance, thinner skin and infiltration, especially around the face and hands, dilative cardiomyopathy and pericardial fluid. The heterogeneity of symptoms may mislead the diagnosis and delay the treatment; therefore measurement of TSH levels should be part of biochemical testing in elderly subjects and cases with TSH >10 mIU/L should be treated according to the cause and stage of the disease (2).

CASE REPORT

We present the case of a 66-year-old female patient from an urban area, who was admitted to the Geriatric Department because of severe physical asthenia and weight loss during the past year. The patient was diagnosed 2 years earlier with severe bradycardia possibly due to a sick sinus syndrome but not confirmed by ECG Holter monitoring; she had a type AAI pacemaker implanted anyway. The patient was also known with congestive gastritis and secondary anemia, treated at home with iron supplements.

On admission she presented severe physical asthenia, underweight (body mass index = 17), and the skin was pale, dry, hyper pigmented and thickened at face level. She had bradycardia (58/min) and a blood pressure of 90/60 mmHg. On palpation the abdomen was sensitive in the epigastrium where we found a mobile tumor of 3/4 cm. The deep tendon reflexes were bilaterally diminished and she presented paresthesia in her limbs.

The Comprehensive Geriatric Assessment (CGA) showed: malnutrition (MNA= 14/30), minor cognitive deficit (MMSE= 14/30) and moderate depression (GDS=7).

Blood tests showed mild anemia (Hb=10.2g/dl), dyslipidemia (Chol=281mg%, TG=160 mg%, LDL=180 mg%, HDL=30 mg%), stage 3 chronic kidney disease (creatinine clearance = 40.6ml/min/1.73m^2) and a lower tract urinary infection.

At that time we thought that the main cause for patient's symptoms was anemia due to several possible causes, such as: digestive tumor, chronic inflammatory process, responsible for the absence of response to iron supplements, or secondary to chronic kidney disease. While waiting for the examination of the digestive system, the patient presented sudden aggravation of the neurological symptoms (severe headache and delusions) associated with vomiting and severe secondary hyponatremia (Na=128.8 mEq/l). The absence of any obvious cause for the digestive manifestations and the persistence of neurological manifestations after the correction of sodium levels determined us to extend the investigations; cardiac ultrasound revealed the presence of fluid in the pericardium with preserved systolic function. Hypothyroidism would have been the ideal candidate to explain all the symptoms and hormone levels (TSH > 100 UI, fT4 = 0.15 ng/ml) and thyroid gland ultrasound (fig.1) (LTD: 1.0/2.3/1.2 cm (1.43 ml), LTS: 1.07/2.51/1.29 cm (1.80 ml), with hypoechogenic structure and low vascularization) confirmed the diagnosis.

Endocrinological assessment confirmed an autoimmune thyroiditis (AAT-TPO= 127 UI/ml vs N: < 34 UI/ml).

The substitution therapy with levothyroxine (Euthyrox) was introduced with gradually increasing doses and the course was favorable: disappearance of abdominal pain, neurological manifestations, depression and cognitive disorder and correction of hemoglobin levels.

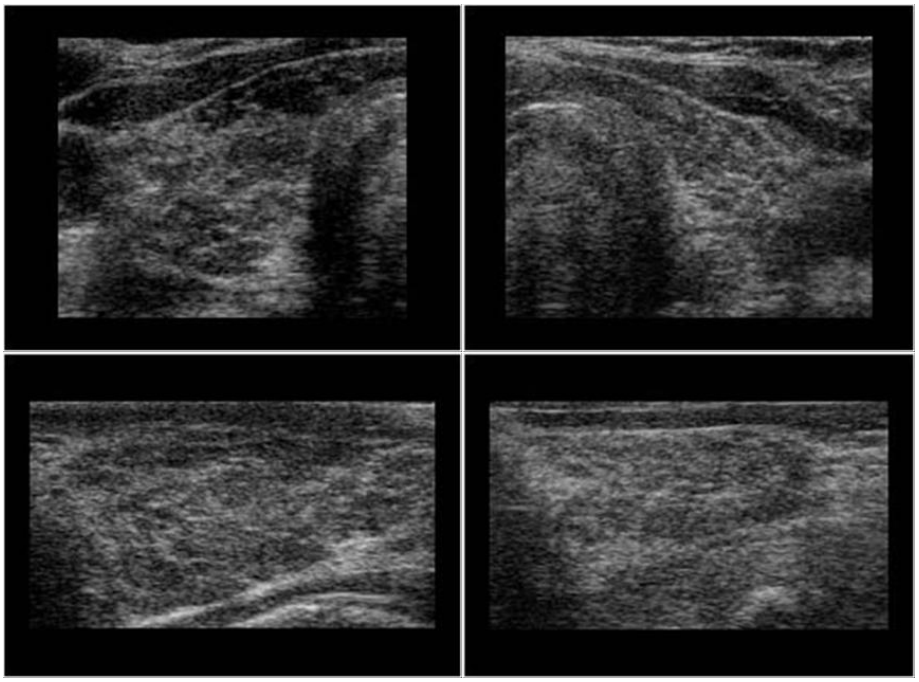

Fig. 1. Thyroid ultrasound – appearance characteristic for autoimmune thyroiditis

DISCUSSION

The first cases of myxedema were reported in the literature more than 90 years ago when the effects of the thyroid hormones upon the heart (heart failure and dilative cardiomyopathy) were discovered. The first case of third degree atrioventricular block secondary to hypothyroidism was described in 1918 (Aub and Stern, 1918, cited by 3), and by 1930 there were 21 reported cases of hypothyroidism with secondary heart diseases (heart failure refractory to digitalis therapy, atrioventricular blocks of different degrees, pericarditis and cardiomegaly) (4). Schantz and Dubbs (cited by 5) reported a similar case in 1951, with adequate sinus rhythm after hormone replacement therapy. Over the years there were few reports of isolated cases of overt hypothyroidism, and they focused mainly on episodes of myxedema coma.

Hypothyroidism mainly affects women and the elderly (6). In elderly population this disorder is frequently associated with such drugs as amiodarone and lithium (7). The symptoms may be atypical and might be considered more likely secondary to aging than due to hypothyroidism, especially if there is no previous medical or pharmacological information available. The neurological manifestations occur in most of the cases: physical asthenia, bradypsychia or even different degrees of cognitive dysfunction, paresthesia, and depression. The carpal tunnel syndrome is very frequent, and the deep tendon reflexes are usually modified (8). The cardiac manifestations are insidious, manifested by the progressive increase in size of the heart cavities, episodes of left ventricular failure and pericarditis, which are unresponsive to

diuretic and digitalis treatment. ECG appearance varies from sinus bradycardia with nonspecific modifications of ST segment and T wave to third degree atrioventricular block or the torsades de pointes (9, 10, 11). Due to this heterogeneity of symptoms, the disease can progress for a long time under various other diagnoses and therapeutic schemes as it was the case of our patient, who was even treated for a possible sick sinus syndrome with the help of a pacemaker. Moreover, the persistence of digestive symptoms needed further investigations, such as endoscopy and colonoscopy, as persistent anemia highly suggested the presence of a digestive tumor.

Unlike the severe form, subclinical hypothyroidism is relatively frequent in the general population (15% in women and 10% in men) (12). Unfortunately, the onset of the disease in the geriatric patient may be wrongly attributed as manifestations of other diseases such as depression, Parkinson disease (7) or Alzheimer disease. The existence of co-morbidities and poly pharmacy may mask the symptoms and signs of hypothyroidism and delay the correct diagnosis and proper treatment.

The most important feature of subclinical hypothyroidism is the increased likelihood (2-5%) to progress to overt hypothyroidism, the progress rate being proportional to TSH level. It was hypothesized that subclinical hypothyroidism would be a risk factor for cardiovascular disease (13).

The decision to treat the elderly patients with subclinical hypothyroidism is still controversial. A review published in 2007 (13), including 12 trials involving 350 patients monitored for 6 to14 months, concluded that hormone replacement did not result in improved survival or decreased cardiovascular morbidity. The present consensus (1) for the elderly patients is that replacement therapy should be carefully considered due to the high risk for cardiovascular side-effects: tachycardia or aggravation of stable angina. Patients with subclinical hypothyroidism should be informed about the disease and its clinical manifestations and serum TSH level should be measured once or twice a year.

The recommended and appropriate replacement therapy for overt hypothyroidism is levothyroxine sodium. In elderly patients the initial dose should be smaller if there is any concomitant heart disease: 12.5 to 25 µg/day. Replacement dose titration should be cautious with close monitoring of patient symptoms and thyroid function tests. Because of the long half-life of levothyroxine sodium, small dosage adjustments may be performed at 6-week intervals by an increment of 12.5 to 25 µg until serum TSH is in the mid-normal range. After that, its measurement may be done every 6 to 12 months to monitor the dose and compliance (14). Thyroid hormone therapy has no beneficial effect above placebo in elderly individuals with normal serum TSH and T4 levels. The major risk of levothyroxine sodium therapy is over-replacement, with anxiety, muscle wasting, osteoporosis and atrial fibrillation as adverse effects.

CONCLUSIONS

This article draws attention on the polymorphism of signs and symptoms associated with myxedema in elderly patients. The risk to overlook hypothyroidism is high and may lead to unnecessary procedures, as was in our patient the pacemaker implantation for an AV block which could be managed only with thyroid hormone therapy.

Hypothyroidism should be the first diagnostic assumption should we deal with patients under treatment with amiodarone and/or lithium, largely used in treating cardiovascular and neurological diseases highly characteristic for elderly. Hypothyroidism should be suspected in all patients with second and third degree atrio-ventricular block of unknown cause, refractory heart failure and idiopathic pericarditis.

Treatment for overt hypothyroidism was initiated immediately after the diagnosis was confirmed with excellent outcome; however, treatment should be closely monitored to avoid over-replacement.

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BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM - A RAPIDLY EVOLVING ENTITY. CASE REPORT

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BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM. - A RAPIDLY EVOLVING ENTITY. CASE REPORT (Abstract): blastic plasmacytoid dendritic cell neoplasm (BPDCN), CD4+/CD56+hematodermic neoplasm was formally known as blastic NK-cell lymphoma. It is in fact a form of acute myeloid leukemia notable for highly aggressive behavior with cutaneous, lymph node and bone marrow involvement. This entity is derived from plasmocytoid dendritic cells and has a predilection for extranodal sites, especially the skin. Elderly male patients are the most affected and the prognostic is poor. The first case was reported in 1994 and sice then, single cases and a few small series have been published. This article presents the case of a previously healthy 56-years-old man, who presented himself to a skin eruption consisting in multiple, large dermal ulcerated tumors, located on the trunk and scalp. The lesions were painless and grew in size rapidly. Physical examination was normal except for the skin lesions. Histological examination of a biopsy specimen and immunohistochemical studies (positive for next markers: CD4, CD 45, CD56, CD68, Ki 67) revealed the rare diagnostic - blastic plasmacytoid dendritic cell neoplasm. **Keywords:** BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM, CD4+/CD56+ HEMATODERMIC NEOPLASM, IMMUNOHISTO-CHEMISTRY, SKIN CANCER.

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) it is a rare disease, more prevalent in Asia and Latin America than in Europe, with predominance in males (1). Over the past decades, the understanding the biology of lymphoid cells has improved and so has the knowledge of primary cutaneous lymphomas. The current World Health Organization/ European Organization of Research and Treatment of Cancer (WHO/EORTC) classifications are based on clinical, pathological, immunopathological, molecular and cytogenetic findings. The hematodermic neoplasm is defined as a separate entity, CD4+/CD56+ hematodermic neoplasm (2). NK are a variety of cytolytic cells capable of killing a different target cells such as bacteria/viruses, infected cells or tumor cells (3). CD4+/CD56+ hematodermic neoplasm is an extremely rare, very aggressive form of acute myeloid leukaemia. Due to its rapid fatal course, no clinical trial has been conducted and the therapeutical options are

based on small retrospective cohorts of patients (4). Generally this type of lymphoma has a skin-tropism, with clinical presentation in the form of papules or nodules that vary in appearance and affects elderly adults, although BPDCN without cutaneous lesions has been described (5).

We report the case of a 56 years-old man, with CD4+/CD56+ hematodermic neoplasm, with rapid degradation of clinical status and severe reaction to the chemotherapy.

CASE REPORT

A 56-years-old man was admitted in the Dermatology Department of "St. Spiridon" Universitary Emergency Clinical Hospital, Iaşi with the suspicion of *Mycosis fungoides.* The clinical examination revealed many cutaneous painless tumors on the trunk and scalp, 5/10 cm in size, ulcerated, which appeared in the last 2 months and which rapidly, grew in size. Bilateral inguinal lymph node enlargement (less than one cm in diameter) was noted in physical examination. The patient was at first assessed in another hospital center, for red macules and joint pain, and was treated with corticosteroids. The lesions reappeared after he interrupted the treatment while the tumors progressed rapidly and became necrotic (fig. 1).

Fig. 1. Clinical aspects of dermal necrotizing tumors, with rapid progression

Laboratory data revealed: white blood cell count was 2.97 x 10^9 cells (neutrophils 9.8%, lymphocytes 63.6%, monocytes 19.5%, basophiles 5.4%), hemoglobin level 12.1 g/dl, albumin level 51.2%, C-reactive protein (CRP) level was 1.52 mg/dl, and lactate dehydrogenase (LDH) level, 602 IU/l.

Computerized tomography showed:

- right parieto-occipital lesions involving the brain (fig. 2);
- dermal tumors on the anterior surface of the chest; note the presence of air bubbles within the tumor volume (fig. 3);
- systemic involvement of lymph nodes, especially in the armpit and inguinal areas;

- many cutaneous, expansive nodules, disseminated on the hole scanned area, but most consistent and larger in chest area;

- heterogenous gas bubbles structure, calcification and contrast enhancement in the solid component of the tumor.

Fig. 2. Brain lesions

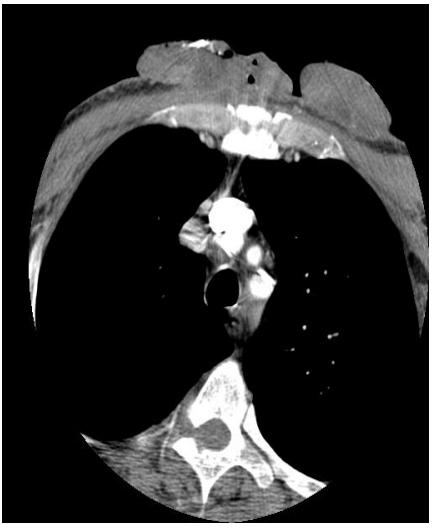

Fig. 3. Dermal tumors on the chest

A skin biopsy was performed and pathologic exam established the final diagnosis. The immunohistochemical examinations showed diffuse dermal infiltration with neoplastic cells, showing blastoid morphology and positive reaction for: CD4, CD45, CD56, CD68, and Ki 67, whereas CD3, CD30, CD5, CD8 and CD15 were negative.

Figure 4-tumor cells with intense CD4+ staining (ob.x 20), figure 5 -tumor cells with intense CD56+ staining (ob.x 40) and figure 6 with skin biopsy showing diffuse dermal infiltration with tumor cells.

Based on pathological findings, the final diagnosis was cutaneous CD4+/CD56+ hematodermic neoplasm, with systemic involvement. The patient began chemotherapy using a regimen combining vinblastine, lomustine and etoposide. After 14 days of chemotherapy, low neutrophil counts were detected, the chemotherapy was stopped and filgastrim treatment was initiated. The second day, the patient was found unconscious, with no reaction to verbal stimulus, and he was transferred to the Intensive care Unit. His recovered was slow after supportive treatment, correction of acid-base and hydro-electrolytic imbalance, mannitol, methotrexate, calcium folinate, phenobarbital and carbamazepine. Two weeks later his neurological status returned to normal, there were no neurological focal signs or evidence for brain bleeding. He was transferred back to Hematology Clinic and the therapeutic program was reconsidered with methotrexate. The oncology committee decided to start radiotherapy but during these sessions the patient became highly agitated, bewildered and aggressive and is treated with anti-edematous, phenobarbital, valium and haloperidol. The skin lesions were diminished rapidly and the patient was discharged.

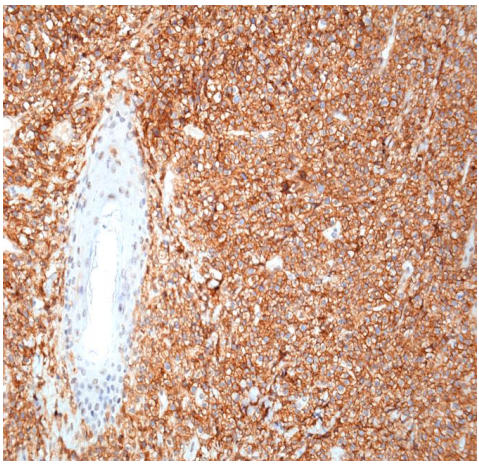

Fig. 4. Tumor cells with intense CD4+

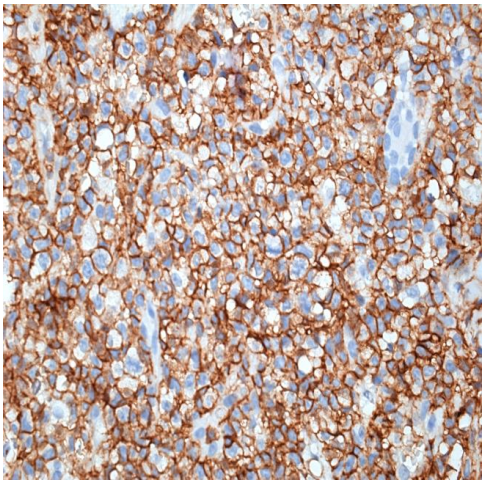

Fig. 5. Tumor cells with intense CD56+

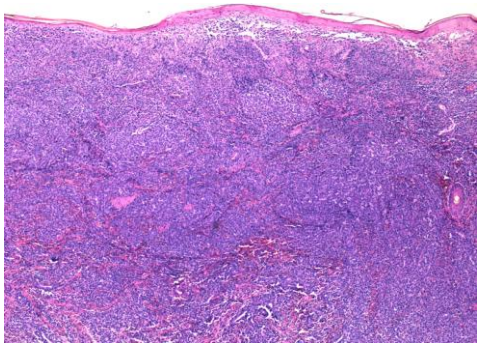

Fig. 6. Skin biopsy showing diffuse dermal infiltration with tumor cells.

After only one month, the patient's state worsens and deceases with multiple organ and system failure.

DISCUSSION

BPDCN is characterized by positive results for CD4, CD43, CD56, CD68 and CD123 and negative results for conventional myeloid and lymphoid T and B-cell markers with involvement of the skin in the most cases. Recently, these immunophenotypical characteristics lead to the new terminology, BPDCN, instead of the blastic NK-cell lymphoma.

It has been thought that these cells arise from NK progenitor cell, but most recent studies suggested that the origin is a lymphoid related plasmocytoid dendritic cell. This idea is sustained by the expression of the blood dendritic cell antigen 2 (BDCA2) and interleukin-3 receptor alpha subunit (CD123) (6, 7).

As in others forms of leukemia, the atypical presentation is very common, the median survival rate is low, in this case about 12-14 months, so recognizing the clinical, pathological and immunohistochemical features are very important for finding a promising treatment (8, 9).

The differential diagnosis must include nasal type NK-cell lymphomas, cutaneous T-cell lymphomas and CD 56^{+} acute myeloid leukemia.

Mycosis fungoides, our first suspicion, is distinguished from BPDCN by the presence of epidermal involvement and $CD3^{+}$, $CD56^{-}$ immunophenotype.

CONCLUSIONS

In our opinion, at this moment there is no consensus for the optimal treatment of BPDCN. Myeloablative treatment with allogenic bone marrow transplantation, within the first episode of remission has

resulted in a better survival (10). Other promising options are immuno-therapy with interleukin-3or CD123 antibody. In conclusion, dermatologists must become aware of this rare form of leukemia and more informed about the use of CD cell markers in the field of cutaneous oncology.

ACKNOWLEDGEMENTS

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INCIDENCE AND RISK FACTORS OF *CLOSTRIDIUM DIFFICILE* INFECTION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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INCIDENCE AND RISK FACTORS OF *CLOSTRIDIUM DIFFICILE* INFECTION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE (Abstract). Recent changes in the epidemiology of *Clostridium difficile* infection (CDI) include the identification of patients with inflammatory bowel disease (IBD) as a group at risk in comparison to the general population. **Aim**: To identify the incidence and risk factors for CDI among patients with IBD. **Material and methods**: Case-control study of 78 patients diagnosed with IBD, hospitalized at the Iasi Institute of Gastroenterology and Hepatology between January 2012 and -July 2014. Demographic data and clinical characteristics were reviewed for all patients. IBD patients with positive *C. difficile* toxins A and B tests were matched by sex, age and type of IBD with IBD patients hospitalized in the same period with negative *C. difficile* toxins tests. **Results:** Both groups were comparable for baseline characteristics. Of the 78 patients diagnosed with IBD included in the study, *C. difficile* was detected in 26 patients (33.33%). There was no statistical difference regarding length of hospital stay (10.42±7.34 *vs.* 8.01±6.14 days, p=0.129) between the two study groups. Risk factors for CDI in patients with IBD were: ulcerative colitis (OR=1.90, CI= 1.320-2.720, p=0.001), use of proton pump inhibitors (OR=1.57, CI=1.133-2.032, p=0.012), previous antibiotic use (OR=2.3, CI=1.587-3.332, p<0.0001), and albumin<3g/dl (OR=1.78, CI=1.023-5.558, p=0.038). Immunosuppressive and anti TNF-α treatment were not risk factors for *C. difficile* development in patients with IBD. **Conclusions**: CDI in patients with IBD is a serious infection and should be treated aggressively with close clinical follow-up. Ulcerative colitis, previous treatment with antibiotics and proton pump inhibitors represent risk factors for CDI development in patients with IBD. **Keywords:** *CLOSTRIDIUM DIFFICILE*, RISK FACTORS, ULCERATIVE COLITIS, INCIDENCE.

Recently there has been an increase in both incidence and severity of *Clostridium difficile* infection (CDI) worldwide (1). Recognized risk factors for infection include advanced age, broad-spectrum antibiotic therapy, prolonged hospitalization, malignancies (2), immunosuppression, the use of proton pomp inhibitors and the presence of multiple co-morbidities (3, 4).

Several studies demonstrated an increasing incidence of CDI in patients with underlying inflammatory bowel disease (IBD), with a more severe course of the disease compared with the non-IBD population (5-9). The incidence of CDI among hospitalized IBD patients increased from

1% in 1998 to 3% in 2007 (10). Both ulcerative colitis (UC) and Crohn's disease (CD) present high-risk for CDI, but patients with UC are more susceptible than those with CD (11,12).

Besides traditional risk factors for CDI, IBD patients present specific risk factors, unique to IBD, in relation to colonic involvement and disease severity, and it seems that IBD itself is an independent risk factor for CDI. In a study by Issa et al., colonic involvement and pancolitis were associated with a threefold greater risk (5), concluding that the extent and severity of the disease may be considered independent risk factors for CDI development in patients with IBD.

CDI has a negative impact on IBD course and is associated with a significant increase in need for colectomy and mortality (13). Therefore, in the present study we aimed to evaluate the incidence and the risk factors associated with CDI in IBD patients admitted in a tertiary center.

MATERIAL AND METHODS

Patients. We conducted a prospective, case-control study on IBD patients admitted to the Iasi Institute of Gastroenterology and Hepatology in the interval January 2012- July 2014.

Demographic data and clinical characteristics like comorbidities, previous antibiotic use, immunomodulators, acid suppression, length of hospital stay, gastrointestinal surgery and outcome were assessed. Patients with indeterminate colitis, other infectious colitis and sepsis were excluded.

Diagnosis of CDI. All patients with flare activity were tested using immunochromatography assays for toxins A and B. Patients who tested positive for *C. difficile* toxins A and B were identified (n=26) and matched with IBD patients with negative *C. difficile* toxins (n=52) hospitalized during the same period of time.

Statistical analysis. The statistical analysis was carried out using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean +SD and categorical data as percentage. The Kolmogorov-Smirnov test was used to determine whether the continuous variables were normally distributed. Analysis of variance and the chi-square test were used for continuous and categorical variables, respectively. The Student t test or Mann-Whitney U test were used to compare groups, with the chi-square test used for categorical variables. Risk factors for CDI in patients with IBD were determined using logistic regression for univariate analysis. A p value of less than 0.05 was considered statistically significant.

RESULTS

Incidence of CDI. Baseline demographic, clinical, and laboratory characteristics of the study population are outlined in Table I. There was no significant difference between the 2 study groups.

During the study period, a total of 78 patients were admitted with the diagnosis of IBD of whom 26 (33.3%) had concomitant CDI. Of the IBD patients, 43(55.1%) had UC, and 35(44.8%) had CD.

We assessed the annual incidence of CDI in patients with IBD and found that it increased from 2.01% in 2012 to 16% in 2014 (fig.1). Likewise, UC patients were more susceptible to CDI than CD patients (fig.2).

TABLE I

Baseline characteristics of study population

Parameter	*C. difficile* n=26	Control n=52	p
Age (yrs) (mean±SD)	50.96±18.16	44.60±16.57	0.126
Sex m/f	16/9	33/19	0.363*
UC/CD	21/5	22/30	**0.001***
Albumin (g/dl) (mean ±SD)	3.63±0.67	4.36±0.83	**0.002**
Hemoglobin (g/dl) (mean ±SD)	12.46±2.25	12.88±2.28	0.450
Platelets (x10^9/mmc) (mean ±SD)	351±16.88	350±15.33	0.966
ESR (mm/h) (mean ±SD)	34.22±17.69	32.02±33.63	0.777
CRP (mg/dl) (mean ±SD)	4.81±6.26	4.81±6.26	**0.045**

Abbreviations: UC, ulcerative colitis, CD, Crohn's disease, SD, Standard deviation, ESR, erythrocyte sedimentation rate, CRP, C-reactive protein.
*test X^2

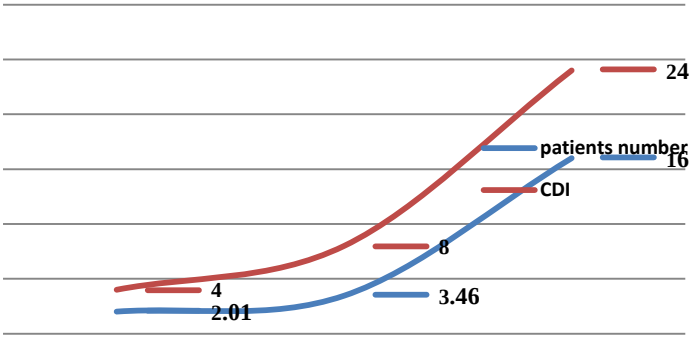


Fig. 1. Annual incidence of CDI in patients with IBD

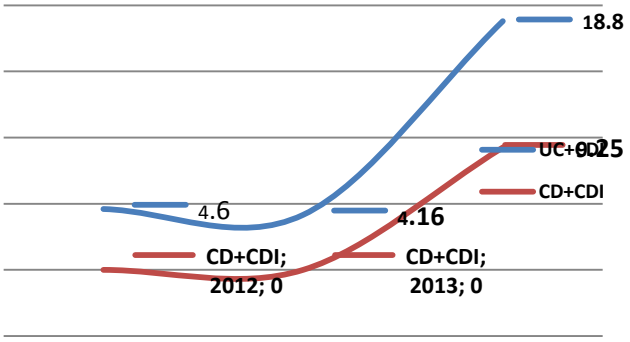


Fig. 2. Annual incidence of CDI in patients with UC and CD

Risk factors for CDI. Antibiotic use prior to CDI was reported in 43 patients (55.1%), the most commonly used antibiotics being ciprofloxacin (36.1%) and ampicillin (19%) for urinary tract and upper respiratory tract infections. In our study, the length of hospital stay was not influenced by CDI (10.42±7.34 vs. 8.01±6.14, p=0.129) (tab. II).

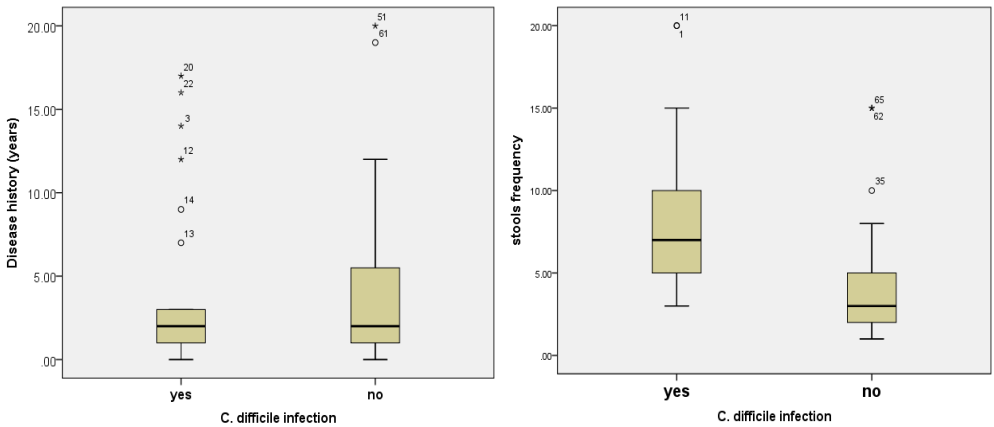
C. difficile infection was more common among patients with a recent diagnosis of IBD. Likewise, patients with an IBD flare-up were more susceptible to CDI (8.07±4.48 *vs.* 3.94±3.13, p<0.0001) (fig. 3). Cardiovascular co morbidities and malignancies did not influence the risk for CDI in our study population.

In our study, IBD patients with CDI were treated with metronidazole 250 mg orally four times daily for 10 days. In case of metronidazole failure vancomycin treatment was initiated. This was required in 2/26 (7.69%) patients. In this study the rate of CDI recurrence was 16%.

TABLE II

Risk factors for CDI

Parameter	OR	IC 95%	p
ulcerative colitis	1.90	1.320-2.720	**0.001**
proton pump inhibitors use	1.57	1.133-2.032	**0.012**
antibiotic use	2.3	1.587-3.332	<**0.0001**
albumin <3g/dl	1.78	1.023-5.558	**0.038**



a. b.

Fig. 3. CDI in IBD patients; a- diagnosis of IBD, b- IBD flare-up.

DISCUSSION

A dramatic increase in CDI has been documented over the last two decades (5-9). Today, CDI is recognized as the main cause of infectious nosocomial diarrhea associated with increased morbidity, mortality and healthcare costs (14).

Several studies have reported a significantly increased incidence of CDI among IBD patients, particularly in those with UC. Thus, a study using nationwide data showed that in US the incidence of CDI among hos-

pitalized UC patients has doubled over a 7-year period (15). Likewise, a Belgian study reported a nearly four-fold increase in CDI in both IBD and non-IBD patients between 2000 and 2008 (8). Our results showed that the incidence of CDI in IBD has increased during the study period for which data were available. Also, CDI is significantly more common in UC than in CD.

The classical risk factor for CDI is exposure to broad-spectrum antibiotics. Antibiotics disturb the normal intestinal flora, allowing *C. difficile* to proliferate (16). In this study, we found that previous antibiotic therapy and previous proton pump inhibitors treatment represent risk factors for CDI development in patients with IBD. Additionally, immunosuppressive and anti-TNF-α treatments were not risk factors for CDI.

Contrasting results have been reported regarding the length of hospital stay in IBD patients with CDI. Jodorkovsky et al. (11), reported a similar mean length of hospital stay for UC patients with and without superimposed CDI (11.7 *vs.* 11.0 days; p = 0.70), while Bossuyt et al. (8) found notably shorter stays in IBD patients with CDI (mean 15.2 days) as compared to non-IBD patients with *C. difficile* (mean 27.7 days) (<0.001). Our data support that the length of hospital stay is not influenced by the presence of CDI.

Our findings suggest that CDI is more frequent in patients who present with IBD flare-ups. Therefore, clinicians should maintain a high index of suspicion for CDI in IBD patients, especially in those with UC.

Recurrence of CDI is present in 10-30% of IBD patients (16). In this study the rate of CDI recurrence was 16%. This is in agreement with previous studies that reported similar findings.

A major limitation of our study may be the small number of patients and the fact that it was a single center study. This could have an influence on the conclusions regarding risk factors. Another limitation of this study is the absence of routine CDI testing in all patients with IBD as in other studies, and this could have influenced the annual incidence rate of CDI.

CONCLUSIONS

Our results show that the risk of CDI in IBD patients is extremely high and the incidence appears to be increasing at a faster rate. IBD patients who present with symptoms of a flare must be tested for *C. difficile* toxins. A rapid and adequate diagnosis and treatment may improve prognosis in IBD patients.

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NEWS

THE STRUCTURE OF IBUPROFEN BOUND TO CYCLOOXYGENASE-2

Cyclooxygenases (COX-1 and COX-2) catalyze the rate-limiting step in the biosynthesis of prostaglandins, prostacyclins, and thromboxanes and are the pharmacological targets of non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors (coxibs). One of the most commonly available over-the-counter pharmaceuticals in the world is ibuprofen. The anti-inflammatory and analgesic properties of ibuprofen are thought to arise from inhibition of COX-2 rather than COX-1. Ibuprofen occupies an area of the enzyme between the substrate channel opening and the apex of the active site. While an X-ray crystal structure of ibuprofen bound to COX-1 has been solved, no such structure exists for the cognate isoform COX-2. Almost all interactions formed between ibuprofen and the active site residues of COX-2 consist of hydrophobic interactions. A group of researchers have determined the crystal structure of muCOX-2 with a racemic mixture of (R/S)-ibuprofen and the study reveals that only the S-isomer of ibuprofen was bound resulting that the S-isomer possesses higher affinity for COX-2 than the R-isomer. The structure clearly reveals that S-ibuprofen is bound with full occupancy in both monomers of COX-2. The results provide the first atomic level detail of the interaction between ibuprofen and COX-2 (Orlando BJ, Lucido MJ, Malkowski MG. The structure of ibuprofen bound to cyclooxygenase-2. *J Struct Biol* 2015; 189: 62-66).

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EXERCISE-BASED REHABILITATION FOR METABOLIC SYNDROME - CASE REPORT

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EXERCISE-BASED REHABILITATION FOR METABOLIC SYNDROME - CASE REPORT (Abstract): Physical activity is a key part of cardiac rehabilitation, especially in patients with metabolic syndrome. Recent reports suggest that high-intensity interval training is more effective than continuous moderate intensity training, classically employed in cardiac rehabilitation programs. However, the implementation of a standard training protocol aimed at achieving a target heart rate during exercise can be limited by exaggerated pressor response, especially in deconditioned subjects. We report the case of a 47-year-old male with metabolic syndrome who was enrolled in a one-year exercise based-cardiac rehabilitation program. We implemented a two-phase protocol, starting with 5 continuous training sessions at 25 W, during which we gradually increased exercise duration up to 30 minutes. The second phase of the program consisted of biweekly interval training sessions of 25-50, 50-75 and finally 75-100 W. The switch to higher workloads was made only after the patient presented appropriate pressor response adaptation to current training intensity. Our program positively influenced our subject`s cardiovascular risk factors and allowed discontinuation of antihypertensive drug therapy. **Keywords**: CARDIAC REHABILITATION, METABOLIC SYNDROME, INTERVAL TRAINING.

At least 3-5 weekly sessions of physical activity (150 minutes of moderate intensity or 75 minutes of vigorous physical exercise) decrease all-cause mortality and positively influence cardiovascular risk factors, including the incidence of metabolic syndrome (MS) (1, 2, 3). MS is not only more prevalent in patients with cardiovascular disease, but also an independent risk factor for higher morbi-mortality rates among these patients (4). It is estimated that half of the patients referred to cardiac rehabilitation suffer from metabolic syndrome, and several studies have shown an improvement of all MS diagnostic criteria after cardiovascular rehabilitation. The fact that short-term effects of cardiac rehabilitation appear to be more spectacular than the long-term results can be due to poor long-term adherence to lifestyle changes (5).

The level of physical activity is inversely correlated to metabolic syndrome incidence, making it one of the most important components of cardiac rehabilitation pro-

grams (6). Performing cardiopulmonary exercise (CPX) testing when enrolling a patient in a rehabilitation program allows an objective assessment of the subject`s physiologic response to exercise and plays a key part in risk stratification and determining optimal exercise training intensity. CPX test results include peak $VO_2$ – a marker of the patient`s functional capacity, the anaerobic threshold – which guides exercise prescription, respiratory exchange ratio and oxygen pulse. CPX testing is particularly useful in patients on beta-blocker treatment, in which the Karvonen method (using heart rate reserve in prescribing target heart rate during exercise) should not be employed (7).

Patients with cardiovascular disease are usually recommended moderate intensity exercise at 60-80% of the maximum heart rate or anaerobic threshold. Recent reports suggest that high-intensity interval training is more efficient than classical moderate-intensity continuous training (8). According to another report, even 51 minutes per week of high intensity interval training was as effective as 150 minutes per week of moderate intensity continuous training in patients with metabolic syndrome (9). Further studies are needed to determine the most effective length of the high intensity interval bout as well as the optimal weekly volume of training.

CASE REPORT

CD, a 47-year-old male, was referred to the local Cardiovascular Rehabilitation Clinic for exercise testing and exercise-based rehabilitation for grade II arterial hypertension. His past medical history also included obesity, mixed dyslipidemia and a positive family history of hypertension and diabetes. The patient met the criteria for metabolic syndrome: class 1 abdominal obesity (Body Mass Index 34.1, waist circumference 118 cm) elevated fasting glucose (112 mg%), hypertriglyceridemia (209 mg%), hypertension (165/100 mmHg on admission), so we decided to start treatment with a low dose angiotensin converting enzyme inhibitor.

During the cardiopulmonary stress test our patient achieved a maximum workload of 120 W (59% of predicted value), with a peak oxygen uptake (VO_2) of 1719 mL/min (62% of predicted value) and anaerobic threshold of 1710 mL/min (17.6 mL/kg/min), suggesting a slight reduction of functional capacity (Weber class A). The test was halted after 11 minutes due to uncontrolled blood pressure (BP) values (230/120 mmHg), although our previous Ambulatory Blood Pressure Monitoring (ABPM) illustrated appropriate blood pressure control (mean 24h-Systolic BP 115 mmHg, mean 24h-Diastolic BP 71 mmHg, max-BP 150/125 mmHg, 24h systolic abnormal ratio 2.8%, dipping index 18%).

Our patient was enrolled in an exercise-based rehabilitation program, consisting of biweekly 30-minute sessions of aerobic resistance training on a bicycle ergometer under the supervision of a certified kinesiotherapist. The first phase of rehabilitation consisted of 5 sessions during which we progressively increased cycling duration from 10 to 30 minutes. This was performed at a continuous workload of 25 W. We started the second phase training sessions with 2.5-minute warm-up followed by five sets of 4-minute cycling at 25 W and 1-minute training at 50 W, ending with a 2.5-minute cool-down. The work-out intensity was gradually increased according to protocol (tab. I). The switch to higher workloads (50-75W and 75-100W after 1 and 5 months, respectively), was determined by patient's pressor response during exercise.

TABLE I

Interval training protocol

	2.5-minute warm-up	5 x 5 minutes training sequences of:	2.5-minute cool down	Exercise duration
25-50W	10 W	4` - 25W + 1` - 50 W 3` - 25W + 2` - 50 W 2` - 25W + 3` - 50 W 1` - 25W + 4` - 50 W	10 W	30 minutes
50-75W		4` - 50W + 1` - 75 W 3` - 50W + 2` - 75 W 2` - 50W + 3` - 75 W 1` - 50W + 4` - 75 W		30 minutes
75-100W		4` - 75W + 1` - 100 W 3` - 75W + 2` - 100 W 2` - 75W + 3` - 100 W 1` - 75W + 4` - 100 W		30 minutes

A second cardiopulmonary stress test was performed after 1 year of exercise-based physical rehabilitation, with significantly improved results (maximum workload 178 W, 86% of predicted value, VO_2 max 2174 mL78% of predicted value, oxygen pulse 17 mL/min). Our patient`s cardiovascular risk markers also showed gradual improvement during the 1-year follow-up (tab. II), allowing the withdrawal of previous angiotensin converting enzyme inhibitor treatment.

TABLE II

Evolution of clinical and biological parameters during the one-year follow-up

	Baseline value	Months 3	6	12
Weight	108	106	103	98
Waist circumference	118	117	114	110
Triglycerides	209	180	103	92
Total cholesterol	209	180	151	160
Low density lipoprotein cholesterol (LDL)	113.2	83	55	57.6
Glycemia	112	108	103	98
Systolic BP	165	132	130	117
Diastolic BP	100	92	79	68
Resting Heart Rate	92	86	72	65

DISCUSSION

High intensity training has proven more effective than continuous moderate intensity training standardly used in cardiac rehabilitation. Although several training protocols have been studied and published (8), the implementation of a standard, universal protocol, aimed at achieving a target heart rate during exercise is often limited by patient`s exaggerated pressor response, as

illustrated in our case report.

During the first CPX testing, our patient rated the perceived exertion as "somewhat hard" (Borg scale 13) but the test was halted after 11 minutes due to high blood pressure (230/120 mmHg). Although according to our subject`s test results (maximum workload 120 W, Weber class A) we could have begun the phase I trainings at a 50 W workload, our patient presented an exaggerated pressor response after just 4 minutes of training (BP 200/110 mmHg) forcing us to decrease initial continuous exercise workload to 25 W. Cycling duration at 25 W was also limited by high blood pressure values (127/72 mmHg at baseline, 160/100 mmHg after 4 minutes and 170/100 after 10 minutes), requiring 5 sessions in which we gradually increased exercise duration from 20 to 30 minutes thus allowing pressor response adaptation.

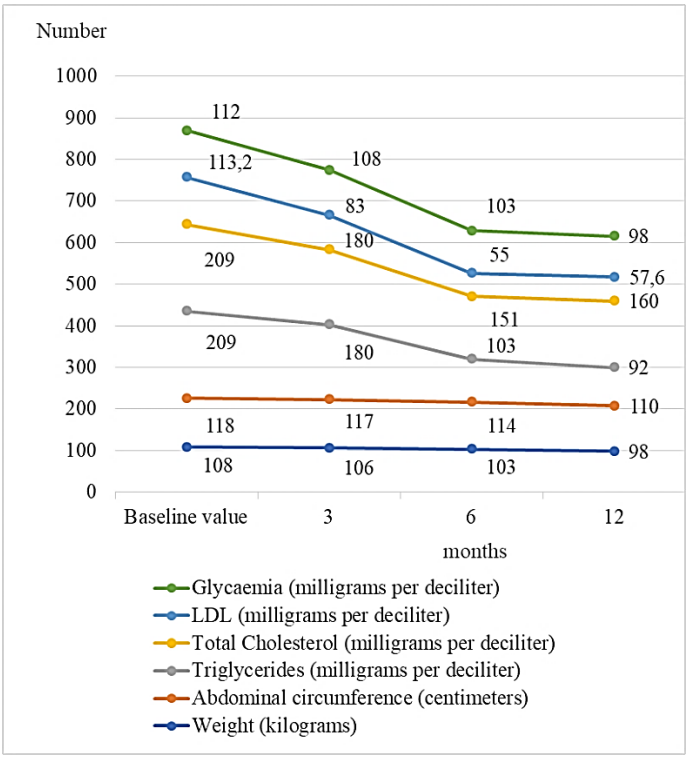

Fig. 1. Beneficial effects during 1 year of exercise rehabilitation: weight, triglycerides, LDL, waist circumference, total cholesterol and fasting glucose have all improved by 9.2%, 55.9%, 49.11%, 6.7%, 23.4% and 12.5%, respectively.

Our two-phased training protocol ensured that the patient maintained adequate blood pressure values during work-out and permitted the switch to higher workload exercise sessions only after achieving appropriate pressor response adaptation to current exercise intensity (blood pressure during peak workload interval below 140/90 mmHg). Our approach has proven as effective as other rehabilitation protocols (5) in

reducing the patient`s resting heart rate (by 29%, 27 beats per minute), systolic and diastolic blood pressure values (by 29 and 32%, respectively), thus allowing withdrawal of the antihypertension medication. After one year of exercise-based cardiac rehabilitation our subject no longer meets the metabolic syndrome criteria, exhibiting a 12.5% decrease in fasting glucose, a 55.9% decrease in serum triglycerides as well as a 6.7% decrease in waist circumference, now below the 102 cm threshold (fig.1).

Patient adherence is still an unresolved issue in cardiac rehabilitation (8). However, our individualized exercise protocol was enthusiastically received by our patient, who was present for 82% of his scheduled sessions, an excellent adherence rate compared to those reported in other studies (8). We believe that our 3- and 6-month check-ups contributed to this high adherence rate as it allowed the patient to acknowledge his progress.

CONCLUSIONS

The intensity of interval training in our patient was limited by exaggerated pressor and chronotropic response to relatively low exercise workload, although according to the cardiopulmonary exercise test our subject presented mildly altered functional capacity (Weber class A). Our patient with metabolic syndrome benefited from long-term exercise-based cardiac rehabilitation but required an individualized exercise protocol.

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BUTYRYLCHOLINESTERASE ACTIVITY – BIOMARKER FOR PREDICTING THE OUTCOME IN ACUTE CHOLINESTERASE INHIBITOR POISONING – A 30-YEAR RETROSPECTIVE ANALYSIS

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BUTYRYLCHOLINESTERASE ACTIVITY – BIOMARKER FOR PREDICTING THE OUTCOME IN ACUTE CHOLINESTERASE INHIBITOR POISONING – A 30-YEAR RETROSPECTIVE ANALYSIS (Abstract): **Aim.** To assess the role of butyrylcholinesterase (BuChE) activity as a predictive biomarker in acute cholinesterase inhibitor poisoning in a cohort from a regional tertiary care hospital. **Material and methods:** Plasma butyryl-cholinesterase activity on admission and at regular intervals during admission and clinical outcomes of cases admitted to the Toxicology Clinic of "Sf. Spiridon" Emergency Hospital Iasi, Romania between 1983 and 2013 were evaluated. **Results:** A total number of 606 patients were included in the study. The mean BuChE-activity level on admission was 1.54 ml NaOH N/100. A correlation between the amount of ingested organophosphates/carbamates (OPs/CMs) and low cholinesterase activity on admission was found. 66.66% of the patients were admitted to hospital within 8 hours after poisoning. The initial, daily and mean total atropine doses administrated were 9.65 mg, 10.51 mg and 69.39 mg, respectively. 67.16% of the investigated patients received Toxogonin for 6.41 days showing a slow increase in BuChE activity afterwards. The average number of hospital days was 11.22. The study revealed that complications occurred in patients with BuChE-activity levels below 1.4 mL NaOH N/100. A positive correlation between mortality rate (3.8% of patients) and the lowest BuChE-activity level on admission (0.89 mL NaOH N/100) was found. **Conclusions:** BuChE activity on admission and its level during hospital stay represent an important predictive factor for acute cholinesterase inhibitors poisoning. **Keywords:** BUTYRYL-CHOLINESTERASE, CHOLINESTERASE INHIBITORS POISONING, TOXICITY

Organophosphates (OPs) and carbamates (CMs) are toxic synthetic compounds that are used as: (a) insecticides, (b) chemical warfare agents, (c) therapeutic agents in veterinary and human medicine (1, 2, 3). Nowadays they are the most commonly used pesticides worldwide.

The World Health Organization (WHO) estimates that every year over 3 million people experience acute poisoning by OPs, with more than 200.000 deaths. OPs self-poisoning is more common than unintentional intoxication, which is very rare, especially in areas where WHO class I toxicity pesticides are used (4). Farmers and people employed in agriculture are considered a

high-risk group of accidental intoxication with OPs (5, 6, 7). There are reports indicating that in many rural areas of Asia, where farmers are constantly using toxic pesticides, OPs are frequently used in suicides (8).

Their primary mechanism of action is the inhibition of esterase enzymes, especially acetylcholinesterase and butyrylcholinesterase. These two enzymes are biomarkers of OPs and CMs systemic toxicity. Acetylcholinesterase inhibition results in accumulation of acetylcholine and overstimulation of both muscarinic and nicotinic receptors. Red-cell acetyl-cholinesterase inhibition is correlated with poisoning severity and can guide the oxime and atropine therapy. Acute butyryl-cholinesterase inhibition does not seem to cause clinical features and is not related to severity of poisoning. It can be used as a marker of exposure to OPs or CMs and for measuring cholinesterase-inhibiting compound elimination from the body. Many OPs pesticides are more potent inhibitors of butyrylcholinesterase than they are of acetylcholinesterase (9, 10).

The main goal of this study was to evaluate BuChE activity as a biomarker of acute OPs/CMs poisoning, in patients admitted to the Internal Medicine and Toxicology Clinic of “Sf. Spiridon” Emergency Hospital Iasi between 1983 and 2013.

MATERIAL AND METHODS

We conducted a descriptive retrospective clinical study on patients with OPs/CMs poisoning admitted to the Internal Medicine and Toxicology Department of the Iasi "Sf. Spiridon" Emergency Hospital aimed at evaluating the phenomenon of acute cholinesterase inhibitor poisoning over a period of 30 years.

Hospital records of patients diagnosed with acute accidental/voluntary OP or CM poisoning between the years 1983-2013 were reviewed.

The obtained data were used to create a complex and complete medical profile for each intoxicated patient.

A total of 53 parameters were assessed. The data on BuChE activity were evaluated by the analysis of following parameters: (a) BuChE-activity level on admission; (b) BuChE activity during hospital stay (c) time to serum cholinesterase return to normal physiological levels.

BuChE activity on admission was expressed as ml of NaOH N/100, not as mU/mL due to the fact that in most patients the Michel method for BuChE determination was used. Reference values were considered between 4-6 mL NaOH N/100. The others patients were assessed using the Ellman method; the obtained cholinesterase levels were expressed as mU/mL and then converted to mL NaOH N/100.

The experimental protocol of this investigation was implemented according to recommendations of the University Committee for Research and Ethical Issues, and to the guidelines stipulated in the EU Charter of Fundamental Rights, regarding clinical investigations on human subjects (10).

Statistical analysis of data was performed using SPSS for Windows version 19.0 and one-way ANOVA method, followed by Bonferroni post hoc test. Chi-square test was used for comparing means and variables and frequency analysis, considering the level of significance for p value less than 0.05.

RESULTS AND DISCUSSION

A total number of 606 patients were included, accounting for 11% of the total number of acute poisonings. The highest number of cholinesterase inhibitor poison-

ing cases was admitted in 1995 (44 cases).

BuChE activities on admission and during hospital stay were assessed using several parameters: (a) means of patient transportation to hospital (ambulance or means of transportation others than ambulance); (b) time interval between poisoning and hospital admission; (c) amount of ingested OPs; (d) age and sex; (e) clinical parameters; (f) treatment (initial, daily and total dose of atropine, Toxogonin® treatment); (g) time to return to normal BuChE levels; (h) course; (i) complications; (j) discharge status.

On admission most patients had a BuChE level around 1.5 mL NaOH N/100 (fig. 1).

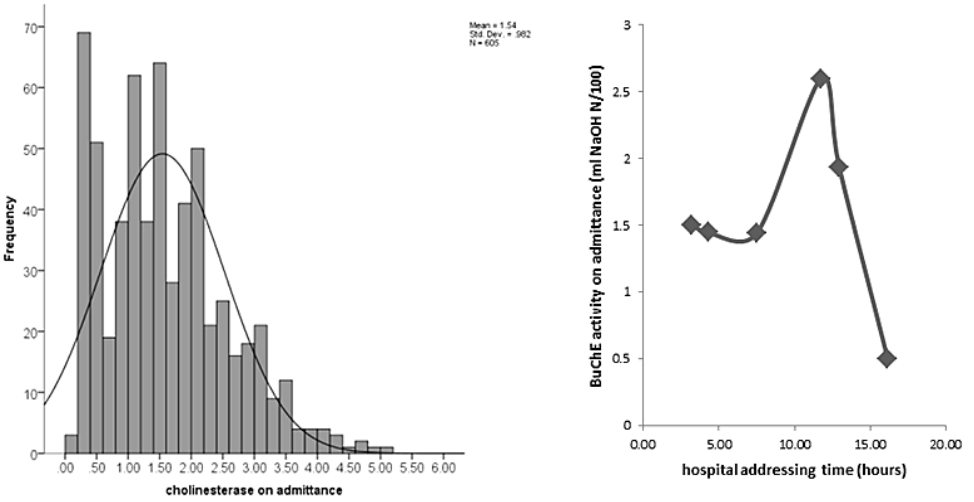

Fig. 1. Cholinesterase activity on admission. Correlations between butyrylcholinesterase activity on admission and hospital addressing time.

The estimated average time for admission to the emergency department after poisoning was 8.8 hours. 66.66% of the patients admitted to hospital within 8 hours after poisoning were using personal means of transportation and had an average serum BuChE level of 1.5 mL NaOH N/100. 6.72% of the patients admitted within 16 hours after poisoning were transported to hospital by ambulance and had an average serum BuChE level of 0.5 mL NaOH N/100 (fig. 1).

It is worth mentioning that patients using personal means of transportation reached the hospital in a shorter time frame than those that transported by the ambulance.

We found a correlation between the amount of ingested OPs/CMs and low cholinesterase activity on admission (p=0.002). More than half of the patients (58.74%) had ingested a small amount of OPs/CMs (average 69.19 mL). In this group, the cholinesterase activity level on admission was on average 1.44 mL NaOH N/100, with a slow increasing tendency. A rapid increase in cholinesterase activity was found in patients that ingested a higher amount of OPs/CMs (84.73 mL). In these patients, the cholinesterase activity on admission was 2.33 mL NaOH N/100 (fig. 1).

In patients older than 80 years, the average BuChE level was 0.22 mL NaOH

N/100. The highest average BuChE level recorded was 1.88 mL NaOH N/100 in patients aged 16 to 19 years.

There were no statistically significant differences (p=0.084) in average BuChE levels on admission between females and males.

The higher the average BuChE level on admission, the less complicated were the clinical manifestations of poisoning (p=0.000).

The initial, daily and total mean atropine doses administrated were 9.65 mg, 10.51 mg and 69.39 mg, respectively. Patients with average BuChE levels around 2.5 mL NaOH N/100 and 0.5 mL NaOH N/100 on admission were given a lower atropine dose. In patients with a plateau BuChE level on admission followed by an increase during hospital stay, the highest total atropine dose - 90.52 mg was administrated. The smallest total atropine dose was administrated in patients that had a dramatic increase in BuChE activity (fig. 2).
The initial and daily atropine doses were almost similar. There were no significant differences between initial and daily doses of atropine when compared with BuChE activity on admission. One significant difference was found between the total dose of atropine and BuChE activity on admission: the lower BuChE activity on admission, the higher the atropine dose administrated to the patient.

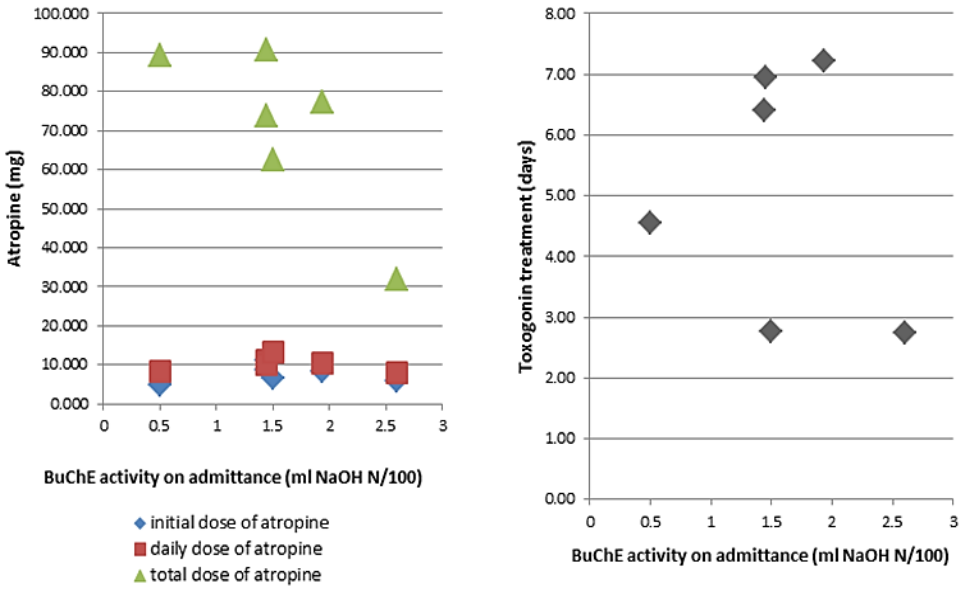

Fig. 2. Correlations between butyrylcholinesterase activity on admission, atropine and Toxogonin therapy.

The mean duration of Toxogonin treatment was 5.74 days. 67.16% of the patients (BuChE on admission 1.44 mL NaOH N/100) received Toxogonin for 6.41 days and showed a slow increase in BuChE activity afterwards. The longest duration of

Toxogonin treatment (7.23 days) was in patients with a decreasing BuChE tendency after admission, followed by an increase in BuChE activity. The shortest Toxogonin treatment (2.74 days) was given to the patients who had a dramatic increase in BuChE activity after admission (fig. 2).

No correlation was found between the total number of Toxogonin treatment days and the initial BuChE level on admission. There was a strong correlation between the total number of Toxogonin treatment days and the further BuChE activity during hospital stay (p=0.000).

Of all patients, 9.75% received exogenous cholinesterase. There were no statistically significant differences regarding BuChE initial activity between these patients and the patients who did not receive exogenous cholinesterase (p=0.361).

The following correlations were found: BuChE activity increased in patients with the highest BuChE levels on admission; BuChE activity was oscillating in patients with the lowest BuChE levels on admission; the return to normal of serum cholinesterase activity was positively correlated with the mean initial BuChE level on admission.

In most of the patients (67.73%) cholinesterase activity returned to normal within 6-7 days after admission, following an ascending slope (fig. 3). In patients with the lowest initial BuChE level on admission (0.5 mL NaOH N/100) cholinesterase activity was oscillating, described by alternated ascending and descending slopes, and was not normal at the time of the discharge from hospital. In patients with the highest mean BuChE level on admission (2.59 mL NaOH N/100) cholinesterase activity had rapidly increased, returning to normal physiological values 3 days after admission (fig. 3, 4).

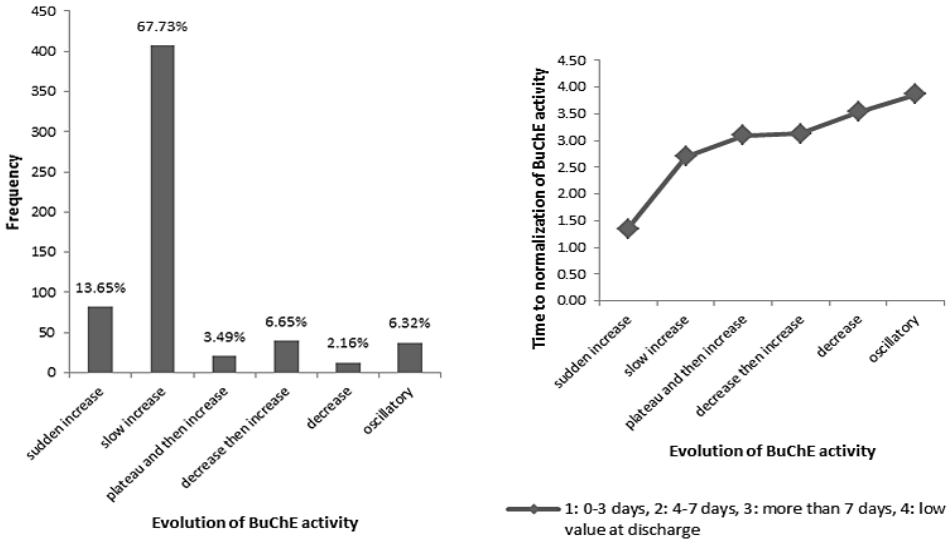

Fig. 3. Correlations between the course of butyrylcholinesterase activity, frequency and time to normalization.

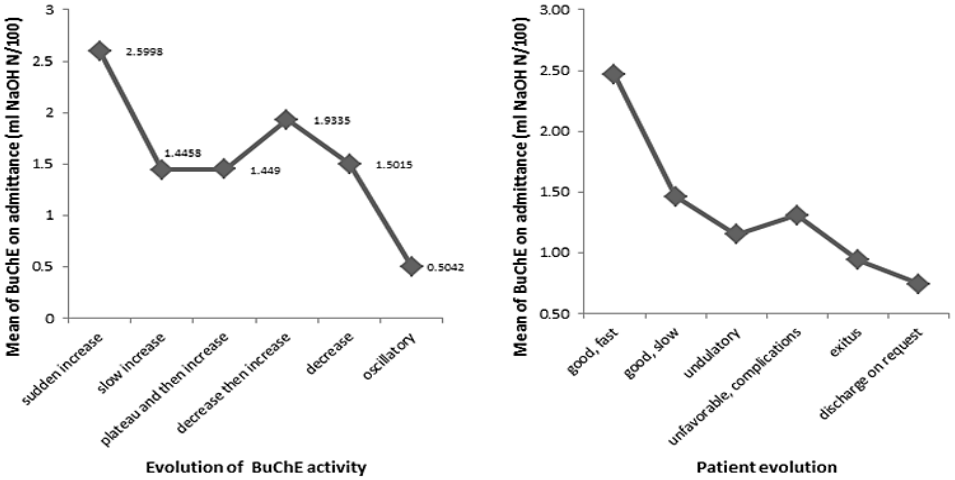

Fig. 4. Correlations between mean BuChE activity on admission, its dynamics and patient course

The higher the BuChE on admission, the better the patient clinical course - p=0.000.

Of the total number of patients, in 67.73%, with a slow increasing tendency for BuChE, the average length of hospital stay of 11.65 days. In patients with a dramatic BuChE increasing tendency, the average length of the hospital stay was 6.7 days. The highest average length of stay was 13.95 days and was recorded in patients with a plateau-like BuChE activity followed by an increase in activity – mean BuChE level on admission 1.44 mL NaOH N/100. The mean length of hospital stay was positively correlated with BuChE course and its normalization time.

Our investigation demonstrated a higher incidence of complications in patients with BuChE levels below 1.4 mL NaOH N/100. In patients with BuChE levels above 1.66 mL NaOH N/100 (73% of patients) no complications were present.

The most frequently recorded complications were acute pulmonary edema (10.6% of cases), in patients with of 1.29 mL NaOH N/100 and toxic myocarditis (3.2% of cases), in patients with a mean BuChE activity of 1.38 mL NaOH N/100 on admission (Fig. 5). A BuChE level of 0.85 mL NaOH N/100 on admission was associated with such complications as: respiratory problems (1.3% of cases) and cardiac rhythm disturbances (0.7% of cases).

Our results indicate a positive correlation (p=0.072) between the presence of both cardiac arrest (3% of cases) and sudden death (0.3% of cases), and the mean level of BuChE activity (1.2 mL NaOH N/100).

A positive correlation was also found between mortality (3.8% of patients) and the lowest average BuChE activity on admission - 0.89 mL NaOH N/100.

Of the total number of patients, 80% were discharged at the recommendation of the treating physician (mean BuChE activity level of 1.63 mL NaOH N/100 on admission), while 10.41% on request (1.13 mL NaOH N/100) (fig. 5).

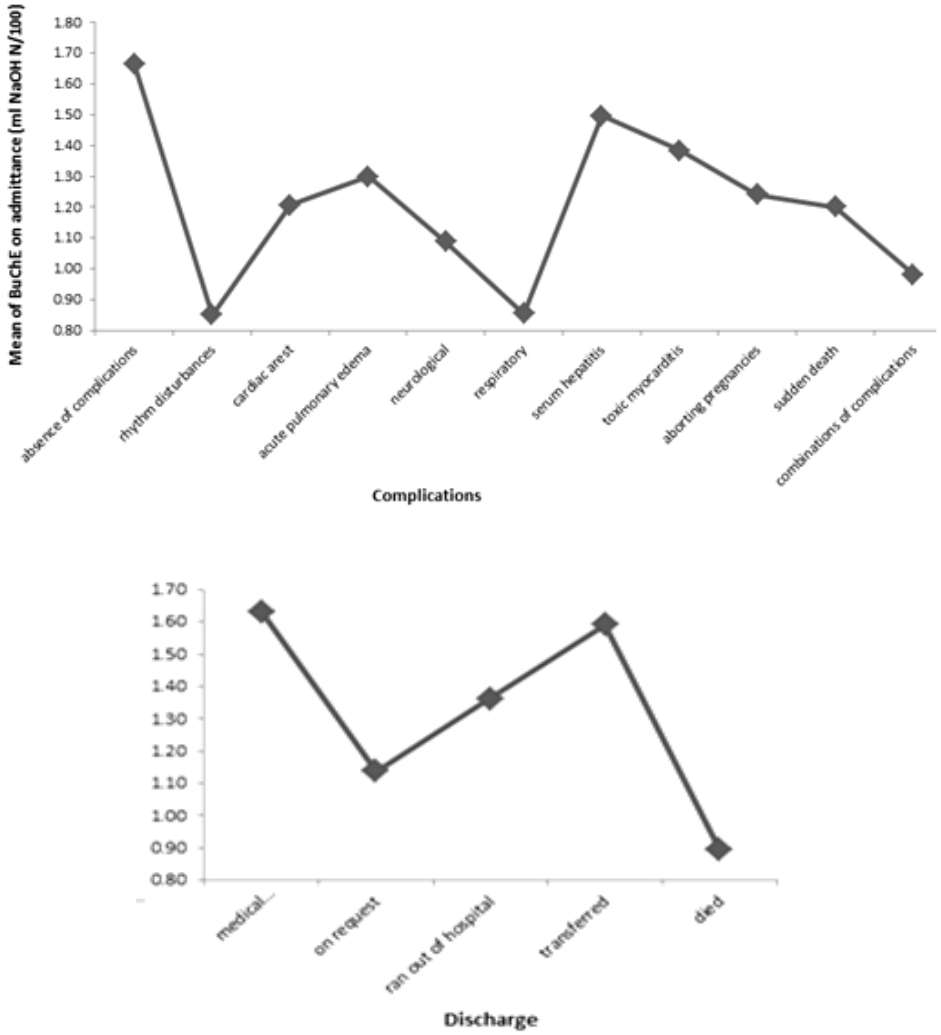

Fig.5. Correlations between **complications**, **discharge** status and butyrylcholinesterase activity on admission

CONCLUSIONS

In our study BuChE activity on admission and its course during hospital stay represents an important prognostic factor for cholinesterase inhibitors acute poisoning. The decrease of BuChE activity was positively correlated with the degree of poisoning. A tendency of BuChE activity to increase in patients with high BuChE levels and an oscillating course in patients with low BuChE activity level on admission were found. Complications were reported in patients with BuChE levels below 1.4 mL NaOH N/100, but not in patients with BuChE levels above 1.66 mL NaOH N/100.The incidence of mortality was correlated with low mean BuChE levels on admission.

ACKNOWLEDGEMENT

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NEWS

BACTERIAL INFECTION - SIDE EFFECTS OF COSMETIC TREATMENT

The lip augmentation and wrinkle smoothening are very commune, but the cosmetic treatment which consists in injection of soft tissue fillers can induce tender subcutaneous lumps and lesions difficult to treat. A recent study published in the journal *Pathogens and Disease*, showed that side effects of filler treatment are caused by bacterial infection. Using a mouse model, the researchers evaluated capacity of fillers for sustaining bacterial infections and the possible treatment strategies. Three type of gel contaminated with *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* were injected in a mouse model. The results showed that the permanent gel was able to sustain bacterial infection more than the semi-permanent gel. Temporary gel did not cause infection. Also, the study revealed that antibiotic treatment become inefficient if bacteria have formed a biofilm. This research emphasizes importance of antibiotics injection together with the filler itself, for prevent bacterial complications induced by cosmetic treatment (Alhede M, Er Ö, Eickhardt S et al. Bacterial biofilm formation and treatment in soft tissue fillers. *Pathogens and Disease*. 2014; DOI: 10.1111/2049-632X.12139).

Luncă Cătălina

SURGICAL TREATMENT OF CHRONIC COLOSTASIS IN CHILDREN: A TEN-YEAR EXPERIENCE

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SURGICAL TREATMENT OF CHRONIC COLOSTASIS IN CHILDREN: A TEN YEAR EXPERIENCE (Abstract) **Objective:** To analyze our ten year experience of surgical treatment of children with chronic colonostasis of different origin.**Material and methods:** The study covers 353 children aged from 1 day to 17 years with colonic stasis who undergo treatment at the Department of Children's Surgery, Bukovinian State Medical University, Cernăuţi, Ukraine. Study design included case history, physical examination, routine clinical and biochemical laboratory tests, endoscopy (sigmoidoscopy, colonoscopy), X-ray (irrigoscopy, irrigography, other radiopaque techniques) methods, and sphincterometry. **Results.** Non-surgical treatment was effective in 261 (73.94 %) patients. The group of the operated patients consisted of 92 (26.06 %) children. The outcome of surgical treatment of Hirschsprung's disease (49 children), dolichosigmoid (29 children), Payr's disease (12 children), and Chilaiditi syndrome in all cases is considered to be satisfactory.The optimal methods of surgical operations are offered according to pathology of colon. **Conclusions:** One-step Soave-Boley operation is considered to be the method of choice for rectal and rectosigmoid forms of Hirschprung's disease. Transversal colon resection and phrenico-colic ligament dissection in Payr's disease, hepatopexia and right colon fixation in Chilaiditi syndrome are effective treatment approaches. **Keywords:** SURGERY, CHILDREN, CONSTIPATION.

Chronic constipation in children is greatly under evaluated problem since this disease affects over 15% of the entire population. Obstipation syndrome appears at birth or early childhood in about 30% of patients (1). Disorders of transit through the colon may be due to (2) anatomical features (elongation, drooping, creases, looping, abnormal mobility of the various parts, abnormal intramural nerve plexus) and functional factors (nutritional, dyskinetic, reflectory, intoxication).

However, underlying mechanisms of pathogenesis are not sufficiently clear. It may be determined by specific polymorphism of candidate gene (3), or similarly to other diseases by combination of different candidate genes polymorphisms attributing into increased risk by means of changed metabolism and regulation, thus causing appropriate switch in genetically determined therapies (4).

However, even introduction of modern sophisticated research techniques (5) did

not clarify important questions related to the problem.

Organic pathology in childhood requires extremely traumatic and difficult colonic surgical interventions.

Tactical and technical errors in performance of surgical treatment of colonostasis in children lead to the formation of a huge number of complications, disability, and social disadaptation (6). The aim of this study is to analyze our ten-year experience of surgical treatment of children with chronic colonostasis of different origin.

MATERIAL AND METHODS

This study conforms to international bioethical standards (European Convention on Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on ethical principles of scientific medical research involving human subjects, GCP, EUC directive #609, etc.) and approved by Commission for Bioethics in Research of the Bukovinian State Medical University, Ukraine. All patients' representatives signed written permissions and obtained full information about the study prior to participation.

The study covers 353 children aged from 1 day to 17 years with colonic stasis who undergo treatment at the Department of Children's Surgery, Bukovinian State Medical University, Cernăuţi, Ukraine (tab. I).

TABLE I

Distribution of children with colonostasis according to the etiology of disease

Pathology	Number of children	Number of operated children
Hirschprung's disease	49 (13.88%)	49 (100%)
Dolichosigmoid (isolated)	144 (40.79%)	15 (10.42%)
Dolichosigmoid+ high position of the left colonic flexure	36 (10.20%)	14 (38.89%)
Payr's disease	29 (8.22%)	12 (41.38%)
Chilaiditi syndrome	2 (0.57%)	2 (100%)
Spastic colitis	11 (3.12)	0
Neural disorders	7 (1.98%)	0
Functional disorders	75 (21.25%)	0
Total	353 (100%)	92 (26.06%)

Study design included case history, physical examination, routine clinical and biochemical laboratory tests, endoscopy (sigmoidoscopy, colonoscopy), X-ray (irrigoscopy, irrigography, other radiopaque techniques) methods and sphinctero-metry.

Hirschprung's disease diagnosis was based on the presence of areas of narrowing in the distal colon with proximal funnel-like expansion at X-ray pictures, absence or abrupt decrease of the contrast passage of in expended parts of the large intestine, anal sphincter hypertonia. Subtotal form of agangliosis was observed in seven children, rectosigmoid in 29, and rectal in 13. The diagnosis was confirmed with a rectal suction biopsy, which presented the absence of ganglion cells and the presence of hypertrophic nerve trunks (7).

Dolichosigmoid diagnosis was based on

the analysis of the sigmoid colon by following criteria: length, width, additional loops of colon, presence/retention of radiopaque mixture in the sigmoid colon and proximal colon after bowel movements and defecation. We clinically distinguished dolichosigmoid with violated evacuation function and without it. The presence of left colonic flexure high position was observed in 36 children and isolated dolichosigmoid in 144 (fig. 1).

Fig. 1. Irrigogramm, patient P., 12 years old, prior to defecation. Dolichosigmoid with left colonic flexure high position (2) compared to right flexure (1)

RESULTS AND DISCUSSION

In 12 patients with Hirschprung's disease, the first stage of surgical treatment was colostomy. The major indications for the *anus praeter naturalis* for Hirschprung's disease were: colon perforation (1 patient); transversal torsion (1 patient), decompensated enterocolitis and colic dilatation with severe fecal intoxication (three children), acute form of Hirschprung's disease (4 patients), ineffectiveness of conservative treatment (3 children). In three patients in the neonatal period,we formed suspended transversostoma, as a first step before performing De la Torre transverso-endorectal pull-through (8). In ninechildren,anus *praeternaturalis* was applied on the transition agangliosis area.

In subtotal agangliosis form of Hirschsprung's disease, colon pull-through maneuver in the left side sinus performed in 3 patients, the bowel pull-through in the right mesenteric sinus holding it in front of the terminal ileum performed in two children, and pull-through of the ascending colon with 180^0 turn performed in two patients.

In 10 children with rectosigmoid form of Hirschprung's disease and in 5 with rectal form modified Soave operation wasperformed. In 17 children with rectosigmoid form and in 7 with rectal form we performed Soave-Boley procedure. In two patients with rectosigmoid form and in one with rectal form a three stage (stage 1– transversostomy, stage 2 – transanal endorectal pull-through maneuver, stage 3– transversostomy closure) endo-rectal pull-through surgery (tab. II).

According to the type of surgery, all patients with dolichosigmoid were divided into two groups: group 1 with traditional sigmoid resection and group 2 with formation of superior sigmoid ligament using our technique (tab. III).

The technique of surgery was as follows. We performed sinistral transrectal laparotomy. Mobilization of the sigmoid colon with dissection of its lateral posterior peritoneal fold. Distal portion of colon was pulled up to determine hypertrophyand level of resection, applying "marking sutures" so that the no prolapsed bowel is left

in pelvis. Sigmoid crossed at the level of the distal portion, 3-4 cm above the promontory and the proximal part of the colon was resected in strained position so that it was left enough for application of anastomosis with already crossed bowel.

TABLE II

Distribution of children with the Hirschprung's disease by type of surgery

Operation	Hirschprung's disease forms			Total
	Subtotal	Rectosigmoid	Rectal	
Pull-through maneuver in the left side sinus	3 (42.9%)	–	–	3 (6.1%)
Pull-through maneuver in the right side sinus	2 (28.6%)	–	–	2 (4.1%)
Pull-through of the ascending colon with 180° turn	2 (28.6%)	–	–	2 (4.1%)
Modified Soave technique	–	10 (34.5%)	5 (38.5%)	15 (30.6%)
Soave-Boley procedure	–	17 (58.6%)	7 (53.9%)	24 (49.0%)
Transanal endorectal pull-through	–	2 (6.9%)	1 (7.7%)	3 (6.1%)
Total	7	29	13	49

TABLE III

Distribution of children operated for dolichosigmoid depending on type of surgery

Technique	Type of dolichosigmoid		Total
	Isolated dolichosigmoid (1 group)	Dolichosigmoid + high position of the left colonic flexure (2 group)	
Sigmoid resection	8 (53.3%)	8 (57.1%)	16 (55.2%)
Sigmoid resection + formation of superior sigmoid ligament	7 (46.65)	6 (42.9%)	13 (44.8%)
Total	15	14	29

End-to-end anastomosis with interrupted inverted sero-muscular sutures was imposed. The upper sigmoid ligament was formed by suturing of section of colon above the anastomosis to the parietal peritoneum by interrupted sutures (fig. 2).

In case of high left colonic flexure and dolichosigm we dissected phrenicocolicum ligament.

Histological examination showed sclerosis of the *lamina propria* of the mucosa. In submucosal tissue a process of sclerosis combined with fatty degeneration, hypertrophy of muscle fibers and atrophic processes (myocyte vacuolization), sclerotic changes of serosa and mesenteric tissues.

After traditional sigmoid resection periodic constipation after surgery was observed in 18.75%, abdominal pain in 37.5%, partial incontinence in 60%. After addition of the upper sigmoid ligament formation during surgery, constipation was absent, abdominal pain persisted in 14.29%, partial incontinence in 16.67%.

Surgery for Payr's disease included upper midline laparotomy, division of adhesions in the region of the splenic flexure and dissection of the phrenico-colic ligament. Excessive transversal was mobilized between Hirsch's and Payr-Strauss' sphincters. Transversal colon resection with restoration of passage by imposing end-to-end anastomosis was performed with interrupted inline inverting sutures (PDS 4/0).

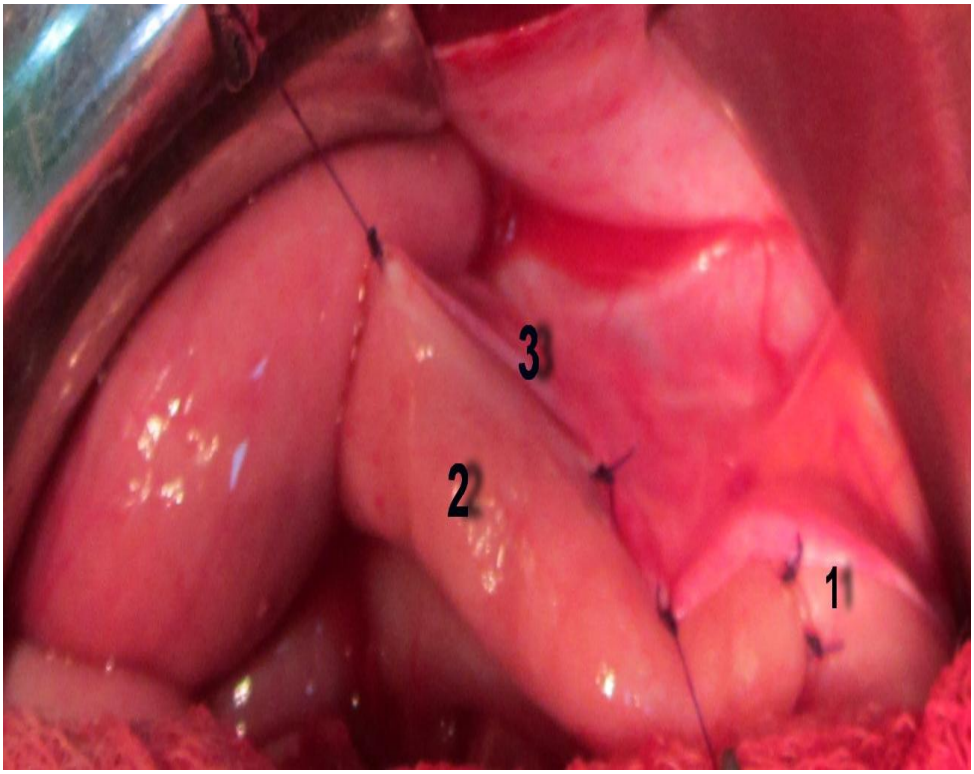

Fig. 2. Resection of the sigmoid colon (2). Intestino-colic end-to-end anastomosis (1). Formation of the upper sigmoid ligament (3)

Histological examination of the resected transversal colon showed no changes. However, there was sclerosis of the lamina *propria* of the mucosa; in submucosal tissue sclerotic process combined with fatty degeneration. There was hypertrophy of muscle fibers, and in some cases (5 children) atrophic processes (vacuolization of myocytes), indicating progression of the disease;rough connective tissue between muscle fibers around the vascular cells and nerve plexus. In several cases (four children) observed serosa and mesenterial tissue sclerosis.

In patients with Chilaiditi syndrome performed right lobular lateral hepatopexia by corrugating falciform and triangular ligaments with their fixation diaphragm; colofixation of right colonic flexure to the parietal peritoneum.

The quality of life of children after surgery for Payr's disease and Chilaiditi syndrome, calculated in SF-36 was evaluated as satisfactory with elimination of pain and

chronic constipation.

CONCLUSIONS

The optimal method for surgical treatment of rectal and rectosigmoid forms of Hirschprung's disease in children is the one-step Soave-Boley operation.

The most rational way to restore the integrity of the intestinal tract after subtotal colon resection is colorectal anastomosis on the right or left mesenterial sinus (depending on the length of the segment).

Sigmoid colon resection with formation of the upper sigmoid ligament can be an operation of choice for surgical treatment of chronic colostasis under dolichosigmoid in children.

Resection of colon *transversum* and phrenico-colic ligament dissection, in Payr's disease, hepatopexia,and right colon fixation in Chilaiditi syndrome are effective treatment methods for these diseases in children.

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AMIODARONE-INDUCED THYROID DYSFUNCTION – CLINICAL PICTURE. STUDY ON 215 CASES

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AMIODARONE-INDUCED THYROID DYSFUNCTION – CLINICAL PICTURE STUDY ON 215 CASES **(**Abstract): **Aim**: The purpose of this study was to investigate the prevalence and the clinical-evolutionary implications of thyroid damage in patients treated with amiodarone in the Cardiology Clinic of the "Sf. Spiridon" University Hospital of Iasi. **Material and Methods:** The study included a group of 215 patients, 90 men and 125 women with ages between 35 and 87, hospitalized in the Cardiology Clinic between 2004 and 2014, who received amiodarone treatment, in most cases for the prophylaxis of various arrhythmias, both supra-ventricular and ventricular. **Results:** In 27.80% of the patients, the assessment of the thyroid function was imposed by the appearance of the clinical picture characteristic for hypo- and hyperthyroidism, and for 72.19% it was carried out as a routine examination. Amiodarone-induced hypothyroidism was clinically diagnosed in 20.85% of the patients. Hyperthyroidism occurring during treatment with Cordarone was found in 6.95% of the patients. The confirmation of the diagnosis of amiodarone-induced thyroid dysfunction was based on hormonal dosages (TSH, FT4 and even FT3 in some cases), on the endocrinological clinical consultation and on the imaging study – i.e. thyroid echography. **Conclusions:** Amiodarone-induced thyroid dysfunction is relatively rare compared to the number of patients treated with this anti-arrhythmic drug (27.8%) from the group under study. Thyroid dysfunction, regardless of the type (with hypo or hyper-function), represents a negative element in the evolution of patients with pre-existing heart diseases, not only by aggravating the clinical picture of the basic illness, but also by the necessity of permanently reviewing the therapeutic scheme imposed also by the association of thyroid dysfunction medication, according to case. **Keywords:** AMIODARONE, HYPOTHYROIDISM, HYPERTHYROIDISM.

Discovered in 1961 by Tondeur and Binon, amiodarone has been widely used especially in Europe, initially as anti-anginal preparation and later on as anti-arrhythmic, proving its effectiveness in the treatment of both atrial and ventricular arrhythmias with a malignant potential, being considered one the most effective anti-arrhythmic agents in preventing sudden arrhythmic cardiac death (1, 9). In 1985, amiodarone was approved by the FDA (*Food and Drug Administration*) for the treatment of atrial fibrillation and the prevention of recurrent ventricular tachy-

cardia. Despite this beneficial activity the prescription of amiodarone started to be limited due to the occurrence of serious side effects, some even disastrous, which were associated with the chronic use of the drug (2, 6, 8). Among these, the effects on the thyroid function raise the biggest issues for both patient and the physician; often it is necessary to reassess the therapeutic scheme because of the need to treat the newly occurred thyroid dysfunction. Thyroid abnormalities have been observed in 14-18% of the patients that received the minimum dose of amiodarone (8). The effects on the thyroid are variable:

- subclinical impairment, i.e. abnormal thyroid function detected only through regular laboratory tests (TSH, FT4, FT3) in the absence of any clinical manifestations (5, 6).

- clinically manifested thyroid dysfunction: amiodarone-induced thyrotoxicosis or hypothyroidism. Both can occur either on a previously normal thyroid gland or in the context of a pre-existing thyroid impairment, which they may worsen (3, 4, 6, 7, 8, 9).

The mechanisms of amiodarone activity on the thyroid are particularly complex: amiodarone inhibits the activity of deiodinase enzyme and thereby it decreases the peripheral conversion of thyroid hormones. It also decreases their renal elimination, increases T4, decreases T3 by about 25% and inhibits the entry of T4 and T3 in the peripheral tissues. (8). Amiodarone together with its metabolite, also has a direct cytotoxic effect on the thyroid follicular cells, the result being a destructive thyroiditis. It acts as a competitive antagonist of T3 at the heart level (3, 4, 7, 8, 9).

Amiodarone-induced thyroid impairment can resemble the clinical and biochemical picture of either hypothyroidism or hyperthyroidism. Amiodarone-induced hyperthyroidism may be of two types: *Type 1* - due to excess iodine in amiodarone, which causes excessive synthesis of thyroid hormones; it usually occurs in subjects with prior thyroid impairment (subclinical or clinical) and *Type 2* – it appears as an inflammatory process followed by the destruction in those with previously normal thyroid. The latter type is far more serious and it requires an urgent specific therapeutic protocol, which also involves the administration of glucocorticoids. Combined forms of thyroid impairment are rare (6, 8, 9). This study aimed to investigate the prevalence and the clinical-evolutionary implications of thyroid impairment in patients treated with amiodarone hospitalized in the Cardiology Clinic of the "St. Spiridon" University Hospital of Iaşi.

MATERIAL AND METHODS

The study included a group of 215 patients hospitalized in the Cardiology Clinic between 2004 and 2014, who received amiodarone treatment, in most cases for the prophylaxis of various arrhythmias, both supraventricular and ventricular. The group included 90 men and 125 women aged between 35 and 87. Patients were examined clinically and paraclinically (12-lead electrocardiogram, 2D echocardiography, M mode and Doppler, chest radiography, and laboratory data common in patients with heart disease: renal function and electrolytes, liver function, myocardial cytolysis enzymes). Since patients were going to receive amiodarone in the treatment scheme, the thyroid function was investigated for all of them, by routine hormone dosing (TSH, FT4, FT3) prior to the beginning of the treatment.

The basic pathology of the patients from the group was represented by the following conditions:

- acute viral myocarditis;
- acute myocardial infarction complicated by supraventricular arrhythmias and ventricular ESV and ventricular tachycardia;
- other forms of ischemic cardiopathy;
- ischemic dilatative and toxic (ethanolic) cardiomyopathy;
- HTA; on the background of this disease, the patients had different atrial arrhythmias (ESA, FA, atrial flutter) and ventricular arrhythmias (ESV), which were considered the expression of hypertensive cardiopathy;
- amiodarone administration pre-and post cardioversion.

RESULTS AND DISCUSSION

The indications for amiodarone administration in the study group were:

- controlling atrial and ventricular arrhythmias in the absence of or after the failure of other therapeutic alternatives;
- preparing for electrical cardioversion in patients with recent atrial fibrillation (onset under 1 year), with clear indication of cardioversion;
- the prophylaxis of atrial fibrillation recurrence after obtaining sinus rhythm;
- the prophylaxis and treatment of malignant ventricular rhythm disturbances occurred on the background of the coronary ischemic disease (i.e. IMA or other clinical forms of chronic ischemic cardiomyopathy);
- the prevention and treatment of severe ventricular rhythm disturbances occurred in the evolution of patients with dilative cardiomyopathy, having various aetiologies (ischemic, alcoholic, acute viral post myocarditis).

All patients in the group had their thyroid function investigated prior to the administration of amiodarone through TSH, FT4 and FT3 dosing, and it was within normal limits. The posology for amiodarone was, in most cases, as load - 800-1200 mg/day for 7-10 days, followed then by the maintenance dose 200 mg/day, 5-7 days/week (1 - 1.4 g/week). The duration of the treatment up to the moment of being included in the study (considered to be the moment that thyroid damage was discovered) was between 6 months and 8 years. The evaluation of the thyroid function, after starting the treatment, was made in 187 patients from the group (86.97%). In 52 patients (27.80%), the evaluation of the thyroid function was imposed by the appearance of the clinical picture characteristic for hypo- and hyperthyroidism; in the remaining 135 (72.19%) a routine examination was carried out. Amiodarone-induced hypothyroidism was clinically diagnosed in 39 patients (20.85%), of whom 13 were women and 26 men. Clinical elements suggestive for the hypothyroidism diagnosis were: somnolence, carotenoderma, excessive bradycardia, important physical asthenia and skin infiltration. All cases were confirmed through elevated TSH values and low FT4 values.

Hyperthyroidism occurring during treatment with Cordarone has been encountered in 13 patients (6.95%), 10 men and 3 women. Hyperthyroidism apparition was suggested by the following signs and symptoms (classified as the hyperkinetic syndrome): sweating, tremor, the aggravation of a pre-existing atrial fibrillation by increasing the ventricular frequency that became refractory to all antiarrhythmic therapy, unexplainable weight loss and tachycardia. In 11 cases there was a wors-

ening of the clinical picture of cardiac insufficiency. In all cases of hyperthyroidism, TSH values were lowered with normal or increased FT4. The confirmation of the amiodarone-induced thyroid dysfunction diagnosis of required collaboration with the endocrinologist, and it followed these steps:

1. Laboratory studies – i.e. TSH, FT4 and FT3 dosing in some situations. In case of amiodarone-induced hypothyroidism, laboratory data have objectified increased levels of TSH with low levels of free FT4 in all patients. Serum thyroglobulin was dosed to a limited number of patients and it had high values;

Patients with amiodarone-induced hyperthyroidism had high levels of free FT3 and FT4, and low, or even undetectable, levels of TSH;

2. Endocrinologic clinical examination;

3. Imaging studies – i.e. thyroid echography which, only in 5 patients with amiodarone-induced hypothyroidism, proved the enlargement of the thyroid and the nodular structure with hypoechogenic areas (partially orienting towards type 1 of amiodarone-induced thyroid dysfunction). In the remaining patients, thyroid echography was normal.

The current study revealed the following:

- None of the patients in the study presented clinically obvious thyroid impairment prior to the initiation of the amiodarone therapy;

- The occurrence of thyroid dysfunction induced by amiodarone did not correlate with the underlying heart pathology or the duration of the treatment, nor with other major cardiovascular risk factors. Some correlation was observed in the female group between hypothyroidism and obesity (85% of the women who developed amiodarone - induced hypothyroidisms were previously obese). The clinical picture corresponding to thyroid dysfunction had as a particular feature, according to the case, only the association with the signs of basic heart disease;

- The period of time between the initiation of the amiodarone treatment and the occurrence of clinical signs of thyroid dysfunction, was highly variable, i.e. between 6 months and 8 years. Also, no correlation could be established between the type of thyroid dysfunction (with hypo or hyperfunction) and the length of the treatment with amiodarone. In general, in female patients, thyroid dysfunction was diagnosed earlier because they came more often to the examination;

- There was no correlation between the administered amiodarone maintenance dose and the occurrence of a certain type of thyroid dysfunction;

- Amiodarone-induced hyperthyroidism was more common in male patients, on whom it caused an extremely unfavourable evolution and a prognosis: fibrillation and atrial flutter with rapid ventricular pace (over 150/min) resistant to antiarrhythmic therapy, and fatalities due to the worsening of the cardiac failure phenomena evolving up to cardiogenic shock and refractory acute renal failure.

Patients diagnosed with amiodarone-induced hypothyroidism had a somewhat favourable evolution after beginning the treatment with replacement hormones, i.e. alleviation of the clinical picture together with the correction of the thyroid hormone levels. Most patients who developed hypothyroidism showed an excessive bradycardia (i.e. 40-45/minute ventricular frequencies), symptoms (dizziness), which made

them come to for examination.

CONCLUSIONS

Amiodarone-induced thyroid dysfunction is relatively rare compared to the high number of patients treated with this antiarrhythmic (27.8% of the studied group). Amiodarone-induced hypothyroidism is usually less obvious, in terms of the clinical picture, while the amiodarone-induced hyperthyroidism has a severe clinical picture by worsening the phenomena of cardiac insufficiency, by maintaining an elevated heart rate, generally resistant to antiarrhythmic drugs; some cases can be fatal especially when they occur on a severe basic cardiac pathology. Thyroid dysfunction, regardless of the type (with hypo- or hyper-function), represents a negative element in the evolution of the patients with pre-existing cardiac pathology, not only through the worsening of the clinical picture of the underlying disease, but also through the need to permanently review the therapeutic scheme also imposed by the association of the medication of the thyroid dysfunction, according to case.

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MASSIVE PERICARDIAL EFFUSION ASSOCIATED WITH HYPOTIROIDISM

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MASSIVE PERICARDIAL EFFUSION ASSOCIATED WITH HYPOTIROIDISM (Abstract): The diagnosis of hypothyroidism is difficult because hypothyroidism in adults and especially the elderly, classic, has an insidious onset with a range of nonspecific symptoms which may delay diagnosis for months or even years. Old age seems to represent trigger factor for autoimmune diseases, including hypothyroidism. Clinical features in hypothyroidism, such as weight gain, fatigue, intolerance of the cold, constipation, dry skin, edema and muscle weakness, and decreased osteo-tendinous reflexes are usually subtle and can be overlooked. Thyroid dysfunction may be associated with a negative impact on the cardiovascular system. Pericardial, pleural and peritoneal effusions are common findings in hypothyroidism. This case report represents a typical primary hypothyroidism (autoimmune) and shows the clinical features of this disease. Basically we talked about a severe myxedema with the involvement of internal organs in an elderly woman and the euthyroidism restoration, under thyroid replacement therapy, was correlated with the clinical improvement and cardiovascular and neurological status, with radiographic remission and regression to extinction of pericardial effusion at repeated echocardiographic evaluations. **Keywords:** AUTOIMMUNE THYROIDITIS, HYPOTHYROIDISM, PERICARDIAL EFFUSION, LEVOTHYROXINE.

Thyroid dysfunction may be associated with a negative impact on the cardiovascular system. The prevalence of hypothyroidism increases with age (1,4), especially in women (2,3) and is often difficult to diagnose clinically in elderly patients (1,5). Old age seems to represent a trigger factor for autoimmune diseases, including hypothyroidism (3).

Incidence of pericardial effusion in hypothyroidism is related to the severity and duration of disease, beeing reported to be in approximately one-third of the cases, as low as 3% in the early mild stage and as high as 80% when myxedema is present (6).

Pericardial, pleural and peritoneal effusions are common findings in hypothyroidism. A few cases have been reported in which pericardial fluid evolved in to tamponade. A variety of conditions can cause pericardial effusion. Early recognition of etiologic factors is important to improve outcome and avoid unnecessary invasive investigations. Once a diagnosis set, pericardial effusion slowly regresses and eventually disappears after several months of

replacement therapy. We here present the case of a patient with massive pericardial effusion associated with hypothyroidism, which responded to levothyroxine replacement therapy after 6 months.

CASE REPORT

A 84-year-old woman presented at the emergency department of our hospital, with profound dyspnea, pronounced asthenia, somnolence, vertigo, paresthesia, symptoms emphasized about 2 weeks prior hospitalization, in terms of ambulatory detection by echocardiographic exploration of an important pericardial effusion (fig. 1).

The patient has husky voice, slow speech, and periods of disorientation in time and space and visual hallucinations. Physical examination revealed cool and dry skin, eyelid edema and edema of the extremities, faint heart sounds with a heart rate of 54 beats / minute, TA= 125/88 mmHg, bilateral basal crackles and reduced vesicular murmur, with a respiratory rate of 20 breaths / min and a temperature of 35.4 °C, coprostasis. Neurological examination revealed delayed deep tendon reflexes, dysdiadochokinesia, depressive mood.

Fig. 1. Ultrasound 2D parasternal long axis section, the objectification of pericardial fluid disposed in the posterior wall of the left ventricle in diastole of 18.31 mm.

Laboratory tests revealed blood count and C-reactive protein (CRP) values within normal limits, hypercholesterolemia 373 mg/dl, an LDL-cholesterol of 250 mg/dl, HDL cholesterol 47 mg/dl and triglycerides 193 mg/dl.

An electrocardiogram revealed sinus bradycardia, with a low P wave and QRS complexes and first degree atrioventricular block (PQ was 0.24 sec). Chest radiograph identified cardiac enlargement and bilateral pleural effusion.

The echocardiogram revealed massive pericardial effusion, disposed circumferentially with fibrin deposits in fluid or on epicardial surface, with measurement of the

pericardium separation foils, with a maximum of 28 mm and an approximate estimated liquid volume 600 ml, without signs of tamponade (fig. 1, 2, 3).

Fig. 2. Ultrasound 2D parasternal short shaft section outlining the pericardial fluid in large quantities ordered in the posterior wall of the left ventricle, with dimensions of 21.17 mm, containing fibrin deposits.

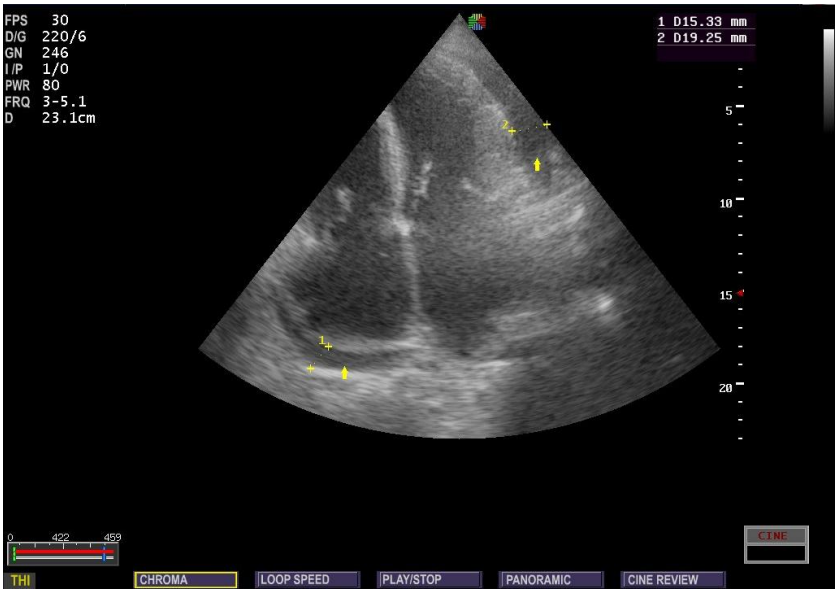

Fig. 3. Ultrasound 2D apical 4-room section, which highlights the pericardial fluid disposed in the anterolateral wall of the left ventricle of 19.25 mm and the free wall of the right atrium of 15,33 mm without ultrasound resemble tamponade.

A computed tomography (CT) scan of the chest revealed no evidence for acute lung injury, aortic dissection, or tumor lesions. Infectious, neoplastic, traumatic, uremic, post heart attack pericarditis or aortic dissection being excluded, thyroid dysfunction was taken into account and, in this regard, we dose serum thyroid-stimulating hormone (TSH) was 96.8 μUI / ml, free T3 2.9 ng/dl, free T4 0.3 pg/ml, anti-peroxidase (TPO) 372U/ml, and anti-thyroglobulin 834U/ml with a thyroid ultrasound without changes of position, size or structure. Once diagnosis settled, in the absence of signs of tamponade, pericardiocentesis was not performed (fig. 3).

Treatment was started with low doses of levothyroxine initially with 12.5μg and increased at 25μg after the first week and fitting according to thyroidian parameters. The patient was evaluated at 1, 3 and 6 months.

Restoring euthyroidism was correlated with clinical improvement and cardiovascular and neurological status, radiographic remission and regression to extinction of pericardial effusion on repeated echocardiogram.

DISCUSSION

Chronic autoimmune thyroiditis may eventually cause hypothyroidism, mainly via destruction of thyrocytes. The serological hallmark of Hashimoto's disease is the presence of high titers of thyroid peroxidase (TPO) auto antibodies. Elevated titers of anti-TPO simply show the presence of an autoimmune reaction against the thyroid and may be associated with hypothyroidism (most common) as well as with hyperthyroidism.

The diagnosis of hypothyroidism is difficult because hypothyroidism in adults and especially the elderly, classic, has an insidious onset with a range of nonspecific symptoms which may delay diagnosis for months or even years. Clinical features in hypothyroidism, such as weight gain, fatigue, intolerance of the cold, constipation, dry skin, oedema and muscle weakness, decreased osteo-tendinous reflexes (due to thyroid hormone action on the nervous system) are usually subtle and can be overlooked (7). The diagnosis is rarely made solely on clinical grounds, especially the elderly, who are often faced with either depression or dementia.

The occurrence of pericardial effusion in hypothyroidism appears to be related to the severity and duration of disease. The incidence of pericardial effusion is reported as 3% in the early mild stage reaching up to 80% of cases when myxedema is present (6).

In contrast to the frequent occurrence of pericardial effusion in patients with hypothyroidism, massive pericardial effusions are rarely associated with tamponade. Cardiac tamponade in patients diagnosed with hypothyroidism is probably as rare as it is due to pericardial distensability and the slow accumulation of fluid, allowing significant fluid accumulation without hemodynamic compromise (8). A variety of medical conditions, including malignant tumors, may lead to pericardial effusion. The presumptive mechanism underlying exudative pericardial effusions is extravasation of hygroscopic mucopolysaccharides into the pericardial space along with increased capillary permeability, decreased lymphatic drainage, and increased retention of salt and water (8). Due to its insidious onset, a pericardial effusion secondary to hypothyroidism frequently occurs without hemodynamic change, and is apparent only on echocardiography (9). Such findings in

an asymptomatic elderly patient can be misleading and may lead to unnecessarily invasive procedures such as pericardiocentesis (9). Using echocardiography it can be detected a pericardial effusion with a sensitivity of 96%, specificity of 98% and overall accuracy of 97.5% (10). Suggestive clinical signs of hypothyroidism and thyroid function evaluation in patients are crucial for an early diagnosis.

Therefore, hypothyroidism should be excluded in all patients with unexplained pericardial effusion etiology, not only in patients with clinically evident hypothyroidism or elder. Finally, once the diagnosis has been established, treatment with thyroid replacement hormones usually leads to the resolution of the effusion over 2–12 months without sequel.

CONCLUSIONS

This case report represents a typical primary hypothyroidism (autoimmune) and shows all the clinical aspects of these diseases. Basically we talked about a severe myxedema with the involvement of internal organs in an elderly woman and the euthyroidism restoration, under thyroid replacement therapy, was correlated with the clinical improvement, cardiovascular and neurological status, with radiographic remission and regression to extinction of pericardial effusion, at 6 months without sequelae.

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EFFECT OF ORAL LOW DOSE CLONIDINE PREMEDICATION ON POSTOPERATIVE PAIN IN PATIENTS UNDERGOING ABDOMINAL HYSTERECTOMY: A RANDOMIZED PLACEBO CONTROLLED CLINICAL TRIAL

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EFFECT OF ORAL LOW DOSE CLONIDINE PREMEDICATION ON POSTOPERATIVE PAIN IN PATIENTS UNDERGOING ABDOMINAL HYSTERECTOMY: A RANDOMIZED PLACEBO CONTROLLED CLINICAL TRIAL (Abstract): This study evaluated the effect of oral low dose clonidine premedication on postoperative pain and hemodynamic status of the patients during abdominal hysterectomy under general anesthesia. **Methods:** This study is a randomized, placebo controlled double-blinded clinical trial. After approval of the study protocol by the Ethics Committee 60 patients were recruited and written informed consents were obtained. Two hours before surgery, patients in the treatment group (n=30) received a single oral dose of 100 microgram of clonidine and those in the placebo group received an oral dose of placebo (n=30). The severity of postoperative pain was assessed at 2nd, 6th, 12th and 24th hours after the operation using the visual analog scoring system (VAS score). Also the total dose of administered analgesics during 24 hours after the surgery and the interval between the surgery and the first request of analgesic were compared between the two groups. Systolic blood pressure and pulse rate were recorded during the surgery and at the post anesthesia care unit. Drug related adverse effects were also evaluated. **Results:** Postoperative pain VAS during 24 hours after the surgery was significantly (P=0.001) lower in clonidine group (3.86±0.89) compared to the placebo group (4.86±0.93). Also, the interval between surgery and the first request of analgesic in clonidine group was4.13±3.27 hours on of longer duration (P=0.02) compared to 2.88±3.74 hours in placebo group. The mean heart rate and systolic blood pressure were lower in the clonidine group. **Conclusion: A single** oral 100 µg dose of clonidine administered 2 hours before abdominal hysterectomy significantly reduces the severity of postoperative pain. **Keywords:** POSTOPERATIVE PAIN, CLONIDINE, HYSTERECTOMY

Prevention and treatment of postoperative pain and associated complications like nausea and vomiting are critical issues in postoperative care. Unless managed adequately, acute postoperative pain can adversely affect the outcome of surgery and may lead to a condition of chronic pain. Opiates such as morphine are the standard drugs used to alleviate postsurgical pain. However, although opiates produce antinociception through their µ receptor agonist activity, they also activate the N-methyl-d-

aspartate (NMDA) receptor, resulting in hyperalgesia and the development of tolerance to opiates. In addition, opiates have several side effects which can result in significant complications including hyperalgesia. For these reasons we prefer to use adjuvant drugs that alleviate pain and reduce postoperative consumption of the opioids.

Postoperative pain management is accomplished by different methods including the systemic (oral, intramuscular, or intravenous), regional (epidural or intrathecal), or transdermal administration of analgesic agents, and less commonly cutaneous electrical stimulation (1, 2).

Although the mechanisms responsible for the genesis of acute pain are not completely understood, it is believed that sensitization of the neurons in the dorsal horns of the spinal cord contributes significantly to postoperative pain. Acute pain is known to trigger a cascade of messages from the spinal cord and the release of aminoacids and neuropeptides from afferent fibers. The resulting stimulation of the NMDA receptor causes hyper stimulation of the spinal dorsal horn neurons and prolongation of postoperative pain (3, 4). In patients with history of ischemic diseases, postoperative pain increases the sympathetic tone and enhances the risk of myocardial ischemia and myocardial infarction (5). Consequently, morbidity and mortality are increased. Hysterectomy is one of the most prevalent surgeries in women and is the definite treatment for abnormal uterine bleeding (AUB) because it yields the most satisfactory results compared to alternative therapies. Abdominal hysterectomy with or without salpingo-ophorectomy is associated with moderate to severe pain (6).

Clonidine is an alpha-2-adrenergic receptor agonist (7) used chiefly as an antihypertensive agent. Through stimulation of the α-2 receptor and the negative feedback at the presynaptic neurons, significant reductions in the sympathetic activity occur at the level of the heart, the kidneys, and the peripheral blood vessels lowering cardiac output, vascular resistance, and blood pressure. Clonidine is also effective in the prevention of vascular headaches by blocking vasomotor reflexes. In addition, clonidine is used in the treatment of dysmenorrhea and the control of opioid withdrawal symptoms. It is also used as an adjuvant in the management of attention deficit hyperactivity disorder (ADHD). Its half-life is 12-16 hours and its side effects include drowsiness, sedation, headache, vertigo, nausea and vomiting.

The aim of the present study is to corroborate and expand the observations reported in previous studies (8, 9; 10, 11, 12) which evaluated the use of clonidine as a premedication in the management of postsurgical pain.

MATERIAL AND METHODS

After approval by our institutional Ethics Committee, the study was registered in Iranian registry of clinical trials (http://irct.ir) as IRCT201108202963N3. Sixty women with ASA physical status I or II who were candidates for abdominal hysterectomy under general anesthesia were recruited in this randomized, double blinded placebo controlled clinical trial. Randomization was done by an epidemiologist using a computer generated table of random numbers. Patients were randomly allocated to either to the placebo group or the clonidine group (30 patients each). Sample size estimation was based on α=0.05 and ß error=0.8 and based on previ-

ous studies S=2. Assuming possible 10% loss to follow-up, 30 patients were needed for each group. The patients with chronic pain, opium addiction, or allergy to clonidine were excluded from the study. At the beginning of the study, patients in the two groups were similar in terms of age, body weight, systolic blood pressure and heart rate (tab. I). Two hours before surgery patients received a single oral dose of either placebo or 100 µg of clonidine. The drug administration occurred under supervision of an anesthesiologist who was not aware of patients' group assignment. Induction and maintenance of anesthesia was with 2 mg/kg propofol and also 2 mg/kg atracurium and then infusion of propofol 150 microgram/kg/h and O2 – N2O 50% - 50% for all patients. 5 mg morphine was given to all patients after induction of anaesthesia. After administration of 0.5 mg /kg atracurium IV, patients were intubated. All surgeries were done by same surgeon using the same surgery method. The intensity of postoperative pain was evaluated by researcher using patient` self report and quantified using a 10 cm visual analog scale (VAS) where zero indicates no pain at all and a score of 10 indicates very severe, intolerable pain. VAS was recorded at 2nd, 6th, 12th and 24th hour after the operation. Morphine was the only analgesic drug used in the first 24 hours after surgery. If the VAS score was more than 4, a 5 mg morphine dose was administered intramuscularly to maximum dose of 5 mg/6 hours. The two groups were compared with respect to the total dose morphine administered during the first 24 hours after the surgery and the time interval between the end of surgery and the first request of analgesia. Systolic blood pressure and heart rate were recorded before induction of anesthesia, immediately, 5 minutes, and 30 minutes after laryngoscopy and intubation and also at the end of the surgery and in the post anesthesia care unit (PACU). Patients were also monitored for drug related side effects. All measurements and data records were done by a resident of anesthesiology who was not aware of patients` group assignment. Statistical analyses were performed using SPSS 15.5 software and differences were evaluated using Students` T-test, Mann Whitney and Fishers exact tests. P-values <0.05 indicate statistically significant differences.

TABLE I

Patients' ages, body weights, and hemodynamics at the beginning of the study

	Clonidine	**Placebo**	**P-value**
Age	45.86 ± 4.06	48.16 ± 4.48	NS
Body Weight	64.56 ± 13.29	68.80 ± 12.88	NS
Systolic BP	116 ± 11	120 ± 20	NS
Heart Rate	91 ± 16	93 ± 17	NS

NS = not significant

RESULTS

A total of 71 patients were evaluated for the study, 11 patients were excluded before randomization because they did not meet the inclusion criteria and 60 patients were recruited and randomly assigned to the placebo or the clonidine group (30 patients per group).

VAS scores at the 2nd and 6th hours after the operation were not significantly different between the groups (P>0.05), but at the 12th and the 24th hours after the operation the VAS scores were significantly lower in the clonidine group (tab. II).

Compared to placebo group, the patients in the clonidine group had lower mean heart rate and mean systolic blood pressure before induction of anesthesia, immediately after laryngoscopy and 5 minutes after tracheal intubation (P<0.05). Also mean systolic blood pressures at the 30th minute after tracheal intubation was lower in the clonidine group (P<0.05) (tab. III). Although VAS scores at 12th and 24th hours after the operation were 10% and 20% lower in the patients who received clonidine, the consumption of opioid in the first 24 hours after the operation was not different (fig. 2). The time interval between the end of operation and 1st request for analgesia was 40% longer in the clonidine group (4.13±3.27 hours in clonidine group vs. 2.88±3.74 hours in placebo group, Mann-Whitney Test, P=0.02). No difference was detected between the two groups in terms of the common side effects related to clonidine, and none of the patients had pruritus or bleeding at the field of surgery.

TABLE II

Mean ±SD of VAS pain scores at different times after surgery.

Measurement Time / Group	2nd hour after surgery	6th hour after surgery	12th hour after surgery	24th hour after surgery
Clonidine	6.76±1.33	6.20±0.71	5.83±0.91	3.86±0.89
Placebo	7.43±1.5	6.6±1.1	6.46±1.16	4.86±0.93
P-Value	NS	NS	**0.023**	**0.001**

Repeated measures

TABLE II

Mean ±SD of Pulse Rate and Systolic Blood Pressure (SBP) and related P-Values in study groups

	Time / Group	Before anesthesia induction	Immediately after larygoscopy	5 minute after intubation	30 minutes after intubation	End of surgery	Recovery room
Pulse	Clonidine	81±11.9	82.5 ±19.41	77.4±12.11	74.96±11.62	75.9±10.25	78.7±10.07
	Plocebo	92.4±14.32	95.3±14.85	82.5±12.21	75.8±11.92	77.6±10.68	79.3±9.89
	P	0.001	0.002	0.045	NS	NS	NS
SBP (mmhg)	Clonidine	115.89±15.09	114.9±22.13	110.5±16.15	115.1±16.34	120.4±12.83	121.3±11.35
	Plocebo	131.7±12.72	133.3±18.82	122.2±16.23	123.3±13.06	124.6±10.15	124.6±10.45
	P	**0.001**	**0.001**	**0.003**	0.015	NS	NS

Repeated measures, NS = not significant

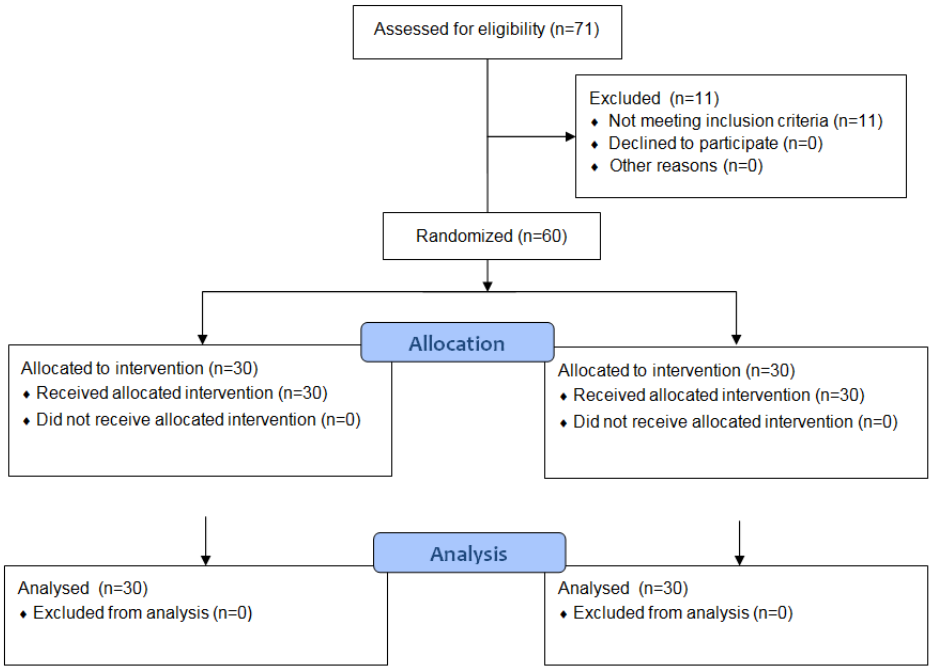

Fig. 1. Flow of the study

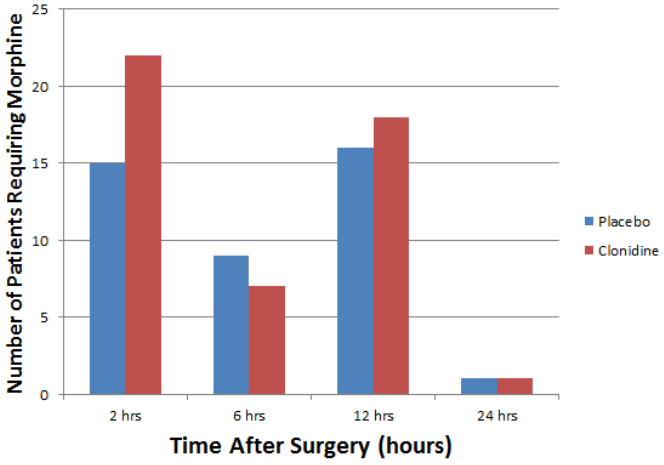

Fig. 2. The number of patients requiring analgesia at different time points after surgery

DISCUSSION

Prevention and treatment of postsurgical pain is of critical importance in postoperative care.

Perioperative pain is thought to involve multiple mechanisms, including sensitization of peripheral somatic and visceral nociceptive nerve terminals as well as cen-

tral neurons, nociceptive transduction, , and loss of local and descending inhibition of neurons in the brainstem and spinal cord (13).

There is ample evidence that perioperative systemic administration of α2-agonists decreases postoperative opioid consumption, pain intensity, and nausea (14). Alpha2-agonists inhibit the release of P-substance in dorsal root neurons (15). Also stimulation of α_2-adrenoceptors in neural terminals may decrease pain by preventing norepinephrine release (16).

The results of the present study demonstrate that a single oral dose of clonidine (100 μg) administered two hours before surgery can lower postoperative pain scores at 12th and 24th hours after the operation in the women who underwent abdominal hysterectomy. Time interval between the end of surgery and need for first dose of analgesic was significantly longer without notable side effects. Heart rate and systolic blood pressure after laryngoscopy and intubation were lower in the patients who received clonidine compared to those who received placebo. Surprisingly the patients in the clonidine group had lower incidence of nausea than those in the placebo group.

Hidalgo MP et al in 2005 showed that three doses of 100 μg clonidine (at the night before surgery and first hour and 24 hours after surgery) can decrease post hysterectomy pain (8). In this study the patients received a total of three 100- g doses of clonidine compared to a single dose in our study. Hidalgo and co-workers did not discuss possible complications that might be associated the higher dose of clonidine.

In a study in 2006 administration of 2-4 mg/kg oral clonidine as premedication in children can decrease postoperative pain and anxiety (9). These results were confirmed by Toru Goyagi et al. In this study after adding oral clonidine to epidural morphine, postoperative pain was decreased without any complications (10). In a study in 2007 Persec J concluded that administration of clonidine before surgery will decrease postoperative pain more than administration of clonidine after surgery or placebo (17). Our study supports this conclusion. However, in our study there was no significant difference between groups with respect to the total consumption of morphine. This difference may be due to lack of cooperation by the nursing staff on the ward who may inject the analgesic without considering VAS score.

Clonidine can decrease stress response and improve the patient's hemodynamic status by decreasing the level of circulating catecholamine. Our study shows that clonidine decreases systolic blood pressure (SBP) and Heart rate (HR) during surgery and recovery.

Shivering Singh et al in 2011 concluded that in patients who were candidates for laparoscopic cholecystectomy, 150 μg oral clonidine as premedication administered 90 minutes before the induction of anesthesia can improve the patient's hemodynamics and prolong the time between surgery and the first request for analgesia (11). Nausea is one of the most prevalent complications after surgery. In our study nausea and vomiting occurred at a significantly lower rate in the clonidine group, but the two groups were similar with respect to other adverse effects. In this respect our results were similar to the findings reported by Farmery et al (12).

In our study the administration of morphine was based on the patient's request,

and there was no significant difference in morphine consumption between the two groups at any point during the first 24 hours after surgery.

The long lasting effect of clonidine despite its 12 hours half-life may be due to its diverse actions modifying not only the adrenergic component to pain perception but also the neurohumoral response to tissue injury (18). A study in China on rats showed that the effects of clonidine were blocked by the α2-adrenoceptor antagonist yohimbine and partially reversed by the μ-opioid receptor antagonist naloxone (19).

CONCLUSION

A single oral dose of 100 μg of clonidine as a premedication in patients undergoing abdominal hysterectomy improves perioperative hemodynamic stability and reduces postoperative pain intensity without any considerable side effects. Clonidine is likely to play an increasing role in clinical practice in perioperative pain management.

CONFLICT OF INTEREST

This study was financially supported by the Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The study is part of Fayezah Niknam dissertation required for graduation as General Practitioner. Other authors declare no conflict of interest.

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NEWS

IS INTRAOPERATIVE HISTOLOGY IMPORTANT IN PREDICTING INFECTION IN PATIENTS UNDERGOING REVISION ELBOW ARTHROPLASTY?

A recent study by Ahmadi and co. brings new informations regarding the role of intraoperative histology in the diagnosis of infection in patients undergoing revision elbow arthroplasty. In the study were included 296 consecutive revision elbow procedures, from witch 227 intraoperative histology and operative samples for culture were obtained. The number of procedures with histology read as consistent (acute inflammation) was 33 (14.5%). Intraoperative cultures were positive in 39 procedures (17.2%). Intraoperative histology was considered true positive (both histology and cultures positive) in twenty arthroplasties (8.8%), true negative (both histology and cultures were negative) in 175 arthroplasties (77.1%), false positive (the histology was positive but the culture was negative) in thirteen arthroplasties (5.7%), and false negative (the histology was negative but the culture was positive) in nineteen arthroplasties (8.4%). With regard to intraoperative histology, the sensitivity was 51.3%, the specificity was 93.1%, and the accuracy was 85.9%. The positive predictive value was 60.6% and the negative predictive value was 90.2%. The study concluded that intraoperative histology had a high specificity and negative predictive value, but a low sensitivity and positive predictive value for predicting infection in the setting of revision elbow arthroplasty and it should be used in conjunction with other studies to definitively establish the diagnosis of infection in the setting of revision elbow arthroplasty. (Ahmadi S, Lawrence TM, Morrey BF, Sanchez-Sotelo J. The value of intraoperative histology in predicting infection in patients undergoing revision elbow arthroplasty. *J Bone Joint Surg Am.* 2013; 95:1976-1979).

Simona Tucaliuc

EBSTEIN'S ANOMALY

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EBSTEIN'S ANOMALY (Abstract): Ebstein's anomaly is a rare congenital heart disease, accounting for less than 1% of all congenital heart diseases, characterized by a wide clinical, electrocardiographic, echocardiographic, anatomic and prognostic polymorphism. The disease can be fatal since birth or may remain asymptomatic until adulthood, sometimes being associated with septal defects, transposition of great vessels, preexcitation syndromes, or left ventricular noncompaction. The genetic changes underlying this syndrome are not fully known, but in the cases associating left ventricular nonompaction a mutation in MYH7 gene encoding the β-myosin heavy chain was recently detected. The authors present 2 cases of Ebstein's anomaly with different onset and course and discuss the current clinical, electrocardiographic and echocardiographic criteria used for prognostic stratification of Ebstein disease in relation to international literature. **Keywords**: EBSTEIN'S ANOMALY, TRICUSPID VALVE, ELECTROCARDIGRAPHY, ECHOCARDIOGRAPHY.

Ebstein's anomaly is a rare congenital cardiac malformation characterized by a combination of pathological anatomical and functional changes of the tricuspid valve, right ventricle and atrium. The diagnosed cases account for less than 1% of all congenital cardiac malformations, the prevalence being approximately 1 in 20,000 live births (1-4). The malformative complex affecting the tricuspid valve and right ventricle is characerized by many variations and degrees of severity, conferring a significant clinical electrocardiographic, echocardiographic and evolutive polymorphism to the disease. The main lesion is represented by the apical displacement of the septal and posterior tricuspid valve leaflets by over 8 mm/m^2 body surface area. Functionally, the malformation causes atrialization of part of the right ventricle, and different degrees of tricuspid regurgitation, sometimes associated with stenosis. The disease can be associated with other cardiac malformations, most commonly with interatrial septal defect or patent foramen ovale (80-94% of patients) and rhythm and conduction disorders, due to the discontinuity of intracardiac excito-conductor system (3-6). The authors discuss the peculiarities encountered in two cases of Ebstein's anomaly diagnosed in adults and compare them with the literature data.

CASES PRESENTATION

Case 1. A 51-year-old man was admitted for cardiovascular symptoms that occurred about 2 years earlier and gradually worsened, including NYHA class II exertional dyspnea, atypical chest pain without specific radiation, enhanced by mental strain and fatigue. History did not reveal other cases of congenital heart disease in consanguineous relatives, potentially teratogenic drug use, or pregnancy-related disease of his mother. The patient was nonsmoker, and physical examination showed longilin, hypoasthenic constitutional type, poor nutritional status (body mass index – BMI, 17 kg/m^2), jugular pulse synchronous with carotid pulse, apex shock in the V-th left intercostal space 1 cm outside the midclavicular line, normal blood pressure, at auscultation heart sounds I and II were duplicated, there was a mesosystolic click followed by a short systolic murmur, telesystolic, at the apex, with highest intensity in the V-th left intercostal space, parasternally, and at the xiphoid appendix, accentuated by post-inspiratory apnea. The antero posterior chest radiograph revealed a slight bulging of the right lower and left middle arches with a cardiothoracic index (CTI) at limit, of about 0.44. Electrocardiography showed a sinus rhythm (SR) with heart rate of 60 beats/min, QRS axis at +30°, PQ interval = 0.14 s, and prolonged QRS duration (0.14 s in standard leads and 0.18 s in precordial leads) suggestive of right bundle branch block (RBBB) (fig. 1).

Cardiovascular assessment continued with the study of standard sections used for transthoracic echocardiography, 2D mode, color Doppler, continuous and pulsed: parasternal long axis - normal-sized left heart (left ventricle – LV, 39/26 mm, interventricular septum – IVS, 9 mm, left ventricular posterior wall – LVPW, 9.5 mm), while the right heart has dilated cavities (right atrium – RA, 69/39 mm, right ventricle – RV, 79/49 mm), paradoxical IVS movement, the position of the tricuspid valve is diverted to the anterior part and is visible concomitantly with the mitral valve and its excursions are large and closure delayed; anterior mitral valve presents a 6mm telesystolic prolapse and minor mitral regurgitation; apical four-chamber view: septal insertion of tricuspid valve at 26.8 mm inferoapical to the mitral valve, Doppler grade III tricuspid regurgitation (fig. 2); the calculated atrialized RV portion represented approximately 25% of total RV area.

Contrast echocardiography excluded the presence of interatrial communication. Infectious endocarditis prophylaxis, limited physical activity, and low dose diuretics were recommended.

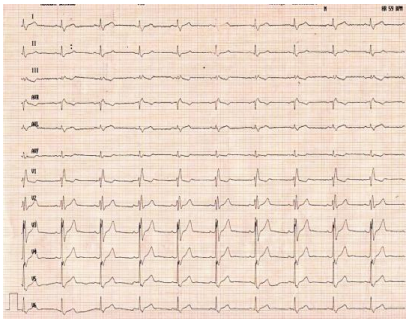

Fig. 1. Case 1 electrocardiogram. SR 60/min, PQ=0,14 s, RBBB

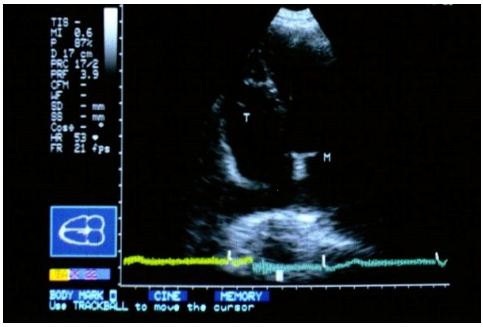

Fig. 2. Case 1 ecocardiography Apical four-chamber view

Case 2. A 34-year-old female patient presenting since childhood repeated fainting spells, intermittent headache, and decreased execise tolerance was diagnosed at age 14 with Ebstein's anomaly, severe form. The patient was recommended antiplatelet therapy and limited physical exertion; her condition had improved and, later on she carried two pregnancies to term, delivery by cesarean section. This year, the patient's condition worsened again, with rest and exertional dyspnea, chest pain, cyanosis, dizziness, headache and more episodes of loss of consciousness. Emergency craniocerebral computed tomography revealed a moderate diffuse cerebral atrophy. Cardiovascular assessment showed: grade I obesity (BMI 31.5 kg/m^2), increased cross-sectional cardiac dullness, sound I diminished, sound II duplicated, systolic murmur grade IV/VI in the III-rd and IV-th left intercostal space; electrocardiogram: 60/min SR, QRS axis at + 30°, first-degree atrioventricular block (AVB) (PQ = 244 ms), major right bundle branch block, RA and RV overload (fig. 3); echocardiography: low insertion of the tricuspid valve (at 47 mm from the insertion of mitral valv, cusp inserted into the free wall of the right ventricle, separately from tricuspid ring) causing atrialization of a large RV portion (38% of RV area) (fig. 4); left cavities were compressed by the right ones, paradoxical IVS motion; Doppler grade III tricuspid insufficiency and Doppler grade I aortic insufficiency; in the interatrial septum (IAS) foramen ovale was open and had a small bidirectional shunt at color Doppler.

Given the worsening of cardiac dyspnea, presence of syncopes, cyanosis and echocardiographic appearance, the patient met the indication for tricuspid valve prosthetic replacement or valvular reconstruction, but until then the continuation of antithrombotic and diuretic therapy was recommended.

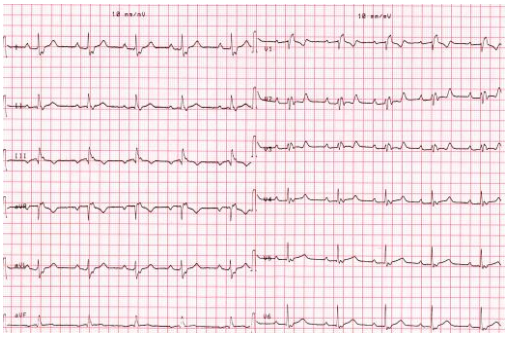

Fig. 3. Case 2 electrocardiogram. SR 65/min, AVB grade I, QRS fractionation

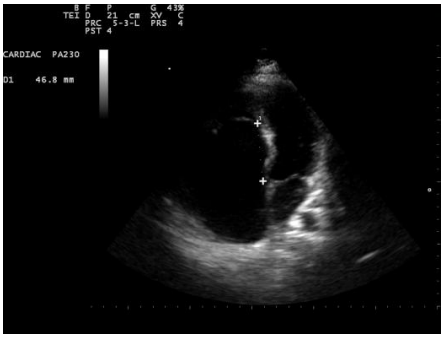

Fig. 4. Ecocardiography case 2 – apical 4 chamber view, dilatation of right chambers

DISCUSSION

In Ebstein's anomaly, tricuspid valvular disease is highly variable and causes a wide anatomic, clinical and laboratory spectrum, although the malformation is quite uncommon. The presented cases illustrate this variability. The first patient is a man in which the disease has progressed discretely and passed almost unnoticed until the age of 51 years, the second case is a relatively

young female who, despite two childbirths, has been symptomatic since childhood and last year symptoms worsened so much that surgery was indicated. This prognostic difference in is related to the echocardiographic changes: the apical displacement of the tricuspid valve was more significant in the second case and determined an over 30 percent RV atrialization, considered a sign of severity.

For practical reasons, the studies and anatomical models have gradually been correlated with the echocardiographic findings and classifications of the disease occurred. The best known were the 1988 Carpentier's anatomo-functional classification (7) and the echocardiographic classification developed by Celermajer in 1994 (8), its goal being to determine, based on the calculation of echocardiographic score, the severity of this condition since birth. According to Carpentier classification, the anomaly may fall into one of four types:

- Type A – the atrialized RV portion is small, anterior leaflet of tricuspid valve is mobile and the septal one is minimally displaced;

- Type B - RV retains functionality, anterior leaflet of tricuspid valve is still mobile, without tethering, but the septal and posterior leaflets are displaced apically more than 25 mm;

- Type C – agenesis of the tricuspid valve posterior leaflet, hypoplasia and severe displacement of the of tricuspid valve septal leaflet, and tricuspid valve anterior leflet partially attached to RV wall, can cause obstruction of the right ventricular outflow tract;

- Type D – the right ventricle is almost completely atrialized, tricuspid valve anterior leaflet is attached to the ventricular wall, forming a second endocardium (5, 7).

Celermajer proposed calculating an echocardiographic ratio of the area of right atrium and the atrialized RV portion compared with that functional RV and left heart, resulting the following classification: grade 1 ratio < 0.5; grade 2 - ratio of 0.5 to 0.99, grade 3 - ratio 1 to 1.49, grade 4 - ratio above 1.5 (4, 8). With reference to these classifications, both presented cases fit Carpentier' type B, but the calculation of echocardiographic ratio made possible the first patient to be classified as Celermajer grade 2, and the second Celermajer grade 3, indicating a more severe prognosis. The second case also presented a patent foramen ovale. In more than half of the patients with Ebstein's anomaly an interatrial communication of secundum atrial septal defect type or patent foramen ovale is associated (4).

Although electrocardiography is a classical method that did not seem first line for stratifying the severity of Ebstein's anomaly, some very recent research support a close relationship between the malformative complex morphology assessed by echocardiography or nuclear magnetic resonance and more elecrocardiographic parameters. Assenza GE et al. evaluated several parameters that can be measured on surface ECG: P wave amplitude in DII, PR interval, QRS duration and morphology and correlated them with morphological and magnetic resonance data. For the first time they propose the systematic measurement of S-wave upstroke and revealing a particular pathological aspect present in the terminal portion of the QRS complex or initial ST segment as a small negative wave, below 0.5 mV, aspect called "QRS fractionation". Increased QRS duration

especially in its terminal portion and its fractionation are parameters that correlate well with disease severity, decreased exercise capacity, systolic dysfunction, and RVatrialization (3). In our study, the first patient presented an early repolarization syndrome (known to accompany Ebstein's anomaly in a variable percentage, between 0 and 44%) (11) and QRS prolongation with RBBB aspect, while the second patient presented AVB grade I and QRS fractionation, elements of severity and poor prognosis (3, 4). For patients with Ebstein disease and asymptomatic preexcitation syndrome, Pappone et al. have proposed the assessment of arrhythmic risk by electrophysiological studies and ablation procedures for high-risk patients (9, 10).

The presented cases had isolated Ebstein's anomaly, but in the literature numerous complex cases associating other cardiac malformations (pulmonary valve stenosis or atresia, and ventricular septal defects, transposition of great arteries, noncompacted LV) are reported. More cases of Ebstein's anomaly associated with LV noncompaction are the result of mutations in sarcomere encoding genes, especially MYH7 gene encoding β-myosin heavy chain (11).

Given the discussed severity criteria, the indication for surgery is limited only to the second case. The literature reported medium and long-term favorable results following both prosthetic intervention and plasty consisting in creating a unicusp valve from the anterior leaflet, plicature of the free wall of atrialized portion and posterior tricuspid annuloplasty (12-14).

CONCLUSIONS

Ebstein's anomaly is one of the rare congenital heart malformations with a highly variable evolutionary spectrum, ranging from fatal cases since childhood to adult asymptomatic cases discovered fortuitously. Patients should be assessed periodically by cardiologists experienced in the diagnosis of congenital heart diseases using electrocardiography, echocardiography, electrophysiology studies and magnetic resonance imaging.

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NEWS

FACTORS RESPONSIBLE FOR RELATIVE POSITION OF INTERPROXIMAL

PAPILLA IN HEALTHY SUBJECTS

A group of Korean researchers conducted a study that examined the factors that can be associated with the appearance of the interproximal papilla.Were examined one hundred and forty-seven healthy interproximal papillae between the maxillary central incisors (male, 74; female, 73; average age, 25.36±7.58 years). A digital photograph and periapical radiograph of the interdental embrasure were taken for each subject using a 1-mm grid metal piece. The recorded parameters were: the amount of recession of the interproximal papilla, contact point-bone crest distance, contact point-cemento-enamel junction distance, cemento-enamel junction-bone crest distance, inter-radicular distance, tooth shape, embrasure space size, interproximal contact area, gingival biotype, papilla height, and papilla tip form. The amount of recession of the interproximal papilla was associated with: 1) increase in contact point-bone crest, contact point- cemento-enamel junction, and cemento-enamel junction -bone crest distance; 2) increase in the inter-radicular distance; 3) triangular tooth shape; 4) decrease in the interproximal contact area length; 5) increase in the embrasure space size; 6) flat papilla tip form. The amount of gingival recession was not associated with the gingival biotype or papilla height. In the triangular tooth shape, the contact point-bone crest distance and inter-radicular distance were longer, the interproximal contact area length was shorter, and the embrasure space size was larger. The papilla tip form became flatter with increasing inter-radicular distance and cemento-enamel junction-bone crest distance. The relative position of the interproximal papilla in healthy subjects was associated with the multiple factors and each factor was related to the others. A triangular tooth shape carries a higher risk of recession of the interproximal papilla because the proximal contact point is positioned more incisally and the bone crest is positioned more apically. This results in an increase in recession of the interproximal papilla and flat papilla tip form. (Kim JH, Cho YJ, Lee JY, Kim SJ, Choi JI. An analysis on the factors responsible for relative position of interproximal papilla in healthy subjects. *J Periodontal Implant Sci*, 2013; 43 (4): 160-167)

Irina Grădinaru

CLINICAL AND THERAPEUTIC ASPECTS OF AMIODARONE INDUCED THYROID DYSFUNCTION

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CLINICAL AND THERAPEUTIC ASPECTS OF AMIODARONE-INDUCED THYROID DYSFUNCTION (Abstract): Discovered in 1961 by Tondeur and Binon, two Belgian chemists, amiodarone was widely used, especially in Europe, initially as an antianginal agent, and later as an antiarrhythmic one (following the experiments conducted at the University of Oxford by Dr. Bramah Singh). Thus, the role of amiodarone was reconsidered, becoming, together with Sotalol, a class III antiarrhythmic – a group characterized by the action of extending the potassium channels refractory period. In time, amiodarone has proven its effectiveness in the treatment of both atrial and potentially malignant ventricular arrhythmias, being considered one of the antiarrhythmic agents involved in the prevention of sudden cardiac death due to arrhythmias. In December 1985, amiodarone was approved by the FDA (Food and Drug Administration) for the treatment of atrial fibrillation and the prevention of recurrent ventricular tachycardias. Despite this positive effect, the prescription of amiodarone began to be limited because serious side effects occurred, some of them disastrous, which were associated with the chronic use of the substance. **Keywords:** AMIODARONE, ANTIARRHYTHMICS, THYROID DYSFUNCTION.

Amiodarone is a class III antiarrhythmic (according to the classification of Vaughan and Williams) whose effectiveness in the treatment and the prophylaxis of atrial and ventricular rhythm disorder (especially of those with life-threatening risk) is unanimously accepted (1). Over time, its beneficial effects, however, have been far outweighed by the severe side effects occurred on various organs, the most important of them being the fact that it affects the thyroid, the liver, the lungs, the eyes, the kidneys, the skin, and the nervous system.

As chemical structure, the amiodarone molecule contains a benzofuran cycle and a phenyl ring with two iodine atoms and two aliphatic radicals (fig.1).

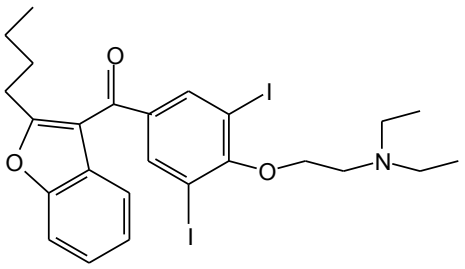

Fig. 1. Amiodarone

Its action is that of blocking at the same time the sodium, potassium and calcium channels. It presents obvious structural similarities with thyroxine, a hormone produced and released by the thyroid gland (2).

Pharmacokinetics

Amiodarone is a lipophilic substance, with a high volume of distribution, long and unpredictable half-life (estimated at 100 days), with the possibility of accumulation in the lungs, liver, skin, fat tissue, nervous system and the thyroid. Accumulation in the kidneys is slightly lower compared to other organs. The release from these tissues is much slower (it may last up to 9 months). These effects are due to its major metabolite that is created in the liver by dealkylation (CYP 3A4-mediated) – up to the final product - desethylamiodarone (DEA), which remains pharmacologically active for a long period of time (half-time: 57±27 days) (2).

Between 66-75% of the quantity of amiodarone that is administered is eliminated from the body via the bile and the stool, instead the DEA intra-thyroidian concentration remains high (2).

THYROID DAMAGE

Thyroid abnormalities were observed at a rate between 14-18% of the patients receiving the minimum dose of amiodarone. The effects at the thyroid level are variable:

- abnormal thyroid function detected only by periodic laboratory tests (TSH, FT4, FT3) in the absence of any clinical picture;

- clinically manifested thyroid dysfunction: under the form of amiodarone-induced thyrotoxicosis, or hypothyroidism. Both can occur on a previously normal thyroid gland and in the context of a pre-existing thyroid damage that they can worsen (3, 4, 5).

Regarding thyroid damage, the mechanisms of action of amiodarone are particularly complex: amiodarone inhibits the action of an enzyme – deiodinase - and thereby decreases peripheral conversion of thyroid hormones. It also decreases their renal elimination, it increases T4 and it decreases T3 by about 25% and it inhibits the entry of T4 and T3 in peripheral tissues. The level of T4 increases by 40% within 1-4 months of treatment with amiodarone. The inhibition of deiodinase activity in the pituitary gland can be noticed after the first 3 months of treatment and it leads to the increase of the level of thyroid stimulating hormones. Amiodarone together with its metabolite has a direct cytotoxic effect on the thyroid follicular cells, the result being a destructive thyroiditis. It acts as a competitive antagonist of T3 at the heart level.

Amiodarone-induced thyroid damage can take the clinical and biochemical picture either of hypothyroidism or hyperthyroidism.

An emergency might be, in some cases, only amiodarone-induced hyperthyroidism which in its turn can be of two types:

Type 1 - due to iodine excess in amiodarone which causes excessive synthesis of thyroid hormones; it usually occurs in subjects with prior thyroid damage (subclinical or clinically manifested);

Type 2 – it appears as an inflammatory process followed by destruction in patients with previously normal thyroid. A destructive thyroiditis appears with the release of hormones preformed from damaged thyroid follicular cells. This manner of evolution requires an emergency specific therapeutic conduct that involves glucocorticoids.

Combined forms of thyroid damage may rarely appear. From a biochemical

point of view, amiodarone-induced hyperthyroidism is characterized by very low levels of TSH, greatly increased FT4, and decreased FT3 during initial stages.

Causes and risk factors

The risk of developing thyroid damage under amiodarone treatment is independent of the daily or cumulative dose of amiodarone. There are studies that have shown that autoimmune thyroid damage is the main risk factor for developing hypothyroidism. At the same time, obesity and family history regarding thyroid disease could be considered predisposing factors. The presence of anti-thyroglobulin or thyroid antiperoxidase gives an estimated risk of 13% for developing hypothyroidism in women.

CLINICAL ASPECTS

The clinical picture is variable – from the absence of any symptom (it is the case of subclinical forms of disease), up to forms with dramatic and life threatening clinical manifestations. As "alarm" clinical elements for thyroid dysfunction which appeared under treatment with amiodarone can be included: signs and symptoms of cardiac decompensation, tachyarrhythmias, worsening angina phenomena, weight loss, and unexplained fatigue. Thyrotoxicosis can occur both during treatment with amiodarone, but also after several months of its discontinuation.

Amiodarone-induced thyrotoxicosis may result in the following symptoms:

- weight loss, heat intolerance, sweating;
- muscle weakness, permanent fatigue;
- emotional lability, hyperkinetic status (palpitations, newly emerging arrhythmias or the worsening of pre-existing ones);
- frequent stools, diarrhea;
- nervousness, anxiety, oligomenorrhea (4, 5, 6).

Amiodarone-induced hypothyroidism can result in:

- fatigue, lethargy, severe drowsiness, cold intolerance;
- weakness, constipation, slowness (in severe cases it can lead to mixedematous coma);
- menorrhagia in women;
- dry, rough skin;
- pericardial effusion (4, 5, 6).

It is worth mentioning that these symptoms are not specific to thyroid damage caused by amiodarone.

The thyrotoxic crisis is an acute and extremely serious complication which may occur in the evolution of the hyperthyroid patient (rare but possible in the context of amiodarone-induced thyrotoxicosis), which consists of massive, brutal body intoxication with thyroid hormones, associated with acute suprarenal insufficiency explained by excessive degradation of cortisol (4,5,7). Clinically manifested by agitation, delirium, fever, tachycardia, vomiting, diarrhea, severe arrhythmia (or the aggravation of the pre-existing ones), hypertension initially, then collapse and coma. Listed as therapeutic alternatives might be: synthetic antithyroid drugs, intravenous beta-blockers, corticotherapy (4, 5, 7, 8, 9).

Manifest or subclinical hyperthyroidism, particularly the forms with undetectable levels of TSH serum is correlated with a substantial increase in cardiovascular mortality (up to 20%). Factors associated with the increase of mortality in these situations are: arrhythmias (AF), embolic strokes (systemic), the aggravation of cardiac insufficiency, hypertension, pre-existing coronary disease (3,7). After

treatment with synthetic antithyroid drugs, certain symptoms, arrhythmias and the negative hemodynamic balance can sometimes persist despite the normalization of thyroid hormones. Studies have also shown that treatment with radioactive iodine may increase cardiovascular mortality. In patients with hyperthyroidism, a status of hypercoagulability and hipofibrinolisis which would increase the risk of thromboembolic events (especially the brain) (3, 4, 7, 8).

Laboratory diagnosis

Amiodarone-induced thyroid damage must be confirmed through laboratory tests that basically consist of specific hormonal determinations. They may be modified even in the absence of a clearly contoured clinical picture (subclinical affecting) (1, 5, 8).

For amiodarone-induced hypothyroidism, laboratory tests are similar to those of spontaneous hypothyroidism and they include: low levels of free T4 and elevated levels of serum TSH. Serum thyroglobulin is also elevated (due to stimulation by TSH) (4, 5, 6, 10).

In the case of amiodarone-induced thyrotoxicosis, laboratory tests objectify: undetectable levels of serum TSH and highly elevated levels of free T4 and T3 (4, 5, 6, 10).

Imagistic studies are necessary for recognizing the right type of thyrotoxicosis, on which the choice of treatment modality relies.

Thyroid ultrasound - may indicate in Type 1 ahypoechoic or nodular aspect with thyroid size increase (6).

Radioactive iodine uptake studies, normal or elevated, also direct to type 1 (6).

If thyroid biopsy is performed, histological changes specific for amiodarone therapy are: diffuse follicular fibrosis, epithelial atrophy, apoptosis, necrosis, vacuolated cells arranged in the form of intraluminal aggregates, lymphocytic infiltrates (6, 9).

THERAPEUTIC ASPECTS

Therapeutic conduct for patients with amiodarone-induced thyroid dysfunction, regardless of type and form, remains a challenge because there is insufficient data on the optimal treatment (6). For patients who associate cardiac damage, collaboration between cardiologist and endocrinologist is essential.

In the case of thyrotoxicosis, making the difference between the two types is mandatory for the choice of therapy.

The decision to interrupt or not the treatment with amiodarone usually belongs to the endocrinologist, but he will take into account the severity of concomitant cardiac damage, which could highly obscure the prognosis. The evolutionary ways after stopping amiodarone can be extremely polymorphic. Thus, usually, after stopping amiodarone, mild forms of thyrotoxicosis regress spontaneously in approximately 15 - 20% of the cases (6). Some of the patients with type 2 become euthyroidia affected within 3-5 months after stopping the treatment, while in other cases thyrotoxicosis may last up to 8 months even after stopping the drug administration (6, 9). There are also situations in which stopping amiodarone may worsen the symptoms, and in severe forms of thyrotoxicosis it may lead to the emergency thyroidectomy (6).

In type 1 – therapeutic conduct involves, besides stopping amiodarone, the administration of synthetic antithyroid drugs (higher doses than the usual ones) to block the synthesis of thyroid hormones. In type 2, long-term corticosteroid therapy is

indicated (to inhibit the activity of deiodinase), and even thyroidectomy in the cases that do not respond to the above mentioned treatment (4, 6 10).

As alternative treatments, in the case of thyrotoxic crises there are mentioned: synthetic antithyroid drugs, intravenous beta-blockers, corticotherapy.

Hypothyroidism usually remits after the elimination of amiodarone. In some cases amiodarone associated with thyroid hormones can be continued (4, 6, 10).

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NEWS

USE OF NANOTECHNOLOGY TO INHIBIT BIOFILM FORMATION IN PATHOGENIC BACTERIA

Gholap *et al.* examined the possibility to use nanotechnology to development an agent that inhibits biofilm formation mediated by quorum sensing. They tested a nanocomposite, CdTe-TiO2, using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) to evaluate biofilm inhibition. SEM analysis showed the exposed cells without the surrounding matrix, while the control showed matrix-covered cells. CLSM showed spatially distributed fluorescence as indication of biofilm inhibition, while the control revealed continuous fluorescence. As a result of CdTe-TiO2 use, the inhibition of biofilm was around 57%. The study also demonstrated that the nanocomposite has antibacterial properties due to formation of reactive oxygen species inside the cells (Gholap H, Patil R, Yadav P et al. CdTe-TiO2 nanocomposite: an impeder of bacterial growth and biofilm. *Nanotechnology* 2013; 24(19):195101).

Teodora Vremera

CELIAC DISEASE WITH NEUROLOGIC MANIFESTATIONS IN CHILDREN

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CELIAC DISEASE WITH NEUROLOGIC MANIFESTATIONS IN CHILDREN (Abstract): Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals and neurologic manifestations may be one of the presentations form. **The aim** of this study was to report the incidence of neurologic manifestations in children with CD. **Material and methods:** Between 2000-2010, 48 children aged 2-18 years diagnosed with CD have been monitored. The diagnosis of CD was made by serological tests and intestinal biopsy. The study protocol included: measurement of weight and height, biological and immunological tests, histological examination, questionnaires filled out by parents about their child motor development and some neurologic signs, psychological exam, electroencephalogram, and brain CT-scan. **Results:** 16 of the 48 children presented one or more neurologic symptoms as the onset manifestation of CD. The neurologic signs in order of frequency were: headache/migraine, attention-deficit/hyperactivity disorder, epileptic seizures, mental retardation, cerebellar ataxia and behavior disorders. Brain CT-scan showed cerebral calcifications in 3 patients with epilepsy, and atrophy in 2 cases with cerebellar ataxia. All children received gluten free diet, but a favorable course was noticed only in the children with migraine and epilepsy, in the other patients this diet having no influence on neurologic symptoms. **Conclusions:** This study proved the variety of neurologic symptoms that can be included in the clinical signs of celiac disease in pediatric patients. That is why in the presence of different neurologic symptoms of unknown etiology and resistant to treatment, celiac disease must be taken into account and laboratory investigations have to include intestinal biopsy and immunological test. **Keywords:** CHILDREN, CELIAC DISEASE, EPILEPTIC SEIZURES, CEREBRAL CALCIFICATIONS, MIGRAINE, SCHOOL PROBLEMS.

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. Its prevalence is difficult to estimate because many cases are asymptomatic or present atypical signs. The classical form of CD is characterized by gastrointestinal manifestations, including the malabsorption syndrome, steatorrhea, failure to thrive, and histopathological changes (villous atrophy, crypt hyperplasia, lymphocytic inflammatory infiltrate) found in intestinal mucosa biopsy specimens. In the past few years, the clinical spectrum of CD has been widened, atypical forms with late onset or oligosymptomatic being reported. The diagnosis of these forms is made by the identification of antigliadin (IgA-

AGA), antiendomysial (IgA-AEM) and antitissue transglutaminase IgA (IgA-ATTG) antibodies in the blood (1).

The first study on the association of neurologic manifestations in patients diagnosed with BC was published in 1966 by Cooke and Smith (2). The neurologic symptoms appear in 6-10% of the patients with CD, but it was demonstrated that a high percentage of the patients with neurologic signs of unknown cause are also sensitive to gluten (3). The most frequent neurologic symptoms described especially in adults with CD were cerebellar ataxia, epilepsy, myoclonus, neuropathy, dementia, headache, depression and multifocal leucoencephalopathy (2). These symptoms are generally chronic and progressive. At present there are not enough data on the association of neurologic symptoms with gluten sensitivity in children and adolescents.

The aim of this prospective study was to evaluate the neurological manifestations in children diagnosed with CD between 2000-2010.

MATERIAL AND METHODS

The authors conducted this prospective study with the approval of the Hospital Ethics Committee. In this study were enrolled 48 children (27 girls and 21 boys) aged 2 to 18 years who were diagnosed with CD. Informed consent was obtained from their parents. The classical onset symptoms (chronic diarrhea, anorexia, vomiting, and failure to thrive) were present in 32 patients (66% of the cases). The neurologic manifestations were the first sign of CD in 16 cases (33.33%). In all these cases the diagnosis of CD was made by increased IgA-AGA, IgA-ATTG, and IgA-AEM levels and confirmed by intestinal biopsy. IgA deficiency was excluded in all cases.

The study protocol included: physical evaluation; biological and immunological tests; intestinal biopsy with histological exam; questionnaires filled out by parents about motor development and neurologic signs (epileptic seizures, headache, gait disturbances, disorders of limb movement, school problems); neurologic exam; psychological test; electroencephalogram; cerebral CT-scan.

The diagnosis of neurobehavioral conditions, specific learning disabilities, attention-deficit/hyperactivity disorder (ADHD) was based on the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (3), the diagnosis of migraine was based on the International Headache Society Classification (4), and the diagnosis of epileptic seizures was based on the 1989 diagnostic criteria of Commission on Classification and Terminology of the International League Against Epilepsy (5).

RESULTS

The spectrum of neurologic manifestations in the 16 patients with atypical CD onset was heterogeneous. Two or more neurologic symptoms were detected in 9 patients (tab. I). In order of frequency the neurologic and psychiatric manifestations were: headache (6 patients), learning disabilities/ADHD (5 patients), epileptic seizures (4 cases), mental retardation (4 cases), cerebellar ataxia (4 cases), and behavior problems (2 cases). All patients received gluten free diet (GFD). A favorable course was noticed only in the patients with epilepsy and headache. GFD had no influence on the neurologic symptoms in 6 children.

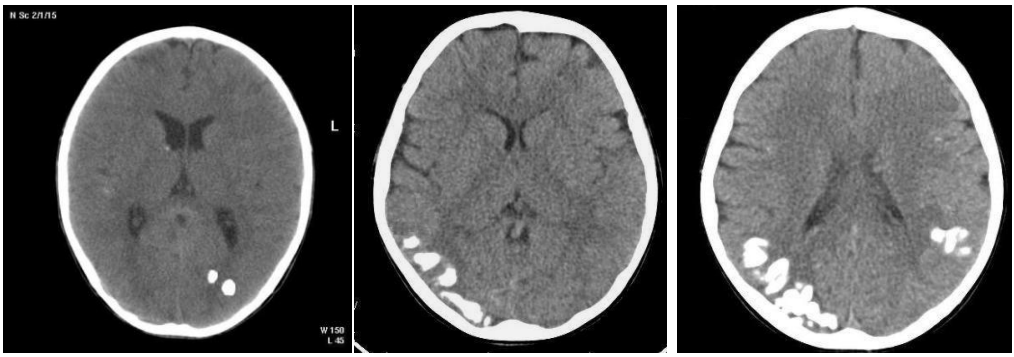
TABLE I

Characteristics of CD patients with neurologic manifestations

Case /sex /age	Neurologic findings	Other clinical symptoms	Delay in CD diagnosis-months)	Serological tests/ histological findings in duodenal biopsy specimens	EEG findings	Brain CT-scan	Course of neurologic symptoms after GFD
1/M 15 years	epileptic seizures	failure to thrive, puberty delay	12 months	anemia, IgA-AGA, moderate villous atrophy	focal discharge	OC	favorable
2/F 11 years	migraine	pallor, fatigue, diarrhea	10 months	anemia, IgA-TTG, partial villous atrophy with crypt hyperplasia	Normal	normal	favorable
3/M 8,2 years	CA, learning disabilities / ADHD	ST, failure to thrive	24 months	anemia, IgA-EMA, villous atrophy with mononuclear submucosal infiltrates	Normal	cerebellar atrophy	no improve-ment
4/M 7,11 years	ADHD	pallor, chronic diarrhea	32 months	anemia, IgA-EMA, villous atrophy with increased IEL	Normal	normal	no improve-ment
5/F 4,3 years	unspecific headache	nausea, loss of appetite, diarrhea	12 months	IgA -AGA, villous atrophy, crypt hyperplasia and increased mitotic index in crypts	Normal	normal	favorable
6/F 14,2 years	migraine	short stature, puberty delay	20 months	IgA- TTG, villous atrophy with submucosal lymphocytic infiltrates	Normal	normal	favorable
7/M 10,4 years	epileptic seizures, SMR	pallor, failure to thrive	22 months	anemia, IgA-AGA, mild villous atrophy, crypt hyperplasia and inflammatory infiltrates in lamina propia	focal discharge	OC	favorable
8/M 7,2 years	MMR, behavior problems	pallor, loss of appetite, altered bowel habits	18 months	anemia, IgA -TTG, villous atrophy with IEL infiltrates	normal	normal	no improve-ment
9/M 16 years	epileptic seizures, ADHD	nausea, loss of appetite, short stature, pallor	23 months	anemia, IgA -EMA, villous atrophy, crypt hyper-plasia and lymphocytic, eosinophilic and basophilic infiltrates in lamina propria	generalized discharge	OPC	favorable
10/M 5,1 years	unspecific headache	none reported	16 months	IgA -EMA, villous atrophy, crypt hyperplasia and IEL infiltrates	normal	normal	favorable
11/F 2,8 years	CA, SMR	nausea, vomiting, diarrhea	12 months	IgA -EMA, partial villous atrophy with increased IEL	normal	cerebellar atrophy	no improve-ment
12/F 17 years	migraine, behavior problems	puberty delay, failure to thrive	16 months	IgA -TTG, villous atrophy, crypt hyperplasia, mononuclear submucosal infiltrates	normal	normal	favorable
13/F 15,9 years	migraine	short stature, puberty delay	10 months	IgA-AGA, partial villous atrophy, crypt hyperplasia	normal	normal	favorable
14/M 9,3 years	ADHD	loss of appetite, diarrhea, pallor	12 months	anemia, IgA -AEM, IgA-AGA, villous atrophy, mononuclear submucosal infiltrates	normal	normal	no improve-ment
15/F 12,8 years	epileptic seizures, ADHD	diarrhea, pallor	14 months	anemia, IgA-AEM, partial villous atrophy, crypt hyper-plasia, IEL infiltrates	generalized discharge	normal	favorable
16/M 4,9 years	CA, MMR	ST	18 months	IgA-EMA,IgA-AGA, villous atrophy, crypt hyperplasia	normal	normal	no improve-ment

CA= cerebellar ataxia, ADHD= attention-deficit/hyperactivity disorder, SMR=severe mental retardation, MMR=moderate mental retardation, ST=steatorrhoea, OC= occipital calcifications, OPC= occipito-parietal calcifications, IEL=intraepithelial lymphocytes

The epileptic seizures preceded the gastrointestinal manifestations in all 4 epilepsy cases. Two of them presented complex partial seizures and the remaining 2 generalized tonic-clonic seizures. Brain CT-scan revealed numerous cortico-subcortical occipital calcifications in cases number 1 (fig. 1a) and 7 (fig. 1b), and occipito-parietal calcifications in case 9 (fig. 1c). Electroencephalogram revealed generalized spike-wave complexes in occipital regions in case 1, parieto-occipital spikes with slow waves in case 7, and generalized spike-wave complexes and polyspikes with great amplitude in occipital derivations in cases 9 and 15. All patients received gluten free diet and treatment with antiepileptic drugs (oxcarbazepine in cases 1 and 7, and sodium valproate in cases 9 and 15). After antiepileptic treatment and gluten free diet these patients had a favorable course without seizures.



1a **1b** **1c**

Fig. 1a. Brain CT-scan case 7: calcifications in left occipital region. **1b**. Brain CT-scan **1c.** Brain CT-scan case 9: numerous bilateral occipito-parietal calcifications case1: numerous cortico-subcortical calcifications in the right occipital region.

Cerebellar syndrome was described in 3 CD patients with a history of neuromotor development delay; one of them presented ADHD and the other two mental retardation. The neurologic exam evidenced disorders of posture (hypotonia), postural instability, gait impairment, disorders of movement coordination. CT-scan revealed cerebellar atrophy in cases number 3 and 11.

Headache was present in 6 CD cases; the diagnostic criteria for migraine were met in cases 2,6,12 and 13, and for unspecific headache in cases 5 and 10. In all patients both the neurologic exam and electroencephalogram were normal. CT-scan was normal in all cases. Jejunal mucosa biopsy confirmed the diagnosis of CD. Patients were fed gluten free diet associated with folic acid and B12 vitamin with favorable course. All children are still monitored, and the repetition of intestinal biopsy confirmed the mucosal recovery. Psychiatric disorders, severe enough to affect the social and occupational functioning of children, were present in 10 CD patients: ADHD/learning problems (5 cases), mental retardation (4 cases), and behavior disorders (2 cases).

DISCUSSION

The neurologic complications are a possible manifestation of CD. The central and

peripheral nervous systems are affected in different degrees (2). According to Collin et al. (6), 16% of the neurologic diseases of unknown etiology represent symptoms of undiagnosed CD.

A higher prevalence of gluten sensitivity was noticed in neuro-degenerative diseases such as hereditary spinocerebellar ataxia and Huntington disease (2). Some studies have reported the onset of CD with extra-gastrointestinal symptoms (7). In our study the neurologic signs were the first symptom of CD in 16 patients. Zelnik (1) reported neurologic complications in 51.4% patients with CD. Briani (9) remarked the association of neurologic manifestations in 22.5% of the 71 patients with CD. On the other hand, Cakir (10) made different neuro-physiological tests in CD pediatric patients and reported subclinical neurologic signs in 11%. In our study 33.33% of children diagnosed with CD presented neurologic symptoms.

The first cases of epilepsy, and intracerebral calcifications associated with CD were described by Gobbi in 1992 (10). Even though the association between epilepsy and CD was just a coincidence, the increasing number of cases reported afterwards in the literature and a higher than expected incidence of BC in patients with epilepsy and intra-cerebral calcifications suggest a correlation. Besides, the progressive growth of intra-cerebral calcifications before de introduction of GFD and the onset of epilepsy with advancing age if CD remains undiagnosed are suggestive of a correlation between these three diseases (11). Some authors claim that the toxicity of gluten may trigger seizures in patients at high risk (1). Diaz et al. (11) believe that all patients with epilepsy and CD must be investigated for CD, even in the absence of digestive symptoms. In our study intracerebral calcifications were evidenced by CT-scan in 3 patients who also presented epileptic seizures. In all patients the association of GFD to antiepileptic medication caused the disappearance of seizures. Some authors consider that in the case of the triple association intracerebral calcifications, epilepsy and CD the favorable response to treatment is possible only if GFD is initiated early (10). Other studies report a good seizure control even if epilepsy was diagnosed after some years of gluten exposure (12).

In Bushara's opinion headache can be an atypical manifestation of CD (2). Some authors suggest that malabsorption is not involved in the pathogenesis of headache crisis in patients with CD, but the inflammatory and autoimmune mechanism may be responsible for its occurrence (1). Roche-Herrero (14) reports the presence of migraine, tension or unspecific headache in 39.5% of children and teenagers with CD. In our study 6 of the 48 patients presented headache. In all the cases the initiation of GFD determined the disappearance of migraine and headache episodes.

Many authors reported the presence of cerebellar ataxia in CD patients (14). There are studies that report an increased incidence of CD and sensitivity to gluten in patients with idiopathic cerebellar ataxia (15). Also, some authors noticed cerebellar symptoms in patients with CD (1,14). Zelnik (1) diagnosed intracerebral calcifications in 10.25% of the patients with CD, and in our study 3 of 48 patients with CD presented signs of cerebellar ataxia.

Developmental delay is relatively frequent in the history of children with CD, in our study being found in 3 children with cerebellar ataxia and CD, probably due to

nutritional deficiencies and toxic effects of severe malnutrition.

Psychiatric signs may be frequent in CD patients. Rodrigo (16) claims that these symptoms may be present in about 1/3 of the cases. In the study of 111 patients with CD, Zelnik (1) noticed the learning and behavior problems in 20.7%. In our study 10 of the 48 cases presented a delay in mental development, ADHD, learning and behavior problems, these data being comparable with those reported in the literature.

The mechanism by which gluten intolerance contributes to the occurrence of neurologic manifestations remains unclear. It is supposed that the pathogenic mechanism of neurologic and psychiatric manifestations associated with CD is determined by the toxic effects of gluten or an immune reaction initiated by gluten or its metabolites in the intestinal mucosa, as well as the severe and prolonged lack of thiamine, pyridoxine, ciancobalamine (due malabsorption) and folic acid deficit (14). Some authors believe that the toxicity of gluten induces by an autoimmune mechanism irreversible lesions of vasculitis, inflammatory lesions and lymphocytic infiltrates at neuronal, glial and axonal level, reason why the clinical picture is minimally improved by GFD, and the disappearance of different antibodies in blood or cerebrospinal fluid (2).

The identification of the same phenotype HLA (DQW2DR3 and/or DR7) in patients with neurologic manifestations suggests immune system disturbances (2).

The authors who have used GFD in patients with CD and neurologic symptoms reported very different results. Siqueira stated that such a diet does not improve the neurologic symptoms (7). On the other hand, Diaz reported a favorable course after GFD in patients with CD and epilepsy (11). In our study the GFD gave good results in cases number 2, 5, 6, 10, 12 and 13 who presented migraine and headache episodes.

CONCLUSIONS

Neurologic and psychiatric symptoms may be present not only in some diseases primarily affecting the brain, but also in gastrointestinal diseases, in our case CD. That is why, CD must be considered in patients with different neurologic symptoms of unknown etiology that are resistant to treatment, because in many cases CD types are atypical, monosymptomatic or latent. In all these situations the laboratory investigations will necessarily include intestinal biopsy and immunological tests, and in patients with confirmed diagnosis GFD will be initiated, even though GFD does not always influence in a favorable way the course of neurologic symptoms.

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NEWS

IDENTIFICATION OF PERMETHRIN RESISTANCE LOCI IN MALARIA VECTORS

Resistance to the limited insecticides used for malaria control is a growing threat, thus, identification of the major loci responsible for resistance is in need. Witzig and co. used quantitative trait loci (QTL) mapping to identify resistance phenotypes in the major malaria vector, *Anopheles gambiae*. The study shows the results of mapping of pyrethroid resistance in 3 isofemale pedigrees established from wild *A. gambiae* and makes suggestions for future genetic mapping of insecticide resistance. Two QTL were identified on chromosomes 2L and 3R, in similar genomic sites as those previously identified in laboratory strains (Witzig C, Wondji CS, Strode C, Djouaka R, Ranson H. Identifying permethrin resistance loci in malaria vectors by genetic mapping. *Parasitology*. 2013;28:1-10). [Epub ahead of print])

Teodora Vremera

EFFICACY OF RIFAXIMIN VERSUS LACTULOSE FOR REDUCING THE RECURRENCE OF OVERT HEPATIC ENCEPHALOPATHY AND HOPITALIZATIONS IN CIRRHOSIS

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EFFICACY OF RIFAXIMIN VERSUS LACTULOSE FOR REDUCING THE RECURRENCE OF OVERT HEPATIC ENCEPHALOPATHY AND HOSPITALIZATIONS IN CIRRHOSIS (Abstract): Both rifaximin and lactulose have been reported to be effective for maintenance of remission from hepatic encephalopathy (HE) in patients with cirrhosis. The **aim** of this study was to compare the efficacy of different treatment strategies for preventing recurrences and HE-related hospitalizations. **Materials and methods:** Seventy-eight cirrhotic patients who recovered from HE were grouped according to one of the following therapies: rifaximin intermittently 14 days/month (RI- group), rifaximin 400 mg three times daily (RD-group) and lactulose 30 to 60 ml in 2 or 3 divided doses daily (L-group) for a 6-month period. The follow-up lasted 12 months. A Kaplan-Meier analysis was performed to determine the probability of developing recurrent overt HE episodes. The frequencies of HE- related hospitalizations in the three groups were evaluated comparatively. **Results:** Over a 12-month follow-up period, 10 (26.31 %) of 38 patients in the RI-group, 7 (25%) of 28 in the RD-group and 4 (33.33 %) of 12 in the L-group experienced recurrent bouts of HE, the differences not being significant. More L-group patients experienced more severe episodes of overt HE in spite of mild or moderate disease. In the rifaximin groups overt HE episodes were similarly frequent, and the severity of bouts was associated with Child-Pugh score. Fewer hospitalizations were reported in the rifaximin groups. **Conclusions:** According to our data, rifaximin and lactulose are equally effective for the maintenance of remission from overt HE. However, rifaximin is superior for reducing the risk of HE-related hospitalization. **Keywords**: HEPATIC ENCEPHALOPATHY, LACTULOSE, RIFAXIMIN, HOSPITALIZATION.

Hepatic encephalopathy (HE) is a common complication of either acute or chronic liver disease, potentially reversible, or progressive. The annual risk of developing overt HE in cirrhotic patients is 20% (1). An estimated one-third to one-half of hospitalizations for cirrhosis are related to overt HE, and the frequency of hospitalization for overt HE has nearly doubled over the last decade.

HE is characterized by disturbances in consciousness and behavior, personality changes, fluctuating neurological signs, as-

terixis, and electroencephalographic changes (2). The neuropsychiatric abnormalities range from cognitive deficits (referred to as subclinical HE, minimal HE, or more recently, covert HE), which can only be diagnosed by specialized testing to clinically apparent neuropsychiatric complications consisting of alterations in consciousness and motor disturbances (referred to as overt HE) (3, 4).

Gut-derived toxins such as ammonia are thought to play a central role in the pathogenesis of HE (5). Treatment strategies are based on the principle of reducing the production and absorption of ammonia in the gut.

The development of HE is considered a sign of a poor prognosis and patients should receive therapy for an indefinite period of time or until they undergo liver transplantation (6). Therefore, prevention of overt HE is warranted. The term secondary prophylaxis was proposed to define the therapy administered to prevent recurrence of overt HE in patients with a history of overt HE episodes (7). Currently, the options for a long-term treatment of HE are lactulose, a non-absorbable disaccharide, and rifaximin, a minimally absorbed oral antibiotic. Several studies have demonstrated the efficacy of rifaximin and lactulose for the maintenance of remission from HE and decrease in hospitalization requirement (7, 8).

In this prospective study conducted over a 12- month period we evaluated comparatively the efficacy of rifaximin and lactulose for prevention of overt HE recurrence and frequency of HE-related hospitalizations in patients with previous episodes of overt HE.

MATERIAL AND METHODS

Patients with cirrhosis who were followed up in our outpatient clinic or hospitalized between October 2010 and December 2011 were considered eligible. The study was performed in accordance with the ethical principles of the Declaration of Helsinki. The protocol was explained to at least one relative of each patient and written informed consent was obtained from all selected patients or their relatives.

Inclusion criteria were: patients with a definite diagnosis of cirrhosis, at least one episode of overt HE in the history (grade 2 or more according to West-Haven criteria) and remission at baseline (grade 0 or 1 according to West-Haven criteria), different grades of liver failure (Child-Pugh A-C, MELD 10-25), and a written informed consent to participate. Exclusion criteria were: gastrointestinal bleeding, spontaneous bacterial peritonitis or other conditions associated with HE precipitation within 3 months before the enrollment, diagnosis of a neurological or psychiatric disease, and current use of psychotropic medications, systemic uncontrolled disease (cardiac, respiratory, kidney failure), recent (< 3 months) active alcoholism, and absence of informed consent. Seventy-eight patients were evaluated. There were no significant differences in the distribution according to gender and etiology of the cirrhosis. Most patients had mild to moderate liver failure. Esophageal varices 2nd degree or more were detected in 54 of the 78 patients (tab. I).

Before enrollment, all patients had a score 0 or 1 according to West-Haven scale.

In order to evaluate the efficacy of different treatment strategies our patients were divided into three groups. RI Group = 38 patients (48.8%) was treated with rifaximin intermittently (14 days/month for 6 months), RD Group = 28 patients (35.9%) received rifaximin daily (400 mg three times daily), and L Group= 12 patients

(15.3%) treated with lactulose 30 to 60 ml in 2 or 3 divided doses per day, for a 6-month period. No significant differences in terms of age, gender, etiology of cirrhosis and severity of liver disease were found in the three groups.

TABLE I
Patients characteristics

CHARACTERISTIC	
Age (yrs)	56.3 [41-75]
Gender (M/F)	44/34
Etiology of cirrhosis:	
Alcoholic	29
HCV/HBV/HBV+HDV	37/2/4
HCV+alcohol	6
MELD score	15.69 [10-25]
Child-Pugh class (A/B/C)	27/43/8
Esophageal varices (No/I/II/III/IV)	20/38/12/4

All patients were followed up every 3 months or at any presentation in outpatient clinic or hospital in a period of one year. Patents were monitored for occurrence of recurrent overt HE, the incidence and severity of episodes and number of hospitalizations involving any episode of overt HE being recorded.

HE was assessed using West-Haven mental status scale and asterixis grade. West-Haven criteria are defined as follows: grade 0: no detectable changes in behavior or personality; grade 1: trivial loss of awareness, euphoria or anxiety, shortened attention span, impaired performance of addition; grade 2: lethargy or apathy, impaired performance of subtraction, minimal disorientation to time or place, subtle personality change, inappropriate behavior; grade 3: confusion, gross disorientation, somnolence to semi-stupor(may respond to verbal stimuli); and grade 4: coma (no response to verbal or noxious stimuli) (9). Asterixis was graded as follows: 0, no tremors; 1, few flapping motions; 2, occasional flapping motions; 3, frequent flapping motions; and 4, almost continuous flapping motions (10).

An episode of overt HE was defined as an increase from a baseline West-Haven score 0 or 1 to a score of 2 or more, or baseline West-Haven score and asterixis grade each increasing by 1 point. HE–related hospitalization was defined as hospitalization directly caused by HE or a hospitalization during which a HE event occurred.

RESULTS

Recurrence of overt HE

Of the 78 patients, overt HE episodes occurred in 21 (26.92%) during the 12-month study period. The distribution of total cases of overt HE was as follows: 10 of 38 patients in RI group, 7 of 28 patients in RD group and 4 of 12 patients in L group. Probability of developing recurrent overt HE, illustrated by a Kaplan-Meier analysis, was not significantly different in the three groups: 26.31% in RI group, 25% in RD group versus 33.33% in L group (fig. 1).

Severity of the overt HE episodes

Patients in L group experienced more

severe episodes of overt HE (grade 3/4) in spite of mild or moderate disease. In the rifaximin groups overt HE episodes were similarly frequent and associated with the severity of liver disease according to Child-Pugh score.

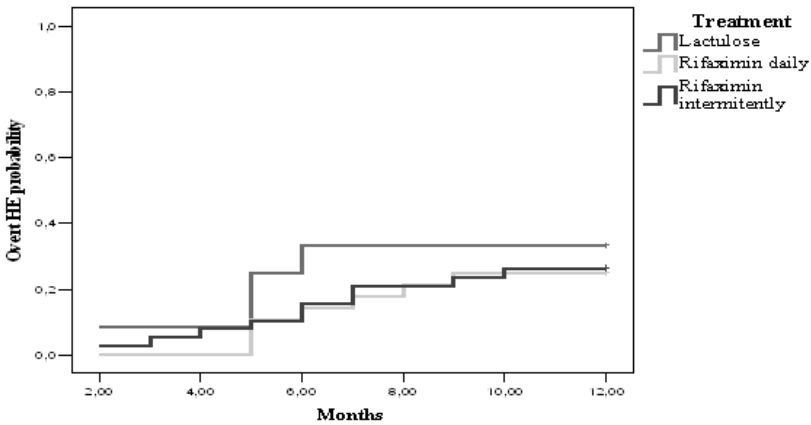

Fig. 1. Probability of developing recurrent overt HE in patients receiving therapy with lactulose following an episode of overt HE compared with patients receiving daily or intermittently rifaximin.

Safety

No serious adverse events related to either treatment were found during the study. There was no significant difference between rifaximin and lactulose on overall patient compliance (100% versus 92%). The most common adverse events with rifaximin were abdominal pain (5 patients-7.57%) and dizziness (3 patients-) showing no consistent pattern or dose relationship. In the lactulose group, 2 patients (16.66%) complained of abdominal pain and 4 patients (33.33%) experienced diarrhea.

TABLE II

Requirement for EH-related hospitalization

Number of EH-related hospitalizations	Rifaximin intermittently group (10 patients)	Rifaximin daily group (7 patients)	Lactulose group (4 patients)
Overall	**12**	**8**	**6**
One admission	8	6	2
Two admissions	2	1	2

Hospitalizations

A total number of 26 HE-related hospitalizations were recorded during follow-up. The 10 patients in RI group required 12 hospitalizations: two patients required two hospitalizations and eight patients one hospitalization. The 7 patients in RD group required 8 hospitalizations: one patient two admissions and six patients one. In the L group, the 4 patients experienced 10 hospi-

talizations: two patients one admission and two patients two admissions (tab. II). The frequency of HE-related hospitalization was comparable between RI group and RD group (31.57% *vs.* 28.57%), but significantly lower than in L group (50%).

DISCUSSION

Recurrent bouts of HE that require evaluation and hospitalization cause a significant burden to patients, their families and caregivers as well as to the healthcare system.

Currently, lactulose and non-absorbable antibiotics such as rifaximin are the only two medications effective when used for secondary prophylaxis against recurrence of overt HE in patients who have recovered from an episode of overt HE (7, 8, 10).

Systematic reviews of randomized trials comparing disaccharides *vs.* antibiotics for the treatment of HE has shown contradictory results. Whilst Nielsen et al. found superior outcomes with the use of antibiotic therapy (11), a meta-analysis of seven randomized controlled trials concluded that rifaximin was not superior to non-absorbable disaccharides in the long-term or short-term treatment except that it was better tolerated (12). Recently, another meta-analysis found that rifaximin has similar effectiveness to other oral therapies with a better safety profile (13).

Both rifaximin and lactulose demonstrate superior efficacy versus placebo for maintenance of remission from HE and lowering the frequency of HE-related hospitalizations. Sharma et al.(7) published the results of a recent open-label study of lactulose versus placebo for preventing recurrence of overt HE in cirrhotic patients showing the utility of lactulose as a secondary prophylactic therapy. Over a median follow-up period of 14 months, the probability of developing recurrent overt HE in patients receiving lactulose was 19.6% compared with 46.8% in the placebo group. Bass et al.(10) found rifaximin 550 mg twice daily more effective than placebo for maintenance of remission from HE over a 6 month period (rifaximin 22% breakthrough HE episodes versus placebo 46%). Further evaluation in a 24 months open-label maintenance trial concluded that long term treatment with rifaximin provide continued protection from HE and reduce the hospitalization rate (8). One of the main limitations of the study was the lack of a real placebo group, concomitant administration of lactulose being permitted. Hence this limited the evaluation of the efficacy of rifaximin alone.

Our study confirmed the efficacy of the two drugs in the maintenance of remission from overt HE, in agreement with the published data (7, 8, 10). Lower frequencies of HE hospitalizations were observed in the rifaximin groups, in line with the results of retrospective chart reviews (14, 15), which have shown that rifaximin, as compared with lactulose, is associated with a significantly lower rate and duration of hospitalization and lower hospital costs. We found a better tolerability of rifaximin which coincides with previous data (14, 16). However, our results are in contradiction with a recent meta-analysis which reported no significant difference between rifaximin and lactulose on diarrhea, but a significant difference in favor of rifaximin on abdominal pain (12).

It appears that rifaximin monotherapy could be a suitable option for long term treatment. However, given the high treatment cost and the current available data, it may be prudent not to recommend rifaximin as long

term treatment in all cirrhotic patients. Our results could represent a starting point for evaluating in future studies the cost-effectiveness and budget impact of competing therapies in hepatic encephalopathy.

The current study differs from the previous randomized studies in that it compared rifaximin and lactulose in terms of prevention the recurrences of overt HE and HE-hospitalizations; we did not have a placebo group since the lack of the treatment in patients at high risk for hepatic encephalopathy was considered unethical. In addition, we evaluated two dosage regimens for rifaximin (daily/intermittent) which proved to be similar for secondary HE prophylaxis. As in a previous study, we chose to define remission as West-Haven grade 0 or 1 because of the difficulties and variability in diagnosing mild HE, in addition with a known fluctuating course between 0 and 1.

One limit of our study is related to the small number of enrolled patients. Also patients with highly advanced liver disease (MELD score >25) were not evaluated.

CONCLUSIONS

We demonstrated that over a 12-month period, rifaximin in daily or intermittently dosage and lactulose are effective in the prevention of overt HE recurrences. No marked difference between the three therapeutic regimens was found in terms of maintenance of remission from overt HE. However, rifaximin reduces the risk of hospitalization involving HE better than lactulose.

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NEWS

EFFECT OF LAYERING METHODS, COMPOSITE TYPE, AND FLOWABLE LINER ON THE POLYMERIZATION SHRINKAGE STRESS OF LIGHT CURED COMPOSITES

The polymerization shrinkage and its associated stress still remain a major drawback of dental composite materials, resulting in bond failure, cuspal flexure, enamel microcracking and bacterial infiltration. Different materials and techniques have been suggested to minimize shrinkage stress of composite resins. The aim of this study was to investigate the effect of layering methods, flowable composite liner and use of low shrinkage silorane-based composite on the polymerization shrinkage stress of light cured composites. Aluminum blocks were used to prepare MOD cavities and divided into four groups. A universal hybrid methacrylate-based composite (Z250), a flowable composite (Z350 flowable), and a silorane-based composite (P90) were used to fill the cavities. Cavities were restored using the bulk technique with Z250, an increment technique with Z250, an increment technique with Z250 and a Z350 flowable lining, and an increment technique with P90. The axial shrinkage strain and flexural modulus of the three composites were determined, and cuspal deflection of each group was measured with linear variable differential transformer probes and compared among groups using ANOVA and Tukey's post hoc test ($\alpha = 0.05$). The incremental filling technique yielded significantly lower cuspal deflection than the bulk filling technique. Flowable composite lining under universal composite (Z250) layering showed higher cuspal deflection than that without flowable composite lining. Silorane-based (P90) composite exhibited lower cuspal deflection than metacrylate based (Z250) composite. These results suggest that cuspal deflection resulting from polymerization shrinkage stress may be reduced by an incremental filling technique and by the use of low shrinking composite. On the other hand, flowable composite lining under conventional composite layering dd not reduce the polymerization shrinkage stress in this study. The cuspal deflection of the first increment layered with a flowable composite liner (Z350 flowable) was considerably higher than the first increment layered with a conventional hybrid composite (Z250). However, the authors suggest that using flowable composite liners with different elastic modulus, shrinkage values, and varying thickness might produce different results (Kwon Y, Ferracane J. Effect of layering methods, composite type, and flowable liner on the polymerization shrinkage stress of light cured composites. *Dental Materials* 2012; 28(7): 801-809).

Gianina Iovan

RISK FACTORS AND THEIR IMPLICATIONS IN THE EPIDEMIOLOGY OF PEDIATRIC OBESITY

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RISK FACTORS AND THEIR IMPLICATIONS IN THE EPIDEMIOLOGY OF PEDIATRIC OBESITY (Abstract): Obesity is the most common food disorder in children from developed countries, its frequency alarmingly increasing in Romania. **Aim:** To evaluate the epidemiological dynamics of obesity and associated risk factors. **Material and methods:** Retrospective and prospective study of 146 obese children admitted to the 2nd Iasi Pediatric Clinic between 2008-2012 aimed at evaluating the epidemiological dynamics of obesity and associated risk factors. Most subjects were teenagers (33%), followed by school age children (21%). **Results:** A growing tendency towards obesity in the study children was noticed during the 5-year follow up. Genetic factors, short breastfeeding, early onset of obesity, and eating disorders were the main obesity-associated risk factors, along with obesity in 1st degree relatives. **Conclusions:** Early infantile and juvenile obesity remains a medical and social problem in our geographic area, and unfortunately a neglected reality. Genetic factors, absence of breastfeeding, inappropriate food habits in the early years of life lead to permanent disorders, with serious consequences in adult life. **Key words**: OBESITY, CHILDREN, RISK FACTORS.

In the last century, there were major changes in the epidemiology of obesity: thus, if between 1950 and 1960 this condition occurred at older ages in Western and some Northern European countries, now its spread is pandemic, reason why scientists have named it "globesity". It has become the main health problem of the 21st century (1, 2), mostly in the United States, population studies demonstrating the increasing prevalence of overweight and obesity in children.

Epidemiological data of pediatric obesity worldwide

Approximately 50% of the obese children grow up to become obese adults. WHO expectations for 2025 are over 300 million obese people worldwide (1). In Australia, China, Brazil, and United States, the growth rate of pediatric obesity highly exceeds that of adult obesity.

According to the NHANES study (1963-1995), the number of obese patients has doubled, increasing by 40% in the age group 12-17 years and by 54% in children aged 6-11 years. Prevalence of overweight in adolescents increased from 5% to 10.5%, and in children aged 6-11 from 6.5% to 11.3%. In the US, 1 of 4 children aged 6 to 17 years and 1 of 2 adults are overweight,

10% of preschool children are obese, many with decreased glucose tolerance (3). During the last two years, over 30% of the American children became obese, doubling the estimated percentage. The prevalence of obesity in the 6-14 years age group is increasing, from 4% during 1963-1965 to 13% in 1999, and in adolescents aged 12-19 years from 5% (1966-1970), to 14% in 1999 (4). Prevalence is growing rapidly being now 50% higher than in 1960. International Association for the Study of Obesity (IASO) estimated that, worldwide, 10% of school children, that is 120 millions, are overweight or obese. According to WHO, globally, obesity has exceeded the prevalence of malnutrition, 15-18% of children being affected by overweight and obesity. Clinical studies showed an increased prevalence of the metabolic syndrome associated with ageing (5,6), but also found in childhood.

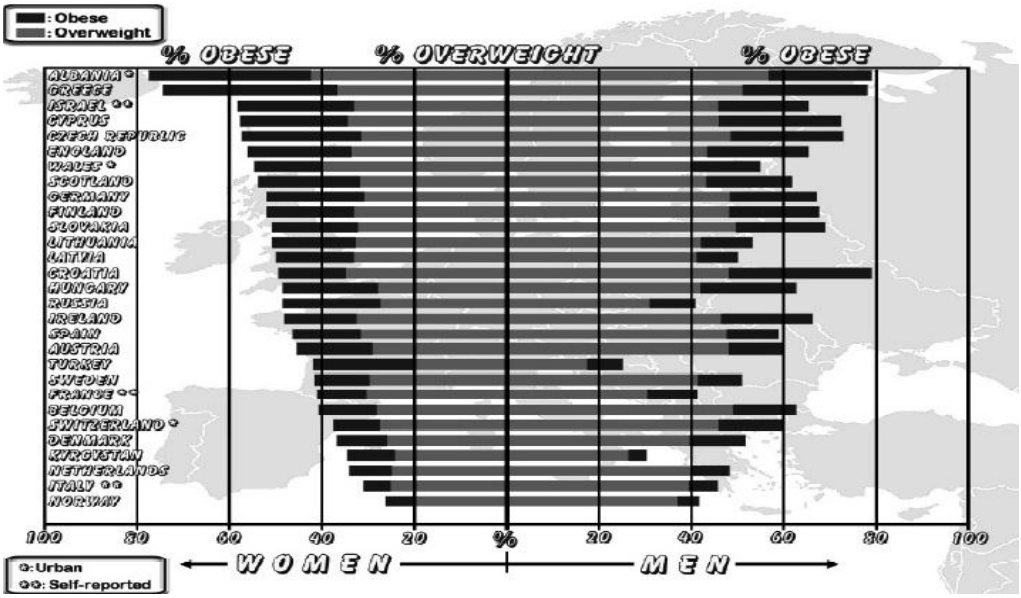

Fig. 1. Prevalence of obesity and overweight in Europe (IOFT, October 2008).

At the end of the last decade, International Obesity Task Force (IOTF) was commissioned by WHO to coordinate anti-obesity measures worldwide and to submit a report on the prevention and management of global obesity (fig. 1).

In 2004-2005, in Iran an epidemiologic study on obesity was conducted in subjects aged 15 to 65 years; the aim was to estimate the prevalence of overweight in that area and to predict the future obesity rate. The results pointed out that the prevalence of overweight and obesity was 57% in women and 42.8% in males; underweight was 5.2% in women and 6.3% in male population (7). In Mexico, there was an increase of 50% of obesity prevalence in children and adults in the last ten years. The highest prevalence of overweight and obesity was reported in the Pacific Islands and Saudi Arabia. According to NHANES II study (1976-1980) there is an increased prevalence of overweight in all age groups: 2-5 years (preschool children), from 5% to

12.4%, 6-11 years, from 6.5% to 17%, and 12-19 years, from 5% to 17.6% (8). More recent data (NHANES 2003-2006) show a higher prevalence of obesity in the boys aged 12 -19 years (27.7%) than in age-matched girls (19.9%). African American and Hispanic children have a higher risk for developing overweight compared to Caucasian children. In 2008, the prevalence of obesity increased in children of American Indian. Alaskan native (21.2%) and Hispanic (18.5%) children, and decreased in Asian children (12.6%) (9). Even in developing countries there was an increased prevalence of obesity in children under 5 years, from 12.4% in 1998 to 14.5% in 2003 and only to 14.6% in 2008. The incidence is still increasing in all age groups including infants and preschool children. In England, childhood obesity rate is 7 %, and infantile obesity rate 27.7%. In the U.S., the frequency in preschool, and school children and teenagers is of 14.1%, 3-20% and 10-30%, respectively. In Chile, studies showed that 7% of preschool and 12% of school children are obese. In many other countries, more than half of people are overweight and obese. It is estimated that 97 million U.S. adults are overweight or obese. Most of them were obese as children. In the past decade, the prevalence of obesity increased by almost 40% in most European countries, especially in the male population (10) (fig. 2). Currently, it is estimated that about one quarter of the world's population is overweight before the age of 20 years, and half will be obese by the age of 20. In 2000, in France, 16% of the children aged 5 to 16 years were obese, and in Italy 25% (6, 11).

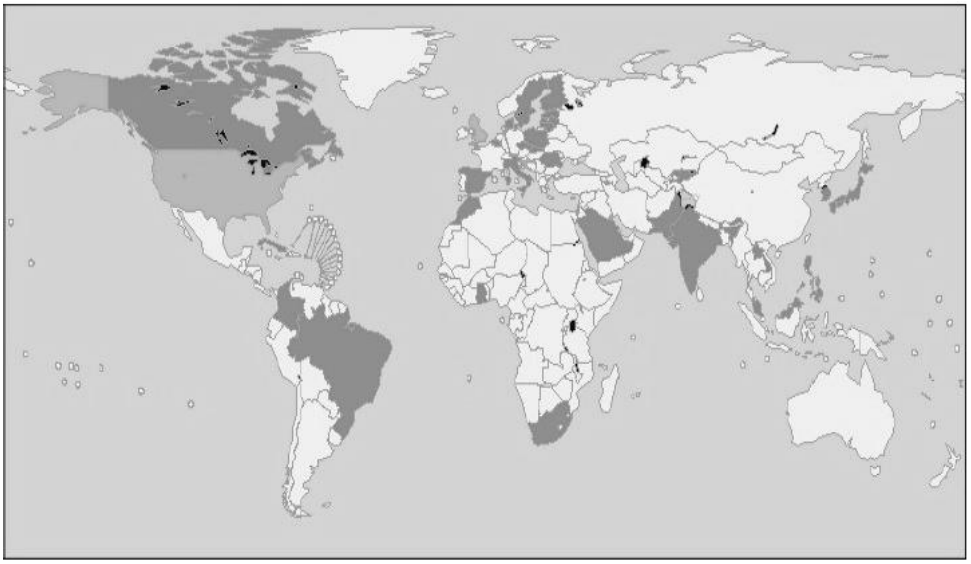

Fig. 2. Prevalence of obesity (WHO, April 2008). Legend: Open white areas - lack of data, gray areas - the prevalence of 30-40% black areas – prevalence over >40%.

In Europe, childhood obesity has tripled in the past 20 years; in the Mediterranean and Eastern Europe countries, up to 40% of the women were obese In Western European countries, obesity is about 25%, in Northern Europe the prevalence of overweight chil-

dren is 10-20%, while in the Southern countries it is 35% and is increasing. It is alarming that the onset of overweight and obesity is at a young age, and in certain geographical areas, some preschool children have decreased glucose tolerance, being at higher risk for type 2 diabetes. The highest prevalence was recorded in Malta (31% boys and 28% in girls) and the lowest in Lithuania (10.3% boys and 4.7% in girls). In Belgium, between 1980 and 1990, the prevalence of obesity increased from 9.2% to 14.5% (12). Reports of the International Obesity Task Force (IOTF) showed the same trend in Southern Europe, where 1 out of 10 children are overweight. In England (1999), the prevalence of overweight increased from 22% at age 6, to 31% at 15 years, and obesity from 10% to 17% in the same age ranges (13). In Germany, a 20 year-study (1975-1995) showed an increase in overweight from 10% to 16.3%, more common in girls, and in obesity from 5.3% to 8.2 % (9).

In France, a study conducted in children in the interval 2000-2001 showed a 3.8% prevalence of obesity and overweight of 3.8% and 14%, respectively (8). An Italian study (2000 – 2002) reported a higher prevalence of overweight of 27.2% in boys and 29.5% in girls aged 6 to 11 years, and obesity of 6.5% in boys and 7% in girls. Recent studies show that in Italy 36% of the 9-year-old children are overweighed or obese, while in Greece the prevalence is 19% in the age group 6-17 years (14). In Spain, 27% of children and adolescents are obese. Epidemiologic studies carried out in the interval 1998-2000 showed a prevalence of overweight of 31.4% in boys and 32.4% in girls, and of obesity of 10.4% and 10.2% in boys and girls, respectively.. Between 1998 and 2004, Bulgaria reported an increase in the prevalence of overweight from 19% to 22% in boys and from 16% to 18% in girls (15).

Obesity in Romanian children

There are few studies on obesity in Romanian children. In 1980, a group of children from 3 months to 16 years of age from Western Romania was measured for assessing the nutritional status; 14.7% were identified to be obese, with a slightly higher frequency of school-age girls. In the 5250 children, obesity had a prevalence of 18.62% in infants, 15.05% in preschool children, and 14.20% in school-age children (4). "Alfred Rusescu" Institute for Mother and Child Care (IOMC) in Bucharest launched the National Nutritional Surveillance Program (1993-2002). The epidemiological studies showed a prevalence of overweight in children aged 0 - 4 years of 6.4% in girls and 5.5% in boys. In 2008, in Cluj-Napoca, a study on 7904 schoolchildren, reported a prevalence of overweight of 12.8% and of obesity of 8.2%. The highest prevalence was recorded in the age group 6-10 years, both for overweight - 15.9%, and obesity-13.3%, and the lowest prevalence in adolescents: 7.6% for overweight and 3.8% for obesity (16).

Risk factors that need to be identified and avoided

Although the risk factors for obesity are clearly represented by heredity (90%), there are many phenomena that target feeding behavior, regulation of appetite and bad eating habits, which can be identified and avoided. Some authors noted that early adiposity rebound (at 3 years of age), high birth weight or prematurity (with a subsequent rapid growth rate), lack of physical activity, correlated with the occurrence of early obesity, The earlier the rebound is, the higher the adiposity will be at adult

age. Thus, dynamic assessment and recording of BMI rebound may allow initiation of preventive measures. Rapid weight gain in the first six months of life is a predictor factor for developing obesity by the age of 3 years (17). Avoiding obesity both before pregnancy and after delivery is crucial for the future baby weight. Low birth weight for gestational age associated with a rapid weight gain after birth increases the risk of obesity, metabolic syndrome and diabetes. Newborns of obese mothers are often overweight and they can develop obesity on long term basis. Children with higher gestational age because of maternal insulin resistance and decreased glucose tolerance are at risk for obesity. Positive family history for obesity and early onset of overweight increase the risk of obesity up to 80% for those with both parents obese and up to 40% for children with one obese parent. Rapid weight gain during the first 4-6 months is associated with higher risk of childhood obesity and with its abdominal distribution in children (18). Breastfeeding should be recommended as a protective factor against obesity in adolescence. The risk of obesity decreases proportionally with the duration of breastfeeding (up to 9 months), each month of breastfeeding decreasing by 4% the risk of obesity. Artificial feeding and excess protein intake in the first year of life are considered risk factors for subsequent development of obesity, with pancreatic and kidney overload. Cow milk proteins specifically stimulate massive IGF-1 release (19). Impaired nutrition in pregnant woman has an important role in childhood obesity and its impact involves some risk factors such as: low birth weight, increased amount of insulin in the amniotic fluid, hypothalamic activity, stimulating and inhibitory peptides. The relationship between obesity and age involves alteration of appetite regulating systems (hunger/satiety balance), which develop in pregnancy and neonatal period. Fetal and neonatal life are critical for the maturation of the complex systems involved in the pathology of metabolic syndrome and obesity. Fetal malnutrition is the result of maternal malnutrition, placental insufficiency, which can add more maternal abnormalities. This leads from low birth weight to later overweight and therefore to obese adult. It has been suggested that genes play an important role, parental obesity being a major risk factor for childhood obesity (17).

MATERIALS AND METHODS

In this study, the authors evaluated a group of patients admitted to the Iasi " St. Mary" Children Hospital in the interval 2008-2012. The aim was to investigate retro-prospectively the epidemiological dynamic of obesity, age-group distribution, and the main risk factors involved in its occurrence.

RESULTS AND DISCUSSION

The study showed an annual increase in the incidence of obesity; thus, during the 52-month study interval, 146 new cases of obesity were detected. (fig. 3). Most children were from urban areas (77%) and 58 patients had first degree relatives with obesity.

Age-distribution of the cases revealed a high obesity rate in teenagers (one third of the cases), with long-term severe consequences, followed by school-age and preschool children (8% of the study patients were obese infants) (fig. 4).

Most study children were from urban areas (71%), with some well-known predisposing factors: less strict meal plan, access to "fast food", large amounts of refined sugar consumption, and reduced

levels of physical activity. By contrast, rural area children had a more balanced diet, with a higher intake of fresh fruits and vegetables, along with more types of physical activity, (residential houses with a backyard, household and farming activities, entertaining activities).

History of the first year of life showed that one third of the children were not breastfed, and 15% were breastfed for a short period of time, because of low milk supply, early resumption of professional activity, appetite and satisfying weight gain after baby food diversification (fig. 5).

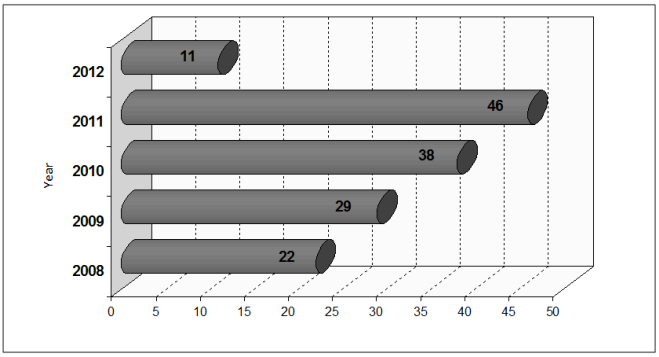

Fig. 3. Number of new obesity cases in the study children

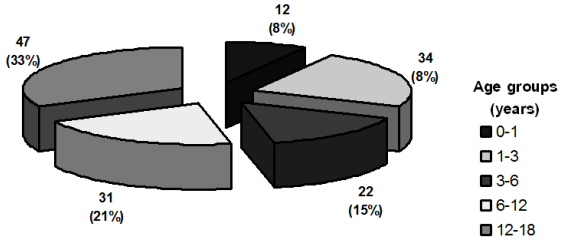

Fig. 4. Distribution of obesity in the study children

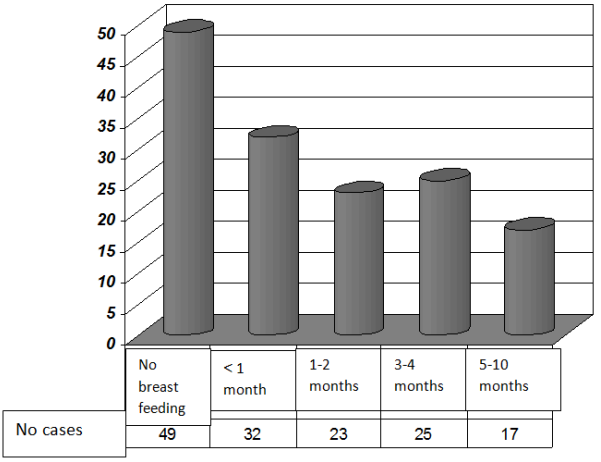

Fig. 5. Nutrition in infancy.

CONCLUSIONS

Childhood and juvenile obesity remain an important medical and social problem worldwide, sadly an ignored reality. The major risk factors are food behavior, poor breakfast, heavy dinner, low fiber diet, lack of exercise. The onset is earlier and more obvious for overweight, as breastfeeding duration is shorter, so young mothers should be encouraged to breastfeed for a longer period despite some minor inconveniences. The higher the prevalence of obesity in the general population, the higher the health system costs. Implementation of prevention and curative measures will have a major impact on this epidemiological phenomenon and expansion of obesity worldwide.

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SPONDILO-DISCARTROZA CU SINDROM LOMBAR RADICULAR ETAJAT HIPERALGIC

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DISCHARTHROSIS WITH HYPERALGIC LUMBAR MULTILEVELED RADICULAR SYNDROME (Abstract): The problems at the level of intervertebral discs are producing dysfunctions and important functional regression at the level of lumbar column, at a stage at which the patient could remain blocked in an anterior or lateral flexion position or producing an antalgic position of scoliosis that could incapacitate the patient to perform activities of daily living. The medical rehabilitation, in such cases, must seek not only the relief of local pain through different methods of obtaining it, but also the functional reeducation of the intervertebral articulations through specific analytical mobilization in order to achieve the biomechanical harmonization of the rachis. **Material and methods**: We report the case study of a 66 year-old patient who presented to our clinic for medical consult and physical therapy when he was diagnosed with discharthrosis, hyperalgic lumbar multileveled radiculopathy at L4 – L5 and L5 – S1. The lumbar x-ray showed osteophytes, disc narrowing at the level of L5 – S1 and inter-apophysis arthrosis. The clinical examination revealed difficulty walking with pain in the right sacroiliac articulations and right sciatic emergence with plantar paraesthesia. The patient developed pain induced scoliosis on the right side that restricted the lumbar range of motion and prevented the right flexion blocking him into an left flexion, any attempt of straightening inducing pain. The condition was treated using specific analytical lumbar mobilization for the realignment of the vertebrae complex. **Conclusions**: In this case study, we found that functional reeducation in cases of pain induced deviations of the rachis of the column should be centered on the harmonization of inadequate pressure and position of the complex intervertebral articulations. **Key words:** FUNCTIONAL REEDUCATION, LUMBAR DISCOPATHY, DISCHARTHROSIS, RADICULAR SINDROM, ANALYTICAL MOBILIZATION

Problemele discale lombare produc disfuncţionalităţi şi restanţe funcţionale importante la nivelul mobilităţii coloanei lombare, pacienţii putând rămâne blocaţi în diferite grade de anteroflexie şi lateroflexie sau scolioze antalgice neredresate ducând la incapacitatea efectuării ADL-urilor (*Activities of Daily Living*) (1). Recuperarea medicală în astfel de situaţii trebuie să-şi îndrepte atenţia nu numai spre îndepărtarea durerii locale, prin diferite metode de analgezie ci şi spre o reeducare funcţională discală, şi articulară prin manevre specifice, cu posibilităţi de rearmonizare a com-

plexului biomecanic al rahisului (2). Studiul de caz prezentat vine să sublinieze importanța refacerii mobilității analitice a coloanei lombare.

MATERIAL ŞI METODE

Pacientul S. V. în vârstă de 66 ani de sex masculin, din mediu urban, s-a prezentat la Centrul de Fiziokinetoterapie şi Recuperare pentru consult şi tratament de specialitate unde a fost diagnosticat cu discartroză, sindrom radicular hiperalgic lombar etajat la nivelul L4 – L5 si L5 – S1 şi tulburări de statică vertebrală. Examenul radiologic toraco-lombar a evidențiat osteofite dorso-lombare cu pensare discală la nivel L5 – S1, punți osoase marginale, scleroza platourilor corpilor vertebrali lombari, importantă artroză interapofizară şi interspinoasă lombară, cu accentuarea lordozei lombare şi articulații sacroiliace şterse bilateral. Examenul clinic a evidențiat un mers posibil sprijinit cu bastonul pe partea stângă, foarte dificil pe călcâi şi pe vârfuri, cu o mică dificultate în stațiune bipodală. Mersul se executa cu dureri în articulațiile sacro-iliace bilaterale, cu precădere pe partea dreaptă şi algii la palparea emergenței sciatice drepte, cu parestezii la nivel plantar, predominant pe partea dreaptă. Coloana vertebrală prezenta în regiunea dorsală o scolioză dextroconvexă cu sensibilitate la palparea apofizelor spinoase cervico-dorso-lombare. Testul Schöber edificator pentru mobilitatea coloanei lombare a fost de 10/11,5cm pacientul prezentând o lateroflexie sever limitată spre partea dreaptă.

DISCUȚII

Tratamentul fizical-kinetic s-a desfăşurat pe durata a 20 de şedințe şi a avut drept obiectiv realizarea analgeziei locale, refacerea staticii, ameliorarea mobilității coloanei şi a constat în electroterapie de tip TENS premodulat hiperalgic, pe zona coloanei lombare, laser pe articulațiile sacroiliace drepte, masaj terapeutic, kinetoterapie individuală şi mobilizări articulare specifice lombare. După 5 şedințe de terapie mobilitatea coloanei lombare pe flexia anterioară a crescut fiind evidențiată prin testul Schöber 10/15cm, iar durerea fiind evaluată pe scara EVA (0-10) la 2. Pacientul nu a mai invocat nici un fel de durere la nivel de anteroflexie, mişcarea putând fi efectuată cu uşurință şi chiar fără a fi nevoie să facă uz de poziții protective ale coloanei lombare în dinamica mişcării, cum ar fi flexarea genunchilor înainte de orice inițiere a mişcării înspre anteroflexie din coloana lombară. Poziția coloanei lombare în decubit dorsal pe masa de tratament putea fi redresată din flexia laterală stânga fără nici o durere, însă odată cu încercarea revenirii la ortostatism pacientul acuza algii în partea dreaptă, coloana ajungând într-o poziție deficitară de flexie laterală stângă, moment în care pacientul nu mai acuza dureri. La măsurarea unghiului de flexie cu goniometrul liniar pacientul avea 25º flexie lateral stânga (fig. 1), făcând posibilă deplasarea doar cu un baston de sprijin.

Fig. 1. Goniometria lombară

Pentru redresarea atitudinii vicioase de flexie laterală stângă, ce ducea şi mai mult la solicitări ale structurilor discale în partea dreaptă, datorită migrării nucleului pulpos spre partea cu presiune redusă (cu creşterea stresului mecanic asupra discului ceea ce producea o agravare a durerilor), s-a recurs la tehnici de mobilizări specifice ale coloanei lombare şi la exerciţii de conştientizare şi redresare activă a poziţiei vicioase (3).

Mobilizările specifice au fost următoarele: a) tehnica rotaţiei lombare după Niederhoffer, cu şi fără facilitare musculară (4) (fig. 2); b) tehnicile mobilizării cu tracţiune bilaterală şi unilaterală după Sohier (5) (fig. 3); c*) hold-relax* cu întindere pe ischio-gambieri (*P.N.F. stretching technique*) (6); d) exerciţiu activ de redresare după conceptul McKenzie (3) (fig. 4).

După 15 şedinţe de efectuare a tehnicilor de mobilizare specifică coloana lombară s-a redresat la 5º de flexie laterală stângă, amplitudinile câştigate făcând posibilă mobilizarea în ambele direcţii de latero-flexie, pacientul acuzând doar o mică jenă în momentul redresării coloanei spre ortostatism.

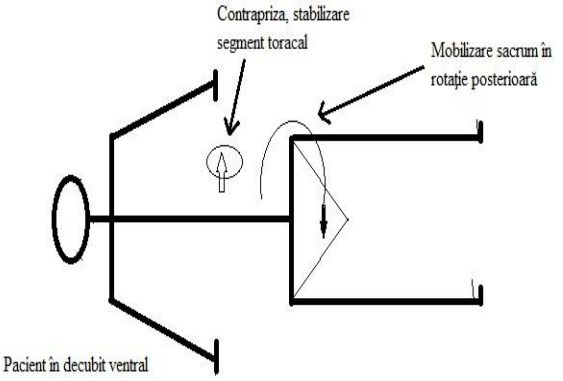

Fig. 2. Mobilizare Niederhoffer

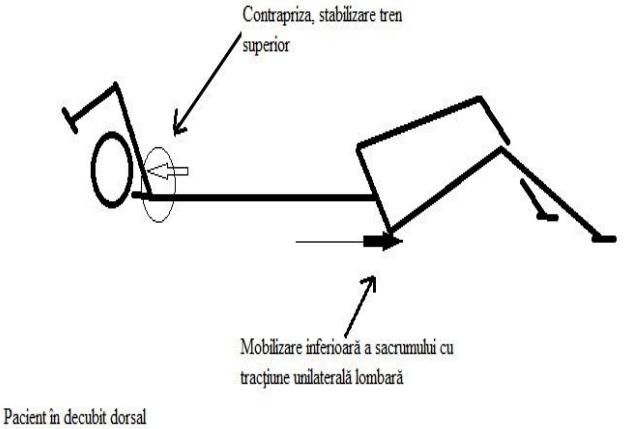

Fig. 3. Mobilizare concept Sohier

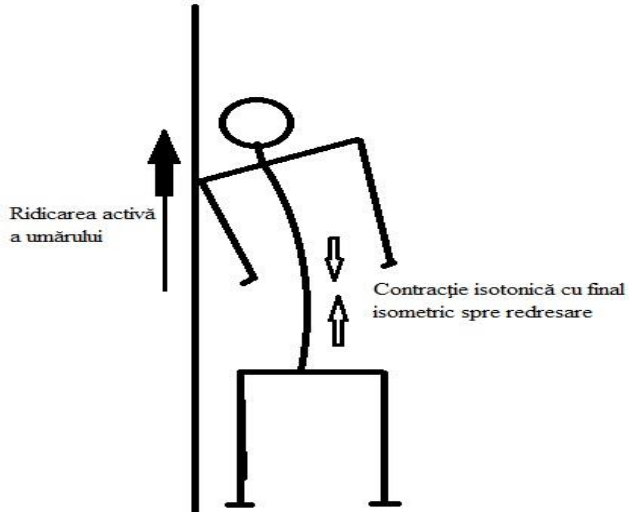

Fig. 4. Redresare activă McKenzie

CONCLUZII .

Studiul nostru a evidenţiat că eşecul restabilirii echilibrului biomecanic la nivelul complexului vertebră-disc-articulaţii interapofizare, deformările şi presiunile inegale păstrate datorită unui restant funcţional de flexie laterală pot duce în timp chiar ele la provocarea de algii importante care să nu mai fie legate de lezarea discală ce a iniţiat dezechilibrul articular.

Mobilizările analitice specifice coloanei lombare rearmonizează complexul articular crescând amplitudinile articulare şi per ansamblu funcţionalitatea.

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PREVALENȚA SIMPTOMELOR DE APNEE OBSTRUCTIVĂ ÎN SOMN ÎNTR-O POPULAȚIE RURALĂ

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PREVALENCE OF OBSTRUCTIVE SLEEP APNEA SYMPTOMS IN A RURAL POPULATION (Abstract): **Aim:** Obstructive Sleep Apnea Syndrome (OSAS) is an under diagnosed condition with significant consequences on health. The aim of this study is to estimate the prevalence of symptoms and key features of OSAS in a rural population. **Material and methods:** 254 subjects were enrolled. We measured height (H) and weight (W), calculated body mass index (BMI) and determined glycated hemoglobin (HbA1c). Information about age, gender, medical history (diabetes, hypertension) was collected. All the participants filled in the Berlin questionnaire, which has separate questions regarding the symptoms of OSAS. **Results:** Snoring was reported by 44.1% of the subjects, observed apneas by 6.7% and excessive daytime sleepiness by 36.6%. All symptoms were more frequently reported by women. A higher percentage of obese, hypertensive and diabetics reported snoring ($p<0.001$). When analyzing gender differences, the significance was maintained, except for diabetic men. Witnessed apneas were more prevalent in hypertensive subjects ($p=0.034$), but were significantly associated with hypertension only in ($p=0.003$). No correlation was found regarding excessive daytime sleepiness and the risk factors studied. **Conclusions:** Prevalence of OSAS symptoms and risk factors in this rural population was noticeable and efforts for early diagnosis and treatment are recommended. **Key words:** OBSTRUCTIVE SLEEP APNEA, SLEEP RELATED SYMPTOMS, PREVALENCE.

Sindromul de apnee obstructivă în somn (SAOS) este definit clinic prin apariția repetată a unor episoade de obstrucție completă sau parțială a căilor aeriene superioare în timpul somnului, asociată cu sforăit puternic şi somnolență diurnă (1). Toate aceste trei caracteristici reprezintă simptomele cardinale de apnee.

Acest sindrom are consecințe medicale multiple, cu un impact major asupra sănătății populației. Există o multitudine de evidențe care atestă legătura dintre SAOS şi procesele aterosclerotice (2) sau morbiditatea cardiovasculară (3), în special hipertensiunea arterială (HTA) (4), fiind demonstrată însă şi asocierea acestui sindrom cu insuficiența cardiacă (5), aritmii (6), infarct miocardic (7), accident vascular cerebral (8). Mai mult decât atât, SAOS se asociază cu obezitatea (9) şi tulburări ale metabolismului glucidic (10). De asemenea, simptomele de SAOS se corelează puternic cu

alterarea calității vieții (11), precum şi cu scăderea capacității de muncă (12). Există date care indică faptul că aproximativ 80% dintre pacienții cu SAOS rămân nediagnosticați şi că numai 10% din populație este supusă unui screening adecvat pentru acest diagnostic (13). Recunoaşterea simptomelor şi semnelor de apnee reprezintă primul pas în managementul eficace al acestui sindrom, mai ales la nivelul medicinii primare, unde pacienții cu HTA, obezitate, diabet zaharat (DZ) sau acuze simple precum oboseala sunt foarte frecvent întâlniți. Scopul acestui studiu este de a identifica frecvența simptomelor cardinale de apnee raportate de participanți şi de a evidenția relația acestora cu anumiți factori de risc pentru SAOS (obezitatea, HTA şi tulburările metabolismului glucidic).

MATERIAL ŞI METODE

Am efectuat un studiu transversal pe un lot format din 254 de femei şi bărbați, cu vârste peste 18 ani, aleşi aleator de către computer din 3248 de persoane aflate în bazele de date ale medicilor de familie, alcătuite cu ocazia Programului Național de Sănătate din 2007-2008. Studiul a avut loc la Deleni, cea mai mare comunitate rurală din județul Iaşi. Înainte de începerea studiului s-a obținut avizul Comisiei de Etică a Universității de Medicină şi Farmacie "Grigore T.Popa" Iaşi, fiecare participant completând un formular de consimțământ informat înainte de înrolare.

Datele pentru studiu au inclus vârstă, sex, istoric medical (HTA, DZ). S-au efectuat măsurători antropometrice: greutate, înălțime, utilizând echipamente standardizate. S-a calculat indicele de masă corporală (IMC) prin formula greutate/înălțime^2 (kg/m^2) şi s-au definit categoriile de status ponderal utilizând criteriile OMS: subponderal (IMC< 18,5 kg/m^2), normoponderal (IMC=18,5-24,99 kg/m^2), supraponderal (IMC=25-29,99 kg/m^2) şi obezitate (IMC≥30 kg/m^2). S-a determinat hemoglobina glicată (HbA1c) din sânge capilar. Valoarea HbA1c considerată normală a fost <5.7%. Între 5,7-6,5% s-a stabilit diagnosticul de prediabet, iar o valoare ≥6.5% a diagnosticat DZ. În cadrul aceleiaşi vizite s-a măsurat tensiunea arterială, cu un tensiometru aneroid, şi a fost completat chestionarul Berlin, un instrument folosit pentru evaluarea riscului pentru SAOS (14). Chestionarul include 10 itemi organizați în 3 categorii. Prima categorie se referă la sforăit şi apneele cu martori (întrebările de la 1 la 5), a doua categorie face referire la somnolența diurnă (4 întrebări), iar cea din urmă categorie conține 2 itemi care evaluează existența HTA şi a unui IMC peste 30 kg/m^2. Din analiza separată a întrebărilor conținute în chestionar, s-a evaluat prevalența simptomelor cardinale ale SAOS. Pentru analiza statistică s-a utilizat programul *SPSS* (Statistical Package for Social Sciences) versiunea 16 pentru *Windows*. Semnificația statistică a diferențelor dintre variabilele continui a fost evaluată prin testul *t-Student* şi ANOVA One-way, iar pentru variabilele categoriale s-a utilizat testul χ pătrat (χ^2). O valoare a lui $p<0,05$ a fost considerată ca semnificativă statistic.

REZULTATE

Lotul studiat a fost format din 152 femei (59,84%) şi 102 bărbați (40,15%). Vârsta medie a populației din studiu a fost 55±16,28 ani şi IMC mediu 27,55±5,29 kg/m^2. Din punct de vedere al IMC, 34,5% au fost normoponderali, 36.5% supraponderali şi 29% obezi. În ceea ce priveşte vârsta, majoritatea subiecților s-au încadrat în grupul de vârstă 40-65 ani (47,2%).

Proporția fumătorilor a fost de 21,7%. Hipertensiunea arterială a fost prezentă la 35% dintre participanții la studiu, iar 6,7% aveau diagnostic anterior de DZ; IMC mediu a fost similar la femei şi bărbați. A rezultat că 40,2% dintre subiecți aveau HbA1c normală, 50,4% aveau prediabet şi 9,4% au avut DZ.

Prevalența simptomelor de SAO în lotul studiat a fost de 44,1% pentru sforăit, 6,7% în cazul apneei cu martori şi 36,6% pentru somnolența diurnă. Toate cele trei simptome analizate au fost mai frecvente la femei comparativ cu bărbații (58,9% *vs*. 41.1% pentru sforăit, 88,25 *vs.* 11,8% pentru apneea cu martori şi 66.7% *vs.* 33.3% pentru somnolența diurnă). La 34,3% dintre subiecți s-a găsit prezența atât a sforăitului, cât şi a pauzelor respiratorii în timpul somnului. Dintre sforăitori, 17,9% sunt normoponderali, 34,8% sunt supraponderali, iar 47,3% sunt obezi (p<0,001). Semnificația statistică s-a păstrat şi la analiza separată pe sexe (p<0,001) (tab. I).

TABELUL I

Relația dintre sforăit şi IMC în lotul studiat

Sforăit	Sex	IMC			*p*
		NP (%)	SP (%)	OB (%)	
Da	Bărbați	17,4	34,8	47,8	<0,001
	Femei	18,2	34,8	47,0	<0,001
	Total	17,9	34,8	47,3	<0,001
Nu	Bărbați	46,4	44,6	18,5	<0,001
	Femei	48,8	33,3	17,9	<0,001
	Total	47,9	37,9	14,3	<0,001

Nu au fost relevate diferențe semnificative în ceea ce priveşte apneea cu martori la categoriile statusului ponderal, deşi s-a observat o tendință mai mare a obezilor de a avea pauze respiratorii (12,3%), comparativ cu supraponderalii (5,4%) şi normoponderalii (3,4%) (p=0,068). Această tendință se menține numai în cazul femeilor, dar fără semnificație statistică (p=0,125). Somnolența diurnă a fost identificată la 43,7% dintre normoponderali, 31,5% dintre supraponderali şi 34,3% dintre obezi, fără semnificație statistică (p=0,280).

TABELUL II

Relația dintre HTA şi sforăit în lotul studiat

HTA	Sex	Sforăit (%)		*p*
		Da	Nu	
Da	Bărbați	63,0	37,0	0,026
	Femei	59,7	40,3	0,001
	Total	60,7	39,3	<0,001
Nu	Bărbați	38,7	61,3	0,026
	Femei	32,2	67,8	0,001
	Total	35,2	64,8	<0,001

În ceea ce priveşte relația dintre prezența HTA şi sforăit, rezultatele au arătat că 60,7% dintre hipertensivi sforăie (p<0,001). La bărbați, 63,0% dintre hipertensivi prezintă sforăit, comparativ cu normotensivii (p=0,026). În cazul femeilor, sforăitul s-a întâlnit la semnificativ mai multe femei hipertensive față de cele cu tensiune arterială normală (59,7% *vs.* 32,2%, p=0,001) (tab. II).

Apneea cu martori a fost identificată la 58,8% dintre hipertensivi (p=0,034). HTA a fost găsită la 66,7% dintre femeile la care s-a identificat apneea cu martori, comparativ cu 38% dintre femeile care nu prezentau acest simptom (p=0,003). La nici un bărbat cu HTA nu s-a relevat prezența pauzelor respiratorii în timpul somnului (tab. III). Somnolența diurnă a fost prezentă la 42,7% dintre hipertensivi, comparativ cu 33,3% din normotensivi, fără semnificație statistică (p=0,090). Nu s-au evidențiat diferențe semnificative

între sexe, deşi s-a observat că un procent mai mare de femei cu HTA au somnolență diurnă față de cele fără HTA (48,4% *vs.* 35,6%, p=0,079).

TABELUL III

Relația dintre apneea cu martori şi HTA în lotul studiat

Apnee cu martori	Sex	HTA (%)		*p*
		Da	Nu	
Da	Bărbați	0	100	0,539
	Femei	66,7	33,3	0,003
	Total	58,8	41,2	0,034
Nu	Bărbați	27	73	0,539
	Femei	38	62	0,003
	Total	33,3	66,7	0,034

În ceea ce priveşte HbA1c, 62,5% dintre sforăitori au avut DZ, 49,2% au avut prediabet şi respectiv 33,3% au avut valori normale (p=0,009). Semnificația statistică s-a păstrat doar cazul femeilor, deşi s-a evidențiat şi în cazul bărbaților că un procent mai mare de diabetici sforăie (tab. IV). HbA1c nu s-a corelat cu prezența pauzelor respiratorii în timpul somnului. Mai multe dintre femeile cu DZ au acest simptom față de cele cu prediabet sau cu metabolism glucidic normal, dar nesemnificativ statistic. La nici un bărbat cu DZ nu a fost identificat acest simptom.

TABELUL IV

Relația dintre sforăit şi HbA1c în lotul studiat

Sforăit	Sex	HbA1c			*p*
		Normal (%)	Prediabet (%)	Diabet (%)	
Da	Bărbați	40,0	46,9	62,5	0,468
	Femei	28,1	50,6	62,5	0,009
	Total	33,3	49,2	62,5	0,009
Nu	Bărbați	60,0	53,1	37,5	0,468
	Femei	71,9	49,4	37,5	0,009
	Total	66,7	50,8	37,5	0,009

Cât privește relația dintre somnolența diurnă şi HbA1c, nu s-au găsit diferențe semnificative între grupuri, deşi procentual mai mulți diabetici au somnolență față de prediabetici sau cei cu echilibru glicemic normal (45,8% *vs.* 33,6%, respectiv 38,2%). Prevalența somnolenței la femeile diabetice a fost de 5 ori mai mare față de bărbații diabetici (62,5% *vs.* 12,5%).

DISCUȚII

Apneea obstructivă în somn este un sindrom frecvent subdiagnosticat (13) atât în populația generală, cât şi în grupuri aflate la risc crescut, cum ar fi obezii, hipertensivii sau diabeticii. Se impune detecția precoce a persoanelor care suferă de această boală pentru că astfel s-ar putea preveni apariția unor boli sau îmbunătăți prognosticul acestora, mai ales că există un tratament cu be-

neficii substanțiale pentru aceşti pacienți.

În lipsa tratamentului adecvat, SAOS reprezintă o cauză de mortalitate crescută, existând studii care au investigat influența simptomelor de SAOS asupra mortalității (15, 16). Prevalența simptomelor şi semnelor SAOS variază în funcție de regiunea şi populația studiată (17-19).

În studiul nostru prevalența simptomelor de SAOS a fost de 44,1% pentru sforăit, 6,7% în cazul apneei cu martori şi 36,6% pentru somnolența diurnă. Netzer et al. (17) a găsit rezultate asemănatoare într-un studiu pe 6.223 pacienți ai medicilor generalişti, în Statele Unite ale Americii şi Europa. Sforăitul frecvent şi pauzele în respirație au fost egal raportate (44%) în cele două continente, diferența statistică fiind dată de somnolență (32,4% *vs.* 11,8%), obezitate şi/sau HTA (44,8% *vs.* 37,1%). În cadrul aceluiaşi studiu, s-a găsit că probabilitatea de avea risc înalt de apnee a fost de 5 ori mai mare (OR 5.60; IC 95%, 4,68-6,71) printre persoanele care au raportat istoric de HTA. Când rezultatele au fost ajustate pentru vârstă şi obezitate, corelația pozitivă s-a menținut între HTA şi sforăit. În ceea ce priveşte somnolența diurnă, femeile au raportat-o ca fiind de două ori mai mare față de bărbați. În studiul nostru, rezultatele au fost similare (66,7% *vs.* 33,3%).

Rezultatele studiului efectuat de Bousculet et al. (19) arată o prevalență mai mare a sforăitului (60,2%) şi a apneelor observate (12,3%), dar în cazul somnolenței diurne s-a înregistrat un procent mai mic (16,4%). Un alt studiu pe o populație americană a relevat că 59% din subiecți sforăie, 6% prezintă pauze respiratorii în timpul somnului, iar 26 % au raportat simptome legate de somnolență (18).

Rezultatele noastre au arătat că toate cele trei simptome analizate au fost mai frecvente la femei comparativ cu bărbații, spre deosebire de alte studii care au găsit la bărbați prevalențe mai mari ale sforăitului şi somnolenței (19), sforăitului şi apneei cu martori (17) sau doar a pauzelor respiratorii în timpul somnului (18). Fitzpatrick et al. (20) a raportat o rată a prevalenței net mai mare la bărbați (16%) față de femei (7%). Un studiu recent a identificat o frecvență mai mare a sforăitului la femei (21). În acelaşi studiu, persoanele considerate la risc au avut istoric de DZ (15,3%).

Martikainen et al. (22), într-un studiu pe 5 ani, a găsit că prevalența simptomelor posibil indicatoare ale prezenței apneei în somn (combinația de sforăit şi pauze respiratorii în timpul somnului) s-a ridicat la 4,7% în 1985 şi 7% în 1990. În lotul studiat de noi, prevalența asocierii dintre sforăit şi apneea cu martori a fost de 34,3%.

Bixler et al. (4) a arătat că 37,0% dintre bărbații şi 36,2% dintre femeile care sforăiau aveau HTA, demonstrând o asociere independentă între sforăit şi această afecțiune. Şi în lotul nostru, prezența HTA s-a corelat semnificativ cu sforăitul, atât la femei, cât şi la bărbați. Sivertsen et al. (12) a arătat că persoanele ce aveau simptome de SAOS au prezentat valori semnificative statistic mai mari ale tensiunii arteriale sistolice şi semnificativ mai mulți indivizi aveau DZ. În studiul nostru 62,5% dintre sforăitori aveau DZ.

CONCLUZII

Rezultatele noastre au arătat o prevalență mare a simptomelor precum şi a unor factori de risc pentru SAOS în populația studiată. Toate simptomele analizate au fost mai frecvent raportate de către femei.

Hipertensiunea arterială, obezitatea şi diabetul zaharat s-au asociat semnificativ statistic cu prezența sforăitului, atât la bărbați, cât şi la femei. Apneea cu martori s-a corelat cu HTA, dar numai în cazul femeilor hipertensive a fost identificat mai frecvent acest simptom.

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PANCREATIC INVOLVEMENT IN PEDIATRIC HEMATO-ONCOLOGY DISEASES

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PANCREATIC INVOLVEMENT IN PEDIATRIC HEMATO-ONCOLOGY DISEASES (Abstract): Pancreatic tumors in children and adolescents are rare and represent a different spectrum of cancers in pediatric age. Pancreatic involvement in pediatric hematology and oncology can occur in primary malignant pancreatic tumors or secondary to chemotherapy regimens. Early diagnosis and complete surgical resection of most pancreatic malignancies is associated with long-term survival. Also, childhood cancer treatment increases significant the risk of pancreatic involvement. **Keywords:** PANCREATIC, PEDIATRIC, ONCOLOGY.

Primary malignant pancreatic neoplasms are exceedingly rare in pediatric age and have a better outcome than the adult pancreatic tumors. There is a paucity of data on malignant pancreatic neoplasms in children and the literature consists predominately of single institution case series spread on long periods of time (1). An incidence of all tumors is 0.18 case per million people of malignant pancreatic tumors in 0-19 age group was reported in United States (2). According to the World Health Organization pediatric tumors of the pancreas are classified into exocrine (pancreatoblastomas, solid pseudopapillary tumor, pancreatic adenocarcinoma) and gastroenteropancreatic neuroendocrine tumors.

Pancreatoblastomas (PB) are considered of embryonic origin, as the result of the persistence of pluripotent pancreatic stem cells after the 8 weeks of gestation. PB are very rare neoplasms that usually present in early years of life, most common in children aged 1 to 8-year-old, with a mean age of 4 years (1, 3).

The incidence is 0.004 cases per 100.000 population of all ages per year (4). Over a thirty-year period, ten cases of pancreatoblastoma were recorded in North American population-based Surveillance, Epidemiology and End Results (SEER) (5) and eleven cases in United Kingdom National Registry of Childhood Tumors (6). European cooperative study group for pediatric rare tumors (*EXPeRT* group) collected 20 cases of pancreatoblastomas between 2000 and 2009 (7).

Typically, these tumors are well encapsulated, with a nodular surface. Microscopic appearance consists in characteristic squamous nest (8).

Symptoms include weight loss, upper

abdominal pain, abdominal mass, diarrhea. Because these tumors are asymptomatic most of the time, they may be quite large at the time of the diagnosis. Elevation of serum alpha fetoprotein may occur in most of the cases, but there are no specific markers for pancreatoblastomas.

PB can behave aggressively, distant metastases are usually present at the time of diagnosis and are in liver, lungs, lymph nodes. Also, the invasion into the vessels or nerves may occur. Because of its rarity, standardized diagnostic and therapeutic guidelines are not available yet. The tumor management depends of tumor localization, size and the presence of metastasis. Main stray of therapy is surgical resection, even in high stages PB. PLADO (cisplatin and doxorubicin) regimen is effective for patients diagnosed with PB. Also, other chemotherapy regimens including etoposide, ifosfamide, vincristine, bleomycin can be used. In patients with resected pancreatoblastomas the 5-year survival rate is between 43% - 80% (7, 9).

Solid pseudopapillary tumor (SPT), also known as papillary cystic tumor, is a rare pancreatic neoplasm with a low potential for malignancy. SPT behaves indolently compared to other malignant pancreatic tumors. SPT accounts for 1%-3% of solid pancreatic malignancies and 22% of SPTs occurs in children, mainly in adolescent females, with a male-female ratio of 1:6.5 (10, 11).

Although SPT are solid tumors, they present cystic degeneration secondary to the loss of cellular cohesion (fig. 1). The pseudopapillary pattern is determinate by the relatively preserved cellular cohesion around the microvasculature (fig. 2). Also, aggregates of foamy macrophages, extensive fibrosis, hemorrhage, calcifications may be seen. Immunohistochemical - chromogranin is negative (12).

Fig. 1. Solid pseudopapillary tumor.

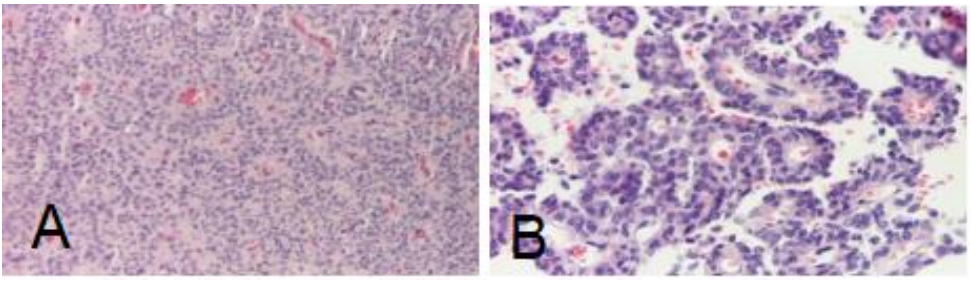

Fig. 2. Solid pseudopapillary tumor – islands of viable tumor with pseudo-papillary, rosette-like architecture (x100 HE) (A); Pseudopapillary pattern (x200 HE) (B).

Clinical findings are nonspecific: palpable abdominal mass, abdominal pain, pancreatitis or dyspepsia. Laboratory tests results, pancreatic exocrine and endocrine

function are preserved. There is no preoperative serum tumor marker predictive for solid pseudopapillary tumor (13). On ultrasound, SPTs appear as encapsulated, well demarcated masses, with solid and cystic components. Computed tomography and magnetic resonance imaging are needed for staging (14). The metastatic lesions are uncommon, in a small percentage of patients are present in the liver, omentum and peritoneum at the time of diagnosis (1). Depending on location, the suitable treatment with excellent results for solid pseudopapillary tumor is complete surgery. Local invasion limited metastatic lesions or recurrence do not contraindicate surgical resection (10). Chemo-radiotherapy and oral retinoid therapy were successfully used in patients with SPT, but their role in SPT treatment is not defined (15, 16). Adjuvant therapy is indicated when surgical approach is inappropriate (16). SPTs are usually resectable and associated with excellent prognosis. Several studies have shown high cure rate, around 95% of patients, that underwent complete surgical excision (17).

Pancreatic adenocarcinomas, ductal adenocarcinoma and acinar cell carcinoma, are the less common pancreatic pediatric tumors, with a poor prognosis.

Ductal adenocarcinoma it is exceptionally found in children, it has been reported in teenaged patients. It common affects the head of the pancreas (18).

Presenting symptoms are obstructive jaundice, abdominal pain, weight loss (19). Ultrasound of the abdomen often show a small hypo vascular mass, with inconstant dilatation of the pancreatic duct due to the tumoral mass. Ductal adenocarcinoma has a similar prognosis for children and adults (20, 21).

Imagistic studies of acinar cell carcinoma describe well circumscribed mass, with area of necrosis. Metastatic lesions are common at the diagnosis. The prognosis seems to be favorable in children than in adults (22).

Pancreatic islet cell tumors are endocrine tumors seen in infants and children. They can be classified as functioning (insulinomas, gastrinomas, ACTHomas, VIPomas) or nonfunctioning.

Gastrinomas are more frequently malignant tumors. The diagnosis is based on hypergastrinemia and gastric hypersecretion. Because of their extra pancreatic localization, indium octreotide scintigraphy is used (23). Surgical resection is indicated in all cases. Chemotherapy has been used in several cases of metastatic gastrinomas.

Insulinomas are quite rare among children, 4 cases per million individuals per year and only ten percent of them are malignant. Insulinomas are functional insulin secreting neuroendocrine tumors. The hypoglycemia induced symptoms consist in dizziness, sweating, headaches and Whipple's triad is described in these patients (24, 25) (fig. 3).

The biological investigations of insulinomas includes glucose levels quickly dropping, increased C-peptide levels and high levels of circulating insulin and proinsulin.

Surgical resection is recommended in all patients diagnosed with insulinoma. Chemo-radiotherapy, chemo-embolization, biotherapy is also indicated in malignant forms of insulinomas (26). In adult population, in addition to chemo-radiotherapy, there seems to be positive effects associated to several natural polyphenols used to induce apoptosis in some types of cancer (27). Nonfunctioning pancreatic endocrine tumors have no specific symptoms and delay the diagnosis. Often, patients present with abdominal pain secondary to local invasion or metastases (28).

Chemotherapy drugs used in the treatment of many pediatric solid tumors and hematological malignancies can induce severe pancreatic complications which may discontinue the chemotherapy regimens and prolong hospitalization.

Acute pancreatitis (AP) is known as a possible complication of high-risk chemotherapy regimen. A variate number of chemotherapeutics such as L-asparaginase, Mercaptopurine, Cytosine arabinoside, ifosfamide, cisplatin, doxorubicin is considered implicated in chemotherapy induced acute pancreatitis (29).

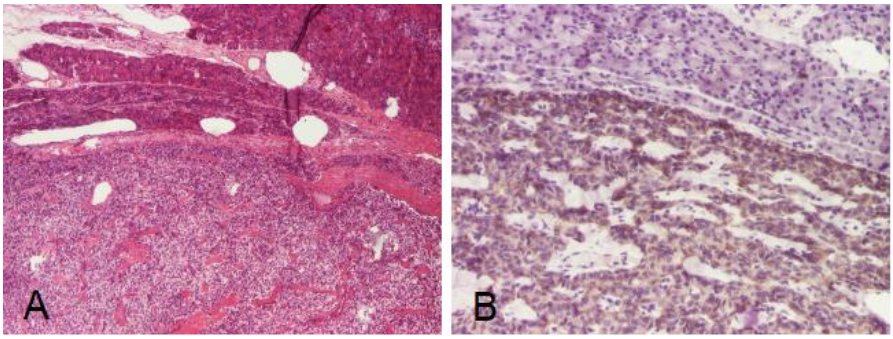

Fig. 3. Pancreatic insulinoma: solid pattern of pancreatic insulinoma (x40 HE stains) (A); Immunohistochemistry positive for chromogranin A (x100) (B).

CONCLUSIONS

Pancreatic involvement in pediatric hematology and oncology can occur in primary malignant pancreatic tumors or secondary to chemotherapy regimens used in childhood cancers. Prognosis of pediatric patients with malignant pancreatic tumors is variable and depends of histologic type. Also, monitoring of pancreatic toxicity and initialization of treatment as early as possible is recommended in children who undergo chemotherapy.

ACKNOWLEDGEMENTS

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ASPECTE PRIVIND CONSUMUL DE PRODUSE ETNOBOTANICE

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ISSUES ON THE USE OF ETHNOBOTANICALS (Abstract). **Aim**: To identify the risk factors for the use of ethnobotanicals and the people at risk to becoming users. **Material and methods**: This questionnaire basez objective population study included 89 subjects aged 14 to 42 years. **Results**: 19.10% of the subjects admitted using ethnobotanicals, most of them being 20-25 years old (35.29%), males (82.35%), with an average educational level, and unmarried. It should be noted that 23.52% of the users were underage. 85.15% of the subjects first used ehnobotanicals out of curiosity, 29,41% under the influence of friends (94.11% of them having friends that use these products). 11.76% of the respondents stated that they did not know what they used, the product being offered to them by friends as a cigarette or candy. Favorite places for consumption were: at home (64.70%), parks and public areas (23.52%), coffee shops and night clubs (11.76%). Cigarette was the preferred method of use (70.58%), followed by joint (23.52%) and "salt bath" (5.88%). The described effects were: hallucinogen (35.29%), aphrodisiac (29.41%) and relaxing (23.52%). To strengthen these effects, most users associated alcohol and tabacco (70.58%), or other illegal drugs (7.14%). According to this study all users were familiar with the risks associated with the use of these products, but only 64.70% agree with closing the weed-shops. **Conclusions**: The obtained data are of help in initiating prevention and rehabilitation programs in school and colleges. **Key words**: ETHNOBOTANICAL PRODUCTS, DRUGS, CONSUMPTION, RISK FACTORS.

România se situează pe locul IV în lume la consumul de plante etnobotanice, după Anglia, Germania şi Olanda (1,2,3).

Prezenta lucrare este o secțiune dintr-un studiu mai amplu efectuat la Spitalul Clinic Județean de Urgențe „Sf. Spiridon" Iaşi, şi are drept scop identificarea principalilor factori de risc care duc la consumul de produse etnobotanice.

MATERIAL ŞI METODE

S-a întreprins o anchetă populațională, realizată pe data de 25 iunie 2011, cu ocazia Zilei Mondiale Antidrog, în oraşul Iaşi, în diferite puncte cu caracter informațional. Lotul inclus in studiu a cuprins 89 subiecți cu vârsta între 14 şi 42 ani, evaluati pe bază de chestionar (19 itemi) care a vizat înregistrarea feedback-ului subiectiv cu privire la produsele etnobotanice şi implicit la consumul acestora, iar rezultatele au fost analizate statistic. Cercetarea clinică a permis evaluarea percepției subiective a participanților referitor la consumul de produse etnobotanice, starea psihică, relații sociale şi starea generală de sănătate. Prelucrarea

statistică s-a realizat prin crearea unei baze de date computerizată cu ajutorul programului *SPSS 18.0*, ulterior realizându-se interpretarea rezultatelor.

REZULTATE ŞI DISCUŢII

În urma prelucrării datelor reiese că, majoritatea persoanelor din lotul de studiu se încadrează în categoria de vârstă 20-25 (47,19%), 18-20 ani (25,84%) şi minori (13,48%), iar distribuţia pe sex arată o repartiţie relativ egală a subiecţilor (44,94% femei şi respectiv 55,06% bărbaţi) (fig.1, 2).

Nivelul educaţional situează majoritatea persoanelor cu studii în curs de desfăşurare (58,42%), studii liceale (17,97%) şi universitare (20,22%).

Din totalul persoanelor chestionate, 19,10% au recunoscut că au consumat produse etnobotanice, ponderea cea mai mare revenind sexului masculin (82,35%). De remarcat este faptul că, 23,52% dintre consumatori sunt minori, un procent mai mare revenind categoriei de vârstă 20-25 ani (35,29%) (fig. 3).

Majoritatea consumatorilor evaluaţi prin ancheta populaţională sunt necăsătoriţi (82,35%) şi doar 11,76% au studii universitare (studii gimnaziale 5,88%, studii liceale 58,82%, studii postliceale 23,52%) (fig. 4).

Dintre consumatori, 64,70% au achiziţionat produsele de la „*weed-shop*”, 5,88% de la dealeri, 29,41% fiindu-le oferite de prieteni. Consumul produselor etnobotanice din curiozitate a fost semnalat la 82,35%, influenţa prietenilor fiind responsabilă de 23,52% din cazuri, iar 11,76% au declarat că nu au ştiut ce consumă, produsul fiindule oferit sub formă de bomboane sau ţigări.

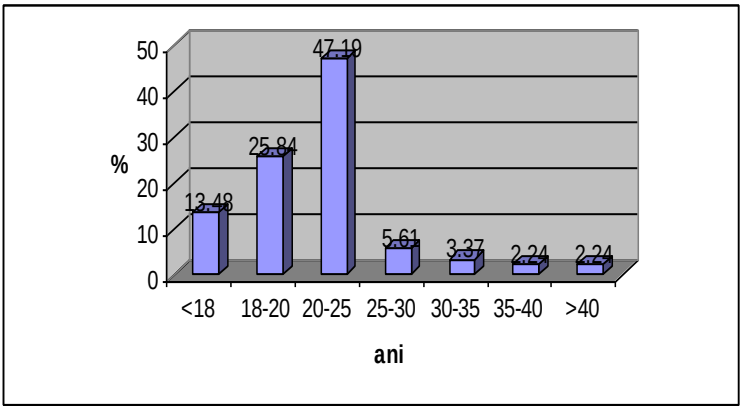

Fig. 1. Distribuţia pe grupuri de vârstă a persoanelor incluse în studiu

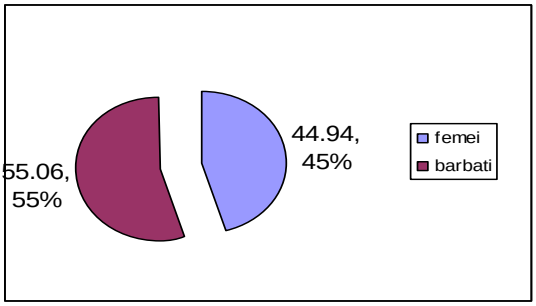

Fig. 2. Distribuţia persoanelor incluse in studiu în relaţie cu sexul

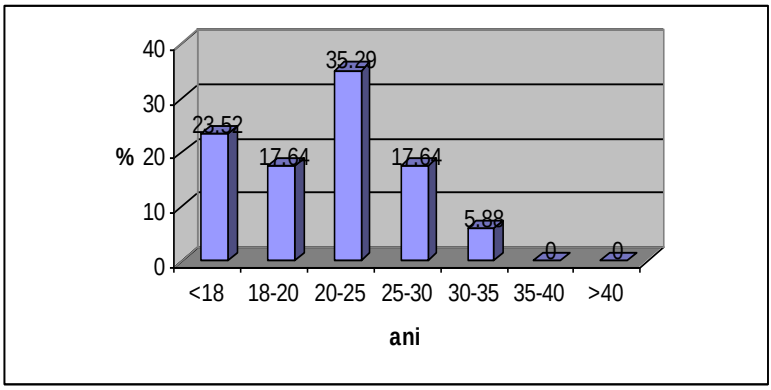

Fig. 3. Distribuția pe grupuri de vârstă a consumatorilor de produse etnobotanice

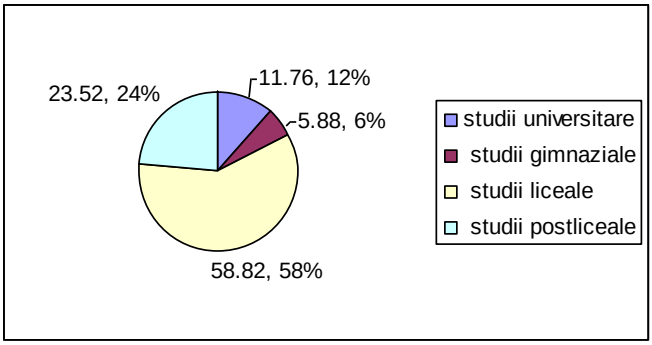

Fig. 4. Distribuția consumatorilor de produse etnobotanice în relație cu nivelul educational

Locurile preferate de consum al acestor produse sunt: locuința proprie (64,70%), parcuri, spații publice (23,52%), cafenele şi cluburi de noapte (11,76%).

Modalitatea preferată de consum este țigara (70,58%), urmată de *joint* (23,52%) şi "sare de baie" (5,88%). Efectul perceput de consumator a fost descris ca fiind halucinogen (35,29%), afrodisiac (29,41%) şi relaxant (23,52%). Pentru potențarea acestor efecte majoritatea consumatorilor asociază produsele etnobotanice cu alcool şi tutun (76,47%), sau cu alte droguri ilegale (11,76%). Cele mai cunoscute şi implicit consumate produse etnobotanice sunt "Pure by Magic" şi "Flower Magic" (68%), ambele produse fiind cunoscute ca îngrăşăminte pentru plante. Modalitatea de consum a acestor produse este administrarea intranazală.

Analiza cromatografică a produsului "Pure by Magic" a dus la identificarea a două pirovalerone: MDPBP [1-(3,4- metilendioxifenil)-2-(pironidil)-1-butanonă] şi MDPV (metilendioxipirovalerona), droguri psihoactive cu proprietăți stimulente (similare cocainei şi amfetaminei) (fig. 5, 6). Aceste substanțe determină creşterea concentrației extracelulare a norepinefrinei şi a dopaminei, implicit creşterea neurotransmițătorilor andrenergici şi dopaminergici, efect responsabil de o serie de manifestări adverse care pot duce la decesul persoanei consumatoare (4,5) .

Începând cu anul 2010, MDPV şi

MDPBP au fost interzise în majoritatea țărilor. În România aceste pirovalerone au fost introduse pe lista de substanțe interzise, proiect de lege care nu a fost încă aprobat.

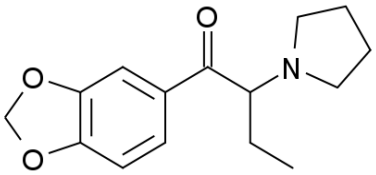

Fig. 5. MDPBP [1-(3,4-metilendioxifenil)-2-(pirodinil-1-butanona]

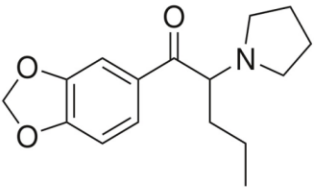

Fig. 6. MDPV (metilendioxipirovalerona)

Studiul a evidențiat faptul ca toți consumatorii cunosc riscurile la care se expun atunci când folosesc aceste produse, însă, doar 64,70% sunt de acord cu închiderea magazinelor care le comercializează .

CONCLUZII

Lotul de studiu a cuprins 89 de respondenți cu vârste între 14-42 ani. Din totalul persoanelor evaluate, 19,10% au recunoscut că au consumat produse etnobotanice, ponderea cea mai mare aparținând grupului de vârstă 20-25 ani (35,29%), fiind de sex masculin (82,35%), cu un nivel educațional mediu şi necăsătorite.

Principalii factori de risc care pot duce la consumul de produse etnobotanice s-au dovedit a fi anturajul şi accesibilitatea la achiziționare a acestor produse.

Rezultatele cercetărilor pot fi utilizate pentru elaborarea unor programe de prevenție şi reabilitare a diferitelor grupuri de vârstă expuse la riscurile consumului de produse etnobotanice.

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STUDIUL DENSITĂŢII MINERALE OSOASE LA PACIENTE CU POLIARTRITĂ REUMATOIDĂ

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BONE MINERAL DENSITY IN PATIENTS WITH RHEUMATOID ARTHRITIS (Abstract): Patients with rheumatoid arthritis (RA), an immune-mediated inflammatory rheumatic disease with peripheral and systemic involvement, are at increased risk of bone loss and fractures. There are many reasons for the high prevalence of osteoporosis (OP) in RA, including both traditional and specific risk factors such as pain and loss of joint function, medication (corticosteroids, methotrexate), and increased proinflammatory cytokines. **Aim:** To evaluate bone mineral density status in RA patients, focusing on potential relation with classical risk factors for OP. **Material and methods:** One-year prospective observational study on 83 consecutive postmenopausal women, 43 diagnosed with RA (group I), and 40 healthy controls (group II) with no previous condition and medication known to affect bone metabolism and turnover. Bone mineral density (BMD) and T-score evaluated by dual X-ray absorbtiometry (DXA) at three standard skeletal sites (L1-L4 lumbar spine, hip and forearm) (Hologique QDR 100 device), and classical risk factors for osteoporosis were assessed in all patients according to a predefined protocol. Data were analyzed in SPSS-13 using ANOVA, t-Student, chi-square and ROC (Receiver Operator Characteristic). **Results:** Decreased BMD was reported in the majority of RA cases, mainly in the spine and femoral neck (86%), but also in total hip (72%); moreover, osteoporosis was commonly demonstrated in lumbar spine and osteopenia at hip level. Statistically significant differences between diagnostic categories (normal, osteopenia, osteoporosis, WHO 1994) ($p<0.05$), while no significant differences between mean BMD levels in women with and without RA at different skeletal sites were found (t-student, $p>0.05$). However, considerable BMD variation (51.7% to 102.3%) was suggested in RA as compared to non-RA patients (14.3% to 27%) (ANOVA). Significant differences in mean T-score at total hip and forearm (mainly 33% radius) were noted in patients with and without RA ($p<0.05$). No relation between osteodensitometric parameters and classical risk factors for OP has been identified in RA, except menopause. Indirect weak statistically significant correlations were found between mean T-score and menopause duration at all skeletal levels (Pearson's rank correlation, $p<0.05$), except for the femoral neck ($r=+0.03$, $p<0.05$). **Conclusions:** Decreased BMD is commonly seen in RA patients. Several characteristics based on DXA assessment have been identified, including preference for distinct skeletal sites (spine, hip, distal forearm), and the particular intervention of menopause. **Key words**: BONE MINERAL DENSITY, OSTEOPOROSIS, RHEUMATOID ARTHRITIS, MENOPAUSE

Poliartrita reumatoidă (PR) este o boală dizabilitantă de tip autoimun, cu evoluţie periferică, la nivel articular, şi sistemică (1). Studiile din literatura de specialitate au demonstrat că bolnavii cu PR au risc crescut pentru pierderea de masă osoasă şi fractură de fragilitate (2, 3). În prezent se recunosc multiple mecanisme inductoare ce includ: durerea şi limitarea funcţională, medicaţia (glucocorticoizi, AINS, metotrexat), precum şi inflamaţia ce caracterizează evoluţia bolii (1, 4). Dat fiind faptul că osteoporoza la bolnavul cu PR este o complicaţie redutabilă, generatoare de durere şi fractură, apare necesitatea particularizării ei sub raportul factorilor de risc generaţi de boala de bază, clinici şi terapeutici (5, 6).

Studiul de faţă îşi propune măsurarea comparativă a densităţii minerale osoase (DMO) prin metoda absorbţiometriei duale cu raze X (DXA) la femei în etapa postmenopauzală, cu sau fără diagnosticul de PR, cu scopul evidenţierii unor particularităţi impuse de boala de bază, dar si pentru a evidentia eventualele relaţii particulare cu factorii tradiţionali de risc pentru osteoporoză în cazul pacientelor cu PR, cu semnificaţie pentru practica clinică.

MATERIAL ŞI METODĂ

Am realizat un studiu prospectiv observaţional cu durata de 12 luni asupra unui lot format din 43 paciente consecutive diagnosticate cu poliartrită reumatoidă (criteriile ACR, 1987) (lotul I) spitalizate în Clinica Reumatologie, Spitalul Clinic de Recuperare Iaşi, în perioada 2009-2010, la care s-a urmărit densitatea minerală osoasă (metoda DXA) şi factorii de risc clasici pentru osteoporoză, respectiv aportul de calciu şi vitamina D, excesul de noxe (alcool, cafea, fumat), istoricul familial de fractură de şold, indicele de masă corporală (IMC), menopauza precoce şi corticoterapia cronică (7, 8). Evaluarea osteodensitometrică DXA (aparat Hologic QDR) a fost realizată pe situsuri scheletale multiple, la nivel vertebral (L1-L4), şold (col femural, şold total) şi antebraţ (treimea distală, medie, proximală, antebraţ total), interesându-ne atât T-*score* cât şi densitatea minerală osoasă (DMO, g/cm^2); au fost utilizate valorile prag validate internaţional (OMS, 1994) pentru a defini categoriile diagnostice, şi anume : $\pm$1 deviaţie standard (DS) pentru T-*score* în limite normale, -1 DS < T-*score* <-2.5DS pentru a defini osteopenia şi T-*score*<-2.5 DS pentru osteoporoză. Au fost luate în atenţie doar paciente cu poliartrită reumatoidă aflate în etapa postmenopauză.

Lotul martor (lotul II), comparabil ca număr, vârsta şi sex cu lotul de studiu, a fost alcătuit din 40 femei în postmenopauză nediagnosticate cu patologie reumatismală inflamatorie cronică (poliartrită reumatoidă, lupus, spondilartrite) sau patologie osoasă metabolică. Determinările DXA au respectat şi în acest lot protocolul standard propus pentru lotul I.

Datele au fost analizate şi interpretate statistic în programele EXCEL, EPIINFO, SPSS, interesându-ne testele ANOVA, t-Student, chi-pătrat şi balanţa de sensibilitate/ specificitate - curba ROC (Receiver Operator Characteristic).

REZULTATE

Parametri osteodensitometrici

Repartiţia pe categorii diagnostice la pacientele cu şi fără PR

La pacientele diagnosticate cu PR, analiza frecvenţelor diferitelor catogorii diagnostice raportat la parametrul central de analiză, DXA, a relevat următoarea distri-

buţie pe regiunile scheletale investigate: (i) T-*score* în limite normale - coloană lombară 14%, şold total 27,9%, col femural 14%, (ii) paciente cu osteopenie - coloană lombară 32,6%, şold total 58,1%**,** col femural 53,5%, (iii) paciente cu osteoporoză - coloană lombară 53,5%, şold total 14%**,** col femural – 32,6%. Astfel, în majoritatea cazurilor de PR, reţinem afectarea densităţii minerale osoase indiferent de prezenţa sau nu a factorilor clasici de risc pentru osteoporoză; procente maximale (86%) sunt decelate atât la nivelul segmentului lombar cât şi la nivelul colului femural, în timp ce şoldul total este afectat în procent de 72%. Mai mult, se pare că la pacientele cu PR osteoporoza predomină la nivel vertebral, în timp ce valori ale DMO şi T-*score* în interval de osteopenie sunt reprezentate preferenţial la nivelul şoldului (total, col femural).

Evaluarea DXA la pacientele fără PR a evidenţiat următoarele aspecte: (i) T-*score* în limite normale - şold total 7,5%, (ii) paciente cu osteopenie - coloană lombară 37,5%, şold total 70,0%, col femural 55,0%, (iii) paciente cu osteoporoză - coloană lombară 62,5%, şold total 22,5%, col femural 45,0%.

Studiul comparativ pe cele două loturi selectate a evidenţiat frecvenţa mai mare a afectării DMO la pacientele fără PR; ca şi în lotul de paciente cu PR, predomină însă osteoporoza la nivel vetebral şi osteopenia la nivelul şoldului. Mai mult, am înregistrat diferenţe semnificative statistic între frecvenţele diferitelor categorii diagnostice la paciente cu şi fără PR ($p<0.05$) pe regiunile scheletale investigate. Datele prezentate sunt sumarizate în figura 1 şi în tabelul I.

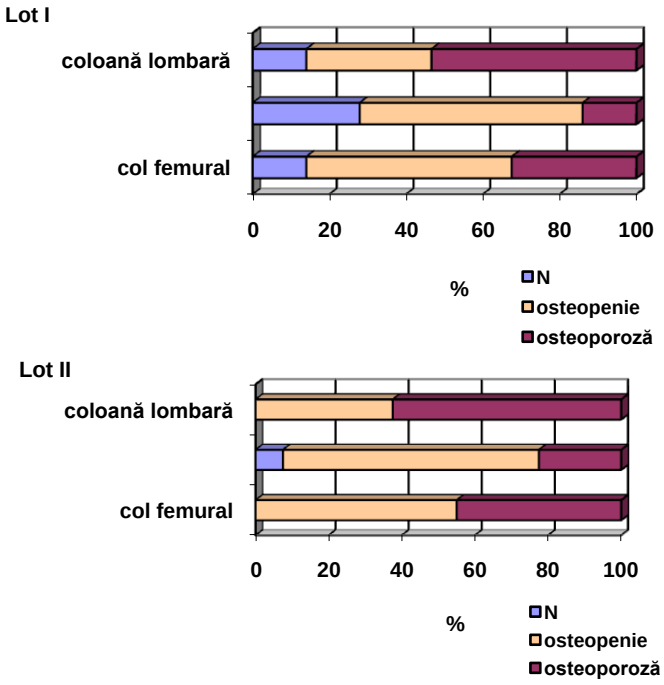

Fig. 1. Distribuţia pacientelor din loturile I şi II în funcţie de clasificarea DXA

TABELUL I

Diferenţe statistice în funcţie de clasa de boală pe loturi de studiu

Clasificarea OMS	Lot I		Lot II		p
	n	%	n	%	
Coloană lombară					
Normal	6	14,0	0	0	**0,042**
Osteopenie	14	32,6	15	37,5	
Osteoporoză	23	53,5	25	62,5	
Şold total					
Normal	12	27,9	3	7,5	**0,033**
Osteopenie	25	58,1	28	70,0	
Osteoporoză	6	14,0	9	22,5	
Col femural					
Normal	6	14,0	0	0	**0,042**
Osteopenie	23	53,5	22	55,0	
Osteoporoză	14	32,6	18	45,0	

Densitatea minerală osoasă la pacientele cu şi fără PR

Valorile medii ale densităţii minerale osoase la toate nivelurile investigate la pacientele cu PR au fost următoarele: coloana lombară (0,780 ± 0,140), şold total (0,780 ± 0,130), col femural (0,680 ± 0,100), antebraţ cu valori diferenţiate pe cele trei regiuni: antebraţ total (0,460 ±0,080), 1/3 radius distal (33% radius) (0,530 ±0,090), treimea medie a antebraţului (0,460 ± 0,090), antebraţ ultradistal (0,360±0,090).

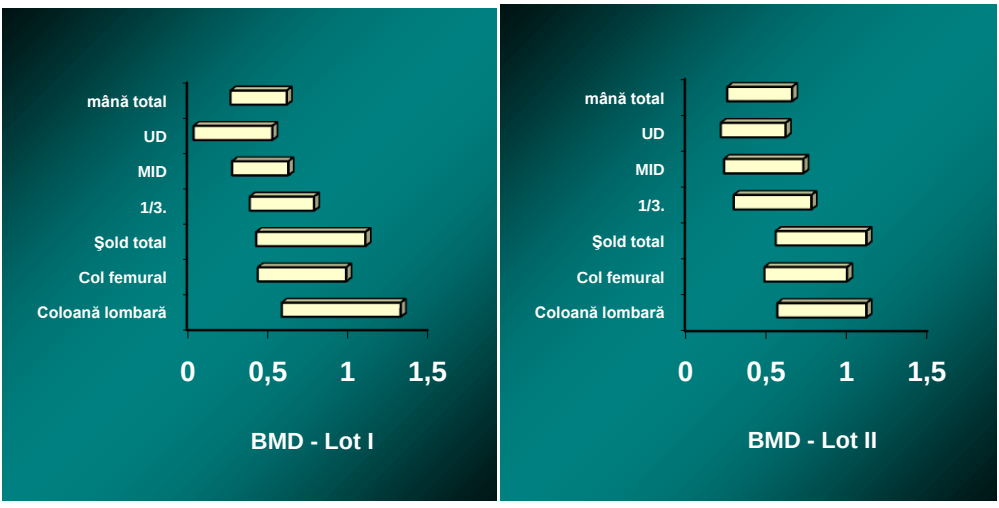

Fig. 2. Seriile de valori ale DMO pe niveluri analizate comparativ pe loturi de studiu

Valorile medii ale densităţii minerale osoase pentru pacientele fără PR au fost următoarele: coloana lombară (0,080± 0,150), şold total (0,790±0,130), col femural (0,700±0,130); pentru antebraţul total valorile înregistrate au fost de 0,480±0,100, pentru regiunea 1/3 radius distal (0,570± 0,130), pentru treimea medie a antebraţului (0,490±0,120), iar pentru antebraţul ultradistal valorile au fost de 0,390±0,100.

Nu s-au înregistrat diferenţe semnificative statistic între valorile medii ale DMO pe diferite regiuni scheletale la pacientele celor două loturi. S-au obţinut astfel următoarele rezultate pentru testul *t-Student*: 1/3 radius distal (1,62), treimea medie a antebraţului (1,28) şi antebraţ ultradistal (1,43).

Cu toate acestea, dacă în lotul de PR se constată variaţii foarte ample ale DMO (de la 51,7% până la 102,3%), pacientele fără PR se definesc prin valori mai omogene ale DMO (14,3% - 27%) (test ANOVA).

T-score la pacientele cu şi fără PR

Valorile individuale medii ale T-*score* pe nivelurile scheletale investigate au fost următoarele, în cazul lotului de PR: coloana lombară (-2,32±1,20), şold total (-1,46±1,29), col femural (-2,13±1,04); pentru regiunea antebraţului avem: antebraţ total (-2,22±1,57), 1/3 radius distal (-2,69±1,55), treimea medie a antebraţului (-2,59±1,58), antebraţ ultradistal (-1,28± 1,31).

Pentru pacientele fără PR au fost raportate următoarele valori medii ale T-*score*: coloana lombară (-2,52±0,41), şold total (-2,04±0,55), col femural (-2,33± 0,52), antebraţ total (-2,59±0,42), 1/3 radius distal (-3,14±0,45), treimea medie a antebraţului (-2,97±0,48), antebraţ ultradistal (-2,87± 0,42). Datele sunt prezentate sintetic în figura 3.

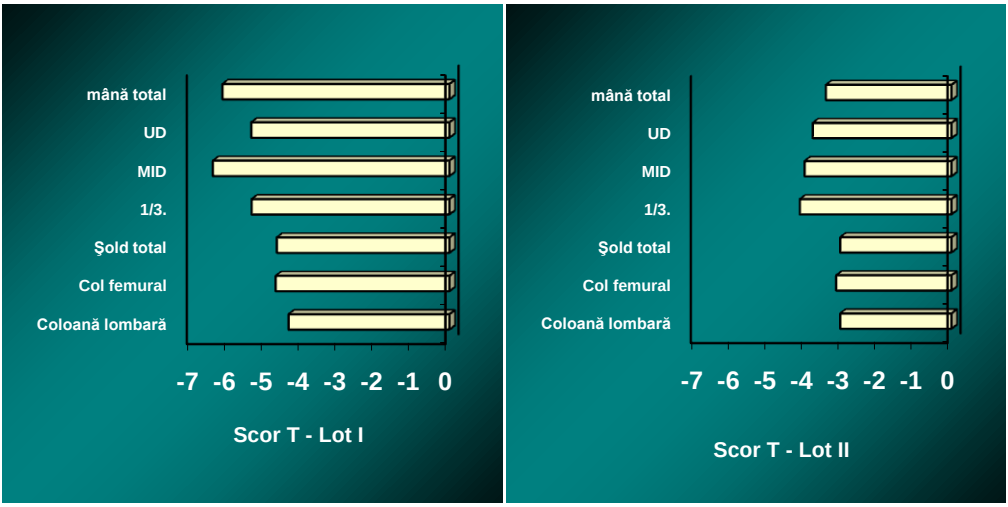

Fig. 3. Seriile de valori ale T-*score* pe niveluri analizate comparativ pe loturi de studiu

Reţinem faptul că valorile medii ale T-*score* confirmă caracteristicile de frecvenţă enunţate anterior pentru ambele loturi, şi anume predominanţa osteoporozei la nivel de coloană lombară şi a osteopeniei la nivelul şoldului (total, col femural).

Am înregistrat diferenţe semnificative statistic între valorile medii ale T-*score* între

cele două loturi de studiu doar la nivelul şoldului total şi la antebraţ, la nivelul antebraţului ultradistal. Datele obţinute sunt următoarele: *t-Student* pentru coloana lombară este de 1,03, pentru şoldul total 2,70, col femural 1,12, antebraţ total 1,49, 1/3 radius distal 1,82, treimea medie a antebraţului 1,50, antebraţ ultradistal 7,55 ($p<0,05$). Celelalte regiuni scheletale nu au înregistrat diferenţe semnificative statistic pentru T-*score* mediu.

În general, valorile medii ale parametrilor osteodensitometrici nu prezintă diferenţe semnificative între cele două loturi de studiu.

Factori clasici de risc pentru osteoporoză

Variaţia seriilor de valori individuale ale subiecţilor din cele două loturi a indicat valori comparabile raportat la aspectele legate de vârstă, greutate, talie şi IMC. Astfel, pentru pacientele cu PR, în ceea ce priveşte valorile medii, acestea au fost de 60,67±7,35 , iar pentru IMC – 26,10±4,18. Pentru pacientele din lotul II valorile sunt, respectiv, de 59,38±4,71, pentru vârstă, şi de 25,50±2,33 pentru IMC.

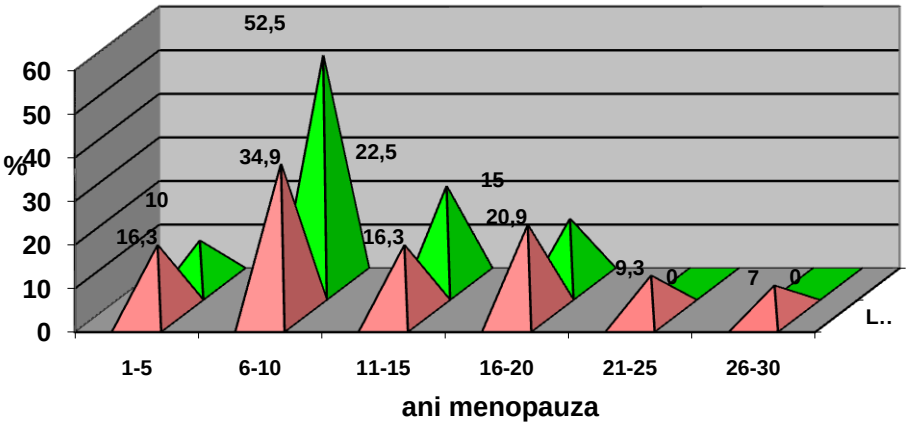

Fig. 4. Distribuţia pacientelor în funcţie de anii de menopauză

Distribuţia în funcţie de anii de menopauză este descrisă în fig. 4, susţinând predominanţa intervalului 5-10 ani postmenopauză în ambele loturi de studiu (35%, respectiv 53%).

Corelaţii între parametrii osteodensitometrici şi factorii de risc clasici pentru osteoporoză

T-score şi durata menopauzei la pacientele cu PR

În general au fost observate doar slabe corelaţii indirecte între *T-score* şi vechimea menopauzei (r Pearson, $p<0,05$); astfel notăm următoarele valori ale parametrului "r", funcţie de sediul scheletal investigat: coloana lombară (r=-0,10), col femural (r=+0,03), şold total (r=-0,13), antebraţ total (r=-0,22), treimea medie a antebraţului (r=-0,26), antebraţ ultradistal (r=-0,20), 1/3 radius distal (r=-0,14). Datele prezentate evidenţiază scăderea T-*score* cu atât mai mult cu cât vechimea menopauzei este mai mare.

Excepţie de la corelaţia între durata menopauzei şi T-*score* se observă la colul

femural (r = +0,03); cele mai puternice asocieri indirecte între T-*score* cu vechimea menopauzei se observă la mână: r = -0,22 la nivelul 1/3 radius distal; r = -0,26 în treimea medie a antebraţului; r = -0,20 la nivelul antebraţului ultradistal.

Nu au fost decelate relaţii cu semnificaţie statistică între parametrii osteodensitometrici şi alţi factori clasici de risc ai OP la pacientele cu PR.

DISCUŢII

Studiul osteodensitometric efectuat oferă o imagine complexă asupra statusului densităţii minerale osoase la paciente cu poliartrită reumatoidă aflate în etapa de postmenopauză. Datele obţinute se înscriu între cele menţionate în literatura de specialitate a ultimilor ani, care precizează faptul că poliartrita reumatoidă se asociază atât cu pierderea localizată (9, 10) cât şi cu cea generalizată (11) de masă osoasă, creşterea riscului de osteoporoză şi fractură de fragilitate (12, 13)**.**

Studiul de faţă precum şi cele prezentate în literatura internaţională demonstrează că măsurarea pierderii de os la pacienţii cu PR prin DXA la nivelul diferitelor segmente (atât central cât şi periferic) este o metodă de diagnostic importantă pentru detectarea osteoporozei (14)**.**

Retinem în studiul de faţă o serie de particularităţi legate în principal de procentul mare de paciente care prezintă scăderea densităţii minerale osoase (osteopenie, osteoporoză) în poliartrita reumatoidă şi situsurile scheletale cele mai afectate (listate în ordinea descrescătoare a frecvenţei, coloană, şold, antebraţ distal). Acest lucru este semnificativ în special pentru pierderea periferică de masă osoasă, care se găseşte în relaţie directă cu variabilele specifice bolii, precum şi factorii de risc cunoscuţi pentru osteoporoză.

Spre deosebire de cele demonstrate în cercetarea noastră, Haugeberg şi colab, 2007 (10) nu au identificat diferenţe în ceea ce priveşte densitatea minerală osoasa la nivel lombar şi şold total; mai mult, au fost evidenţiate valori semnificativ scăzute ale densităţii la nivelul antebraţului prin tehnica DXA. Scăderea DMO la nivelul regiunii distale a antebraţului a fost raportată în PR de Iwamoto şi colab, 2002 (3), în timp ce o scădere semnificativă a DMO la pacienţii cu PR la nivelul tuturor regiunilor scheletale examinate cu excepţia segmentului vertebral, a fost demonstrată şi de Shibuya şi colab., 2002 (15).

Faptul că pacientele cu PR înrolate în studiul nostru au prezentat scăderea DMO în majoritatea cazurilor independent de existenţa factorilor de risc clasic recunoscuţi pentru osteoporoză, sugerează participarea altor factori la afectarea DMO la această categorie de paciente. Intervenţia dezechilibrului citokinic (predominanţa citokinelor proinflamatorii), a activităţii PR, afectarea funcţională cu limitare semnificativă a exerciţiului fizic cotidian precum si medicaţia cu impact asupra metabolismului osos (corticoterapie, metotrexat) sunt factori recunoscuţi în determinismul OP secundare PR (16,17).

CONCLUZII

Poliartrita reumatoidă înregistrează pierderea de masă osoasă ca o complicaţie frecventă care necesită diagnostic şi conduită terapeutică specială. Studiul comparativ al încărcării minerale osoase centrale (coloană, şold, antebraţ) prin metoda DXA pune în valoare o serie de particularităţi legate de boala de bază, care se referă în principal la sediile cele mai afectate precum şi la relaţia cu menopauza.

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ASPECTE TERAPEUTICE ÎN INSUFICIENȚA RENALĂ ACUTĂ LA COPIL

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THERAPEUTIC ASPECTS OF ACUTE RENAL FAILURE IN CHILDREN (ABSTRACT): This article presents the results of a study on 20 children diagnosed with acute renal failure and treated with renal substitution methods at the Iasi IVth Clinic of Pediatric Nephrology în the interval 2003-2008. Treatment by intermittent hemodialysis IHD), peritoneal dialysis (DP), and continuous replacement – hemofiltering (HF) depends on the patient's features and unit resources. The advantages and limitations of every method of treatment are discussed. Future therapies for acute renal failure may find strategies for eliminating the inflammatory response. **Key words:** ACUTE KIDNEY FAILURE, TREATMENT, CHILDREN

Leziunea renală acută are o contribuție semnificativă în morbiditatea şi mortalitatea copiilor în stările critice, în special la cei cu insuficiență organică multiplă.

În ultima decadă s-a schimbat epidemiologia leziunii renale acute, accesul la tratament, obiectivele şi tehnologiile rafinate ale echipamentelor de monitorizare şi de supleere renală (1,2).

Înțelegerea fiziopatologiei insuficienței renale acute, utilizarea biomarkerilor precoce şi o mai bună clasificare, necesită dezvoltarea strategiilor terapeutice de succes pentru leziunea renală acută la copil (3,4,5,13).

a. Prevenirea leziunii renale acute are un larg impact asupra ratei mortalității. Cea mai obişnuită cauză de insuficiență renală în copilărie este depleția volemică şi aceasta poate fi prevenită. Măsurile terapeutice profilactice constau în evitarea deshidratării şi medicamentelor nefrotoxice concomitent, în special la copiii cu afectare renala preexistentă.

Terapia de atenuare a leziunii renale şi de promovare a recuperării

Deşi nu există o terapie specifică pentru prevenirea leziunii renale la om, au început să fie studiate mai multe terapii potențiale (6,7). Managementul leziunii renale acute poate să includa antioxidanți, terapia cu molecule împotriva adeziunii şi administrarea de mediatori vasculari sau celule stem mezenchimale care să prevină leziunea şi să promoveze recuperarea (6,8,10, 12,15).

- Hormonul de stimulare a melanocitelor (MSH) are activitate antiinflamatorie şi s-a dovedit că protejează tubulii renali de leziune (9).

-Pe modele animale cu leziune renală acută, infuzia postischemică de factori de creştere (incluzând insulin-like factor de creştere, factori epidermici de creştere şi factori de creştere hepatocitari) are ca rezultat accelerarea recuperării funcției re-

nale, prevenind alterarea histologică severă şi scăderea ratei mortalităţii (2,8,11). S-a dovedit că înlăturarea radicalilor liberi, oxigenului reactiv şi moleculelor de nitrogen ar scădea gradul leziunii pe modele animale cu leziune renală acută (11).

- Celulele stem multipotente pot avea rol în promovarea recuperării leziunii renale acute pe modele animale, aşa cum au demonstrat studii recente. În schimb studiile pe oameni au fost larg dezbătute (10,12)

- Există studii cu utilizarea peptidului natriuretic atrial şi IGF1 (11).

Pe modele animale s-a demonstrat că administrarea de magneziu cu N-acetilcisteină protejează rinichiul de efectele IRA postischemice.(14)

În momentul în care diagnosticul de insuficienţă renala este stabilit este oportun să se intervină, intervenţia precoce având impact asupra recuperării.

b. Abordarea terapeutică conservatoare la pacientul pediatric cu leziune renală acută include măsuri simptomatice cum ar fi controlul balanţei fluidelor, managementul dietei şi presiunii sanguine care conduc la îmbunătăţirea timpului de supravieţuire. Încărcarea lichidiană este un factor de risc pentru mortalitate, independent de severitatea bolii. Perturbarea balanţei fluidelor sau metabolismelor necesita adesea iniţierea terapiei de substituţie (5,7).

c. Recentele validări ale criteriilor RIFLE pediatrice au permis revoluţionarea alegerii metodei de abordare a tratamentului de substituţie renalaă (3). Avem pentru prima dată o difiniţie comună care permite compararea seturilor de date între instituţii. Aceasta revoluţionează metoda de abordare a terapiei sustitutive şi ghidează pe viitor studiile făcute la întâmplare.

Exista câteva probleme pe care clinicienii trebuie să le rezolve în momentul în care iau decizie cu privire la modalitatea de supleere renală în insuficienţa renală acută pediatrică:

- stabilirea momentului începerii dializei;

- aprecierea gravităţii bolii şi alegerea metodei care deserveşte cel mai bine nevoile pacientului;

- compararea metodelor disponibile în centrul respectiv;

- expertiza locală pentru stabilirea modalităţii de tratament (5,7).

Având în vedere specificitatea şi caracteristicile pacienţilor, aceştia pot fi trataţi prin hemodializa intermitentă (IHD), dializa peritoneală (DP) sau metode de supleere renală continuă, hemofiltrarea (HF).

Paralel cu schimbarea epidemiologiei s-au produs schimbări dramatice şi în abordarea terapiei de substituţie renală la pacientul cu leziune renală acută.

Datele pediatrice mai recente au sintetizat rezultatele şi au clarificat diagnosticele folosind scorurile de severitate a bolilor şi preconizând intervenţia precoce pentru îmbunătăţirea rezultatelor. Din ce în ce mai multe centre de terapie intensivă sunt echipate cu mijloace de supleere renală, ceea ce le face să devină standardul îngrijirii în astfel de unităţi. În ultima decadă se raportează că HF a devenit prima modalitate de tratament. Alegerea PD, IHD sau HF este dependentă de o varietate de factori, din perspectiva pacientului sau a centrului.

Alegerea celei mai potrivite metode de tratament depinde de statusul clinic al pacientului şi de indicaţiile dialitice. Iniţial trebuie evaluat pacientul, ţinând seama că în insuficienţa organică multiplă se ajunge frecvent şi la insuficienţa renală. Invers, la pacientul care iniţial prezintă leziune renală se poate întâlni şi evoluţie spre insuficienţa organică multiplă.

Indicatorii pentru inițierea tratamentului de supleere includ: modificările electroliților, uremia cu sau fără encefalopatie, hipervolemia cu edem pulmonar, insuficiența respiratorie, intoxicatiile, erorile congenitale de metabolism şi suportul nutrițional.

Factorii ce hotărăsc alegerea celei mai potrivite modalități de terapie:

- caracteristicile pacientului, incluzând vârsta, talia, gravitatea bolii şi comorbiditatile;
- caracteristicile organizatorice, incluzând resursele folosite, tipul instituției, tipul unității de terapie intensivă, tehnica;
- condiția socială a aparținătorilor, înțelegerea costului terapiei.

Indicațiile specifice

Îndepărtarea rapidă din organism a ureei sau corectarea anomaliilor electrolitice (creşterea nivelului potasiului) au o importanță extremă în leziunea renală acută. PD, IHD şi HF pot rapid corecta hiperpotasemia şi uremia (2,5,15).

Epurarea devine urgentă în sindromul de liza tumorală, erorile înnăscute de metabolism, hiperamoniemia, hiperpotasemia simptomatică sau ingestia de toxine dializabile, necesitând PD sau HF. Înlăturarea urgentă a fluidelor este necesară la pacienții cu edem pulmonar sau dificultăți în ventilație, putând fi realizată numai prin IHD sau HF.

La pacienții instabili hemodinamic, cu stări septice, supraîncărcare cu fluide, compromişi respirator, care necesita tratament vasopresor, este preferabil HF pentru controlul statusului fluidelor. Pacienții după intervenții chirurgicale cardiace cu încărcare lichidiană minimă şi instabilitate hemodinamică pot fi mai bine tratați prin PD.

Caracteristicile pacienților

Condiția fizica a pacientului, vârsta, procedurile chirurgicale anterioare şi stabilitatea globală contribuie cel mai adesea la alegerea tratamentului.

Contraindicațiile DP includ: herniile diafragmatice, intervențiile chirurgicale intraabdominale recente, sepsisul şi malignitatile intraabdominale, lezarea suprafeței peritoneului.

Hipotensiunea severă contraindică IHD.

La pacienții nou-născuți, unde accesul vascular este dificil de obținut, se foloseşte PD. La aceştia cateterul poate fi plasat în vasele ombilicale.

Prezența coagulopatiilor face dificil accesul vascular, având impact asupra performanțelor IHD sau HF.

Caracteristici organizatorice

În unele zone geografice, resursele constituie un factor de limitare. PD poate fi suficientă şi alte forme de supleere să nu fie folosite. Abilitatea pentru IHD sau HF depinde de personal şi de experiență.

Considerentele ce pot fi luate în calcul pentru stabilirea unei terapii potrivite vor fi:

- dacă pacientul are nevoie numai de epurare;
- soluția şi numărul de cicluri care pot îndeparta mai multe fluide;
- stabilitatea hemodinamică;
- factorii ce limiteaza condițiile pacientului.

MATERIAL ŞI METODĂ

În Clinica a IV-a de Nefrologie Pediatrica Iaşi în perioada 2003-2008 au fost internate 51 de cazuri diagnosticate cu leziune renală acută conform criteriilor RIFLE pediatrice. Pacienții au fost evaluați atent din punct de vedere clinic şi paraclinic. Parametrii stării de hidratare (tensiune, edeme, greutate, diureză, pierderi extrarenale, aport hidric oral şi parenteral) au fost evaluați zilnic. Retenția azotată şi iono-

grama s-au determinat zilnic în prima etapă şi chiar mai frecvent la bolnavii din terapie intensivă.

Din totalul de 110 pacienți cu IRA, 90 au fost tratați conservator şi 20 au necesitat terapie de substituție renală. Au fost utilizate:

- hemodializa 6 cazuri;
- dializa peritoneală 2 cazuri;
- hemofiltrarea 12 cazuri.

Cauzele ce au determinat leziunea renală acută în cele 20 de cazuri dializate:

TABELUL I

Etiologia la bolnavii tratați prin metode de supleere

Nr. crt.	Cauze	Număr de cazuri	Procente %
1.	SHU	11	55
2.	Disfuncție organică multiplă	4	20
3.	Intoxicații	2	10
4.	Șoc hemoragic	1	5
5.	Necroză corticală	1	5
6.	Glomerulonefrită	1	5

Pe primul loc se situează sindromul hemolitic uremic urmat de disfuncție organică multiplă. Urmărind evoluția cazurilor la bolnavii studiați se remarcă supraviețuire în 16 cazuri şi decese la 4 cazuri. Cauzele deceselor au fost: sepsisul 2 cazuri, hemoragia cerebrală, necroza corticală. Dintre copiii tratați cu hemodializă au decedat 2 şi dintre cei tratați prin hemofiltrare 2. După externare pacienții au fost urmăriți ambulator, la intervale strânse, inițial săptămânal, apoi la o lună, 3 luni, 6 luni, 1 an. După 2 ani au supraviețuit 14 şi 3 au evoluat spre boala renală cronică.

Indicațiile dializei au fost următoarele:

- încărcarea lichidiană cu producerea edemului pulmonar acut sau insuficiența respiratorie - 4 cazuri;
- tulburări metabolice severe: acidoză severă - 5 cazuri, hiperpotasemie - 1 caz, hipernatremie - 1 caz.
- intoxicații: etilenglicol - 1 caz, medicamente dializabile (ciprofloxacin) - 1 caz;
- sângerare - 1 caz
- encefalopatie uremică - 6 cazuri

Dializa peritoneală a fost folosită în 2 cazuri.

Din punct de vedere istoric, PD a fost prima modalitate de terapie substitutivă continuă folosită în unitățile de terapie pediatrică. Mulți clinicieni au avut un grad de confort relativ ridicat prin folosirea acestei terapii. Rapoarte recente din Nigeria, India şi Brazilia au demonstrat în continuare eficiența ei în tratamentul leziunii renale acute pediatrice (5). PD oferă o varietate de avantaje în populația pediatrică cu insuficiență renală acută:

- datorită aspectelor tehnice, accesul a fost rapid şi sigur de obținut, chiar la pacienții cu instabilitate hemodinamică, cum ar fi coagulopatiile.
- s-a realizat cu puțin echipament şi costuri mici.
- se impune în cazul în care nu există IHD sau HF sau în cazul în care există zone rurale sau țări mai puțin dezvoltate;
- PD nu necesită acces vascular iar pacienții în stări critice pot fi dializați cu prezervarea vaselor necesare pentru procedurile viitoare;

Accesul chirurgical în forma clasică plasează cateterul Tenckoff pentru PD în stratul subcutanat la pacienții incapabili să tolereze plasarea chirurgicală, făcând ca terapia să fie rapid instituită. Tehnica a fost bine tolerată de micii pacienți.

Dezavantajele pot include:

- pierderi de dializant în jurul cateterului sau în spațiul pleural, conducând la

hidrotorax;

- infecțiile;

- inițierea unui volum mic de dializat (10mL/kg corp) limitând clearance-ul adecvat.

La copil PD manuală este performantă. Dextroza folosită în PD poate fi o sursă suplimentară de carbohidrați pentru nutriție dar totodată poate conduce la hiperglicemie, necesitând corecție insulinică. Suplimentarea cu natriu şi aminoacizi poate fi necesară.

PD este limitată pentru anumite categorii:

- pacienți cu disfuncție pulmonară la care induce agravarea simptomatologiei prin creşsterea volumului de dializat abdominal;

- pacienți cu şunt ventriculo-peritoneal sau sindrom Prune-Belly, prezentând o creştere a complicațiilor;

- pacienții cu intervenții chirurgicale abdominale nu sunt apți pentru această metodă;

- pierderi semnificative de Ig,cu risc de infecție.

Complicații

- sângerarea;

- peritonitele pot duce la creşterea pierderii de proteine, compromisuri nutriționale, pierderea capacității de ultrafiltrare şi leziuni ale membranei peritoneale;

- complicații mecanice: fisuri, pierderi de lichid, obstrucția cateterului cu cheaguri de fibrină.

Cele mai mari costuri asociate cu PD, în afară de asistentă, sunt cele ale soluției de dializă.

Hemodializa intermitentă

Prezintă avantajul ultrafiltrării rapide. La pacientul stabil hemodinamic este modalitatea cea mai potrivită. Este importantă în intoxicații, sindromul de liză tumorală şi hiperamoniemie întâlnite la populația pediatrică. Exista posibilitatea de a ajusta compoziția dializatului pentru a trata anumite anomalii ale electroliților (hiperpotasemia, hipernatremia) fiind un avantaj major în comparație cu PD sau HF. Este o modalitate valabilă în spitalele mari. Accesul vascular este cel mai important component şi contribuie la realizarea satisfăcătoare a terapiei. Utilizarea venelor cervicale poate fi preferabilă. Amplasarea cateterului poate fi făcută de catre nefrologul pediatru sau de medicul de terapie intensivă.

Accidente care au apărut în cazurile noastre:

- infecția de cateter-septicemie cu bacil cereus - 1 caz;

- hemoragie - 2 cazuri.

Amplasarea cateterului sublavicular ar trebui evitată (pericol de stenoză, făcând imposibilă formarea unei fistule în viitor).

Avantaje :

- rapiditatea înlaturării fluidelor.

Dezavantaje :

- schimbările rapide osmolare conduc la edem cerebral;

- pacienții hipotensivi tolerează rău dializa;

- pacienții non uremici (cu boli congenitale de metabolism şi intoxicații) riscă să prezinte hipofosfatemie.

Hemofiltrarea

Hemofiltrarea a fost instituită la copiii care au beneficiat de supleere renală fără complicații.

În ultimii 5 ani s-au stabilit principiile HF la copii de către un consorțiu pediatric ce cuprinde 13 centre din USA. Consorțiul de Prospectivă Pediatrică (ppCRRT) care a redefinit datele demografice, rezultatele şi stategiile (1,2,5,7). Una dintre cele mai importante variabile a fost gradul de încărcare a pacientului.

Dezavantaje

Cel mai mare dezavantaj al HF este

complexitatea şi pretul crescut comparativ cu cel al PD sau IHD.

Noile aparate folosite au un grad de siguranţăa mai mare, impunând şi creşterea costurilor. Folosirea corectă necesită asistenţă specializată şi suport farmacologic. În ciuda folosirii circuitelor mici în anumite împrejurări, volumul mare extracorporeal necesar pentru HF şi IHD (în particular la nou născuţi) este un dezavantaj major, făcând să fie utilizat la pacienţii peste 10 Kg. Volumul de sânge din circuitul extracorporeal depăşeşte 10 % din volumul de sânge al pacientului. Asociat cu instabilitatea hemodinamică, pacientul are un risc evident. Pacienţii cu insuficienţă organică multiplă pot să nu tolereze HF. Necesită atenţie la tipul hemofiltrului utilizat, datorita reacţiilor bradikininei, activării complementului, cascadei coagulării, degranulării neutrofilelor şi speciilor de oxigen reactive (2,5).

Avantaje

- mimează efectele funcţiei renale, realizând o ultrafiltrare continuă, fiind terapia ideală pentru pacienţii instabili;

- ultrafiltrarea este separată de îndepărtarea soluţiei dializate, oferind mai multă flexibilitate pentru prescripţie, în funcţie de nevoile pacientului;

- îndepărtarea continuă a fluidelor este performantă, permiţând nutriţia adecvată;

- HF poate rapid şi predictibil să restaureze homeostazia;

- HF întreţine controlul uremiei mai bine comparativ cu PD sau IHD;

- înlocuirea filtrului permite clinicianului controlul nivelului de electroliţi; acesta este important în special în hipernatremii, în controlul creşterii presiunii intracraniene precum şi în stările hiperosmolare;

-HF poate avea un beneficiu suplimentar, restaurând homeostazia imunologică, prin îndepărtarea moleculelor proinflamatorii.

În timp ce indicaţiile pentu dializă sunt destul de bine înţelese, datele privind momentele începerii sunt limitate (5,7,15). Consortiul Pediatric de Prospectivă a terapiei de substituţie renală a demonstrat că încărcarea cu fluide constituie un factor de risc important al mortalităţii. Aceasta sugereaza ca intervenţia precoce este esenţială pentru îmbunătăţirea rezultatelor. S-a constatat la copiii cu intervenţii chirurgicale pentru malformaţiile congenitate de cord că PD precoce constituie un avantaj pentru supravieţuire. Aceste date susţin utilizarea intervenţiei înainte de acumularea unei cantităţi semnificative de fluide (> 15% din greutatea corporală).

Pe lângă aceasta, doza de dializă a devenit o variabilă cu importanţă din ce în ce mai mare. Doza de dializă inegală dintre diferitele metode le face dificil de comparat între ele, chiar şi variaţia dozelor în cadrul aceleiaşi metode este subiect de investigat.

Rascue şi colab., în studiile pe adulţi a demonstrat că o doza de 35 mL/Kg/h a fost mai eficientă decat 20 mL/Kg/h pentru supravieţuirea pacientului (8). La pacienţii cu sepsis le-a fost necesară o doză mai mare, 45 mL/Kg/h (5).

Este necesar un scor al severităţii bolii. Swartz a demonstrat că atunci când a fost controlată severitatea bolii, HF a fost superioară IHD în ceea ce priveşte supravieţuirea pacienţilor (16).

CONCLUZII

Utilizarea corectă a mijloacelor de epurare extrarenală contribuie la ameliorarea prognosticului.

Epurarea extrarenală se utilizează in extremis, dupa eşecul terapiei conservatoare.

În centrele cu rezerve limitate, ţelul este optimizarea şi implementarea tratamentului disponibil.

Iniţierea dializei trebuie efectuată precoce, hemofiltrarea fiind superioară celorlalte metode de supleere.

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MULTIFACTORIAL ETIOLOGY OF LOWER LIMB EDEMA IN ELDERLY PATIENT WITH HEART FAILURE. CASE REPORT

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MULTIFACTORIAL ETIOLOGY OF LOWER LIMB EDEMA IN ELDERLY PATIENT WITH HEART FAILURE (Abstract): Aging population and increasing life expectancy has led to an exponential increase in the incidence of heart failure. In the elderly patient with heart failure lower limb edema is most commonly considered in the context of decompensated heart disease, causes such as malnutrition, kidney and liver disease or secondary drug effects being rarer. We report two cases of edema of noncardiac origin. In the first case, kidney damage was identified, edema resulting from hypoproteinemia and proteinuria, and in the second case it had an iatrogenic cause, namely the adverse effect of dihydropyridine calcium channel blocker. In the latter case, investigations centered on the assessment of body composition revealed the tendency to dehydration vs. hyperhydration, which led to a different therapeutic approach compared to the general tendency to administer *per primam* a diuretic treatment. The elderly patient with comorbidities that associate heart failure and lower limb edema requires careful investigations of the possible alternative causes. **Keywords:** ELDERLY, HEART FAILURE, LOWER LIMB EDEMA, MULTIFACTORIAL ETIOLOGY.

Symmetrical lower limb edema is a common clinical sign in the elderly, but also a major problem in geriatric medicine (1). This sign is so common in daily clinical practice because it is the symptom of a wide range of acute or chronic illnesses, and therefore particular attention should be given to the differential diagnosis centered on the elderly patient (2).

The cause of lower limb edema in the geriatric patient is frequently multifactorial (2). Although venous insufficiency and heart failure are the most common causes (3), an important role in this issue is given to protein calorie malnutrition and drug-induced edema: dihydropyridine calcium blockers (4), non-steroid anti-inflammatory drugs (2, 5), corticotherapy.

The general trend in an elderly patient with history of heart failure of various causes and lower limb edema is to establish a causal relationship between them, without further investigation of other causes for edema (6). Therefore, additional systematic investigations are not performed, and patients receive routine diuretic treatment (1), with severe functional and metabolic consequences such as secondary falls (2), renal impairment, hypokalemia, alkalosis and volume depletion.

CASES REPORTS

To exemplify, we report two cases of

elderly patients with heart failure in whom lower limb edema detected on physical examination was due to noncardiac causes.

Case 1. An 81-year-old female the medical history of whom revealed, among others, cardiovascular disorders represented by stage 3 essential hypertension, severe aortic valve stenosis, mild mitral regurgitation and heart failure with preserved ejection fraction. She associated vertiginous syndrome and cervical-lumbar spondylosis. For the afore-mentioned conditions the patient followed drug treatment with loop and anti-aldosterone diuretics (fixed-dose combination of spironolactone 50 mg and furosemide 20 mg), beta blocker (bisoprolol 5 mg), dihydropyridine calcium blocker (Amlodipine 5 mg) and betahistine 24 mg.

The patient presented to a geriatric medical ward with lower limbs edema, declaring the onset of symptoms a month earlier.

The cardiovascular exam showed a 4/6 systolic murmur in the aortic valve area and the echocardiographic examination revealed: normal-size heart, pulmonary artery pressure (PAP=27 mmHg) within normal range, normal inferior vena cava size with inspiratory collapse present and left ventricular ejection fraction (EF)=50%, findings inconsistent with a cardiovascular cause. To rule out iatrogenic-induced edema, treatment with amlodipine was discontinued, but with no result.

Further investigations revealed: high creatinine level corresponding to an eGFR of 44 mL/min/1.73m^2, hypercholesterolemia and hypertriglyceridemia, hypoproteinemia and non-specific inflammatory syndrome (erythrocyte sedimentation rate=130 mm/h). Urine tests were suggestive of glomerular nephrotic syndrome with 24-hours urine protein of 6.48 g.

Considering the diagnostic elements of nephrotic syndrome, further investigations were performed to establish its ethology. In this regard, we investigated a possible immunological (pANCA and cANCA antibodies were negative), and infectious cause (Ag HBs, Ac anti VHC negative), paraneoplastic manifestations - pulmonary, digestive, gynecological, hematological (thoracic X-Ray without suggestive lesions, abdominal ultrasound, kappa to lambda light chain ratio, gynecological examination - within normal limits). In the absence of an obvious cause, renal injury was interpreted in the context of a chronic glomerulopathy secondary to cardiovascular damage, and edema was considered secondary to hypoproteinemia and proteinuria. In addition to cardiovascular medication, maximal antiproteinuric treatment with angiotensin converting enzyme inhibitor was initiated, without diuretic dose adjustment. The outcome was favorable, with lower limb edema and serum proteins correction.

Case 2. An 84-year-old female patient with multiple comorbidities: cardiovascular (stage 3 essential hypertension, paroxysmal atrial fibrillation chemically converted to sinus rhythm, ischemic heart disease), renal (single left kidney after right nephrectomy for kidney tuberculosis), gynecological (total hysterectomy), endocrinological (thyroid nodules and total thyroidectomy) and orthopedic (left hip replacement following femoral neck fracture, right coxarthrosis). The patient followed drug treatment with amiodarone 200 mg/day, amlodipine 10 mg/day, carvedilol 12.5 mg/day, apixaban 2.5 mg x 2/day (adapted to renal function), levothyroxine 100 micrograms/day and alfacalcidiol 0.5 mg/day

(with both metabolic and renal protection due to the antiproteinuric effect).

The patient was symptomatic by dyspnea on low physical exertion, fatigability, and physical examination revealed symmetrical lower limb edema.

Cardiovascular examination showed rhythmic heart beats and blood pressure controlled with the current antihypertensive medication. Chest X-ray showed leftward displacement of the left heart border, right basal pachypleuritis. Transthoracic echocardiography revealed a normal-sized heart with preserved left ventricular systolic function (volumetric EF=57%), delayed relaxation diastolic dysfunction, mild tricuspid regurgitation, normal caliber vena cava, and full inspiratory collapse and moderate pulmonary hypertension (PAPs=60 mm Hg). Clinically, varicose veins were not found, and venous echo Doppler revealed some slightly dilated calf perforator veins.

Blood tests revealed atherogenic dyslipidemia, high creatinine levels (creatinine=1.36 mg% from 1.1 mg% a month earlier), with eGFR of 35.6 mL/min/1.73m^2 from 46.1 mL/min/1.73m^2 a month earlier and normal thyroid status. We did not find any possible cause of the acute deterioration of chronic kidney disease: prerenal (absence of nephrotoxic medication, euvolemic status-determined by bioimpedance), intrinsic renal (urine tests with no pathological findings) and post-renal (absence of ultrasound signs of obstruction and absence of radiographic signs of pelvis compression).

The assessment of body composition by bioimpedance revealed a dehydrated patient, and paradoxically, parenteral hydration was initiated with consequent correction of renal function. Discontinuation of dihydropyridine calcium-channel blocker treatment resulted in remission of edema.

DISCUSSION

The diagnosis of heart failure in the elderly can be difficult due to the increased prevalence of atypical signs and symptoms, presence of concomitant diseases (they may alter the classic manifestations of heart failure) and, on the other hand, due to the different perceptions of patients on the changes usually associated with it (7). Confusion, delirium, cognitive deficit, physical and mental asthenia which are a characteristic of the elderly can alter the symptoms or even mask them completely (8).

In the geriatric population, heart failure is the second most common cause of symmetrical lower limb edema after venous insufficiency (1); this aspect is due to the increased incidence of heart failure with preserved ejection fraction of up to 50% (3), being consistent with the two reported cases.

Among the most common - and less investigated - comorbidities in the elderly patients with heart failure are renal dysfunction, frailty and malnutrition (9, 10), polypragmasy and drug-induced edema (6, 11), lower limb edema being a common symptom of these entities (12). The systematic investigation of these entities will lead to a correct identification of the causes for edema onset, avoiding inappropriate administration and disastrous results of diuretic treatment, most commonly injectable furosemide.

In the first presented case, further investigations revealed the presence of a nephrotic syndrome, superimposed on preexisting heart disease. Considering the wide range of nephrotic syndrome causes, exhaustive parallel investigations were

required to avoid omitting a possible causal treatment. Chronic glomerulopathy is a common clinical syndrome in the elderly with chronic kidney disease (13), and this is the type of kidney damage identified in the first reported case.

Another important aspect is the iatrogenic cause of lower limb edema in the elderly. The incidence of drug-induced edema is seriously underestimated (1). Among the incriminated agents, antihypertensive drugs (3) and non-steroidal anti-inflammatory drugs (2) are the most common drugs. It is estimated that 50% of patients receiving dihydropyridine calcium channel blockers develop lower limb edema at different times of treatment (1). Our second case illustrates the situation of amlodipine-induced edema in a patient with stable cardiac failure and concomitant kidney disease. For the accuracy of the diagnosis in this case, the data in the literature demonstrate the superiority of bioimpedance versus clinical assessment (14).

Pulmonary hypertension is an underdiagnosed cause of lower limb edema, especially in the elderly patients. The substrate is sleep apnea, but it can also be secondary to chronic lung disease (1). The echocardiographic assessment in the second reported case revealed moderate pulmonary hypertension, which confirms the frequently multifactorial etiology of lower limb edema in the elderly patient (15).

CONCLUSIONS

In our cases, strict investigation of alternative causes of lower limb edema in the elderly is therefore necessary, beginning with the most common causes such as heart failure, protein-calorie malnutrition, to the least frequent, represented by iatrogenic cause, nephrotic syndrome and pulmonary hypertension. This strategy may reduce diagnostic confusion and may also reduce inappropriate use of diuretics with severe consequences: renal damage, dehydration, hyponatremia/dyselectrolytemia, increased risk of falls and poor life quality.

A direct consequence of this phenomenon is the increased number of patients experiencing symmetrical lower limb edema, the investigation of this clinical sign being a major but neglected problem of geriatric medicine due to its frequently non-cardiac and often multifactorial etiology.

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NEWS

IMPACT OF LEFT VENTRICULAR DIASTOLIC DYSFUNCTION ON LUNG TRANSPLANTATION

Diastolic dysfunction may influence perioperative outcome, early graft function, and long-term survival. Has been compared the outcomes of double lung transplantation (DLTx) for patients with pulmonary arterial hypertension (PAH) with preoperative left ventricular (LV) diastolic dysfunction with the outcomes of patients without diastolic dysfunction. Fourteen (31.8%) patients with diastolic dysfunction pretransplantation had a higher body mass index (29 [IQR 21.5-32.6] vs 22.4 [IQR 19.9-25.3] kg/m^2) and mean pulmonary arterial pressure (54.6 10 mmHg vs 47 +/- 11.3 mmHg) and right atrial pressure (16.5 +/- 5.2 mmHg vs 10.6 +/- 5.2 mmHg). The patients received extracorporeal life support more frequently, had worse APACHE II scores, and a trend toward worse ventilator-free days. In conclusion, diastolic dysfunction was the only variable that correlated with overall survival and leads to early postoperative morbidity and worse survival in patients with PAH after DLTx (Avriel A, Klement A, Johnson S, *et. al*. Impact of Left Ventricular Diastolic Dysfunction on Lung Transplantation Outcome in Patients with Pulmonary Arterial Hypertension, *American Journal of Transplantation* 2017; 17(10): 2705-2711).

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CORELAŢII ÎNTRE HIPERTENSIUNEA ARTERIALĂ ESENŢIALĂ ŞI GROSIMEA INTIMĂ-MEDIE A ARTEREI CAROTIDE, LA COPIL

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CORRELATION BETWEEN ESSENTIAL HYPERTENSION AND CAROTID INTIMA MEDIA THICKNESS IN CHILDREN (Abstract): Essential hypertension is no longer un uncommon disease in children. This study try to determine if carotid intima thickness is increased in children with essential hypertension. **Material and method**: This subjects are compared with control group with normal blood pressure. The study group consists of 78 children with essential hypertension; there were excluded cases with secondary hypertension. These children had carotid intima media thickness, left ventricular mass index and body mass index performed. In control group there were 53 subjects and there were performed the same investigations for them. **Results**: Sixtyfour cases (82,05%) from study group had obesity (body mass index ≥ 85 percentile). Left ventricular mass index was increased in 40 cases (51,28%). Increased carotid intima media thickness was found in fiftyone subjects (65,38%) in the group of hypertensive children and it range between 0,52 şi 0,69 mm. In the control group carotid intima media thickness varies between 0,32 şi 0,54 mm. The carotid intima media thickness correlated with sistolic blood pressure(P=0,00000), with diastolic blood pressure (p=0,000002), with body mass index (p=0,00000) and with body mass index(p= 0,000001). **Conclusion**: The study could not state if increased carotid intima media thickness is due to hypertension or to obesity, because in children there is a strong association between these two diseases. Anyway, evaluation of this parameter may offer information about early endothelial disfunction. **Key words**: ESSENTIAL HYPERTENSION, CAROTID INTIMA MEDIA THICKNESS, ENDOTHELIAL DISFUNCTION

Incidenţa hipertensiunii arteriale esenţiale (HTAE) la copil este în creştere, pragul vârstei de debut coborând spre vârsta de 10-12 ani (1) . HTAE reprezintă un factor de risc major pentru dezvoltarea aterosclerozei şi a patologiei coronariene la adult. Se consideră că acest risc exista la valori ale tensiunii arteriale (TA) de 115/75 mm Hg şi se dublează cu fiecare creştere cu 20/10 mm Hg acesteia (2). Între HTA şi activitatea endoteliului vascular există o relaţie cauză-efect în ambele direcţii. Pe de o parte, presiunea arterială constatant crescută determină disfuncţie endotelială consecutivă, cu modificări funcţionale si structurale la nivelul vaselor (3). Pe de altă parte, disfuncţia endoteliului vascular contribuie la progresia HTA (4). Mai mult, nu trebuie ignorat faptul că la copil, obezitatea se asociază frecvent cu hipertensiunea arterială .Una dintre cele mai precoce modificări caracteristice acestei asociatii o reprezintă dis-

TABELUL I
Caracteristicile demografice şi paraclinice ale lotului de studiu şi lotului martor

	MARTOR			STUDIU			P
	media	ab.std.	cazuri	media	ab.std.	cazuri	
Vârsta	14,06	2,79	53	13,83	3,16	78	0,673102
Gn	3139,34	562,60	32	3301,02	632,74	49	0,244134
Ga	54,31	17,56	53	64,64	20,14	78	0,478138
T	1,528	0,1386	53	1,547	0,1684	78	0,002925
IMC	23,01	6,65	53	26,37	4,79	78	0,001016
TAS	107,04	6,14	53	133,47	15,41	78	0,000000
TAD	63,75	7,33	53	77,86	11,60	78	0,000000
IMTc	0,37	0,06	50	0,43	0,06	75	0,000000

Gn = greutatea la naştere; Ga = greutatea actuală; T = talia; IMC = indicele de masă corporală; TAS = tensiunea arterială sistolică; TAD = tensiunea arterială diastolică; IMTc = grosimea intimă-medie la artera carotidă comună

funcţia endotelială şi aparitia plăcilor aterosclerotice evidenţiate chiar de la vârsta de 2 ani (5). Modificările structurale de la nivelul arterelor carotide pot fi puse în evidenţă cu ajutorul ecografiei Doppler prin măsurarea grosimii intimă-medie la artera carotidă (IMTc). În studiul de faţă ne-am propus să determinăm corelaţia între hipertensiunea arterială esenţială şi valoarea crescută a IMTc precum şi în ce măsură IMTc crescută se datorează HTA sau obezităţii.

MATERIAL ŞI METODĂ

Caracteristicile clinice

Au fost incluşi în studiu 78 pacienţi internaţi la Spitalul Clinic de Copii Braşov, în perioada ianuarie 2007 – noiembrie 2009, care au fost diagnosticaţi cu hipertensiune arterială. Toţi subiecţii aveau valori ale TA sistolice şi/sau diastolice mai mari de percentila 95 pentru vârstă, sex şi înălţime, confirmate la cel puţin 3 determinări separate, la interval de 2 săptămâni. Nu au fost incluşi în lotul de studiu pacienţii la care au fost identificate cauze secundare de HTA.

Lotul martor a fost alcătuit din 53 de subiecţi cu TA sistolică şi diastolică sub percentila 90 pentru vârstă, sex şi înălţime, la minim 2 determinări separate.

În ambele loturi am măsurat greutatea şi talia şi am calculat indicele de masă corporală (IMC) conform formulei $IMC = G/T^2$ (unde G= greutatea, exprimată în kg, T= talia, exprimată în metri). Tensiunea aterială a fost măsurată respectând recomandările "Celui de al 4-lea raport privind diagnosticul, evaluarea şi tratamentul hipertensiunii arteriale la copil şi adolescent"(6).

Ecocardiografia

Atât la subiecţii cuprinşi în lotul de studiu cât şi la cei din lotul martor s-a efectuat ecocardiografie 2D, mode M şi examen Doppler (ecograf marca Fukuda). Nici un pacient nu a fost diagnosticat cu coarctaţie de aortă sau cu alte anomalii structurale cardiace. La examenul ecocardiografic s-a urmărit depistarea hipertrofiei VS (HVS), considerată ca fiind cea mai frecventă complicaţie a HTA primare la copil. Masa ventriculului stâng (VS) a fost calculată pe baza formulei (6):

$$\textbf{Masa VS (g)} = 0{,}80\,[1{,}04\,(\text{SIV}+\text{DED-VS}+\text{PP-VS})^3-(\text{DED-VS})^3]+0{,}6$$

unde: SIV = septul interventricular, DED-VS = diametrul end-diastolic al VS, PP-VS = peretele posterior al VS

Pentru evaluarea HVS s-a calculat masa ventriculului stâng care a fost indexată cu înălţimea pentru a face corecţia cu suprafaţa corporală. HVS este definită de valori

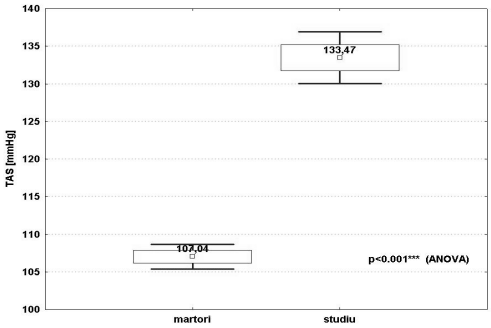

Fig. 1. Variaţia TAS la lotul studiu şi lotul martor

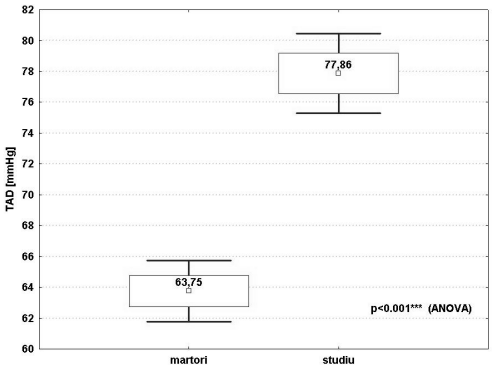

Fig. 2. Variaţia TAD la lotul studiu şi lotul martor

ale indicelui masei VS mai mari de percentila 95, adică, pentru băieţi ≥ 39,36 g/m^2 iar pentru fete ≥ 36,88 g/m^2 (7).

Ecografia Doppler a arterelor carotide

Am determinat grosimea intimă-medie la artera carotidă atât la subiecţii incluşi în lotul de studiu, cu HTA, cât şi la subiecţii din lotul martor. Am folosit ecograful marca Fukuda, iar examinarea s-a efectuat cu transductor liniar cu frecvenţa de 12,5-13 MHz. Subiecţii au fost examinaţi într-un mediu ambiental liniştit, cu confort termic, fără zgomote, după un repaus prealabil de 15 minute. Poziţia de examinare a fost în clinostatism, cu faţa în sus şi capul rotit la 45° de parte opusă arterei examinate. Am efectuat manual câte 5 măsurători pentru fiecare perete (anterior şi posterior), pe o distanţă de 1 cm, pentru artera carotidă dreaptă şi stângă iar în studiu s-a utilizat media aritmetică a valorilor obţinute. Acestea au fost comparate cu valorile considerate normale pe grupe de vârstă (8).

Analiza statistică: am folosit pentru calculul statistic metoda ANOVA pe care am aplicat-o pentru variabilele continue şi testul chi pătrat pentru compararea grupurilor. Am considerat semnificativă statistic valoarea $p < 0,05$.

REZULTATE

Lotul studiu a cuprins 78 de pacienţi la care s-a stabilit diagnosticul de HTA primară. Dintre aceştia, au fost 46 băieţi (58,97%) şi 32 fete (41,02%). Vârstele subiecţilor au variat între 5,9 ani şi 17,11 ani (media de vârstă 13,67 ani). Lotul martor a cuprins 53 de subiecţi, cu 25 băieţi (47,16 %) şi 28 fete (52,83%), cu vârste cuprinse între 5,7 şi 17,10 ani (media de vârstă 14,07 ani). În lotul de studiu, 55 de copii (70,51%) prezentau şi obezitate (IMC > percentila 95), 9 copii (11,53%) erau supraponderali (IMC cuprins între percentila 85 şi 95) iar 14 (17,94%) au fost normoponderali (IMC < 85). În lotul martor 3 subiecţi (1,88%) au fost supraponderali (IMC cuprins între percentilele 85 şi 95) şi 18 subiecţi (33,96%) au fost obezi (IMC > percentila 95). Caracteristicile demografice şi clinice sunt prezentate în tabelul I. Valorile TAS şi TAD în cele două loturi este reprezentată în figurile 1 şi 2. Se observă o diferenţă semnificativă între cele două grupuri; 44 subiecţi (56,41%) din lotul studiu au valori ale TA > percentila 99 pentru vârstă, sex şi talie, ceea ce demonstrează instalarea formelor severe de HTAE încă de la vârstă mică. Ecocardiografia a pus în evidenţă la pacienţii hipertensivi (valori ale TAS şi/sau TAD > percentila 99) prezenţa hipertrofiei VS în 40 de cazuri (51,28%). Urmărind IMTc, am observat că aceasta se corelează pozitiv cu HTA (fig. 3), ea fiind semnificativ crescută la subiecţii hipertensivi faţă de cei

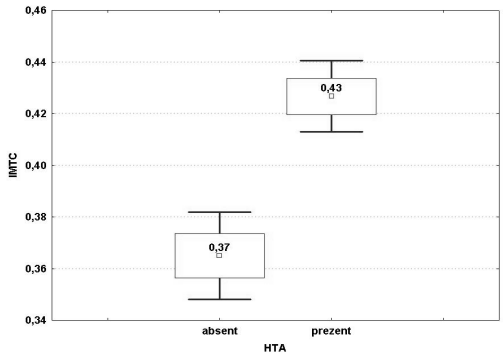

Fig. 3. Corelaţia HTA - IMTc

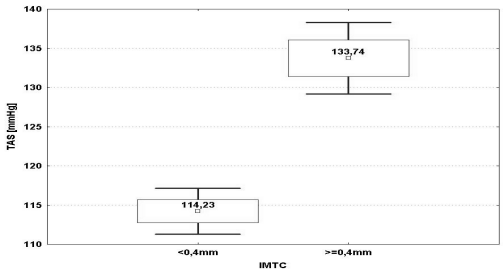

Fig. 5. Variaţia IMTc cu TAS

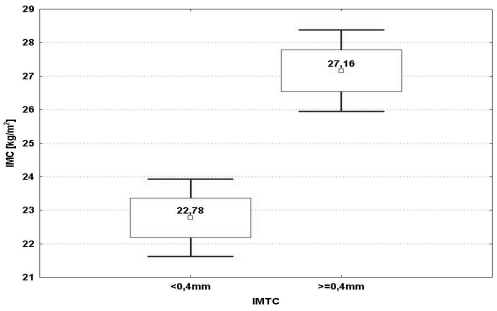

Fig. 4. Variaţia IMTc în funcţie de IMC

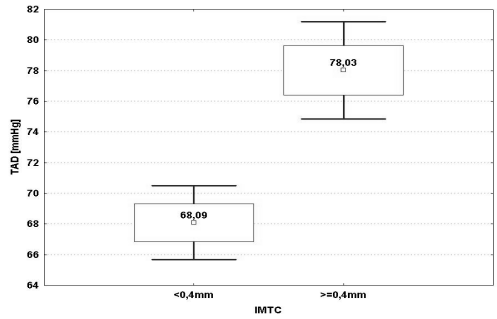

Fig. 6. Variaţia IMTc cu TAD

normotensivi. În lotul studiu IMTc a variat între 0,52 şi 0,69 mm iar pentru subiecţii normotensivi limitele au fost de 0,32 şi 0,54 mm. Toţi subiecţii hipertensivi care au avut HVS au prezentat şi IMTc crescută. 18 subiecţi hipertensivi si obezi(IMC > 95th) nu au avut HVS dar au avut IMTc crescută. De aceea am considerat creşterea grosimii intimă-medie a carotidei ca o consecinţă a obezităţii şi a proceselor aterosclerotice şi nu a hipertensiunii arteriale. Atât în lotul de studiu cât şi în lotul martor, la pacienţii cu obezitate, s-a depistat raport crescut intimă-medie la artera carotidă comună, ceea ce înseamnă că IMTc s-a corelat pozitiv cu IMC (p= 0,000001) (fig. 4). În lotul subiecţilor hipertensivi am constatat că există o corelaţie pozitivă între IMTc şi valorile TAS (p=0,0001) (fig. 5) şi TAD (p=0,0001) (fig. 6). Pacienţii cu valori ale TAS şi TAD mai mari de percentila 99 au fost cei care au prezentat cele mai severe modificări ale raportului intimă-medie (valori mai mari 0,5 mm), probabil, ca o consecinţă asupra peretelui arterial a regimului presional crescut. În mod cert şi-a adus contribuţia şi factorul timp la modificarea IMTc, deoarece la aceşti subiecţi era prezentă şi HVS, dovada unei evoluţii în timp a HTA. Astfel, am constatat că IMTc s-a corelat pozitiv cu indexul masei VS (p=0,0001) (fig. 7).

În lotul martor, 7 subiecţi au prezentat IMTc crescută aceştia fiind normotensivi dar obezi (IMC > 95th) fapt ce sugerează că aceasta este consecinţa modificărilor aterosclerotice.

DISCUŢII

În studiul nostru, vârsta de debut a HTAE a fost între 10 ani şi 17 ani la 69 subiecţi (88,46%) dar, 9 subiecţi (11,53%) aveau vârste cuprinse între 6 şi 10 ani când au fost diagnosticaţi cu HTAE. Cu toate că procentul este mic şi nesemnificativ statistic, trebuie să subliniem vârsta mică la care a debutat boala. Şi în literatura de specialitate

se fac referiri legate de coborârea vârstei de debut sub 10-12 ani. Un număr semnificativ de subiecţi au prezentat valori crescute ale TA şi afectarea „organelor ţintă" (HVS şi IMTc crescută) fapt ce permite încadrarea HTA în stadii avansate şi indică o evoluţie anterioară de cel puţin 6 luni. Procesele patologice care se produc la nivelul peretelui arterial, consecinţe ale regimului presional ridicat, pot să fie puse în evidenţă cu ajutorul ecografiei Doppler, prin măsurarea grosimii intimă-medie arterială. Creşterea indicelui intimă-medie este dovada modificărilor structurale produse la nivelul peretelui arterial. Am aplicat în studiul de faţă această metodă şi am reuşit să punem în evidenţă prezenţa acestor modificări structurale arteriale. Astfel, am găsit că valoarea IMTc este mai crescută la copiii cu hipertensiune arterială primară faţă de copiii cu valori normale ale TA şi că această valoare se corelează pozitv cu valorile TAS şi TAD. În cadrul lotului cu subiecţi hipertensivi valoarea IMTc a fost crescută atât la cei obezi cât şi la cei normoponderali, ceea ce demonsterază că regimul de presiune sanguină ridicată poate determina în timp modificări vasculare structurale. Pe de altă parte nu putem ignora faptul că, în studiul nostru, proporţia subiecţilor obezi a fost semnificativă atât în grupul hipertensivilor cât şi în grupul normotensivilor. Aceştia au avut, deasemenea, IMTc crescută, corelată pozitiv cu IMC. Se observa astfel o asociere strânsă între obezitate şi creşterea IMTc (9,10) şi de aceea, este dificil de precizat în ce măsură, la apariţia ei au contribuit HTA sau obezitatea. Este posibil ca modificările structurale ale endoteliului vascular să fie datorate obezităţii şi ele să fi precedat şi detreminat instalarea HTA. Ar fi interesant extinderea studiului pentru a urmări subiecţii hipertensivi şi obezi, cei hipertensivi dar fără obezitate şi cei obezi dar fără hipertensiune arterială, pe o perioadă mai lungă de timp, pentru a vedea în

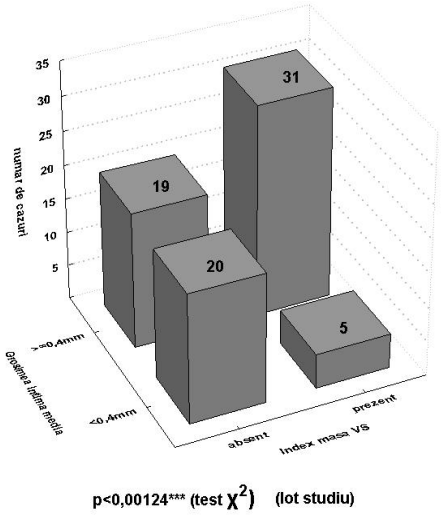

Fig. 7. Corelaţia IMTc cu indexul masei VS în lotul de studiu

ce măsură modificările prezente în copilărie (HTA, obezitatea, disfuncţia endotelială) sunt predictibile pentru evenimentele cardiovasculare din viaţa adultă. De asemenea, ar putea prezenta interes studiere indicatorilor funcţionali vasculari cum sunt distensibilitatea vasculară, geometria arterială coronariană dar şi vasodilataţia mediată de flux.

CONCLUZII

HTA esenţială este prezentă la copil. Raportul intimă-medie crescut corelează cu HTA şi este un indicator al modificărilor structurale endoteliale secundare regimului presional crescut. Deoarece un procent semnificativ din populaţia pediatrică cu HTA studiată a asociat şi obezitate, nu se poate afirma cu certitudine că alterările vasculare se datorează exclusiv HTA. Determinarea raportului intimă-medie prin ultrasonografia Doppler este o metodă accesibilă, neinvazivă, uşor reproductibilă care poate oferi date utile despre procesele structurale de la nivelul arterelor chiar înainte de apariţia semnelor clinice de afectare organică datorată HTA.

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RESPIRATORY FEATURES OF A SYSTEMIC LUPUS ERYTHEMATOSUS IN A SMOKING PATIENT. CASE REPORT

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RESPIRATORY FEATURES OF A SYSTEMIC LUPUS ERYTHEMATOSUS IN A SMOKING PATIENT. CASE REPORT (Abstract): There is increasingly mentioned in literature a respiratory onset of the systemic lupus erythematous (SLE). A 61-year-old female (active smoker, with myocardial infarction history) was admitted in the hospital for respiratory failure, fatigue, nose rash, alopecia, photosensitivity, polyarthralgia, weight loss. Computed Tomography (CT) scan of the chest noticed a pattern of an obliterative bronchiolitis ("tree-in-bud", interstitial fibrosis, and bronchiectasis), kyphosis and advanced atherosclerosis. Immunological investigation revealed antinuclear antibodies that sustained SLE diagnosis. Lung function impairment showed severe mixed ventilatory dysfunction with decreased oxygen saturation (SaO2) below 90% during a minimal effort. Bronchoscopy, chest CT and biomarkers excluded sarcoidosis or lung tumor. Skin biopsy confirmed SLE vasculitis. The patient received immunosuppressive medication, oxygen, bronchodilators, vasoactive agents, platelet aggregation inhibitors, statins. Clinical status improved after 2 weeks and stabilized after 1 year under treatment. SLE may have a long less symptomatic evolution and could evolve apparently subacute with respiratory features. The previous myocardial infarction and the advanced atherosclerosis may be considered early manifestations of SLE vasculitis in a smoker. Early education for smoking cessation in patients with ischemic condition has to be part from the rehabilitation program for respiratory and cardiovascular complication prevention. **Keywords**: SYSTEMIC LUPUS ERYTHEMATOUS, OBLITERATIVE BRONCHIOLITIS, SMOKING.

Systemic lupus erythematosus (SLE) is a severe autoimmune disease with poly visceropathy affecting multiple organs: skin, kidney, vessels, spleen, liver, joints, lungs and the nervous system. The lungs are commonly involved later in the disease evolution with common features: pleural effusion, interstitial lung fibrosis, vasculitis, diaphragmatic dysfunction and upper airway dysfunction (1, 2, 3, 4). The pattern of interstitial fibrosis with obliterative bronchiolitis (OB) is rarely met but possible (less than 5% in SLE) (5). This form of lung involvement in SLE associates obstructive/mixed ventilatory disorder, asthma like symptoms and are close linked with smoking (6, 7, 8). It is well known that smoking by itself is a main risk factor for

various chronic respiratory diseases: chronic bronchitis, idiopathic fibrosis, collagen diseases, tuberculosis and sleep apnea (6, 8, 9). There is increasingly mentioned in literature the late-onset of SLE while clinical/serological aspect seems to be more atypical. In this context, SLE may be considered in people (especially women) of more advanced age starting from any clinical presentations compatible with the disease (10). The case report reviews the particularities of the clinical aspect, pulmonary manifestations of SLE, investigation, and their contribution in SLE diagnosis.

CASE REPORT

We present the case of a late SLE onset in a 61-year-old female patient (active smoker and occupational exposure to chemicals) with a history of pulmonary tuberculosis (PTB) in 2000 and a myocardial infarction in 2008 (minimally treated after the acute episode). The patient was admitted in the Pulmonology Clinic with symptoms began in the last 2 weeks: wheezing, intermittent purulent cough, progressive respiratory failure with rest dyspnea and cyanosis, nose and malar rash, alopecia, asthenia, weight loss, polyarthralgia and diffuse crackles. The chest x-ray revealed a diffuse interstitial fibrosis. Thoracic High Resolution Computed Tomography (HRCT) confirmed the fibrosis and revealed an aspect of obliterate bronchiolitis (OB): "tree-in-bud" bronchioles, centrilobular micronodules (by bronchiolar inflammation and mucus plug), bronchiectasis and air trapping with "mosaic" pattern (fig. 1. a.). Computed Tomography (CT) of the chest excluded lung tumor or pleural complication. Sagittal CT section showed kyphosis and diffuses atherosclerosis of the thoracic and abdominal aorta (fig. 1.b.).

Fig. 1. a. Chest CT: a. Interstitial fibrosis, obliterative bronchiolitis ("tree-in-bud" aspect of bronchioles, centrilobular micronodules) and bronchiectasis.
b. Kyphosis, osteoporosis, advanced diffuse atherosclerosis of the aorta.

Lung function evaluated by spirometry indicated a mixed ventilatory dysfunction with a severe obstructive pattern with forced vital capacity (FVC) of 1.74L (67.9%), forced expiratory flow in 1 second (FEV-1) of 1L (48.7%) and ratio FEV1/FVC of 60.2%. Carbon monoxide diffusing capacity (DLCO) was very low

(57%) and decreased oxygen saturation (SaO2) below 90% during a minimal effort to 86%.

Electrocardiography (ECG) showed an atrial tachycardia, diffuse ischemic changes and sequela of right ventricle myocardial infarction. Transthoracic echocardiogram showed multiples aortic atheroma, right ventricle dilatation and moderate pulmonary hypertension.

Bronchoscopy evidenced a chronic bronchitis, diffuses bronchiectasis and excluded sarcoidosis (negative bronchial biopsy) or a central lung tumor.

Bacteriological investigation performed from bronchoalveolar lavage was negative for Koch bacilli in microscopy and culture, negative for fungi, but positive for the opportunistic infection Pseudomonas aeruginosa.

Several immunological, serological analysis for SLE confirmation were performed revealing low value of C3 Complement (43mg/dl), negative results for anti-neutrophil cytoplasmic autoantibodies (ANCA), serum angiotensin-convertase enzyme, rheumatoid factor, anti-centromere and anti-scl70/anti-topoisomerase antibodies; and positive results for the antinuclear antibodies (ANA) titer (1:40), antibodies anti-double stranded DNA (dsDNA antibodies), anti-Smith antigen, as well as positive cardiolipin antibodies cLA (56 units GPL IgG phospholipid antibodies). Other blood tests revealed mild anemia (Hb-9.86g%, Htc-34%) with reticulocytosis, negative HIV test and anti-streptolysin O antibodies.

The skin biopsy of the scalp confirmed the presence of vasculitis in the context of SLE and permitted differentiation with other lesion: sarcoidosis or “alopecia areata”.

The final diagnosis included SLE (malar and nasal rash, unusual loss of hair, polyarthritis, ANA and dsDNA antibodies, anemia, vasculitis) associated with bronchiolitis obliterans, diffuse lung fibrosis, diffused over-infected bronchiectasis (with Pseudomonas aeruginosa), respiratory failure, moderate secondary pulmonary hypertension, advanced aortic atherosclerosis and ischemic heart disease with sequela of myocardial infarction, mild anemia, dorsal kyphosis, depression and active smoking. The patient was referred to rheumatology and cardiology for further investigations, therapy and monitoring. Under 2 weeks of hospitalization and therapy with immunosuppressive drugs (azathioprine), long term home oxygen, inhaled corticosteroids and long acting-βagonists bronchodilators, vasoactive agents, platelet aggregation inhibitors, statins and antibiotics (fluoroquinolones administered for 10 days), clinical status improved. After 1 year, the patient was stable under chronic treatment.

DISCUSSION

In our reported case, SLE had a slow clinical course with very few symptoms and an apparently late subacute onset with respiratory failure by lung fibrosis and obliterative bronchiolitis (OB) (the diagnostic of SLE was performed in the Pulmonology Clinic). The rheumatologic examination confirmed the suspicion of SLE according to the American College of Rheumatology (ACR) and Systemic Lupus International Collaborating Clinics (SLICC) revised criteria (11). Studies revealed that 3% of SLE cases may occur with respiratory symptoms and have a pattern of obliterative bronchiolitis at the clinical presentation (12). The particularities of SLE with OB and lung fibrosis may be

explained by smoking history and professional exposure (6, 7, 13). So, the risk of lung cancer must be considered in the future (14). The interstitial fibrosis with over infected bronchiectasis evoques a long-time subclinical evolution and an immunodeficient status within the collagen disease. Decreased immunity in association with biological therapy can induced an increased risk of tuberculosis among patients with previous PTB and collagen disease (4,12). OB is a chronic inflammation of the small bronchioles with wall thickening and polypoid proliferation, cell debris, lymphocytic infiltration and peribronchial fibrosis (5) and it may have very heterogeneous causes as viral infections (e. g. respiratory syncytial virus, cytomegalovirus, human immunodeficiency virus), exposure to toxic dust, fumes or drugs (including smoking), collagen diseases, chronic rejection in lung transplantation, primary biliary cirrhosis, some asthma or Chronic Obstructive Pulmonary Disease phenotypes, cystic fibrosis (2, 3, 5, 6, 7, 12). In this reported case, the criteria for OB diagnosis were clinical context of a severe dyspnea in a middle-aged smoker woman, specific HRCT details (hyperinflation, bronchiolar thickening, micronodules, "mosaic" perfusion, diffuse interstitial fibrosis, bronchioles pattern type "tree-in-bud"), severe mixed ventilatory impairment and decreased DLCO. The impact of occupational exposure to chemical products, on lung function, is difficult to evaluate especially related to smoking (1, 2, 6, 7, 14).

The acute coronary accident noticed in the medical history and the advanced aortic atherosclerosis may be considered in relation to smoking and underlying disease. Several studies have established the correlation between SLE and atherosclerosis that is accelerated by the autoimmune vasculitis (15). In this context the treatment with statins was recommended like an immunomodulators of an inflammatory phenomenon (16).

Smoking is an important contributive risk factor that aggravated in the same time fibrosis, obstructive bronchiolitis and vasculitis. Promoting consistent antismoking education would have been required in this case much earlier, especially after the myocardial infarction (17,18). Histopathology from skin biopsy and serologic tests contributed to the diagnosis of vasculitis within SLE and to the differential diagnosis with sarcoidosis or other well-defined vasculitis (19, 20). Vasculitis can be a rare manifestation of tuberculosis or tuberculosis can associate with vasculitis (21). Heavy smoking associated with a collagen disease, as rheumatoid arthritis, can evoques even tuberculosis or lung cancer (22). Bacteriology from bronchoalveolar lavage permitted the diagnosis of the bacterial colonization of bronchiectasis, antibiotic susceptibility test and the targeted antibiotic treatment. Considering the risk of tuberculosis in Romania (22), bacteriological screening must be performed. Negative microscopy for acid fast bacilli stain and cultures for Koch bacilli and nontuberculous mycobacteria excluded tuberculosis and mycobacteria (2-10% prevalence met in immunodeficient patients) (23).

CONCLUSIONS

Our case is distinguished by delayed diagnosing of SLE in a Pulmonology Department. SLE may have a long-time mild symptom masked by a progressive respiratory failure. Vascular and skin main involvement of the SLE did not associated with renal and/or nervous manifestations.

The respiratory features of obliterative bronchiolitis and lung fibrosis allowed the necessity of an urgent broad immunologic investigation for underling collagen disease. The previous early myocardial infarction, round 50-year-old, and the advanced atherosclerosis could be considered in relation both with SLE vasculitis and chronic smoking.

According to the medical history and comorbidities, a close medical surveillance and sustained treatment are required. Early education for smoking cessation in patients with respiratory and vascular ischemic poor condition has to be a main component of a rehabilitation program for preventing the respiratory and cardiovascular complications.

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NEWS

LIPIDOMES OF LUNG CANCER AND TUMOUR-FREE LUNG TISSUES REVEAL DISTINCT MOLECULAR SIGNATURES FOR CANCER DIFFERENTIATION, AGE, INFLAMMATION, AND PULMONARY EMPHYSEMA

The study highlights how health status might be mirrored in the lung lipidome. Little is known about the human lung lipidome, its variability in different physiological states, its alterations during carcinogenesis and the development of pulmonary emphysema. Tissues were sampled for both lipidomic and histological analysis. Using a screening approach, were characterized lipidomes of lung cancer tissues and corresponding tumor-free alveolar tissues. Tumor tissues exhibited elevated levels of triacylglycerols and cholesteryl esters, as well as a significantly lower abundance of phosphatidylglycerols, which are typical lung surfactant components. Adenocarcinomas and squamous cell carcinomas were distinguished with high specificity based on lipid panels. Lipidomes of tumor biopsy samples showed clear changes depending on their histology and, in particular, their proportion of active tumor cells and stroma. Partial least squares regression showed correlations between lipid profiles of tumor-free alveolar tissues and the degree of emphysema, inflammation status, and the age of patients. Unsaturated long-chain phosphatidylserines and phosphatidyl inositol showed a positive correlation with a worsened emphysema status and ageing. This work provides a resource for the human lung lipidome and a systematic data analysis strategy to link clinical characteristics and histology (Eggers LF, Müller J, Marella C *et al*. Lipidomes of lung cancer and tumor-free lung tissues reveal distinct molecular signatures for cancer differentiation, age, inflammation, and pulmonary emphysema. *Scientific Reports* 7, 2017).

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CIRCULAŢIA GERMENILOR ÎN SECŢIILE DE NOU-NĂSCUŢI FACTORI DE RISC PENTRU INFECŢIA MATERNO-FETALĂ

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GERM CIRCULATION IN NEONATAL UNITS – RISK FACTORS FOR MATERNAL--FOETAL INFECTION (Abstract) Infections are the third most important cause of morbidity and mortality in neonatal units. Infection rates are 2-5% in I-II level units, 10% in IIIrd level units and 7-24% in NICUs. **Aim**: To identify risk factors contributing to maternal-foetal and hospital infections in neonatal units of different levels in two maternity hospitals in Romania: Buna Vestire Obstetrics and Gynaecology Hospital in Galati (Lot A) and Cuza-Voda Obstetrics and Gynaecology Hospital in Iasi (Lot B). **Material and methods**: This is a retrospective and prospective study during four years in two neonatal units in maternity hospitals of different levels from Moldova: level II – Lot A and level III – Lot B. **Results**: Neonatal infection rates were significantly higher in preemies compared to term newborns (5.91% - Lot A vs. 9.9% - Lot B). Predominant maternal colonization and infection bacteria were *E. coli* (16.12% - Lot A vs. 12.6% - Lot B) and *S. aureus* (1.3% - Lot A vs. 2.03% - Lot B). Personnel colonization, predominantly with MRSA, presented little hazard for neonatal infection (RR 0.9 – Lot A, RR 0.7 – Lot B). Hospital environment contamination also presented minor risk for neonatal infection (RR 1.7 – Lot A, RR 0.2 – Lot B). **Conclusions**: The main risk factors for neonatal infections were gestational age (premature infants have immunological underdevelopment) and maternal colonization. Personnel colonization and hospital contamination are of little importance in the development of neonatal infections. **Key words**: NEONATAL INFECTION, MATERNAL COLONIZATION, GESTATIONAL AGE, HOSPITAL ENVIRONMENT CONTAMINATION

Infecţiile sunt a treia cauză de morbiditate şi mortalitate în secţiile de nou-născut (1). Rata infecţiilor diferă în funcţie de nivelul secţiei (nivel I-II comparativ cu secţiile de nou-născuţi de nivel III şi terapie intensivă neonatală). Astfel, rata de infecţie în secţiile de nivel I-II este de 2-5%, 10% în secţiile de nivel III şi între 7-24% în secţiile de terapie intensivă neonatală (TIN) (2,3). Factorii majori implicaţi în producerea infecţiilor materno-fetale şi nosocomiale ţin în special de gazdă care este nou-născutul la termen şi mai ales prematur (imaturitate imunologică), de gravidă şi lehuză colonizată cu germeni gram negativi sau pozitivi, de condiţiile de îngrijire în secţiile de nou-născuţi normali (supraaglomerare, circuite inadecvate, personal insuficient) şi terapie intensivă neonatală (proceduri diagnostice şi terapeutice, ventilaţie mecanică, alimentaţie parenterală, cateterizări vasculare etc.) (1). Germenii cei mai implicaţi în producerea infecţiilor neonatale în maternităţile din România sunt germenii gram ne-

gativi (*E. coli, Klebsiella, Enterobacter* etc.) iar dintre gram pozitivi *S. aureus* şi mai rar stereptococul beta hemolitic de grup B care este predominant în infecţiile nou-născutului în SUA.

OBIECTIVE

Identificarea factorilor de risc cu rol în producerea infecţiilor materno-fetale şi nosocomiale în secţii de nou-născuţi de nivele diferite din două maternităţi din România : Spitalului Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi (Lot A) şi Spitalului Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi (Lot B).

MATERIAL ŞI METODĂ

Studiu retrospectiv şi prospectiv pe o perioadă de 4 ani în două maternităţi de nivele diferite din regiunea Moldovei, nivel II – lot A şi nivel III – lot B, în secţiile de nou-născuţi.

Secţiile de nivel II şi III au fost definite conform OM 910/2001 secţiile de nivel II îngrijesc nou-născuţi normali în special şi nou-născuţi prematuri peste 34 săptămâni de gestaţie cu patologie uşoară, iar secţiile de nivel III îngrijesc toată patologia neonatală severă şi nou-născuţi normali.

În perioada analizată (2006 – 2009) în Spitalului Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi din numărul total de nou-născuţi (4267 nou-născuţi) 381 au prezentat infecţii neonatale, aceştia reprezentând lotul A de studiu. În aceeaşi perioadă în Spitalul Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi din numărul total de nou-născuţi (28112 nou-născuţi) 2791 au prezentat infecţii neonatale (Lot B).

Au fost luate în studiu : incidenţa infecţiilor pe grupe de vârstă (prematuri şi nou-născuţi la termen) ; factorii de risc în producerea infecţiei : colonizare maternă, colonizarea neonatală, colonizarea personalului medical pe tipuri de germeni ; identificarea germenilor în testele de sanitaţie ; corelarea colonizării materne cu colonizarea nou-născutului şi infecţia nou-născutului ; corelarea colonizării personalului cu colonizarea nou-născutului şi infecţia nou-născutului ; corelarea normelor de igienă cu colonizarea nou-născutului şi infecţia nou-născutului ; identificarea germenilor la mama, la personalul medical şi prin testele de sanitaţie a fost făcută aleatoriu. Prelucrarea statistică a datelor a fost realizată în SPSS 16, prin teste parametrice şi neparametrice specifice tipului de date, evaluarea rezultatelor făcându-se pe baza indicatorilor statistici descriptivi.

REZULTATE ŞI DISCUŢII

Incidenţa colonizării şi infecţiei la nou-născut în perioada studiată

În perioada 2006 –2009 din numărul total de 4267 nou-născuţi din Spitalului Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi, 804 cazuri au prezentat colonizări bacteriene iar infecţii materno-fetale şi nosocomiale au fost identificate la 381 nou-născuţi reprezentând 8.93% din numărul total de naşteri în perioada studiată. În lotul B de studiu (Spitalului Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi) din numărul total de naşteri (28112 naşteri, în perioada 2006-2009) s-au înregistrat 5504 cazuri cu colonizări din care 2791 nou-născuţi au prezentat infecţii (9.93%).

Studiul evidenţiază faptul că incidenţa prezenţei germenilor a fost semnificativ mai mare ($p=0.017$, $\chi^2=5.65$, 95%CI) în Spitalului Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi – (Nivel III 19.58% versus 18.84% Nivel II), datorită faptului că în Spitalului Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi funcţionează Centru Regional de Terapie Intensivă care îngrijeşte nou-născuţi proveniţi prin transfer din regiunea Moldovei, nou-născuţi din cadrul maternităţii şi prematuri, însă rata de infecţie între cele două maternităţi nu prezintă diferenţă semnificativă aspect ce poate fi explicat prin

TABELUL I
Factorii de risc implicaţi în apariţia infecţiei neonatale

Factori de risc în infecţia neonatală	Lot A N / %	Lot B N / %	RR Raport de risc		r Coef. de corelaţie		p-Niv, de semnificaţie 95%CI	
Prematuritatea			**Lot A**	**Lot B**	**Lot A**	**Lot B**	**Lot A**	**Lot B**
VG *<29 săpt,*	15/0,35%	715/2,54%	12,98	58,24	-0,7	-0,9	0,027	0,000
VG *29-32 săpt,*	48/1,12%	1168/4,15%						
VG *33-37 săpt,*	189/4,43%	899/3,2%						
VG *peste 37 săpt,*	129/3,02%	9/0,03%						
Colonizare maternă	1165/27,3%	7397/26,31%	8,64	13,32	0,87	0,91	0,000	0,000
Tipul de germeni					0,55	0,75	0,001	0,000
Gram pozitivi	217/5,09%	1997/7,01%	2,07	1,38	0,55	0,48	0,002	0,000
Gram negativi	948/22,2%	5400/19,21%	3,94	4,08	0,89	0,95	0,000	0,000
Colonizare personal (probe: 974-lot A/3992-lot B)	211/21,66%	684/17,13%	0,94	0,7	0,63	0,51	0,005	0,041
Teste de sanitaţie (probe: 512-lot A/5512-lot B)	32/6,25%	64/1,16%	1,77	0,2	0,76	0,32	0,003	0,245 (n.s.)

faptul că procedurile de îngrijire efectuate după protocoale bine puse la punct sunt net superioare fată de nivelul II de secţie unde nu există protocoale de practică.

Factorii de risc implicaţi în colonizarea şi infecţia nou-născutului

În lotul A incidenţa nou-născuţilor prematuri cu infecţie neonatală este semnificativ mai mică (1,48%) comparativ cu lotul B (6,7%). O diferenţă semnificativă între cele două loturi a fost înregistrată de asemenea pentru incidenţă nou-născuţilor cu infecţie şi vârste de gestaţie peste 37 săptămâni, în lotul A aceasta fiind semnificativ mai mare (3,02%) comparativ cu lotul B (0,03%), aceste rezultate pot fi explicate prin faptul că secţia de nivel II conform ordinului 910/2001 nu are competenţe de a îngriji nou-născut cu vârste de gestaţie sub 34 săptămâni. Analiza corelaţională a demonstrat existenţa unei asocieri semnificative între VG şi prezenţa infecţiilor neonatale, acestea fiind frecvente la vârste de gestaţie mici ($r=-0.82$, $p=0.0003$, 95%CI).

Colonizarea maternă - germeni izolat la mamă în urocultură şi lohicultură.

Cazurile selecţionate în lotul studiat au fost diferenţiate în funcţie de tipul de germene, frecvenţa a fost calculată raportându-ne la numărul anual de naşteri. Din analiză reiese faptul că germenii frecvent izolaţi la mama atât în lohicultură cât şi în urocultură au fost gram negativii, aspect regăsit în ambele secţii (secţia de nivel II - 22.2%, secţie de nivel III – 19.21%). Germenii gram pozitivi au prezentat o frecvenţă redusă în ambele loturi de studiu (5.09% - lot A vs. 7.1% lot B).

Cel mai frecvent germen izolat la mama a fost *Escherichia coli* atât pentru Spitalului Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi (16.12%) cât şi pentru Spitalului Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi (12.69%). Colonizarea maternă vaginală sau urinară cu *E.coli* îşi are explicaţia în igiena precară a gravidei şi lehuzei. În secţiile de nivel III o frecvenţă mai mare comparativ cu cele de nivel II a fost semnalată pentru streptococul beta hemolitic de grup D (2.91% nivel III vs. 2.64% nivel

II), *S. aureus* (2.03% nivel III vs. 1.33% nivel II), *Klebsiella pn.* (1.84% nivel III vs. 1.62% nivel II) *Enterococcus* (1.66% nivel III vs. 1.42% nivel II). Frecvenţe de sub 1% au fost înregistrate pentru *Str. agalactiae, Pn. aeruginosa, s*tafilococ coagulazo negativ, Proteus (0.09% nivel III vs. 0.02% nivel II), *Str. viridans* (0.02% - nivel III vs. 0% nivel II), *Acinetobacter spp*. (0.01% nivel III vs. 0.05% nivel II), *Citrobacter spp*. (0.01% - nivel III vs. 0.03% nivel II) şi SBHB (0.02% atât în secţiile de nivel II cât şi în secţiile de nivel III).

Tipul de germen izolat la nou-născut

Analiza tipurilor de germeni la nou-născut a demonstrat faptul că în secţia de nivel II (Lot A) frecvenţa maximă a fost înregistrată pentru *Escherichia coli* (4.98%) fiind urmată de *S. aureus* (3.69%) şi stafilococ coagulazo negativ (1,47%). Valorile frecvenţei întâlnite în aceste secţii sunt semnificativ mai mici faţă de frecvenţele întâlnite în secţiile de nivel III în care *Escherichia coli a* fost identificată la 5.97%, pentru *S. aureus* la 4.76% iar în cazul stafilococului coagulazo-negativ s-a înregistrat o frecvenţă dublă a colonizărilor nou-născuţilor (2.46%). O situaţie diferită este întâlnită în cazul colonizării cu *Klebsiella* şi *Enterobacter* pentru care frecvenţa nou-născuţilor este mai mare în cazul celor proveniţi din secţiile de nivel II (2.97% vs. 1.73% - *Enterobacter* şi 2.87% vs. 2.43% - *Klebsiella pn.*). În secţiile de nivel II o frecvenţa scăzută dar semnificativ mai mare faţă de secţiile de nivel III a fost întâlnită pentru *Enterococcus* (1.12% vs. 0.5%) şi *Citrobacter* (0.5% vs. 0.15%). Colonizarea cu *Ps. aeruginosa*, *Acinetobacter spp.*, *Str. virindas*, streptococ beta hemolitic de grup B (SBHB), *Str. agalactiae* a prezentat o frecvenţă mai mică cu diferenţe mici între cele două spitale (nivel II vs. nivel III).

Cel mai puţin frecvent izolat germen la nou-născut a fost streptococul beta hemolitic de grup B 0.11% în timp ce în ţări ca şi SUA 2% din nou-născuţi pot fi colonizaţi cu streptococ beta hemolitic care ulterior poate să inducă pneumonie congenitală sau septicemie (4, 5).

Rata de infecţie la nou-născuţi

Analiza s-a făcut în funcţie de debutul semnelor clinice de infecţie (sub 72 ore şi peste 72 ore) conform definiţiei infecţiei materno-fetale şi nosocomiale date de CDC. În lotul A semnele clinice de infecţie care au debutat în primele 72ore postnatal au fost în procent de 1.85% (din numărul total de naşteri), cele ce au apărut după 72 de ore au fost de 8.91% (infecţie nosocomială. În lotul B incidenţa infecţiilor nosocomiale înregistrează o valoare mai mică 7.46%.

Cele mai frecvente infecţii la nou-născut au fost cele localizate (8.8%), iar septicemia a prezentat o incidenţă mai mică însă ce diferă semnificativ ($\chi^2=5.81$, $p=0.0159$) între cele două loturi analizate (1.95% - lot A vs. 1.41 – lot B).

În secţiile de nivel II cel mai frecvent germen izolat a fost *E.coli* (3.85%) urmând apoi *Klebsiella pn*. (1.57%) şi *Enterobacter* (1.24%). În secţiile de nivel III la nou-născuţii infectaţi s-a izolat frecvent aceeaşi germeni : *E. coli* (3.7%), *S. Aureus* (2.15%), *Klebsiella pn*. (1.22%) şi *Enterobacter* (0.98%).

Contaminarea mediului de spital

În maternitatea Spitalului Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi nu există o rutină în supravegherea mediului de spital, numărul probelor fiind foarte mic în raport cu cele recoltate în lotul B, rezultatele studiului comparativ între cele două maternităţi din acest punct de vedere are o acurateţe scăzută.

În lotul A din totalul probelor recoltate în perioada 2006-2009 (512), 32 (6.25%) au fost pozitive, acestea determinând apariţia infecţiei neonatale la 1.73% nou-născuţi (tab. I).

În lotul B contaminarea mediului de spital a fost investigat prin recoltări periodice,

astfel din totalul probelor recoltate în Spitalul Clinic de Obstetrică Ginecologie „Cuza-Vodă" Iaşi (nivel III) în perioada 2006-2009 (5512), 64 (1.16%) au fost pozitive, cauzând infecţie neonatală la 0.45% nou-născuţi, pondere semnificativ mai mică comparativ cu valoarea înregistrată în lotul A de studiu (1.73%), aspect ce demonstrează riscul crescut de infecţie neonatală în secţiei de nivel II (tab. I).

În lotul A (nivel II) riscul de infecţie neonatală datorat contaminării suprafeţelor (RR=1.77) este semnificativ mai mare comparativ cu riscul existent în secţia de nivel III (lot B) (RR=0.2).

Colonizarea personalului medical

Germenul cel mai frecvent implicat în colonizarea personalului medical a fost *S. aureus*. Studiul evaluează frecvenţa colonizării personalului cu Stafilococ auriu meticilinorezistent (MRSA) şi stafilococ auriu oxacilinosensibil (OSSA) atât în lotul A cât şi în lotul B.

În perioada analizată (2006 – 2009) în Spitalului Clinic de Obstetrică Ginecologie „Buna Vestire" Galaţi s-au analizat 974 probe de exsudat nazal ale personalului, dintre aceste 211 probe au evidenţiat prezenţa *S. aureus* (21.66%) afectând 177 nou-născuţi (94 -2.2% colonizaţi şi 83-1.95% infectaţii neonatale). În lotul B din 3992 probe exsudat nazal recoltate, din care 684 s-au pozitivat (17.13%). Se remarcă faptul că în secţia de nivel II colonizarea personalului reprezintă o incidenţă mai mare (21.66% vs. 17.13%) (tab. I).

În secţia de nivel II rezultatele demonstrează existenţa unei corelaţii moderate (r=0.63, p=0.0357) între colonizarea personalului şi infecţia neonatală, aceasta nereprezentând un factor de risc major în apariţia infecţiilor la nou-născut (RR, OR=0.94). În secţia de nivel III analizată riscul de apariţie a infecţiei la nou-născut datorate colonizării personalului este nesemnificativ (OR=0.6, χ^2=15.287, p=0.02045, RR=0.7, r=0.51, p=0.041 (tab. I). Comparând valorile raportului de risc şi gradul de asociere al colonizării personalului cu infecţia neonatală se constată faptul că colonizarea personalului nu reprezintă un risc major de apariţie a infecţiilor neonatale însă se poate aprecia faptul că în lotul A de studiu (secţie de nivel II) riscul a fost mai ridicat (RR=0.94) comparativ cu cel întâlnit în lotul B (RR=0.7).

Analiza lotului A a demonstrat existenţa unei corelaţii semnificative între tipul de germene identificat la mamă şi tipul de germene identificat la nou-născutul cu infecţie. (r=0.5587, p=0.001342). În lotul B de studiu corelaţia între tipul de germene identificat la mamă şi tipul de germene identificat la nou-născutul cu infecţie este semnificativă (r=0.75, p=0.0000327, 95%CI).

Comparând gradul de asociere dintre tipul de germene identificat la mamă şi cel identificat la nou-născut în cele două loturi studiate, rezultă faptul că în secţiile de nivel II corelaţia este mai mică (r=0.558, p=0.0013) comparativ cu corelaţia întâlnită în secţiile de nivel III (r=0.755, p=0.00003), aspect ce poate fi explicat prin faptul că în secţiile de nivel II infecţia nou-născutului poate fi determinată şi de alţi factori în afara colonizării materne.

În secţiile de nivel III principalul factor ce influenţează apariţia infecţiei la nou-născut este colonizarea maternă, restul factorilor de risc fiind implicaţi mai puţin. În secţiile de nivel II doar 1/4 din nou-născuţii ce prezintă germeni gram pozitivi provin din mame colonizate cu germeni din acelaşi grup în timp ce în secţiile de nivel III mai mult de jumătate dintre nou-născuţii colonizaţi cu germeni din acest grup provin din mame colonizate cu germeni de acelaşi tip.

Analiza transmiterii germenilor de la mama la nou-născut în cele două maternităţi de nivele diferite evidenţiază aspecte diferite. Acestea constau în faptul că în secţiile de nivel III (Iaşi) din mamele cu infecţii

(15.1% Galaţi, 11.2% Iaşi) deşi avem o colonizare mai mare a nou-născutului (nou-născuţi colonizaţi 5.5% Galaţi vs. 7% Iaşi) frecvenţa infecţiilor la nou-născut (1.41% -Iaşi) este semnificativ mai mică în secţia de nivel III comparativ cu secţiile de nivel II (2.06%-Galaţi), aspect explicat prin procedurile de îngrijire (fig.10).

O situaţie diferită este semnalată în cazul nou-născuţilor cu infecţii ce provin din mame colonizate, pentru aceştia înregistrându-se o frecvenţă semnificativ mai mare în secţia de nivel III – Iaşi (7.04%) comparativ cu secţia de nivel II – Galaţi (5.25%), aspect ce poate fi explicat şi prin faptul că aceşti nou-născuţi prezintă o patologie asociată complexă ce creşte semnificativ riscul pentru infecţie (tab. I).

Analiza riscului pe care colonizarea mamei o prezintă pentru infecţia neonatală a fost evaluată realizând tabelele de contingenţă ce priveşte frecvenţa infecţiei în raport cu numărul total de naşteri în perioada studiată.

În secţiile de nivel II (lot A) riscul de apariţie a infecţiei la nou-născut în cazul colonizării materne a fost de 8.64 ori mai mare (RR=8.64, p<<0.05). Există o corelaţie semnificativă între colonizarea maternă şi prezenţa infecţiei la nou-născut (r=0.87, p<<0.05, 95%CI). În secţiile de nivel III riscul de apariţie a infecţiei la nou-născut datorate colonizării materne a fost de 13.32 ori mai mare (RR=13.32, p<<0.05, 95% CI, (r=0.91, p<<0.05) (tab. I).

Comparând rezultatele se remarcă faptul că în secţiile de nivel III riscul apariţiei infecţiei la nou-născut datorate colonizării materne este semnificativ mai mare (RR= 13.32) faţă de riscul apariţie a infecţiei neonatale în secţiile de nivel II (RR=8.64) aspect justificat prin faptul că în producerea infecţilor intervine şi factorul gazdă.

CONCLUZII

Incidenţa infecţiilor în cele două maternităţi a fost de 1.85% infecţii materno-fetale în lotul A şi 2.75% în lotul B iar a infecţiilor nosocomiale a fost de 8.91% în lotul A şi 7.46% în lotul B.

Factorii de risc implicaţi în infecţia nou-născutului a fost prematuritatea (RR=12.9 -lot A, RR=58.2-lot B), colonizarea maternă (RR=8.6-lot A, 13.3-lot B), colonizările nazale ale personalului medical (RR= 0.94–lot A, RR=0.7–lot B) şi într-o mică măsură mediul de îngrijire (RR=1.7–lot A, RR=0.2–lot B).

Germenii cel mai frecvent implicaţi atât în colonizarea şi infecţia maternă cât şi în colonizarea şi infecţia nou-născutului au fost gram negativii (E.*coli, Klebsiella pn. Enterobacter, Entorococcus*) şi *S.aureus* meticilino reziztent (MRSA).

Există o corelare semnificativ statistică între colonizarea maternă şi colonizarea/ infecţia nou-născutului (r=0.8-lot A, r= 0.9-lot B) şi de asemnea o corelarea între colonizarea personalului medical (*S.aureus*) şi infecţia neonatală (r=0.6-lot A, r=0.5 -lot B).

Cele mai frecvente infecţii ale nou-născutului au fost cele localizate (8.81%-lot A, 8.8%-lot B) iar septicemia a reprezentat 1.9% din nn lotului A şi 1.4% din nn lotului B.

Există diferenţe semnificative intre colonizările şi infecţiile nou-născutului între cele două maternităţi aspect ce poate fi explicat prin faptul că în secţia de nivel III procedurile de îngrijire sunt efectuate după protocoale foarte bine puse la punct acestea fiind net superioare fată de nivelul II de secţie unde încă nu există protocoale de practică.

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NOUTĂŢI

EFICIENŢA ASOCIERII AMOXICILINĂ - METRONIDAZOL ÎN ÎNGRIJIREA PARODONTITELOR AGRESIVE GENERALIZATE

Studiul realizat de un grup de cercetători parodontologi din cadrul Universităţii din Istanbul a avut ca scop evaluarea efectelor combinaţiei metronidazol-amoxicilină asupra parametrilor clinici şi microbiologici la pacienţii diagnosticaţi cu parodontită agresivă generalizată. În studiu au fost incluşi douăzeci şi opt de pacienţi, repartizaţi în 2 loturi : lotul de studiu (n = 12) şi lotul martor (n = 16). Lotul de studiu a primit asocierea amoxicilină-metronidazol alături de detartraj şi planare radiculară. Lotul martor a primit doar detartraj si planare radiculară. Alături de examenul clinic, au fost analizate probe de placă subgingivală pentru bacterii total cultivabile şi prezenţa de *Porphyromonas gingivalis*, *Tannerella forsythia* (anterior *T. forsythensis*), *Treponema denticola*, *Prevotella intermedia*, *Prevotella nigrescens*, *Prevotella pallens*, şi *Aggregatibacter actinomycetemcomitans* (anterior *Actinobacillus actinomycetemcomitans*) folosind reacţia în lanţ a polimerazei (PCR). Rezultatele studiului indică faptul că la ambele loturi, toţi parametrii clinici au fost îmbunătăţiţi semnificativ comparativ cu valorile iniţiale ($P < 0,05$). A existat o reducere semnificativă statistic a profunzimii pungii parodontale şi s-a obţinut un câştig de ataşament la lotul de studiu comparativ cu lotul martor ($P < 0,05$). Numărul total de bacterii, a scăzut în mod semnificativ la 3 şi 6 luni la ambele loturi ($P < 0,05$). *T. denticola* şi *T. forsythia* au fost bacteriile cel mai des întâlnite pe parcursul studiului. *T. denticola* a arătat o scădere continuă de peste 6 luni la lotul de studiu, în timp ce la lotul martor nu a fost observată nici o schimbare după 3 luni. *P. gingivalis* a scăzut în mod semnificativ la 3 luni ($P < 0,05$), în timp ce *T. forsythia* a fost singurul patogen care a fost scăzut sub limitele de detecţie, de terapia asociată, cu o diferenţă semnificativă ($P < 0,05$) comparativ cu lotul martor. Rezultatele acestui studiu arată că asociaţia amoxicilină - metronidazol utilizată ca adjuvant la detartraj şi planare radiculară conduce la o vindecare clinică mai bună comparativ cu tratamentul mecanic singur. Terapia medicamentoasă folosită a avut ca rezultat o scădere semnificativă şi substanţială a *T.forsythia* şi a prevenit recolonizarea sa timp de 6 luni, sugerând astfel faptul că *T.forsythia* poate determina stabilitatea pe termen lung a rezultatelor tratamentului parodontal (Yek EC, Cintan S, Topcuoglu N, Kulekci G, Issever H, Kantarci A. Efficacy of amoxicillin and metronidazole combination for the management of generalized aggressive periodontitis. *J Periodontol* 2010 ; 81 (7) : 964-974).

Irina Grădinaru

PRIMARY GASTRIC LYMPHOMA IN A PROSPECTIVE STUDY

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PRIMARY GASTRIC LYMPHOMA IN A PROSPECTIVE STUDY (Abstract). **Aim**: To assess the of occurrence of primary gastric lymphoma using epidemiological, clinical and biological data. This rare pathology includes more different histological subtypes that can affect different segments of gastrointestinal tract. **Material and methods**: Retrospective analysis of the hospital-based medical records of patients with primary gastrointestinal lymphoma admitted in the Hematology Clinic of the University Emergency Hospital of Bucharest between October 2014 and June 2018. The analyzed data included demographic data, *Helicobacter pylori* infection, disease staging, laboratory tests and objective examination. All the patients made a CT scan of the chest, abdomen and pelvis. The histological diagnosis was based on biopsy specimens in performing upper digestive endoscopy and immunohistochemistry. All data was statistically analyzed using *STATA 13* (Stata Corp LLC US). **Results**: This study included 21 patients that have been diagnosed with primary gastrointestinal lymphoma. It was observed that primary gastric lymphoma frequently affects more females than males. Histopathological, the majority of primary gastric lymphoma (90.41%) were B-cell lineage and diffuse large B-cell occurred in 66.66% of cases. Out of the 21 patients surveyed, 13 (61.90%) presented loss of appetite, 9 (42.85%) had abdominal pain. Anemia was present in 16 cases. **Conclusions**: The majority cases in our study were diffuse large B-cell lymphoma which were related to infection with *Helicobacter pylori* in half the cases. **Keywords**: GASTRIC LYMPHOMA, HELICOBACTER PYLORI, MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA, DIFFUSE LARGE B-CELL LYMPHOMA.

In the last years, the incidence of gastrointestinal lymphoma is rising. This rare pathology includes more different histological subtypes that can affect different segments of gastrointestinal tract (1, 2). The stomach is frequently affected by two subtypes of lymphoma and is related with chronic inflammation made by *Helicobacter pylori* infection (3).

Chronic inflammation leads to a hyperstimulation of T helper lymphocytes, which in turn stimulates the proliferation of B-lymphocytes from the gastric mucosa with mutations and subsequent transformation

into gastric lymphoma (4,5). Gastric MALT (mucosa-associated lymphoid tissue) lymphoma has a higher survival rate that can reach 90% at ten years because is an indolent disease (6). Are cases that can develop from MALT lymphoma in Diffuse large B-cell lymphoma (DLBCL) with an unfavorable prognosis, survival rate dropping until 42% (6). Some substances, such as fungicides, pesticides, solvents, were related to a higher incidence of lymphoma (7) and current screening test are not sensitive enough for early detection of the risk, as also seen for other occupational induced diseases (8).

Taking into consideration these aspects, **the aim** of this study is to assess the occurrence of primary gastric lymphoma using epidemiological, clinical and biological data.

MATERIAL AND METHODS

Population. Variables

In this study were evaluated 51 patients diagnosed with primary gastrointestinal lymphoma, admitted in the Hematology Clinic of the University Emergency Hospital of Bucharest, between October 2014-June 2018. All patients included in the study signed and agreed the participation in the research process before any maneuvers (9, 10).

From those 51 patients, was selected a number of 21 (41.17%) patients that have been diagnosed with primary gastric lymphoma with different histological subtype. All of those patients performed upper digestive endoscopy with biopsy sampling for testing *Helicobacter pylori*. All patient with secondary gastric lymphoma were excluded from our analysis.

Variables analyzed included demographic data, the presence of symptomatology B (fever, weight loss without trying, exceeding 10% of body weight in 6 months, and profuse sweating), histological subtype, *Helicobacter pylori* infection, disease staging, performance status, lactate dehydrogenase (LDH), and the International Prognostic Index (IPI).

For the observed patients, examination included also the presence of general signs, assessment of peripheral adenopathies, hepatosplenomegaly, assessment of the general status of the patient in order to calculate the performance status (ECOG). Laboratory analyzes included complete blood count, transaminases, serum lactate dehydrogenase, albumin, immunoelectrophoresis, uric acid, coagulogram, viral serology such as hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV) and Epstein Barr virus (EBV), antibodies of *Helicobacter pylori*.

The histological diagnosis was based on the biopsy specimens obtained during the upper digestive endoscopy and immunohistochemistry. The histological classification was performed according to the World Health Organization classification (2016) and staging was based on the Ann Arbor modified Musshoff classification (11, 12). Also, it was performed bone marrow aspirate with a biopsy. All the patients performed a CT scan of the chest, abdomen and pelvis. Histological remission according to *Groupe d' Etude des Lymphomes de l'Adulte* (GELA) criteria include the necessity for endoscopic biopsy follow-up (13).

Analysis method

The statistical analysis was performed using STATA 13 software. In order to estimate the survival probability from observed survival times, the Kaplan-Meier (KM) method

is used (14). The survival probability at time t_i, $S(t_i)$ is calculated as follow:

$S(t_i)=S(t_{i-1})(1-(d_i/n_i))$

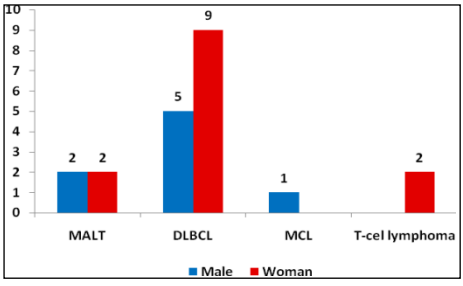
where,

- $S(t_{i-1})$ is the probability of being alive at t_{i-1}
- n_i is the number of patients alive just before t_i
- d_i is the number of events at t_i
- $t_0 = 0$, $S(0) = 1$

The estimated probability $(S(t))$ is a step function that changes value only at the time of each event. It's also possible to compute confidence intervals for the survival probability. The KM survival curve, a plot of the KM survival probability against time, provides a useful summary of the data that can be used to estimate measures such as median survival time.

RESULTS

Gender distribution depending on histological subtype is presented in first figure. From this graphic it can be observed that females were more frequently affected than males. Histopathological, the majority of primary gastric lymphoma (90.41%) were B-cell lineage and diffuse large B-cell occurred in 66.66% of cases. In study group, 47.62% were smokers.



*MCL= Mantle Cell Lymphoma

Fig. 1. Types of gastric lymphoma distribution, by gender

The results obtained by using survival analysis are presented in first table. The results (tab. I) show that, the most frequent symptom was loss of appetite in 13 (61.90%) patients and abdominal pain in 9 (42.85%) patients. LDH was elevated in 80.95% of the cases. Hypoalbuminemia was found in 85.71% of patients. Bleeding at presentation was present in 5 patients and anemia was only present in 16 cases. Localized disease (Ann Arbor stages I or II) was present in 7 (33.34%) of cases and the rest were advanced stages (III-IV). From our study 11 (52.38%) patients died, 9 (42.85%) of them were diagnosed with DLBCL, all with III-IV stage Ann Arbor, high IPI score and ECOG ≥2. All patients were tested for infection with *Helicobacter pylori* using antibodies and biopsy sampling that were positive for 7 patients and respectively 10 patients. Treatment was different for each patient, 5 patients diagnosed with DLBCL and 2 patients with T-cell lymphoma suffered emergent surgery. Patients tested positive for *Helicobacter pylori* received eradication therapy. Chemotherapy regimens consisted in administration of 6-8 application of R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine and prednison) for MALT, DLBCL and MCL and for T-cell lymphoma was administered R-COEP (rituximab-cyclophosphamide, epirubicin, vincristine and prednisone). The only case with MCL was without response to 8 application of R-CHOP regimens and received Ibrutinib 560 mg per day obtaining complete remission. We have 50 months of surveillance and approximately 30% of patients are alive, no statistical differences are noted for MALT and DLBCL (fig. 2).

TABLE I

Baseline characteristics of primary gastric lymphoma patients and their significance level

Parameters	MALT	DLBCL	MCL	T-cell lymphoma	Total number of patients	p-value
Age ≥ 60 years	2 (9.52%)	12 (57.14%)	1 (4.76%)	1 (4.76%)	16 (76.19%)	0.046 (S)
Elevated LDH	1 (4.76%)	14 (66.6%)	1 (4.76%)	1 (4.76%)	17 (80.95%)	0.045 (S)
Low-albumin	4 (19.04%)	12 (57.14%)		2 (9.52%)	18 (85.71%)	0.005 (S)
Hemoglobin <12g/dl	4 (19.04%)	9 (42.85%)	1 (4.76%)	2 (9.52%)	16 (76.19%)	0.16 (NS)
Upper digestive hemorrhage	2 (9.52%)	3 (14.28%)			5 (23.80%)	0.06 (NS)
B symptoms	2 (9.52%)	14 (66.66%)	1 (4.76%)	2 (9.52%)	19 (90.47%)	0.06 (NS)
ECOG						
<2	1 (4.76%)	4 (19.04%)		1 (4.76%)	6 (28.57%)	0.023 (S)
≥2	3 (14.28%)	10 (4.76%)	1 (4.76%)	1 (4.76%)	15 (71.43%)	0.04 (S)
IPI score						
Low	1 (4.76%)				1 (4.76%)	0.36 (NS)
Low/Intermediate	1 (4.76%)	3 (14.28%)			4 (19.04%)	0.39 (NS)
High/Intermediate	1 (4.76%)	2 (9.52%)			3 (14.28%)	0.41 (NS)
High	1 (4.76%)	9 (42.85%)	1 (4.76%)	2 (9.52%)	13 (61.90%)	0.33 (NS)
Ann Arbor Staging						
I-II	2 (9.52%)	5 (23.8%)			7 (33.34%)	0.591 (NS)
III-IV	2 (9.52%)	9 (42.85%)	1 (4.76%)	2 (9.52%)	14 (66.66%)	0.356 (NS)
Death	1 (4.76%)	9 (42.85%)		1 (4.76%)	11 (52.38%)	0.257 (NS)

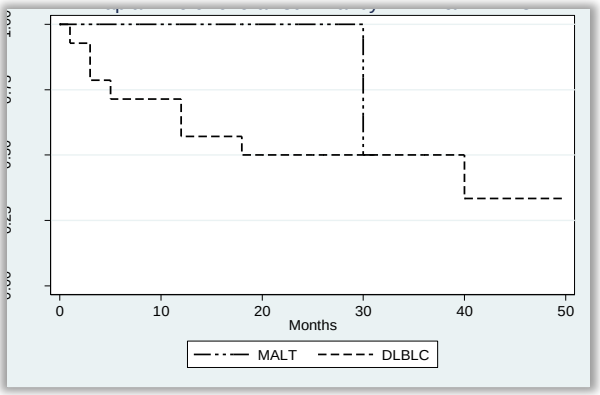


Fig. 2. Kaplan-Meier survival curves for MALT and DLBCL.

DISCUSSION

The results obtained from the data analysis answered to the research objective established. We assumed that in gastric MALT lymphoma is difficult to establish the diagnosis due to normal endoscopy findings in many cases. In our analysis were used 51 patients which were diag-

nosed with primary gastrointestinal lymphoma, admitted in the Hematology Clinic of the University Emergency Hospital of Bucharest. The stomach represents the most common site of gastrointestinal tract affected by lymphoma due the fact that chronic inflammation is caused often by the infection with *Helicobacter pylori*.

Our study shows that, the majority of the patients were females and that a chronic inflammation pathology is hard to diagnose, most cases being diagnosed in late stages, as demonstrated (66.64% cases - stages III-IV Ann Arbor) due to indolent evolution, poor clinical picture, most clinical manifestations occurring late in the disease.

In gastric MALT lymphoma is difficult to establish the diagnosis due to normal endoscopy findings in many cases. Ideally, all patients should benefit from superior digestive endoscopy for biopsy harvesting, even if the mucosa is absolutely normal. Since endoscopic techniques have advanced in recent years, endoscopy can improve treatment strategies. It is also important that biopsy specimens to be examined by anatomo-pathologists in the field for early diagnosis and survival of this patient population.

For histological diagnosis to be correct, about 10-20 biopsies from several regions of the stomach should be obtained (15, 16). For those cases receiving primary surgery for digestive hemorrhages that cannot be managed endoscopically, careful histopathological examination is recommended.

Another endoscopic tool that can be easily used in the staging of this pathology is echoendoscope, which can appreciate the degree of infiltration of the gastric wall, extramural dissemination, and lymph node damage (17).

Besides, the gastro-intestinal site extranodal MALT lymphoma the most frequent sites of infiltration are the salivary glands, lung and thyroid (6) and a differential diagnosis has to be done with primary tumors with this localization (18) in order to achieve this objective. Being meticulous on the patient examination and on the following treatment we can stage the disease and prevent the transforming into DLBCL forms (6). In our country with increased prevalence of tuberculosis (19), the combination of multiple risk factors (20, 21) may increase the risk of hospitalization of these patients and implicitly the incidence of hospital infections (22), especially in patients with comorbidities (23) with associated cardiovascular diseases (24, 25, 26) that may associate gastric lymphoma and other types of cancers (27, 28, 29, 30).

In our study, primary gastric lymphoma affects older females and the common complaints were loss of appetite, abdominal pain and anemia secondary to blood loss. The majority of studies in this domain reflected that this pathology is frequent to males (31).

Recent meta-analyzes have shown that smoking is considered a risk factor for patients diagnosed with gastric cancer, thus counseling for smoking cessation being very important (32, 33). The association between smoking and gastric lymphoma needs to be studied more carefully considering that most patients are smokers (34, 35, 36).

LDH is an important prognostic factor. Although specialty studies did not support hypoalbuminemia as a prognostic factor, it should be taken into consideration because most of our patients (85.71%) experienced hypoalbuminemia. To demonstrate this, additional studies that include a larger number of patients are needed.

In the 50-month period, rate of survival

of for the patients diagnosed with gastric lymphoma, the MALT lymphoma survival was superior to those with DLBCL, with 100% of the former with a survival of up to 30 months, only 75% of those with DLBCL had a survival of 6 months, about 60% had a survival of 10-18 months and only 50% had a survival of 19-40 months. A 30% of those with DLBCL gastric lymphoma showed a survival of 40-50 months.

Most of those lymphomas were present in patients with age ≥ 60 years, diagnosed in advanced stages of the disease and having high mortality.

CONCLUSIONS

In our study we highlighted that the primary gastric lymphomas are a rare pathology, difficult to diagnose, especially because clinical manifestations are unspecific and endoscopic appearance is not typical. In this paper the majority cases of primary gastric lymphoma were diffuse large B-cell lymphoma which were related to infection with *Helicobacter pylori* in half of the cases.

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DIFFERENTIATION OF PATIENTS WITH CHRONIC LOMBOSCIATICA DUE TO DISC HERNIA USING GAIT ANALYSIS TECHNIQUES

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DIFFERENTIATION OF PATIENTS WITH CHRONIC LOMBOSCIATICA DUE TO DISC HERNIA USING GAIT ANALYSIS TECHNIQUES (Abstract): Nowadays gait analysis represent a modern technique used as a useful tool in the assessment of patients with locomotors disease. **Aim**: To make a differentiation of patients with chronic sciatica due to disc hernia using gait analysis techniques. **Material and method**: Study was prospective, with consecutive selection of subjects according to eligibility criteria, using a control group. The number of subjects was 47: 28 patients and 19 healthy subjects. The patients were characterized clinically, imagistically and biomechanically. The biomechanically evaluation was made with VICON MX optical motion capture system. Our data of interest were: temporo-spatial and kinematic parameters. These data were factorial analyzed with a principal component extraction technique, resulting 10 variables, which characterized the system variability in 94.67%. Next step was represented by a hierarchical cluster analysis for sub-group identification. **Results**: We have differentiated two clusters. The two clusters were individualized regarding the temporo-spatial parameters (opposite foot contact, step width) as well as the kinematic parameters (maximum upward rotation in stance of pelvis, maximum adduction of the hip in stance, maximum abduction of the hip in swing, maximum plantar flexion angle in swing, total sagittal plane excursion of the ankle). **Conclusions**: The two clusters can be defined as following: cluster 1: reduced stance phase and mainly distal problems (of the ankle); cluster 2: normal stance phase duration, severely low step width and mainly proximal problems (pelvic belt). **Key words**: GAIT PATTERN, CLUSTER, SCIATICA

A series of previous studies centered on gait analysis, which have focused both on healthy patients (1,2) and on patients with different musculo-skeletal conditions (3) provided a large amount of data of high interest for the improvement of rehabilitation programmes.

Our study focused on patients with chronic lombosciatica due to disc hernia and was motivated by the prevalence of this pathological type which is constantly increasing within general population (4,5,6,7) as well as by the severe socio-economical impact it generates (8,9).

The results of the study will help improving or creating rehabilitation programmes, making them more appropriate for the case.

AIM OF THE STUDY

The aim of our study was to make a differentiation of patients with chronic sciatica due to disc hernia using gait analysis techniques

MATERIALS AND METHOD

STUDY DESIGN

Study was prospective, with consecutive selection of patients according to inclusion and exclusion criteria, using a control group.

Inclusion criteria were : age above 18, mechanical low back pain unilaterally irradiated on radicular territory of L5/S1 ± neurological signs corresponding to the radicular affection (without motor deficit), chronic clinical phenomena with a duration of at least 4 weeks, presence of lumbar disc hernia documented through CT or MRI, patients with independent walking ability. Exclusion criteria were : no systemic disease with relevant influence on walking ability, no orthopedic surgeries on lower limbs, conditions included in "red flags" screening, motor deficit corresponding to radicular involvement L5/S1, counter indications in CT or MRI exploring, presence of the inflammatory biological syndrome.

The number of subjects was 47 : 28 patients with chronic lombosciatica and 19 apparently healthy subjects who represent the control group. The average (± standard deviation) age, mass and height of the patients were 45 years (±12), 67.4 Kg (± 7.5), and 167.7 cm (± 7.5). The average (±standard deviation) age, mass and height of the subjects from control group were 38 years (± 8), 63.7kg (± 6.4) and 168.5cm (± 9.1).

Materials were represented by selected patient and control group, lumbar spine images (CT and IRM), patient's clinical data files and VICON MX optical motion capture system.

Method. The patients were selected and characterized clinically and imagistically. The biomechanical evaluation for patients and control group was performed with Nexus and Poligon programs. Our data of interest were : temporo-spatial parameters (opposite foot off, opposite foot contact, foot off, cadence, step length, walking speed, step time, step width, stride length, stride time) and joint angle values for pelvis, hip, knee, ankle in sagittal and/or coronal plane (fig. 1-5).

DATA ANALYSIS

There are different ways in which information may be extracted from raw data. Cluster analysis is a technique of data mining that assigns records in classes based on a similar measure. Clustering algorithms seek to fragment the entire data set into homogenous subgroups. The similarity of the data within the cluster is maximized while the similarity between the records of different clusters is minimized. As a result studying the newly created subgroups may reveal patterns that can help in defining medical features.

In our cluster analyses, the Ward's linkage method was used, with the purpose of minimizing the error sum of squares. This is related to analyses of variance ANOVA. Thus, we will find homogeneous subgroups.

OUTLIERS' DETECTION

Outliers are extreme values that may generate unstable results on the analysis. It is possible to go against the trend by using them. To eliminate the outliers we have com-

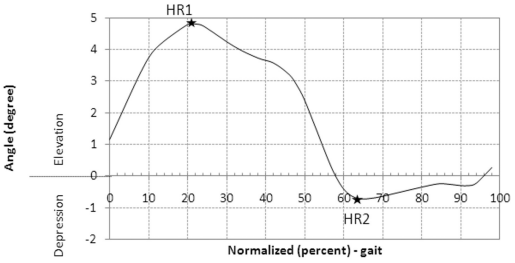

Fig. 1. Pelvis parameters (coronal plane) : HR1 : maximum upward rotation in stance, HR2 : maximum downward rotation in swing

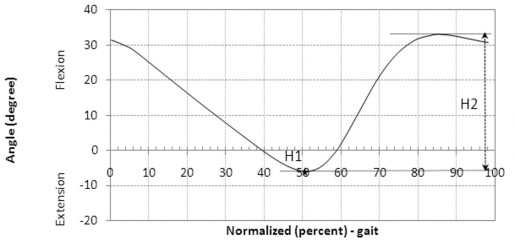

Fig. 2. Hip flexion-extension parameters (sagittal plane) H1 : maximum extension angle in stance phase, H2 : total sagittal plane excursion

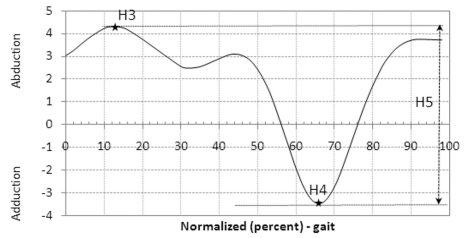

Fig. 3. Hip adduction-abduction parameters (coronal plane) H3 : maximum adduction of the hip in stance, H4 : maximum abduction in swing, H5 : total excursion of the hip

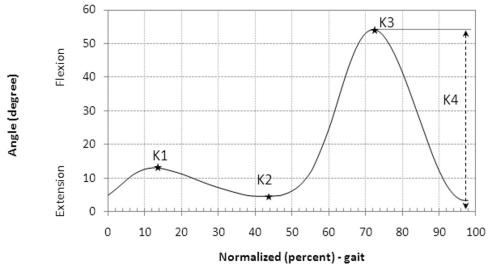

Fig. 4. Knee flexion-extension parameters (sagittal plan) K1 : maximum flexion during loading response, K2 : maximum extension in stance phase, K3 : maximum flexion in swing, K4 : total sagittal plane excursion

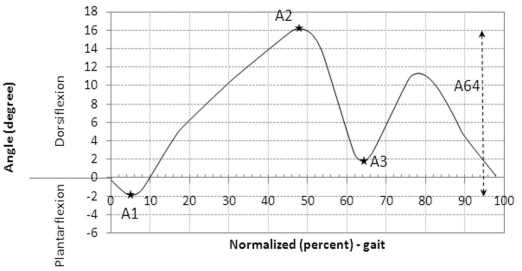

Fig. 5. Ankle dorsiflexion-plantarflexion parameters (sagittal plane) A1 : maximum plantarflexion angle during loading response, A2 : maximum dorsiflexion in stance phase, A3 : maximum plantar flexion angle in swing, A4 : total sagittal plane excursion

puted the Mahalanobis distance for every cases. Next, we have transformed these values in tailed probabilities (Chi-square distribution) and decide to eliminate those that have less than 1‰ probability. This is like the Gaussian distribution of nearly three standard deviations. It is a typical method for detecting extreme values. In our computation we did not find outliers, in fact the cases are close to less than two standard deviations equivalence.

RESULTS

All the 26 variable proposed in the study were compared between the two clusters delivered and each of them with the control group. The student (t) test was utilized and the probability $p=0.05$ was set as a level of significance.

There are the 10 variables selected by the criterion of maximum correlation with the factors derived :

- walking speed,
- maximum extension angle in stance phase of hip (H1),
- total sagittal plane excursion of ankle (A4),
- maximum dorsiflexion in stance phase of ankle (A2),
- maximum flexion angle during loading response of knee (K1),
- maximum upward rotation in stance of pelvis (HR1),
- total excursion of the hip in coronal plane (H5),
- step width, opposite foot contact,
- maximum plantarflexion angle during loading response of ankle (A1).

CHARACTERIZATION AND COMPARISON OF THE TWO CLUSTERS

We have differentiated two clusters, witch were individualized regarding the temporo--spatial parameters (opposite foot contact,

step width) as well as the kinematic parameters: maximum upward rotation in stance of pelvis (HR1), maximum adduction of the hip in stance (H3), maximum abduction of the hip in swing (H4), maximum plantar flexion angle in swing (A3), total sagital plane excursion of the ankle (A4).

RESULTS REGARDING THE TEMPORO-SPATIAL PARAMETERS

In cluster 2, opposite foot contact was achieved at the same value as in the control group. In cluster 1 this event was registered sooner (49.17+/-1.28) than in cluster 2 (50.51 +/-1.49), therefore generating a stance phase with normal duration for cluster 2, and reduced duration for cluster 1.

Both clusters patients have a lower step width compared to the control group ($p < 1‰$). In cluster 2 step, width has significantly statistical lower values than in cluster 1 ($p < 0.003$).

RESULTS FOR KINEMATIC PARAMETERS

Pelvis obliquity. The two clusters share statistically significant differences just regarding the HR1 ($p = 0.002$).

Hip kinematic parameters: the statistically significant differences between the two clusters refer only to H3 and H4

For both clusters H3 is significantly higher than in the control group ($p < 1‰$), yet in cluster 2 it has significantly statistical higher values compared to cluster 1 ($p < 1‰$).

In both clusters H4 is significantly higher than the value of control group ($p < 1‰$), but in cluster 2 the hip abduction is significantly reduced compared to cluster 1 ($p < 0.023$).

Knee. There are no statistically significant differences between the two clusters in terms of knee kinematic data.

Ankle. The two clusters show statistically significant differences in what concerns the A3 ($p < 1‰$) and A4 ($p < 0.03$). Only in cluster 1, A3 and A4 are significantly statistically different compared to

TABLE I

Mean (±standard deviation) temporo-spatial parameters divided into two clusters and control group

No.	Measure	Average +/- StDev			p - significance		
		Cluster					
		1	2	Control (C)	1-2	1-C	2-C
1	Opposite foot off (%)	19.8 (±5.36)	20.6 (±4.32)	11.8(±3.2)	0.711	<1‰	0.001
2	Opposite foot contact (%)	49.17 (±1.28)	50.51 (±1.49)	50.4(±1.1)	0.025	<1‰	0.838
3	Foot off (%)	68.18 (±5.35)	69.85 (±3.94)	61.8(±4.1)	0.434	<1‰	0.001
4	Step frequency (step/min)	74.88 (±14.88)	69.95 (±16.89)	98.2(±5.81)	0.452	<1‰	0.002
5	Double support time(s)	0.68 (±0.31)	0.8 (±0.49)	0.5(±0.034)	0.454	0.018	0.13
6	Step length(m)	0.39 (±0.09)	0.4 (±0.07)	0.59(±0.045)	0.834	<1‰	<1‰
7	Walking speed(m/s)	0.5 (±0.21)	0.48 (±0.19)	1.08(±0.13)	0.865	<1‰	<1‰
8	Step time (s)	0.85 (±0.17)	0.89 (±0.38)	0.11(±0.029)	0.644	<1‰	0.001
9	Step width(m)	0.18 (±0.04)	0.12 (±0.04)	0.25(±0.054)	0.003	<1‰	<1‰
10	Stride length(m)	0.77 (±0.2)	0.85 (±0.16)	1.32(±0.11)	0.336	<1‰	<1‰
11	Stride time(s)	1.67 (±0.34)	1.79 (±0.7)	1.23(±0.073)	0.547	<1‰	0.05

TABLE II
Mean (±standard deviation) pelvic, hip, knee and ankle angles (⁰) divided into two clusters and control group

No.	Measure	Average +/- StDev			p - significance		
		Cluster		Control (C)			
		1	2		1-2	1-C	2-C
12	HR1	2.06 (±2.19)	5.79 (±3.63)	2.62(±3.055)	0.002	0.269	0.043
13	HR2	-1.43 (±1.85)	-0.94 (±1.85)	-2.48(±3.16)	0.534	0.021	0.051
14	H1	2.28 (±7.81)	-2.53 (±5.51)	-11.22(±4.73)	0.125	<1‰	0.003
15	H2	28.83 (±5.3)	32.97 (±6.72)	35.74(±5.5)	0.096	<1‰	0.283
16	H3	6.25 (±2.61)	10.56 (±2.79)	3.5(±4.1)	<1‰	<1‰	<1‰
17	H4	-0.12 (±2.27)	2.45 (±3.16)	-7.05(±3.8)	0.023	<1‰	<1‰
18	H5	6.42 (±2.93)	8.2 (±2.17)	8.92(±2.3)	0.133	0.001	0.386
19	K1	7.47 (±4.64)	9.12 (±6.16)	10.31(±6.4)	0.447	0.013	0.603
20	K2	3.31 (±4.23)	2.78 (±4.99)	2.27(±5.1)	0.779	0.284	0.779
21	K3	45.07 (±7.23)	50.38 (±8.86)	54.06(±5.1)	0.111	<1‰	0.279
22	K4	43.44 (±7.69)	49.6 (±7.12)	51.79(±6.6)	0.062	<1‰	0.414
23	A1	-4.54 (±3.15)	-6.93 (±3.53)	-2.99(±3.95)	0.092	0.039	0.016
24	A2	15.62 (±3.04)	13.5 (±3.13)	13.95(±2.65)	0.111	0.023	0.703
25	A3	1.13 (±5)	-7.21 (±5.43)	-11.47(±5.25)	<1‰	<1‰	0.062
26	A4	20 (±3.04)	23.14 (±3.76)	25.42(±2.8)	0.03	<1‰	0.131

the control group ($p < 1‰$). In cluster 2, these values show similarities with those in the control group.

DISCUSSIONS

The reduction of the stance phase duration in patients belonging to cluster 1 could be explained by human tendency to bring the healthy foot back on the floor more quickly (11).

Both clusters have hyperadduction in stance. Moreover, this aspect becomes more evident in cluster 2 where the adduction in stance is significantly statistically higher, compared to cluster 1. This may explain why step width is much lower in cluster 2 than in cluster 1. Other authors regarding patients with stroke (12) made the same rationality.

Hipoabduction of the hip in swing, possibly in relation with the abductor weakness, is one of the conclusions of our study which keeps within the same lines as in the literature (10).

As previous authors (10) have already exposed it, we have also noted the presence of the so called "false hip extension" which refers to a hip extension restriction during the swing phase.

A possible explanation in the occurrence of sagittal hip extension restriction is the weakness of the gluteus maximus muscle, when tonification of this muscle proves to be successful.

The pelvis changes, in coronal plane, are the results of several disturbances in links of the lower functional kinetic chain (10,13,14). HR2 is limited in the patients of both clusters and even more, there is a higher HR1 in cluster 2.

Step length and stride length are restricted in patients from both clusters compared to the control group. One explanation could be the restriction of the sagittal hip extension, which appears in all patients. Sharon Kinsella et al. (12) presented a similar reasoning.

During the initial double support, patients from both clusters show excessive ankle plantar flexion, which may be partially explained by the occurrence of a weak plantar dorsiflexor muscle eccentric contraction (15).

The swing plantar flexion is statistically significantly reduced in cluster 1, compared to cluster 2. This could be due to a weak

concentric contraction of some of the plantar flexor muscles (the long toe flexors and peroneals) (15).

CONCLUSIONS

Discussing chronic lombosciatica due to disc hernia, in terms of temporo-spatial and kinematic parameters, all patients had problems compared to the normal subjects. Only two of the temporal spatial parameters that have been discussed proved to be useful in identifying the two clusters : step width and opposite foot contact.

Several kinematic parameters have been deducted within the two clusters identification process : HR1, H3, H4, A3, and A4.

Gait characteristics are not homogenous but reveal two clusters :

Cluster 1 : reduced stance phase and mainly distal problems (of the ankle) : decreased ankle plantarflexion in swing phase and decreased total ankle excursion in sagittal plane.

Cluster 2 : normal stance phase duration, severely low step width and mainly proximal problems (pelvic belt) : hyperadduction of hip in stance phase, hypoabduction of hip in swing phase and increased pelvic upward rotation in stance.

The obtained results can be considered as useful in improving the classical rehabilitation treatment schemes applied to patients with chronic lombosciatica due to disc hernia.

In terms of rehabilitation treatment, gait examination in a modern approach, should find wider insertion in current practice.

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COLPOSCOPY IN PATIENTS WITH ATYPICAL GLANDULAR CELLS ON PAP SMEARS

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COLPOSCOPY IN PATIENTS WITH ATYPICAL GLANDULAR CELLS ON PAP SMEARS (Abstract): The aim of this study was to review the risk of preneoplastic cervical lesions or invasive gynecologic cancer in patients with atypical glandular cells (AGC) on Papanicolaou smear and to establish the place of colposcopy in the management of these patients. **Material and methods**: We have analyzed all cases with ACG who attended our colposcopy clinic between 2001-2014. All cases were followed for a period of at least 36 months after the diagnosis of AGC on Pap smear. Medical records were reviewed to determine the clinical data, colposcopy findings and histology results. **Results:** We have analyzed 78 women with AGC on Pap smear. The age of the patients was 41+/-12.3 years old. Normal colposcopy, minor colposcopy changes, major colposcopy changes and suspicion of invasion were encountered in 44 cases (56.4%), 8 cases (10.2%), 12 cases (15.4%), and respectively, 1 case (1.3%) case. The colposcopy was not satisfactory (transformation zone type III) in 13 cases (16.6%). Histology was abnormal in 25 cases (32%) as follow: invasive cancer 5 cases (7.1%), adenocarcinoma in situ in 2 cases (2.6%), high-grade cervical lesion 8 cases (10.6%) and low-grade cervical lesion 10 cases (12.8%). **Conclusions:** The ACG diagnosis needs new strategies in order to reduce the overtreatment but not to increase the false negative results. Colposcopy is useful in cases with associated squamous cervical lesions, but new diagnostic tools are mandatory for a better management. A possible one is represented by Human Papilloma Virus test, but the place of this test should be clarified by further studies.
Keywords: ATIPYCAL GLANDULAR CELLS, HPV TESTING.

Since the introduction of well-organized screening programs, a considerable drop in the incidence and mortality of squamous cervical cancer (SCC) has been reported. The decreased incidence is achieved through detection of early abnormalities, which can be treated, avoiding the progression to invasive cancer. However, despite the screening programs for cervical cancer, in the last decades the incidence of cervical adenocarcinoma has not changed consistently (1, 2). Several studies have reported in the last two decades increasing rates of cervical adenocarcinoma relative to SCC, especially among younger women (3). This may be explained by the fact that adenocarcinomas and adenosquamos carcinomas are less amenable to prevention by screening than SCC of the cervix and cervical cytology is less sensitive and less specific for the identification of glandular neoplastic lesions of the cervix than it is for its squamous anoma-

lies (1, 4).

Because the prognosis of cervical adenocarcinoma is worse than that of squamous cervical carcinoma it is important that the disease is diagnosed as early as possible (1). The cytology plays an important role in the diagnosis and follow-up of the intraepithelial glandular lesions. However, cellular characteristics of endocervical intraepithelial precursor lesions have remained poorly documented with the exception of the adenocarcinoma in situ that has been described in detail.

The term atypical glandular cells of undetermined significance were introduced in Bethesda Nomenclature in 1989 and was defined as morphologic changes in glandular cells beyond those suggestive a benign reactive process, but insufficient for the interpretation of adenocarcinoma (5). In the 2001 Bethesda System, this category has been renamed "atypical glandular cells" (AGC), with the sub classifications "not otherwise specified" (AGC-NOS) and "favor neoplastic" (AGC-FN). The cytology report should define whether the cytological glandular abnormality relates to cervical or endometrial glandular cells or indicate whether the type of glandular cells cannot be clearly identified. Adenocarcinoma in situ has been separated as another distinct category of diagnosis (6). This change has been made in order to better reflect current knowledge and understanding of glandular neoplasia.

Glandular abnormalities represent a challenge not only for the cytologist but also for the colposcopist, with the possibility of missing or misdiagnosing them. This leads to poor sensitivity or specificity of colposcopy in glandular lesions. The clinicians have a difficult task to gain a balance between under or overtreatment of AGC cytological diagnosis. The aim of this study is to document the experience of the colposcopy in cases with AGC in our clinic and to propose new strategies in order to achieve a better management of AGC diagnosis.

MATERIAL AND METHODS

Beginning from 01.01.2001 till 01.01.2014, there were 97 referrals with AGC in our colposcopy clinic. Information regarding age, clinical indication for taking the Babes-Papanicolau smear, clinical history, colposcopy diagnosis and pathological report were extracted from our database. We excluded patients where we had no final report or where the follow-up was not possible (19 patients). All cases were followed for a period of at least 36 months after the diagnosis of AGC on Pap smear. The total number of cases included in the study was 78 (72 cases with AGC-NOS and 6 cases with AGC-FN). Data were analyzed using the statistical package *SPSS*. All patients give informed consent for biopsy. An internal review board for ethic approved the study.

RESULTS

The mean age of the patients were 41 years old +/- 12.3 years SD and the clinical indications for Pap smear are as follows (tab. I) .

TABLE I

Indication for Pap smear

Indication for Pap smear	Number of patients	%
Routine (opportunistic screening)	36	46.2%
Abnormal bleeding	18	23.1%
Cervical pathology in history	24	30.7%
Total	78	100%

The colposcopy exam was satisfactory (columnar squamous junction visible, with transformation zone type I and II) in 65 cases (83.3%). In 13 cases (16.6%) the colposcopy was unsatisfactory (transformation zone type III). All patients with unsatisfactory colposcopy where in menopausal status.

The colposcopy findings are presented are the following (tab. II). In 4 cases (5.1%) we had suspicion of glandular lesion, represented by white areas in the columnar epithelium.

TABLE II
Colposcopy findings

Colposcopy findings	Number of cases	%
Normal findings	44	56.4%
Minor findings	8	10.2%
Major findings	12	15.4%
Suspicion of invasion	1	1.3%
Unsatisfactory	13	16.6%

Histology was obtained through excisional procedures (loop electrosurgical excision procedure or conization) in 24 cases and diagnostic procedures (punch biopsy) in 54 cases. In 34 cases (43.6%) we performed a cervical curettage and in 44 cases (women over 35 years old or with abnormal bleeding) we performed both cervical and endometrial sampling.

The pathological reports are presented in tab. III. In 53 cases the pathology results were normal or had benign changes, while in 25 cases the results showed abnormalities.

TABLE III
Pathological findings

Histology findings	Number of cases	%
Normal findings	53	67.9
Low-grade cervical lesion	10	12.8
High-grade cervical lesion	8	10.3
Adenocarcinoma in situ	2	2.6
Invasive carcinoma	5	6.4

DISCUSSION

Cervical cytology is the only current detection method for cervical glandular neoplasia, but its sensitivity is poor (7). Although the natural history of glandular cervical lesions is poorly documented, several studies in the literature indicate that AGC Pap smear results are associated with a high frequency of underlying high-grade cervical neoplasia or cancer (8, 9). The prevalence or short-term cumulative incidence of invasive gynecologic cancer varies from 1% to 8% in follow-up women with AGC (8, 9, 10).

Because of the risk of a gynecologic neoplasia, direct referral for colposcopy, endocervical or endocervical plus endometrial exploration is indicated when a cytological result of AGC or endocervical adenocarcinoma in situ (AIS) is reported. Endometrial sampling is recommended when: glandular lesion is qualified as being endometrial on cytology report, women is older than 35 years or in cases with abnormal vaginal bleeding (11).

Although in all cases with AGC on Pap smear colposcopy is mandatory, in the literature much debate continues with regard to the colposcopy findings in glandular lesions. There are no obvious colposcopy features that allow definite diagnosis of AIS and adenocarcinoma. Colposcopy is useful to differentiate between normal and abnormal epithelium in squamous cervical lesions. For glandular lesions there are no firm and widely accepted criteria. Many colposcopist consider that glandular intraepithelial neoplasia has no specific colposcopy features and colposcopy with punch biopsy has little role in their precise diagnosis (12). However, everybody agrees that diagnosis by colposcopy, of AIS and adenocarcinoma require a high level of experience and skill. Colposcopists can be trained to suspect where to suspect the presence of an adenocarcinoma in situ or invasive, but confirmation always requires histology.

In our study the association with invasive cancer, adenocarcinoma in situ and high-grade cervical lesions was: 6.4%, 2.6% and respectively 10.3%. Our data are concordant with those from the literature. In cases where colposcopy was satisfactory,

minor and major colposcopy changes were present in 10.2% and respectively 15.4% of cases. The colposcopy was useful in cases where the final diagnosis comprised a squamous intraepithelial lesion. In only 4 cases we observed colposcopy changes suggestive for glandular lesion, and the histology confirmed the glandular involvement in one case. In 13 cases colposcopy was not useful, because the columnar junction was not observed and all of these cases where menopausal patients. In one case colposcopy was normal, but conization revealed adenocarcinoma in situ. This could be explained by the fact that cervical glandular neoplasia may be focal within the endocervical canal rather than arising at the transformation zone (skip lesions). On the other hand, we had 53 patients (67.9%) with negative histology and this may represent an overtreatment induced by the possibility of having CIN.

Patel and col. showed that a normal, satisfactory colposcopy has a negative predictive value of 100% and they can confidently exclude any cervical abnormalities in women under 35 years of age (13). These data are not supported by the study of Chumen because they discovered CIN in patients younger than 35 years old with AGC but normal colposcopy. According to their study the specificity of colposcopy is poor in young patients with AGC (14).

Due to the difficulties that can arrive with colposcopy it is obvious that there is need for new strategies for management of AGC in order to avoid the overtreatment but in the same time not to miss asymptomatic cervical intraepithelial lesions. A possible tool could be represented by Human Papilloma Virus (HPV) typing. While HPV typing is a well and recognized test for detecting squamous CIN, insufficient data are available concerning the performance of HPV testing in glandular cervical lesions. HPV detection was strongly associated with the presence or severity of cervical lesion (CIN 2 or worse) in women referred for AGC or AIS in their Pap smear. These data suggest the use of HPV testing in triage of women with AGC Pap smears (15, 16). In endocervical lesions HPV test could be a valuable tool to decrease the number of diagnostic procedures with its related morbidities but further studies are needed in order to establish the exact place of HPV typing in management of the cytological diagnosis of AGC (17). HPV testing has several limitations and one of them is the inability to diagnose non-related HPV lesions such as endometrial adenocarcinoma. The incidence of endometrial abnormality in AGC Pap smear is ranging from 10% to 40% (14, 18). HPV test is not useful in cases with endometrial abnormalities. A very important role here is the cytologist examination who should specify the origin of the cell: cervical or endometrial. Also, the age could be a predictive factor for the origin of the lesion: younger women most often, have endocervical lesions, whereas endometrial carcinoma generally occurs in older women. Women at risk for endometrial or extra uterine malignancies should undergo appropriate evaluation regardless of HPV status (16). The new cervical screening program introduced in Romania will increase the number of cases with ACG and in clinical practice a proper management of these cases is very important (19, 20).

The main limitation of our study is that we do not have an HPV test applicable to all patients due to the fact that the test is not free of charge and only a small number of patients could afford it. It would be of interest to perform more studies, to see if HPV test could be implemented in the management of AGC, and how this test will reduce, the number of invasive procedures used for the diagnosis.

CONCLUSIONS

The cytological diagnosis and management of AGC represents a challenge not only for the cytologists but also for the clinicians and all women with AGC on cervical cytology require immediate referral for colposcopy and assessment by an experienced gynecologist. Colposcopy is very useful if squamous cervical lesions are associated. New strategies are needed in order to increase the accuracy of the diagnosis and management of glandular lesion and HPV test could be useful for glandular cervical lesions, but the place of this test should be clarified by further studies.

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NEWS

PLACENTA ACCRETA FOLLOWING HYSTEROSCOPIC LYSIS OF ADHESIONS CAUSED BY ASHERMAN'S SYNDROME: A CASE REPORT AND LITERATURE REVIEW

Asherman's syndrome is defined as partial or complete obstruction of the uterine cavity primarily caused by intrauterine procedures and infections. Hysteroscopic adhesiolysis is commonly used to treat Asherman's syndrome. Although the frequency of placenta accreta is known to increase with pregnancy after hysteroscopic adhesiolysis, precise data remain unknown. We report a case of placenta accreta following hysteroscopic lysis of adhesions caused by Asherman's syndrome and IVF treatment and review the literature on placenta accreta following hysteroscopic adhesiolysis. It is necessary to consider placenta accreta as a complication of pregnancies after hysteroscopic adhesiolysis for Asherman's syndrome, particularly in those conceived using IVF. The incidence of placenta accreta is significantly higher in IVF pregnancies than in spontaneous pregnancies. Esh-Broder *et al.* reported that the rate of placenta accreta in spontaneous pregnancies was 12/752 (1.2/1,000), while that in IVF pregnancies was 30/24,441 (16/1,000). The higher rate of placenta accreta in IVF pregnancies may be due to the change in endometrial environment and morphological and structural changes to the endometrium due to the IVF treatment protocol (stimulation protocol). Intrauterine adhesions (IUA) severity is associated with greater reduction in fertility thereby increasing the likelihood that affected patients will need to undergo IVF to have a successful pregnancy. The extent of endometrial loss increases with IUA severity, which likely confers a greater risk of placenta accreta. For this reason, doctors should be aware that IVF pregnancy following hysteroscopic adhesiolysis involves a greater risk of placenta accreta (Yuko Sonan,S Higeru Aoki *et al.*, Placenta accreta following hysteroscopic lysis of adhesions caused by Asherman's syndrome: a case report and literature review, *Case Reports in Obstetrics and Gynecology*, 2018; https://doi.org/10.1155/2018/6968382)

THE USE OF FUZZY LOGIC IN CANCER DIAGNOSIS - REVIEW

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THE USE OF FUZZY LOGIC IN CANCER DIAGNOSIS-REVIEW (Abstract): Cancer has always been a disease difficult to fully comprehend by researchers because both of the genetic and environmental factors as well as the complexity of the human organism. Scientific and technological developments alongside the trend of multidisciplinary approach have enabled the acquisition of new medical knowledge as well as employing new diagnostic or treatment tools. Such could be the example of the use of artificial intelligence in the diagnosis or management of certain diseases. This paper emphasizes on the use of fuzzy logic in cancer diagnosis. The main issue addressed is early diagnosis by identifying at-risk individuals through risk factor assessment. The types of cancer chosen were those with the highest incidence and mortality worldwide: lung, breast, prostate, colo-rectal and gastric cancer. Using an artificial intelligence program along the patient database could quicken and optimize the referral of at-risk individuals to specialists, which in turn could improve the diagnosis of early stage cancer disease, thus reducing mortality and morbidity. **Keywords**: CANCER, DIAGNOSIS, FUZZY LOGIC.

The evolution of science and technology is characterized by an exponential growth in the last centuries alone. Acquiring new scientific knowledge prompted the development of new technologies, which in return, through a positive feed-back, helped enrich scientific knowledge. The physical world around us has been demystified and structured into constituent parts, patterns all governed by clearly stated laws. Yet complex systems still represent an issue to scientist as they are very difficult to describe and rationalize. Examples of complex systems are the living organisms, ecosystems, climate of the earth, social organizations. To be able to describe such a complex system first requires identifying all the separate parts of the system. If we take into consideration the ultimate parts of this system (i.e. atoms) then we encounter one of the first problems: the collection, storage and management of big data. From this derives the second issue: the more constituent parts there are, the higher the computational complexity is. All this without considering the different relationships between the separate parts.

Considering that the complex systems are not static and inside them are multiple patterns and events of variable magnitude,

ever shaping the whole system, it is extremely difficult firstly to correctly identify these events but also to try to formulate universal laws which should apply to the system. In order to tackle these issues and be able to analyze and predict the behavior of a complex system there are two logical solutions. First, which is highly dependent on technological advances, is the improvement of electronic computers to be able to work faster and with immense amounts of data. The other is an interdisciplinary research drawing inspiration from natural computing systems, such as the nervous system. One example is represented by fuzzy logic, which uses both mathematical tools and also words, much alike the human thought process (1).

FUZZY LOGIC

To better understand fuzzy logic, it is necessary first to explain the concept of Boolean sets. These sets, also known as "crisp" sets, are fall under the Law of the excluded Middle formulated by Aristotle, which is also the foundation of the binary system. According to this law, one element can be part of a specific set or not. If we refer to the binary system, 1 means that the element is wholly part of the set or 0 means it is wholly excluded from the set. Fuzzy mathematics was first described in 1965 by Lofti Zadeh. A fuzzy set represents a shift in the binary system paradigm. An element can at the same time belong or not to specific set. This is referred to as a degree of membership. This degree of membership can be a real number included between 0 and 1. If the degree is 0 the element does not belong to the set and if it is 1 then the element completely belong to the set. Anything in between means that the element partially belongs to that set.

In creating a Fuzzy Logic System, multiple fuzzy sets and be connected through AND, OR, NOT operators and afterwards rules must be applied to this logic. These so-called fuzzy rules describe a relationship between input and output fuzzy sets using operators such as IF-THEN (2).

Fuzzy logic systems have structural and functional analogies with the human nervous system and also fuzzy logic mimics the human capabilities of computing with words. The classic examples often used are the sensory system and the fuzzy logic being a model of the human learning via patterns. Because of its unique traits, fuzzy logic systems are optimal for computing data which includes unknown variables and because of the way it derives from binary logic, it does no longer provide a qualitative output, rather a prediction, making it an ideal working tool for such a complex mechanism as the human body (3). The use of fuzzy systems was used with great success in a variety of medical fields. One of the newer uses is the cancer research. This was a logical application of fuzzy logic since variability is keyword in describing cancer. Etiologically we have a complex interaction between genetic factors and risk factors, either environmental or habitual, the correlation between them impossible to predict in a direct manner (with some exceptions such as familial polyposis). Variability is also a key word in disease progression, treatment response and morbidity.

PROSTATE CANCER

Diagnosis of prostate cancer requires three clinical tests: digital rectal examination (DRE), prostatic specific antigen (PSA) test and biopsy. The DRE is able to

raise the suspicion of the disease and can be useful in the staging by observing the local invasion. The PSA test is a quantitative test which measures the amount of this antigen in the patient's bloodstream. Interpreting the test on the other hand is more complicated. While high values of PSA usually correlate with the presence of prostate cancer, normal values do not exclude the presence of the disease. The biopsy is the only test which can provide the exact diagnosis of cancer and bring additional data such as Gleason score, thus revealing the aggressiveness of the cancer (4).

Considering PSA is the only screening tool available at the moment, there have been studies which try to improve the accuracy of this test using artificial intelligence. Such an example is using a hybrid between neural networks and fuzzy logic system in which the input data was PSA, free PSA value and a third data set with the age of the patients. All of the patients had elevated PSA but less than 20 ng/mL. Out of the 1030 men in the study, 195 had diagnosed prostate cancer. The neuro-fuzzy network provided superior predictive value compared to PSA or fPSA levels alone (95% sensitivity with 31.9% specificity) (5). There is also the foresight of FLS which could be able to differentiate between prostatic cancer and benign hypertrophy.

BREAST CANCER

There are many studies which focus on artificial intelligence tools for helping diagnose breast cancer. Image processing employing neural networks have successfully been used in early stage diagnosis. Apart from requiring important amounts of data to be able to train the program, it is also required that patients are referred to these imagistic explorations in the first place. This can be done either by implementing nationwide screening programs or by targeting specific susceptible individuals. One means of identifying at risk individuals is by using clinical data which is relevant in the case of this particular cancer such as age, body mass index, age of the menarche, age of the last menstrual period, age of first pregnancy and smoking. This helped create fuzzy sets to which there are rules applied afterwards and the output data represents the risk probability of breast cancer. The accuracy of this fuzzy system was 95%. Although there are limitations of this study such as patient selection it gives encouraging possibilities of future development of programs able to automatically identify at-risk patients. (6). Another study also used a fuzzy logic-based system to try to identify the patients with breast cancer from a specific group. Aside from patients age, age of menarche, age of first childbirth and age of menopause, data such as nutritional habits, regarding low or high fat diets, alcohol consumption and genetic risk factors were included. This method had 97%, sensitivity, which means that this method correctly identifies at risk patients, 64% specificity, which means the method employed correctly identifies the non-risk individuals and 81% accuracy (7).

COLON CANCER

The relationship between environmental and genetic factors in the etiology of cancer has always been an issue targeted by numerous studies. There are certain gene mutations which have been linked with various degrees of risk for developing cancer during the lifetime of these individuals. There are also environmental factors which

add to this genetic predisposition. But the relationship between these two can be described over a large spectrum. A good example is the hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome. Individuals bearing this autosomal dominant condition have a >70% risk of developing colorectal cancer (CRC) during their lifetime (8). Also, cigarette smokers male patients carriers of hMLH-1 mutations have an increased risk of up 2 times than nonsmokers (9). Using a model-free system, such as fuzzy logic seems ideal for assessing the risk of developing CRC of individuals with Lynch syndrome, with a great phenotypical heterogeneity and with multiple environmental risk factors. Considering members of the kindred are at risk of developing CRC anytime between the age of 25 and 65 and some may never develop it, a proven approach to cancer prevention (colonoscopy prevention versus prophylactic colectomy) is still lacking. A basic fuzzy logic system, with the rules refined using an Adaptive Neural Fuzzy Inference System, was able to identify high risk carriers of the hMLH mutations using as input data the type of mutation, gender, age and smoking status (smoker/non-smoker as well as number of pack-years) (10). Another basic fuzzy logic programs was designed to identify at risk population by using as input data symptoms which are generally ignored by patients such as weight loss, persistent abdominal pain, rectal bleeding and fatigue (11).

LUNG CANCER

Lung cancer represents the most common and primary cause of cancer death in the western world. Thus, early diagnosis through the measurement of biomarkers in target high risk population is of great importance in the management of these patients. The main issue with using biomarkers as screening method for lung cancer is represented by the low specificity or sensitivity of each marker taken individually. Therefore, the first step of improving sensitivity and specificity is to use a biomarkers panel rather that isolated measurements. The data acquired can then be analyzed by using fuzzy logic. This method had specificity of 97.4% and overall sensitivity of 73.6%. In the case of non-small cell carcinoma, this tool identified 70% of stage 1 and 92% of stage II cancers while in the case of small cell carcinoma the limited disease status was identified in 90.7% (12). The early stage diagnosis can be improved by employing other diagnostic tools such as computed tomography and sputum cytology analysis (13).

Apart from diagnosis, using a fuzzy-logic system with a specific biomarker panel (either using two-marker or three marker panels) can be used for the detection of tumor progression in small cell lung carcinoma with sensitivity up to 67% (14).

GASTRIC CANCER

Gastric cancer is ranked sixth among most common cancer types and ranked third among causes of cancer death with 738 000 deaths per year. As is the case of all cancer types, early diagnosis is very important in order to reduce mortality and morbidity. But gastric cancer remains asymptomatic or accompanied by nonspecific symptomatology until late stages of the disease with local advanced disease or in the presence of metastasis. The treatment is also an issue because gastric cancer seems rather resistant to chemotherapy and

even newer, more aggressive surgical options available such as Hyperthermic Intraperitoneal Chemotherapy (HIPEC) are not proving as successful comparing to other cancer types. In areas with higher incidence of gastric cancer, identifying the high and very high-risk population is of key importance for appropriate referral to a specialist and enabling early diagnosis. A fuzzy rule based Mandami expert system developed by Safdari *et al.* was used to identify the risk levels of a group of individuals and then the results compared with experts opinion. The input data consisted of medical history (*Helicobacter pylori* infection, gastric atrophy, gastric ulcer, gastric surgery, pernicious anemia, family history), socio-demographic factors (age, gender, level of literacy), smoking factors, nutritional factors (vegetable and food intake, content of nitrate compositions). The sensitivity of the method was 92.1% and the specificity was 83.1% (15).

DISCUSSION

Globally cancer is responsible for 1 in 6 deaths, being the second leading cause of death. Despite of all research conducted, it is very difficult to reduce these statistics firstly because of the great phenotypical heterogeneity and the lack of symptoms in early stages of the disease. Therefore, an attempt for the prevention of the disease is to identify the at-risk population and introducing screening to this population group. If we consider the multifactorial aspect of the disease and the hard to describe interaction between genetic and environmental risk factors, or the combined effect of various risk factors, the task of correctly identify the patients bearing these disease in the early stages becomes almost impossible. This issue can be easily solved by introducing population-wide screening tests specific for the most common type of cancer in that particular wise, but globally this is an impossible task, firstly considering the expense and the lack of specialists in different areas. A solution can be the use of artificial intelligence, such as fuzzy logic. This model-free system, which is capable of solving problems with a high degree of incertitude, seems a perfect tool to be used for this purpose. Considering it requires a simple patient database, using various input parameters it can assess the degree to which a patient might be at risk. The input parameter, as shown in the examples above, can be usual blood parameters or constitutional traits. Also, artificial intelligence programs can be used in certain grey areas of the diagnostic process, a clear example being a value of PSA between 4 and 10 ng/mL.

CONCLUSIONS

Early stage cancer is difficult to diagnose because of the lack of symptoms or presence of non-specific symptomatology usually ignored by patients. Therefore, patients usually are being diagnosed in late stages, in which treatment both oncological and surgical is limited and with higher morbidity and mortality.

Using an artificial intelligence program along the patient database could quicken and optimize the referral of these patients to specialists, which in turn could improve the diagnosis of early stage cancer disease. Fuzzy logic and neural networks already have proven as valid image processing tools aiding diagnosis, but they can also be used for identifying at-risk patients, with very good sensibility and specificity.

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CLOSTRIDIUM DIFFICILE INFECTION - 30-MONTH RECURRENCE RATE

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CLOSTRIDIUM DIFFICILE INFECTION-30-MONTH RECURRENCE RATE (Abstract) **Aim:** To evaluate the recurrence rate of *Clostridium difficile* infection (CDI) in the patients admitted to the Iasi "Sf. Parascheva" Infectious Diseases Hospital. **Material and methods:** Observational retrospective study of the patients admitted between January 1st, 2017 and June 30th, 2019. **Results:** Of the total of 736 patients diagnosed with CDI and admitted to our hospital 240 were included in this study. According to the number of CDI recurrences, 63.81 % of the patients experienced a first recurrence of CDI, over 20% a second recurrence, and the remaining patients a third to sixth recurrence. **Conclusions:** CDI is still a serious problem and a major burden on both the healthcare system and the patient. The causes of recurrent CDI are unclear. **Keywords**: *CLOSTRIDIUM DIFFICILE* INFECTION, RECURRENCE, TREATMENT.

One of the most common cause of nosocomial diarrhea is *Clostridium difficile* infection (CDI) which increases mortality and morbidity in hospitalized patients (1). Clinical symptoms of CDI range from asymptomatic carrier to mild and severe diarrhea, which can lead to fulminant colitis, toxic megacolon, bowel perforation, sepsis, and ultimately death (2).

Approximately 25% to 33% of antibiotic-associated diarrhea and 90% of pseudo-membranous enteritis are caused by CDI (3).

Although there are guidelines for the diagnosis and treatment of CDI, the infection rate continues to increase in Europe and United States. It is a known fact that there has been an enormous worldwide increase in the incidence and severity of CDI over the past 2 decades despite the prevention programs implemented in many countries (2).

In Europe, CDI costs healthcare services from 5,790 euros to 11,202 euros per episode (4) and the annual rate of recurrent CDI (rCDI) is between 75,000 and 175,000 cases (5).

Major risk factors for CDI in the general population are well known and they include antibiotic exposure (6) proton pump inhibitor (PPI) use, previous and prolonged hospitalizations, (7, 8) chemotherapy, immunocompromising conditions, and advanced age (9).

Studies have shown that the risk of recurrence after a first CDI episode is between 20 and 35%, and of these, 40-60% will ex-

perience a second recurrence (10). The course of the infection is influenced by the personal characteristics of the patient (risk factors, associated diseases) and the speed and accuracy of the treatment (11, 12).

CDI recurrences reduce the quality of life and also can place a major burden on healthcare system. Also, the social burden that comes with CDI has a considerable impact on patient's psychological state.

MATERIAL AND METHODS

We conducted an observational, retrospective study of the patients admitted to the "Sf. Parascheva" Infectious Diseases Hospital over a period of 2 and half years (January 1st, 2017 - June 30th, 2019). Out of a total of 736 patients that were admitted for CDI, we included in our study group 240 patients who had been previously admitted for CDI and presented for the recurrence of more than 3 diarrheic stools.

Data on the number of recurrences, associated diseases, length of hospital stay, presence or absence of the specific toxins, administrated treatment, and if they were transferred from another hospital were collected from the medical records of the patients.

RESULTS

Of the 736 patients admitted to our hospital with CDI from January 2017 to June 2019, 32.61% (240 cases) were admitted for recurrent CDI. Patient age ranged from 1 to 96 years old (mean age 68.5 years old). Patients were primarily urban (60.25%) and predominantly women (58.3%).

After reading the medical records of the patients we found that , 63.81% of the patients were at the first episode of , 20.57% developed a second recurrence, and 15. 62% a third to sixth.

In 92 (38.33%) cases, CDI recurrence occurred while the patients were admitted to other medical departments, fact requiring their transfer to our hospital (fig. 1). We found a high number of referrals by other small infectious diseases hospitals in the territory, gastroenterology, pulmonology, surgery and cardiology departments.

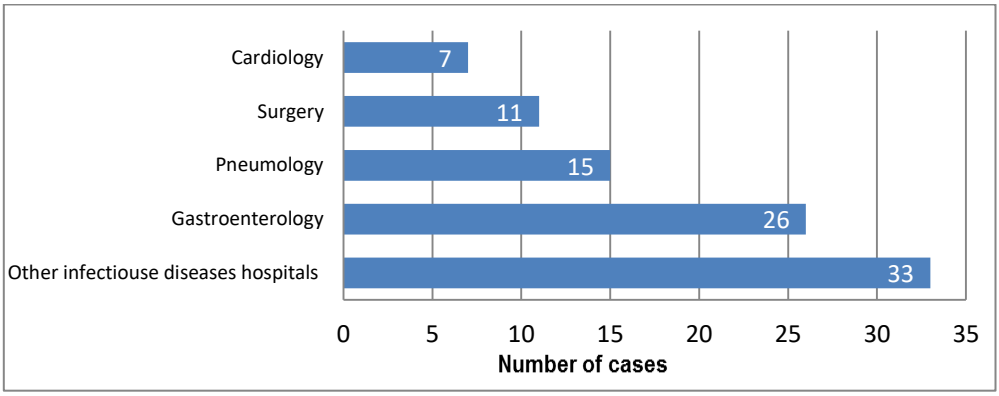

Fig. 1. Distribution of transfers to our clinic

We were interested to find the comorbidities of the studied group. Leading associated pathologies were cardiovascular diseases which were founded as a secondary diagnosis in 60 patients (25%) followed by metabolic diseases in 38 cases, liver diseases in 34 cases and gastroenterological diseases- 31 cases (fig. 2).

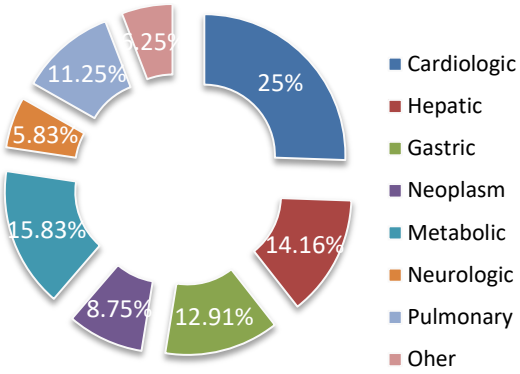

Fig. 2 Comorbidity of the patients

Analyzing the length of hospital stay, most of our study patients were admitted for more than 10 days, with a mean of 12.1 days (Fig 3). Over 50% of the admitted patients required more than 10 days of hospitalization and continuous supportive treatment. In 32 cases more than 15 days of hospitalization were necessary because of the severity of the disease.

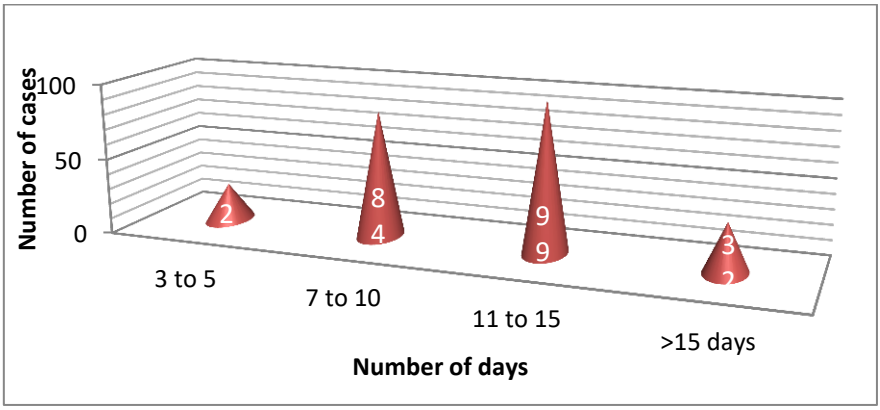

Fig. 3. Number of days of hospitalization

In our study group, 166 of patients received vancomycin (69,16%), followed by vancomycin-metronidazole (8.75%) combination therapy. In 62 cases rifaximin was administered alongside vancomycin treatment.

Cadazolid/vancomycin combination was administered in 3 cases, and fecal microbiota transplantation (FMT) was also performed in 3 cases (fig.4).

The diagnosis was made based on the results of the laboratory tests. In our study group, 26 cases were positive for toxin A alone, and 6 cases for toxin B alone, 8 cases were negative for both A and B toxins, 7 cases had only a positive GDH test, and the remaining 193 patients were positive for both of the toxins (fig. 5).

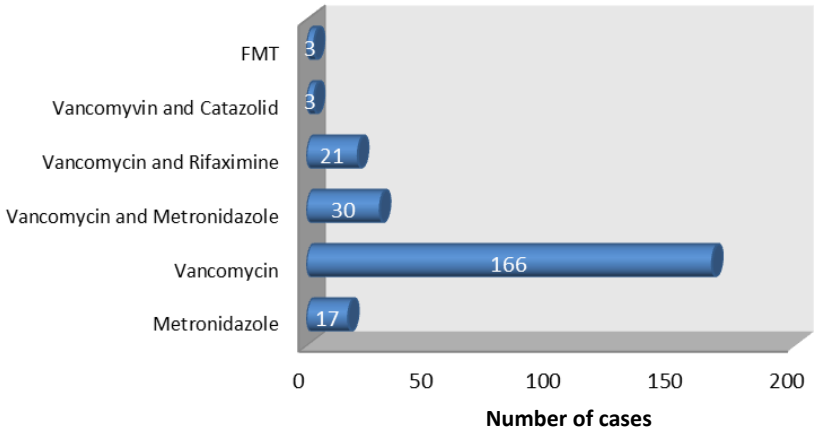

Fig. 4. Administrated treatment

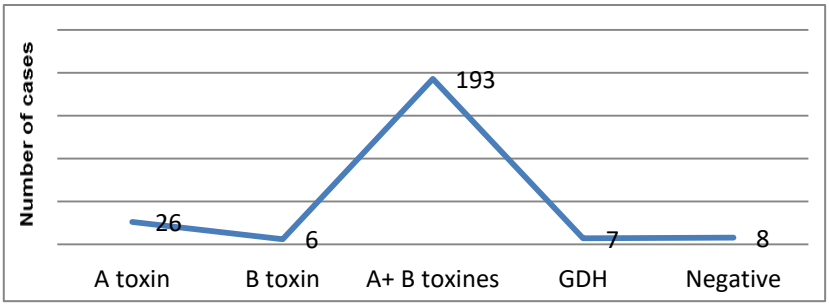

Fig. 5. Results of the laboratory tests

DISCUSSION

Studies have showed that the recurrence rate of CDI is situated between 20% and 35%.

In the presented study the rate of recurrence was closely to the higher value and situated at 32.60% (10). According to the already published data, the female gender has a higher predisposition to CDI (4) data that also applies in our study in which 58.3% of the admitted patients were woman.

Advanced age is one of the most frequent risk factors for recurrence of CDI (17). Even though we included in the study patients of all ages, we could observe that the majority of the patients were people with age over 60 years old with an average age of 68.5 years old and with at least one underlying diseases.

The performed studies (4, 18) correlate that approximately 37% of CDI have the onset on a health-care facility. In our study, 38,3% of the cases admitted in our clinic were transferred from other hospitals. CDI poses a great burden (4) not only on the healthcare system but also on the patient. The patients with multiple recurrences had experienced depression linked to the gravity of the disease directly proportional to the severity of the illness.

In some cases, the intervention of the hospital psychologist was required. The

role of this medical intervention is to minimize the burden of this debilitating disease (14, 15).

More than half of the patients (54.58%) required more than 10 days of hospitalization. The advanced age and the underlying diseases make the patients susceptible to rCDI due to immunocompromised state, which complicates the evolution of the disease. The result of all that is a prologue hospitalization. (16, 19).

After advanced age, comorbidities, use of antibiotics after CDI diagnosis, inadequate immune response, and concomitant receipt of acid-suppressive therapy are considered risk factors for rCDI. In our study the main associated diseases were cardiovascular, metabolic, liver and gastric pathologies. (17)

The primary cardiovascular pathologies that the patients associated in 25% of the cases were hypertension and cardiac insufficiency. The metabolic diseases were represented by diabetes mellitus and obesity and were found as a secondary diagnosis in 15.83% cases. The most frequent hepatic diagnosis was represented by hepatic cirrhosis and chronic hepatitis in 14.16% of the cases. Gastroenterological pathologies were found in 12.91% of the patients and consisted in ulcers, gastritis and acid reflux. We have to mention that 21 patients associated different types of neoplasm (prostate, colon, uterus, breast). Associated diseases are risk factors for CDI, aggravating the progression and prolonging hospitalization (13).

Studies showed that the majority of relapses are due to recurrences of CDI with the original strain rather than reinfection with a different. The management of an initial CDI recurrence includes repeat administration of either oral Metronidazole (500 mg every 8 hours) or vancomycin (125 mg every 6 hours) for10-14 days (10).

In our study group, the majority of patients received vancomycin, followed by vancomycin/ metronidazole combination therapy. In a few cases rifaximin/vancomycin combination therapy was used.

The diagnosis of CDI requires both clinical symptoms and a positive laboratory test. Several methods are recommended for the diagnosis of CDI, including toxicogenic culture, enzyme immunoassays (EIAs) for toxins A and B, and/or glutamate dehydrogenase (GDH) (13). In the studied group both toxins (A and B) were present in 54.48% of the cases (193 cases). In 10.83% of the samples just the A toxin was present and in 2.5% of them only the B toxin was positive. The rest of the sample presented no toxin or a nontoxigenic strain.

Multiple CDI recurrences and prolonged hospital stays can result in patients feeling or being stigmatized by society and even by their close family. A lot of these patients requires psychological support because of the depression caused by this “chronic” illness (14, 15).

Medical world should start focusing on new molecules for treatment of the disease. The presence of multiple comorbidities in the elderly population makes us expect a dramatic increase in the number and severity of CDI cases that could ultimately become resistant to usual treatment (20).

CONCLUSIONS

Over the last 20 years there has been a substantial increase in the number of CDI cases. The first CDI recurrence causes substantial morbidity and is a major burden on healthcare systems. Minimizing the number of recurrences can reduce the social, psychological and medical burden on patients and healthcare systems.

Even if the treatment is administered according to the protocols, approximately one third of the patients experience a recurrence of the infection.

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SOCIOECONOMIC IMPLICATIONS OF UNDERDIAGNOSING TEENAGERS' DEPRESSION

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SOCIOECONOMIC IMPLICATIONS OF UNDERDIAGNOSING TEENAGERS' DEPRESSION (Abstract): The aim of our research was to demonstrate once again that teenagers' depression still remains way too much underdiagnosed, the last one having important socioeconomic implications. Thus it is absolutely necessary to implement depression screening in psychiatric clinics. **Material and method**: The study was carried out in 2 stages, I; a group of 1126 in-patients from "Sf. Maria" Hospital, and II the analysis of the socioeconomic implications of teenagers' depression. **Results**: Only 19, 11% of the depressive patients had a previous depression diagnosis, the rest of 80, 89% were new cases, recently diagnosed; the depressive teenagers are more exposed to drugs and alcohol abuse, to antisocial behavior, and to poorer results in school. **Conclusion**: Screening depression implementation would reduce very much the risk of teenagers' depression underdiagnosing. **Key words**: DEPRESSION, TEENAGERS, PEDIATRICS

ANALYSIS OF THE OUTCOMES OF PREGNANCIES OBTAINED AFTER INFERTILITY TREATMENT, IN "CUZA-VODA" HOSPITAL FROM IASI, ROMANIA

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ANALYSIS OF THE OUTCOMES OF PREGNANCIES OBTAINED AFTER INFERTILITY TREATMENT, IN CUZA-VODA HOSPITAL FROM IASI, ROMANIA (Abstract): The prevalence of infertility is raising nowadays, affecting 15% of couples in their reproductive age. It has significant medical, social, and financial implications. It is estimated that 2-3% of all births in developed countries are the result of IVF procedures. The demand for assisted reproduction techniques is constantly increasing, and there is a continuous need to develop more efficient and less harmful protocols. **Materials and methods:** The descriptive, retrospective analytical study was performed on a group of 657 pregnant women with infertility treatment followed before pregnancy, who gave birth in the Department of Obstetrics-Gynecology of "Cuza-Voda" Hospital in Iasi, between 2010 and 2018. **Results:** No comorbidity was noted as a good predictor in the determinism of low birth weight, which led to the conclusion that infertility treatments do not independently influence the prognosis of birth weight in girls in our study group (AUC<0.600). **Conclusions:** The expected outcome of infertility treatments is highly dependent on the effectiveness of controlled ovarian stimulation, a routine procedure prior to IVF, and the results obtained must be constantly analyzed in order to be improved for the benefit of patients. **Keywords:** INFERTILITY TREATMENT, PREGNANCY, *IN VITRO* FERTILISATION.

Each year around 1.5 million cycles of assisted reproductive medicine (ART) are reported worldwide, with a ratio of 333,000 children born. The figures represent about 70% of all infertility treatments. Thus, about 2.4 million ART cycles are estimated each year, with about 500,000 children born (1). The outcomes after infertility treatment are the result of a multidisciplinary team formed by the specialist in ovarian stimulation treatments, embryologist, and obstetrician, and require careful monitoring in collaboration with different medical specialties in high risk patients (1).

The newborns, in numerous studies, have the same prognosis for development as in the general population, but an important knowledge of maternal comorbidities must be realized, these risk factors being independent of the neonatal ones (2,

3). In current practice, single embryo transfer *in vitro* fertilization protocols is preferred (4, 5, 6), to reduce the risk of these neonatal complications, which are higher in twin pregnancies (7, 8).

MATERIALS AND METHODS.

The descriptive, retrospective analytical study was performed on a group of 657 women, between 18 to 50 years old who underwent infertility treatment in infertility clinics, either from Romania or abroad and became pregnant after controlled ovarian stimulation (COS) protocols. From the total, we excluded stillbirths and we obtained a number of 626 live births. The treatments that infertile patients followed were either mono ovulatory stimulation, intrauterine insemination(IUI), *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), resulting with live birth pregnancies. All the fetuses following these procedures were born in the Department of Obstetrics-Gynecology of "Cuza-Vodă" Hospital from Iasi, between 2010 and 2018.

Statistical analysis was performed in *SPSS 20.0*. The existence of a relationship between variables was evaluated with the Pearson correlation coefficient, p, Skewness and Kurtosis tests.

RESULTS

Analyzing the data from each year, following infertility treatments, we found a slightly increasing trend of number of births (y=14.658+12.08 x), with a frequency peak encountered in 2018 (18.1% of the total study group) (fig. 1), predicting for 2019 a number of approximately 135 births after infertility treatment (fig. 2).

In summary, in 2018, there were 31 singleton natural births (NAT) (1.23% of total live births) and 626 of total caesarean (CST) births (2.24% of total CST) after infertility treatment. Of all, just 11.2% were twins (tab. I, fig. 3).

Distribution by age groups. The corroboration between the mean age of 33.56 ± 4.47 years, the median 33 years, and the results of the Skewness (p=0.287) and Kurtosis (p=0.472) tests, suggest the homogeneity of the various values of mother's age with fertility treatment, showing that it can be applied significance tests for continuous variables (fig. 4). In this study, every year, about 10 to 30% of the mothers with infertility treatment were under 30 years of age (p=0.134).

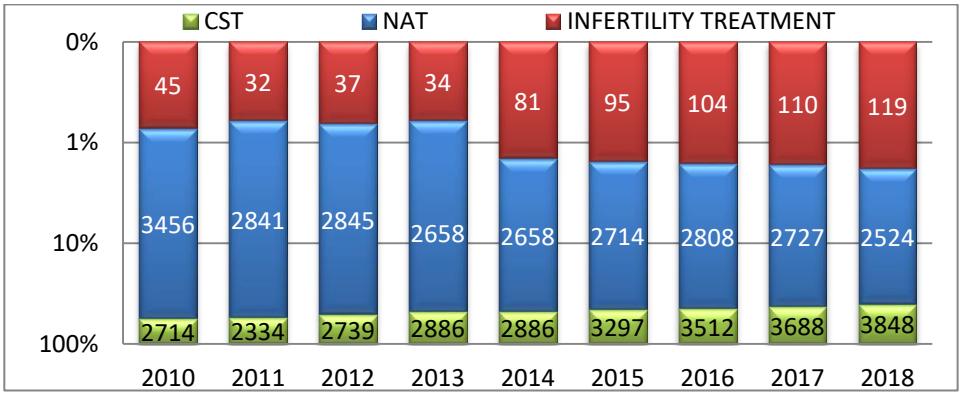

Fig. 1. Distribution of cases with infertility treatment of total live births between 2010 and 2018

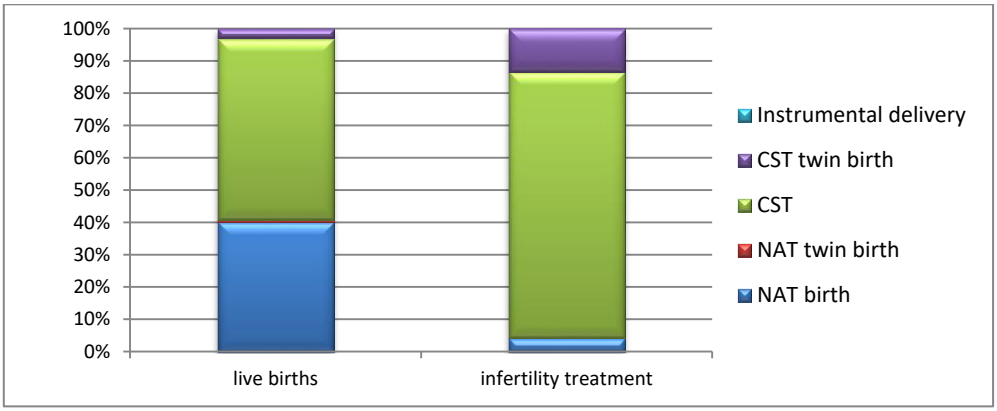

Fig. 2. The incidence of patients that followed infertility treatments from the total of live births in 2018

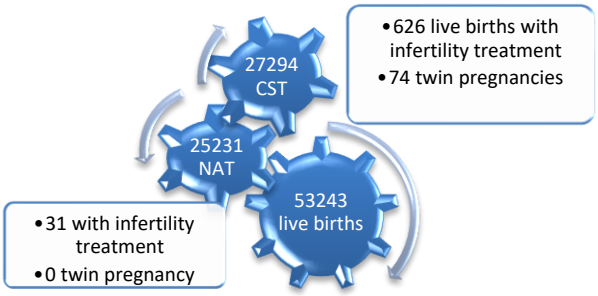

Fig. 3. Distribution of infertility treatments from total live births between 2010-2018

TABLE I

Annual incidence of births after infertility treatment

Year	Infertility treatment	% of patients with primary or secondary infertility	Live births	Patients with Infertility treatment per 1000 live births	NAT		CST	
					Total (% of live births)	Births After infertility treatment (% of total NAT)	Total (% of live births)	Births After infertility treatment (% of total CST)
2010	45	6.8%	6200	7.26	3456 (55.7%)	2 (0.06%)	2714 (43.8%)	43 (1.6%)
2011	32	4.9%	5186	6.17	2841 (54.8%)	2 (0.07%)	2334 (45.0%)	30 (1.3%)
2012	37	5.6%	5591	6.62	2845 (50.9%)	0 (0.0%)	2739 (49.0%)	37 (1.4%)
2013	34	5.2%	5558	6.12	2658 (47.8%)	4 (0.15%)	2886 (51.9%)	30 (1.04%)
2014	81	12,3%	5558	14,57	2658 (47,8%)	3 (0,11%)	2886 (51,9%)	78 (2,7%)
2015	95	14,5%	6022	15.78	2714 (45.1%)	2 (0.07%)	3297 (54.7%)	93 (2.8%)
2016	104	15.8%	6333	16.42	2808 (44.3%)	6 (0.21%)	3512 (55.5%)	98 (3.9%)
2017	110	16.7%	6422	17.13	2727 (42.5%)	7 (0.27%)	3688 (57.4%)	103 (2.8%)
2018	119	18.1%	6373	18.67	2524 (39.6%)	5 (0.20%)	3848 (60.4%)	114 (3.0%)
Total	657	100%	53243	12.34	25231(47.4%)	31 (1.23%)	27904 (52.4%)	626 (2.24%)

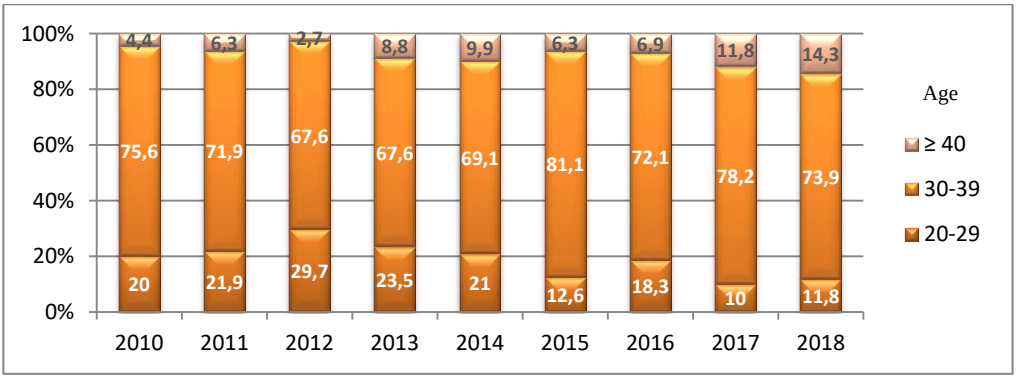

Fig. 4. The distribution of the patients by age and year of study

Distribution by environmental provenance. From the total study group, 74.6% of the mothers with infertility treatment came from the urban area, the ratio of Urban / Rural quotas being 3/1 (fig. 5)

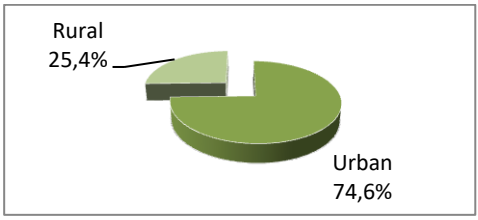

Fig. 5. Distribution of the patients by environmental provenance

Distribution by gestational age. The mean gestational age in mothers with infertility treatment was 37.40 ± 2.14 weeks, close to the median value (38 weeks). These values in corroboration with the results of the Skewness (p=-1.543) and Kurtosis (p=1.876) tests, suggests the homogeneity of the series of values of the gestational age, revealing that these significance tests can be applied for continuous variables (fig. 6)

About 9.7% of natural births and 5.3% of cesarean births in women with infertility treatment, were performed under 32 weeks of gestation (p=0.337) (fig. 7).

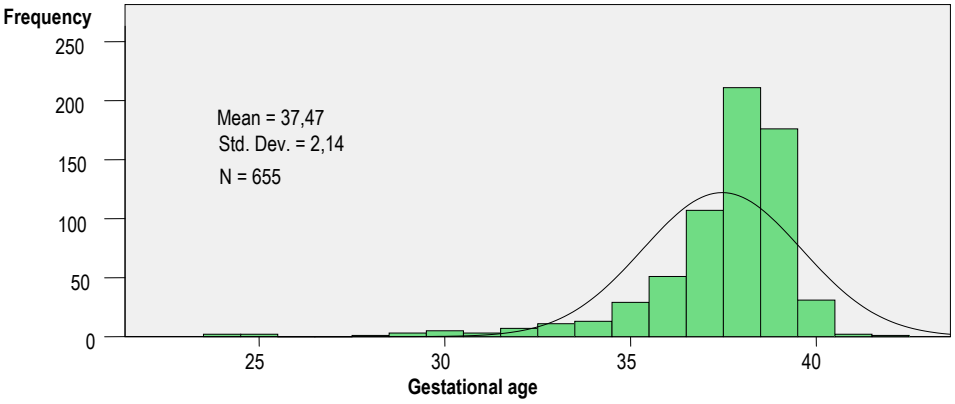

Fig. 6. The histogram of gestational weeks in patients with infertility treatment

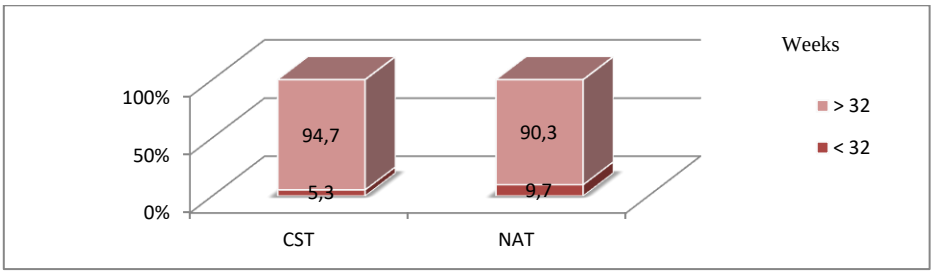

Fig. 7. The group structure patients by gestational age and type of delivery

From the total of patients included in this study, 75% of mothers with infertility treatment gave birth under 32 weeks of gestation and 6 days and 74.1% of these gave birth after 33 gestational weeks. The two groups were represented by women with ages between 30 and 39 years old, showing no statistical significance (p=0.605), thus observing the fact that women after 35 years old requires more often ovarian stimulation in order to achieve a pregnancy (fig. 8).

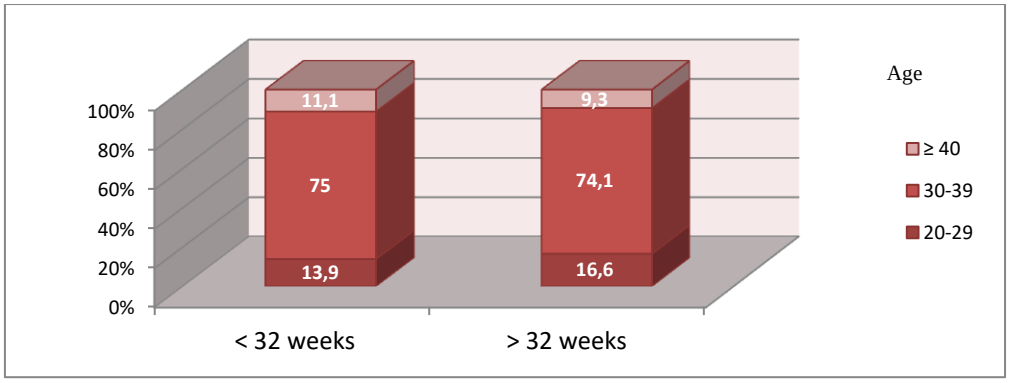

Fig. 8. The group structure by age groups and gestational ages

Applying the Pearson correlation to the case study, shows that the age of IVF mothers and the gestational age of their pregnancies were observed as apparent independent parameters (p=+0.007; p=0.849).

Weight at birth. The mean weight at delivery was 3,077 ± 642 g, closed to the median value (3,100g). Associating these results with the results of the Skewness (p=-0.911) and Kurtosis (p=1.810) tests, suggest the weight homogeneity of the series at delivery, so significance tests can be applied for continuous variables (fig. 9).

Associated pathologies. Of the total of possible comorbidities analyzed, the most commonly identified was hereditary deficiency due to the lack of coagulation factors, in 8.5% of patients, followed by gestational hypertension (6.8%), of which only 0.9% had complicated with various forms of pre-eclampsia, and liver disorders in (5.2%), being objectified by blood tests. Another common association were uterine fibromas that were found in 5.2% of patients (fig. 10).

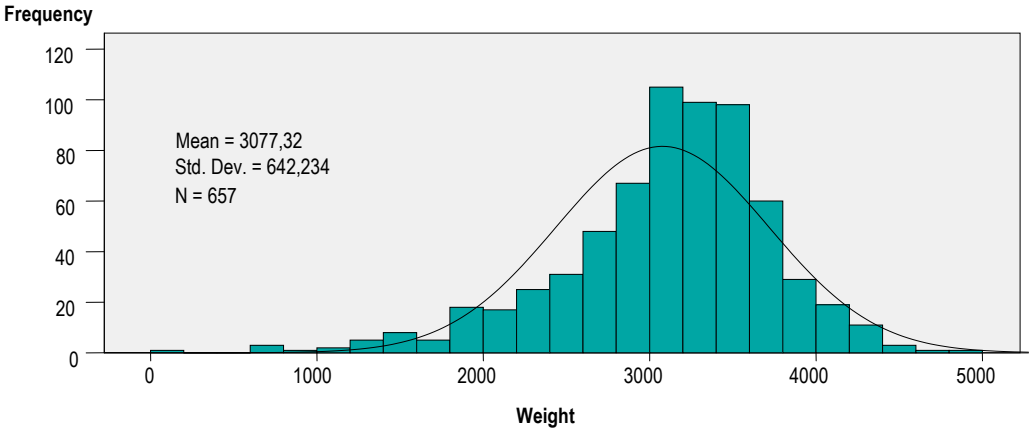

Fig. 9. Histogram of birth weight in the studied group

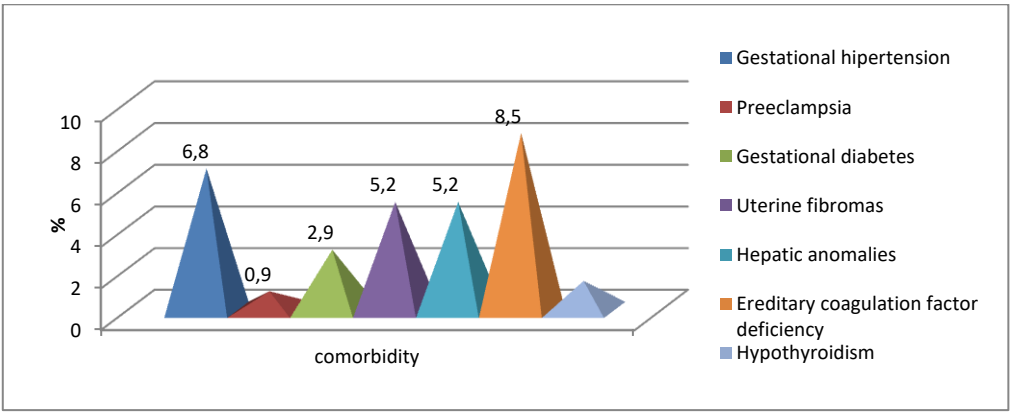

Fig. 10. Frequency of comorbidities

DISCUSSION

Once with the constant improvement of pregnancies and live births after IVF in the last two decades, these procedures has become a high successful treatment for female and male infertility, reaching maximum values of live births between 2001 and 2002 (9, 10). The live birth rates have hit a plateau, subsequently declining in regions of the world. Also, it is important to define how fast "industrialization" is at the global level, the transition from a model of individualized private practice per patient, to a global, generalized one, with greater profit for investors and "commercialization" in IVF practice, respectively the main focus of competition on revenues and not on IVF results (11).

In our study it is important to mention that the overall delivery rate was not lower when single embryo transfer was performed and that the multiple delivery rate (74 from a total of 657 pregnancies) which was 11.2% could have been very much lowered, as twin pregnancies are associated with many maternal-fetal complications.

Worldwide, increased live birth changes were associated with the introduction of new IVF practices, including light stimulation, elective single embryo transfer (eSET), PGS (pre-implantation genetic screening) for aneuploidy detection, total freezing cycles, and embryo banking (12,13). Isolated negative associations were associated with *in vitro* fertilization tasks in pathologies such as high blood pressure, abnormally adherent placenta, genetic abnormalities or isolated birth defects, or birth defects in Japan, Australia, New Zealand and Canada, where there is a consistent research base (14, 15).

CONCLUSIONS

In our study, we wanted to identify factors as good predictors of lower birth weight, below the 10th percentile, of gestational age, and correlations between patient characteristics and associated maternal-fetal complications. The patients that decide to conceive through IVF are becoming younger each year; therefore, this procedure will continue to rise among young couples.

On the other hand, women after 35 years old, have lower chances of conceiving spontaneously, and requires further investigations and infertility treatments. Regarding environmental provenance, we noticed a higher demand of such treatments from patients located in urban area, but we consider that with today's advertisements more women will demand infertility treatments in the nearby future.

We did not identify any co-morbidity as a good predictor in the determinism of low birth weight, which led to the conclusion, of our study, that infertility treatments do not independently influence the prognosis of birth weight and neonatal adaptation. These aspects are important to reinforce in our medical practice, because women that require infertility treatments in order to obtain a pregnancy are more prone to social isolation and marital problems before the conception that can affect their quality of life. It is important to mention that medicine must be redirected from the general approach to the individualized one on the patient, especially in patients that struggle to obtain pregnancies.

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NEWS

TRADITIONAL CHINESE MEDICINE USED ON PATIENTS WITH RHEUMATOID ARTHRITIS: ALTERNATIVE OR COMPLEMENTARY TREATMENT?

The use of traditional Chinese medicine (TCM) on patients with rheumatoid arthritis (RA) had a magnitude of 30% as compared to the general population of Taiwan, according to the results of retrospective cohort study used the Longitudinal Health Insurance Database (LHID) 2000. The authors, Ming Chi-Lu and colleagues, aimed to describe the utilization of TCM and determine the factors related to TCM usage among RA patients. Herbal remedies (49.6%) constituted the most commonly used therapeutic approach, followed by combined therapy (32.7%) and acupuncture or traumatology manipulation alone (17.7%). The profile of the patients who used TCM is female, younger age, higher monthly income and higher Charlson-Deyo co-morbidity index scores, living in urbanized areas, in higher TCM physician density. The top three reasons for seeking TCM services were diseases of the musculoskeletal system and connective tissue, the respiratory system, and the digestive system (Lu M C, Livneh H, Chiu L M, Lai N S, Yeh C C, Tsai, T Y 2019. A survey of traditional Chinese medicine use among rheumatoid arthritis patients: a claims data–based cohort study. *Clinical rheumatology*; *38*(5): 1393-1400).

PSEUDOTUMORAL TUBERCULOSIS AFTER 13 MONTHS OF ADALIMUMAB. CASE REPORT

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PSEUDOTUMORAL TUBERCULOSIS AFTER 13 MONTHS OF ADALIMUMAB. CASE REPORT (Abstract): In patients with chronic immune-mediated inflammatory diseases, biological therapy targeting the components of the immune response acts like suppressive agents of the host immune system, being associated with increased risk of opportunistic infections and tuberculosis (TB). The case of a 35-year-old male patient, active smoker, with chronic psoriasis, previously treated for 13 months with the tumor necrosis factor α (TNF-α) inhibitor adalimumab is reported. The patient was admitted for a peripheral lung mass localized in the left upper lobe, associated with mediastinal lymphadenopathy, highly suggestive of lung cancer. Sputum smears and bronchoalveolar lavage were negative for acid fast bacilli, but tuberculin skin test (TST) results converted from negative to positive in the last 13 months. Histological description of lymph node biopsy revealed sarcoidosis. Due to the discrepancy between positive TST, imaging and histological results, surgical open lung biopsy was considered. Atypical segmental resection was performed, and the final histological diagnosis was TB. In conclusion, all patients with autoimmune diseases should be screened for the presence of latent TB infection (LTBI) before starting biologic therapy, and periodically monitored for active TB disease, which can be atypical because of immunosuppression.
Keywords: BIOLOGICAL THERAPY, ADALIMUMAB, TUBERCULOSIS, PSORIASIS.

Tumor necrosis factor alpha (TNF-α) inhibitors are widely used for the treatment of chronic immune-mediated inflammatory diseases, such as chronic psoriasis (1). TNF-α is involved in the host defense mechanism against Mycobacterium tuberculosis (MTB), in the granulomatous response preventing the dissemination of infection in the body. The use of TNF-α inhibitors may interfere with the immune response to MTB, thus resulting in an increased risk of reactivation of latent tuberculosis foci and active TB disease development (2). The psoriatic patients receiving TNF-α inhibitors are recognized as a new high risk group for pulmonary or extrap-

ulmonary forms of TB, many of them with poor outcomes (1-5). In Romania, TB control is mandatory, prisons included (6). Screening for LTBI is performed systematically and chemoprophylaxis should be provided to all infected patients before starting anti-TNF-α therapy.

CASE REPORT

A 35-year-old male, construction worker exposed to respirable particles, with a 20-pack year history of smoking was admitted to Constanta Pneumophtysiology Clinic for intermittent cough and fatigue with onset 3 weeks before presentation. Chronic psoriasis was diagnosed 13 months earlier, with good clinical response under biological therapy with adalimumab. Before initiating the anti-TNFα therapy, due to the negative TST, LTBI was not confirmed and chemoprophylaxis treatment was not recommended.

Physical exam revealed no abnormality. Routine biological analyses were normal, HIV screening test was negative, but an erythrocytes sedimentation rate (ESR) of 78 mm/hour and mild blood eosinophilia (400 cells/μL; 7%) were recorded. Chest X-ray revealed a pale tumoral opacity in the left middle lung (fig. 1). Chest computed tomography (CT) showed the presence of a peripheral lung mass lesion with irregular shape, spiculated margins and small cavitation inside, localized in the apical segment of the left upper lobe (LUL) (fig. 2). Enlargement of left hilar, left upper paratracheal and subcarinal lymph nodes was noticed.

TST conversion to positive was a red flag for a possible recent LTBI, but sputum smears were constantly negative for acid fast bacilli (AFB). The fiberoptic bronchoscopy appearance was normal, with bacteriological exam negative for bacteria, fungi and AFB, and bronchial wash cytology negative for neoplasia.

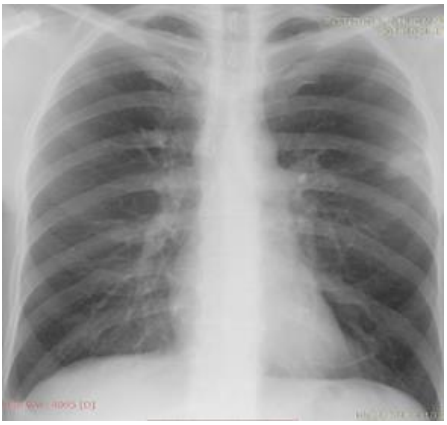

Fig. 1. Chest X-ray showed pale ovalar opacity in the left middle lung.

Fig. 2. Chest CT scan revealed peripheral lung opacity (4.5/5.5 cm), irregular-shaped with spiculated margins and small hyper transparency inside, localized in the apical segment of LUL.

Considering the imaging findings highly suggestive of malignancy, mediastinoscopy with biopsy and histological examination was required. Bioptic samples of paratracheal lymph node revealed joined and isolated non-caseating epithelioid-cell granulomas (fig. 3), suggestive of sarcoidosis.

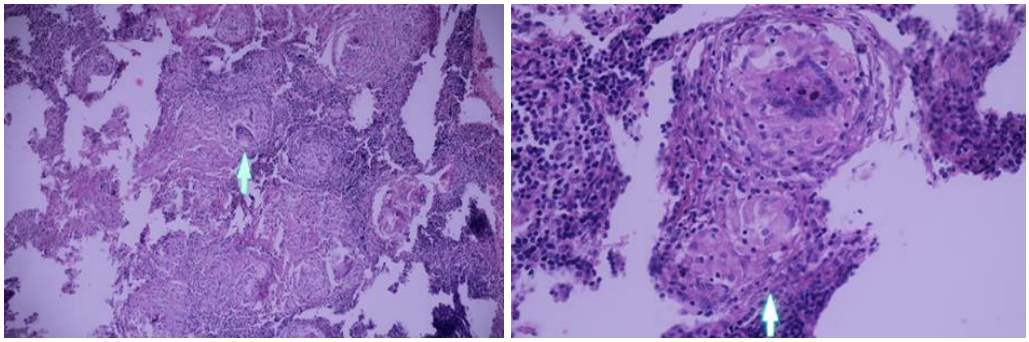

Fig. 3. Mediastinal lymph nodes- Hematoxylin-eosin staining; 10x magnification (left), 40x magnification (right) - non-caseating epithelioid-cell granulomas (arrow)

Due to the discrepancy between CT scan findings and histological result, open lung biopsy was recommended and atypical segmental resection was performed, revealing inflammatory granulomatous lung lesions with caseation necrotic center, histocytes and Langhans-type giant cells, highly suggestive of tuberculoma (fig. 4).

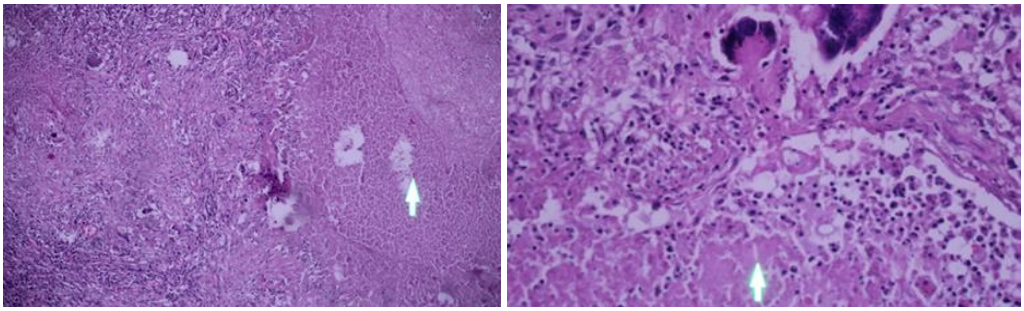

Fig. 4. Tissue Samples of lung biopsy. Hematoxylin-eosin staining - Necrotizing granuloma (left) - 10x magnification - Focal necrosis; arrow: necrosis, arrowhead: epithelioid histiocytes; (right): 40x magnification - arrow: zone of necrosis surrounded by epithelioid histiocytes and Langhans-type giant cells

The presence of necrotizing granuloma with positive AFB staining, along with the 13- month history of anti-TNF-α therapy, as well as TST conversion, together supported a positive diagnosis of TB and the standard first-line regimen of directly observed anti TB therapy was administered for 9 consecutive months, with good clinical, bacteriological and radiological outcomes and complete resorption of lung tuberculoma.

DISCUSSION

Tuberculosis continues to represent a major health problem worldwide, Romania still being one of the high TB burden countries (7, 8). Malnutrition, smoking, alcohol abuse, air pollution, low socio-economic status, along with diverse immunosuppressive conditions are the most common risk factors for TB and for other respiratory disorders (1-4, 9-14).

In this reported case, long term admin-

istration of adalimumab (13 months) for psoriasis increased the TB risk. Recently published data are suggestive for the risk of LTBI progression to TB disease or relapse caused by TNF-α inhibitors. Some monoclonal antibodies, such as adalimumab and infliximab, are associated with a higher risk of TB disease than the soluble TNF-α receptor fusion protein (etanercept) (15). While the monoclonal antibodies bind TNF-α more tightly due to a higher avidity for transmembrane, the soluble receptor may have a higher affinity for the TNF-α molecule (16).

In a smoker, the absence of positive acid-fast smears, the features of lung tumoral mass opacity with associated mediastinal lymphadenopathy were strongly suggestive for lung cancer. Histological pattern of non-caseating epithelioid-cell granulomas in lymph nodes samples collected by mediastinoscopy suggested sarcoidosis. It is well-known the difficulty to differentiate sarcoidosis from TB, especially when caseous necrosis is not seen (17). In our case, positive TST and imaging findings did not support sarcoidosis.

Atypical lung resection was performed revealing tuberculoma. Often, discrepancies are between of lung lesions or interstitial lung disease of unknown etiology required surgical lung biopsy (18). The presence of necrotizing granulomatous inflammation and positive AFB staining supported the diagnosis of TB. Pulmonary tuberculoma may mimic lung cancer or vice versa (19). Usually, in immunosuppressed people, atypical inflammatory tumors (20) or disseminated TB may occur (21, 22). In autoimmune diseases or during prolonged biological therapy, any form of typical or atypical TB can be observed. So, any lung lesion must be systematically screened for TB disease.

CONCLUSIONS

The authors reported this case of false lung cancer tumor in order to emphasize the possible discrepancies between radiological presentation and histological exam in making a positive diagnosis of tuberculosis among patients receiving anti-TNFα agents. Pulmonary tuberculoma may mimic lung cancer. Before initiating treatment with biological agents, negative tuberculin skin test is not enough to exclude TB. Periodic monitoring of these patients is needed.

ACKNOWLEDGMENTS

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DIGESTIVE NEUROENDOCRINE NEOPLASMS: COMPUTED TOMOGRAPHY EVALUATION AND PATHOLOGIC CORRELATIONS

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DIGESTIVE NEUROENDOCRINE NEOPLASMS: COMPUTED TOMOGRAPHY EVALUATION AND PATHOLOGIC CORRELATIONS (Abstract): **Aim:** Our study aims to correlate the enhancement curve (EC) types (obtained after contrast enhanced computed tomography) with tumor grading in case of digestive neuroendocrine neoplasms (NENs). **Material and methods**: Our retrospective analysis included 39 patients with digestive neuroendocrine tumors, evaluated in Iasi with multidetector computed tomography (MDCT) between January 2015 and September 2019, using noncontrast enhanced, arterial and portal venous phases; only 9 patients were evaluated using an intermediate-delayed phase (4 min). Along with the enhancement curve (EC) pattern we evaluated also patient demographics, lesion location, dimensions, homogeneity, presence of calcifications and in case of malignant lesions: local extension, distant and regional lymph nodes metastases. **Results:** The most common location of neuroendocrine neoplasms included in the study was the pancreas (27 cases, 69.23%), followed by the mesentery (6 patients, 15.38%), small bowel (4 patients, 10.25%) and stomach (2 patients, 5.13%). The lesions had the following EC patterns: 27 (69.23%) cases wash-out EC type, 10 cases (25.65%) progressive EC, 2 cases (5.12%) plateau EC. Malignant lesions were found in 9 cases (23.07%) and all had wash-out type EC in the portal venous and intermediate-delayed phases. **Conclusions:** When appropriately combined with other CT features (dimensions, structure, noncontrast CT, arterial, portal venous and intermediate-delayed phases) the enhancement curve types have potential utility in positive diagnosis of digestive neuroendocrine tumors and in differentiating between malignant and benign lesions. **Keywords:** NEUROENDOCRINE TUMORS, WASH-OUT, ENHANCEMENT CURVE.

Neuroendocrine neoplasms (NENs) are proliferative processes that arise from neuroendocrine cells such as enterochromaffin cells or Kulchitsky cells. They can develop throughout the body, the majority occurring in the digestive and respiratory tract. They comprise a large range of neoplasms from the benign to the highly malignant and metastatic ones. In the digestive tract they most commonly occur in the large bowel and in the pancreas. NENs are a heterogeneous group of tumors but they are treated

together because they contain cells witch share common features (have in the cytoplasm secretory granules and produce polypeptides) (1). Historically, the World Health Organization (WHO) classified the neuroendocrine neoplasms differently, according to the site, and this created considerable confusion due to different terminologies and grading criteria in different organs. In 2018 WHO published a new uniform classification for neuroendocrine neoplasms based on a consensus conference held in 2017. NENs are split into neuroendocrine tumors (NETs) and neuroendocrine carcinomas (NECs) based on genetic, clinical, epidemiological, histological and prognostic differences. NETs are graded as G1, G2, G3 on the basis of proliferative activity as assessed by mitotic rate and Ki-67 proliferation index. NEC are considered high grade proliferations and they are split into small cell NECs and large cell NECs. Even though NETs and NECs share neuroendocrine features (demonstrated through synaptophysin and chromogranin A labeling), morphologically they are different. NETs have organoid architecture (nests, cords, ribbons), uniform nuclear features, stippled chromatin and minimal necrosis. NECs have a predominant sheet growth pattern, more atypical nuclear features (often with nucleoli) and usually abundant necrosis (2, 3).

Ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI) and endoscopic ultrasound were in the past common diagnostic tools for diagnosis of NENs. CT scans is almost always the imaging method of choice for patients with suspicion of NENs, being useful for the evaluation of the primary tumor and staging of NECs (4, 5). Primary tumors and their metastases are hyper vascular and are best seen in the arterial phase of a triple phase CT scan (6). MRI is the best conventional study to detail hepatic metastases in NETs (13). MRI acquisition protocol must include diffusion sequences which help the diagnosis especially in non-hypervascular lesions (6, 7). Positron emission tomography (PET) imaging with fluorine-18 fluorodeoxyglucose (FDG) and somatostatin receptor imaging combined with PET may be valuable to image some neuroendocrine tumors (8).

MATERIAL AND METHODS

Our retrospective analysis included 39 patients with abdominal neuroendocrine neoplasms, evaluated at the Radiology Department, "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, between January 2015 and September 2019 with multidetector computed tomography (MDCT) and we correlated the patterns with the histopathological results. The inclusion criteria were age over 18 years old; neuroendocrine neoplasm with intervention and biopsy; availability of preoperatory CT scan with or without IV contrast agent in our institution from 2015-2019, while the exclusion criteria were as follows: pregnancy or history of another malignancy.

We performed MDCT examinations with noncontrast enhanced, arterial and portal venous phases for all patients and only 9 patients (the ones with malignant lesions) were evaluated using an intermediate-delayed phase (4 min). Abdominal MDCT examinations were performed using Siemens Somatom Emotion 16 equipment, with the following protocol:

- patients were asked to sign the informed consent for contrast enhanced CT examinations;

- an intravenous line was placed; the patient was scanned in supine position;
- a scout view for the abdomen was performed;
- noncontrast enhanced phase for the abdomen al pelvis was done;
- we injected intravenously iodinated contrast media (370 mg iodine/mL), at flow rate of 4 mL/s);
- arterial (at 15 seconds), portal venous (at 60-70 seconds) and intermediate-delayed phases (at 4 minutes after intravenous contrast medium injection) were performed.

We measured the tumoral attenuation values (CT densities) on noncontrast enhanced, arterial, portal venous and intermediate-delayed phases and we draw with this values enhancement curves (EC) for each tumor. We considered the following patterns of EC:

- a washout EC type when the density increased in the arterial phase and decreased with more than 40% in the portal venous and intermediate –delayed phases;
- a progressive EC when the density increased continuously in arterial and portal venous phases;
- a plateau EC when the density had variations with less than 10% between the CT phases.

Arterial and venous relative tumor enhancement ratio (RTER) was obtained by dividing tumor densities: arterial or venous postcontrast by noncontrast enhanced.

Tumor tissue sections were obtained for histopathologic and immunohistochemical studies. The tissue samples were fixed in 10% neutral buffered formalin before being dehydrated and paraffin embedded. For histopathologic evaluation, 5-µm sections of formalin-fixed, paraffin-embedded (FFPE) were stained by a standard method with hematoxylin and eosin (HE) with a Leica Autostainer XL (Leica Biosystems, GmbH, Wetzlar, Germany). Immunohistochemical (IHC) staining were performed using the Roche-Ventana BenchMark XT automatic immunostainer (CC1 standard; ULTRA View DAB Detection Kit) following the manufacturer's recommendations (Ventana Medical Systems, Tucson, AZ, USA). Monoclonal ready to use antibodies to the following antigens were used: synaptophysin (rabbit monoclonal, clone SP11 Roche Tissue Diagnostics) chromogranin (mouse monoclonal, clone LK2H10, Roche Tissue Diagnostics) CD56 (mouse monoclonal, clone 123C3, Roche Tissue Diagnostics), Ki-67 (rabbit monoclonal, clone 30-9, Roche Tissue Diagnostics).

RESULTS

We reviewed 39 patients with histologically confirmed NENs (24 females and 15 males - female to male ratio = 1.6:1), with 32-87 years age range (28-71.79% patients in the 6th, 7th and 8th decade) (fig. 1). Each study was initially independently reviewed by two expert radiologists (IJ and MU, each with > 20 years of experience) by using a dedicated radiology imaging software (Siemens Syngo Multimodality Workplace CT Workstation, Siemens Healthcare, Erlangen, Germany). The images were set to corresponding standard window center and width values for evaluation of lung, soft tissue, and bone. For cases with discordant results, a consensus reading with arbitration by a third radiologist was carried out, and the observers were asked to make the final assessment. The intraclass correlation coefficient (ICC) was used to determine interrater and intrarater reproducibility; ICC greater than 0.75 indicated strong agreement and ICC of 0.4-0.75 indicated average agree-

ment. A *p* value of less than .05 was considered to indicate statistical significance. Interrater agreement for the three readers was average-to-strong for tumor location (ICC, 0.88), and the intrarater agreement was strong (ICC, 0.94).

The most common location of neuroendocrine neoplasms included in the study was the pancreas (27 cases, 69.23%), followed by the mesentery (6 patients, 15.38%), small bowel (4 patients, 10.25%) and stomach (2 patients, 5.13%) (fig.2, 3).

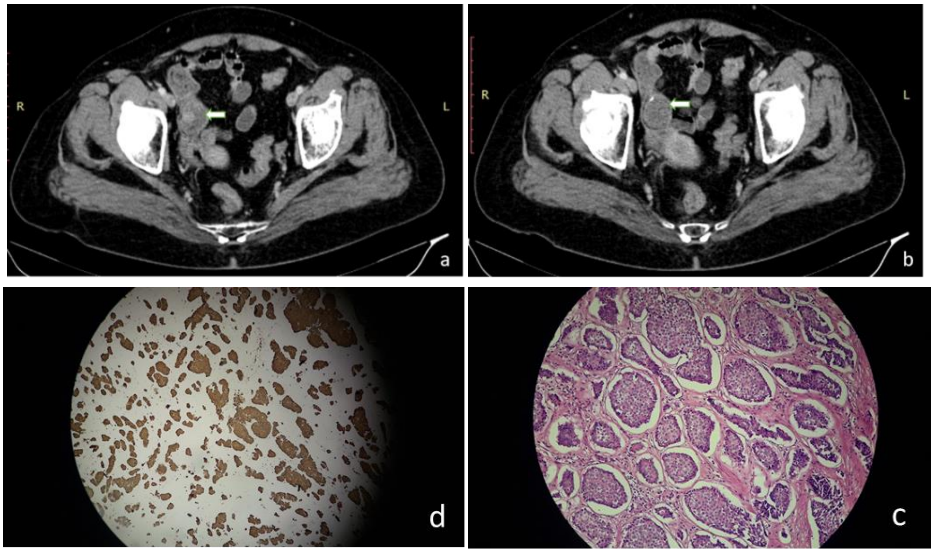

Fig. 1. Small bowel NEN - a, b - contrast enhanced CT, portal venous phase – round, well defined, with inhomogeneous contrast enhancement ileal loop NEN; c – HE staining - the tumor has solid architecture or nested distribution; the cells are monotonous, with coarse salt and pepper chromatin; cytoplasm is abundant, eosinophilic and granular;
d. IHC staining with monoclonal antibody against chromogranin demonstrate diffuse and intense staining, a characteristic feature for neuroendocrine neoplasms.

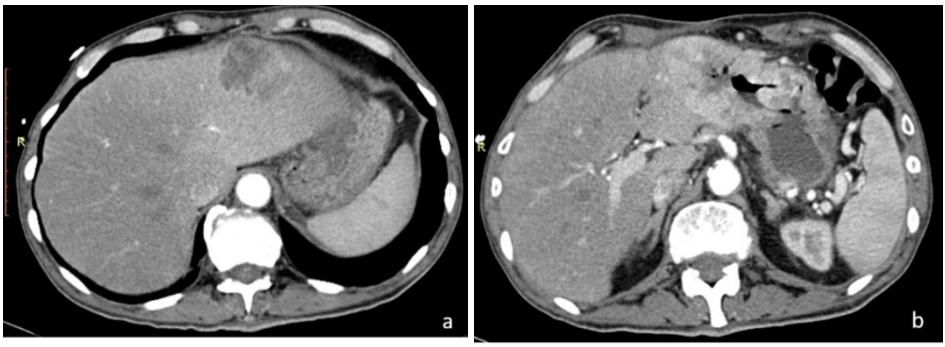

Fig. 2. Stomach NEC - contrast enhanced CT, arterial phase – liver metastasis - round, irregular, poorly defined lesion, with peripheral contrast enhancement (a), gastric mass with intense, inhomogeneous contrast medium uptake (b)

The attenuation values measurements on contrast enhanced MDCT demonstrated that 33 (84.61%) lesions were hypervascular, very well visualized in the arterial phase, 6 (15.39%) tumors were hypovascular, with a medium and low contrast enhancement in portal venous phase. We found the following EC patterns: in 27 (69.23%) cases a wash-out EC type, 10 tumors (25.65%) had a progressive EC and 2 (5.12%) a plateau EC. NECs were found in 9 cases (23.07%) and all had wash-out EC type in the portal venous and intermediate-delayed phases.

MDCT image analysis was focused also on arterial and venous relative tumor enhancement ratio (RTER). The arterial RTER was 2.73 ± 3.5 and the venous RTER was 2.39 ± 4.2 ($p < 0.05$). CT attenuation values and contrast ratios of pancreatic neuroendocrine tumors in the arterial and venous phases were higher than those of mesenteric and small bowel ($p < 0.05$).

Correlating the tumor dimensions and structure with the pathologic results we found:

- 25 (64.10%) tumors had dimensions between 1.5 and 2.5 cm and were homogeneous, and were graded as G1 and G2;

- 14 lesions were larger than 2.5 cm and had an inhomogeneous structure on nonenhanced and contrast enhanced phases; 5(12.82%) lesions were graded as G1, and G2, 9 (23.07%) tumors had G3 grade.

Only 5 (12.82%) large masses had linear, nodular and polymorphic calcifications.

The metastatic potential of neuroendocrine tumors and the correlation with the tumor grading was determined by studying the perilesional extension, regional lymph node involvement and distant metastases (tab. I).

TABLE I

The metastatic potential of neuroendocrine tumors – correlation with the tumor grading

Tumor grading	Perilesional extension		Lymph node metastases		Distant metastases	
	Absent	Present	Absent	Present	Absent	Present
G1, G2 (30 patients)	70% (21/30)	30% (9/30)	83.33% (25/30)	16.67 % (5/30)	86.67% (26/30)	13.33% (4/30)
G3 (9 patients)	33.33% (3/9)	66.67 % (6/9)	55.55% (5/9)	44.45 % (4/9)	44.45% (4/9)	55.55% (5 / 9)

DISCUSSION

There are several possible reasons for the low number of cases included in this study. First, neuroendocrine tumors have a low incidence with a reported value of 5–7 per 100,000 population per year and estimated prevalence of 35 per 100,000 (9). Second, the majority of tumors are nonfunctional and consequently the presentation may be incidental (10). CT scans are often the initial imaging study for a patient presenting with signs or symptoms suggestive of a NET, but in primary NETs, the average sensitivity of a CT scan is 73% (11). CT scans have even better sensitivity in detecting NET metastases, as they demonstrate 80% sensitivity for hepatic metastases (11, 12) and 75% sensitivity for extrahepatic metastases (11).

In our study we found a female to male ratio of 1.6 : 1, compared with the literature which states that neuroendocrine tumors

appear mostly in case of men. Most of the patients were old: 28 (71.79%) of them were between 61 and 87 years old, confirming the literature data which sustained that older person have increased risk of developing a neuroendocrine tumor (13, 14).

The most common location of neuroendocrine tumors included in the study was the pancreas followed by the mesentery and small bowel as compared with the literature were these tumors are mostly located in the small bowel (13, 14).

The density measurements on arterial and venous phases demonstrated that 33 (84.61%) lesions had a high uptake in the arterial phase and 6 (15.39%) tumors had a medium and low contrast enhancement in portal venous phase; this findings were confirmed by many authors who found the same heterogeneity of lesions: benign lesions were hyper vascular, small masses in arterial phase, isodense during portal phase and malignant tumors had heterogeneous enhancement (4, 11).

The study of enhancement curve (EC) patterns demonstrated that 27 lesions had a wash-out EC type, meaning that 18 (60%) benign and 9 (100%) malignant tumors had wash-out EC type.

MDCT image analysis was focused also on arterial and venous relative tumor enhancement ratio (RTER). The arterial RTER was 2.73 ± 3.5 and the venous RTER was 2.39 ± 4.2 ($p < 0.05$). CT attenuation values and contrast ratios of pancreatic neuroendocrine tumors in the arterial and venous phases were higher than those of mesenteric and small bowel ($p < 0.05$).

Correlating the tumor dimensions, structure and the presence of calcifications we found that:

- most of G1 and G2 lesions (25 of 30 – 83.33%) were small with homogeneous appearance;
- G3 tumors were all large, with heterogeneous enhancement and 5 (55.55%) of them had calcifications.

Our results confirmed the literature data concerning of the metastatic potential of neuroendocrine tumors: most tumors with G1 and G2 grades have no perilesional extension (70%), no lymph node involvement (83.33%) and distant metastases (86.67%). On the other hand, most of G3 tumors have perilesional extension (66.67%) and distant metastases (55.55%) but lymph node involvement have been demonstrated only in 44.45% of cases.

CONCLUSIONS

The enhancement curve types provide some opportunity for lesion differentiation between malignant and benign. The diagnostic performance can be improved by appropriate combination with other quantitative CT parameters like lesion dimension and structure, noncontrast and enhanced CT with intravenous contrast material (arterial, portal venous and intermediate-delayed phases).

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THE ROLE OF LYMPHOCYTE-TO-MONOCYTE RATIO AND PLATELET-TO-LYMPHOCYTE RATIO REPORTS AS A PREDICTIVE FACTOR FOR THE OCCURRENCE OF INTESTINAL NECROSIS IN COMPLICATED INCISIONAL HERNIAS

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THE ROLE OF LYMPHOCYTE-TO-MONOCYTE RATIO AND PLATELET-TO-LYMPHOCYTE RATIO REPORTS AS A PREDICTIVE FACTOR FOR THE OCCURRENCE OF INTESTINAL NECROSIS IN COMPLICATED INCISIONAL HERNIAS (Abstract): The current study **aims** to identify the role of lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) reports as a predictive factor for the occurrence of intestinal necrosis in patients with incisional hernias and the cutoff values for LMR and PLR. **Material and methods:** We performed a 4-year retrospective study that included 168 patients in whom we determined MRL and PLR reports. **Results:** From the statistical analysis we noticed that the cutoff value for LMR as a predictive factor in the case of intestinal resections is 2.716 (sensitivity of 54.2% and specificity of 88.4%). For PLT the cutoff level is 124, below this threshold the specificity decreases and the sensitivity increases. Comparing LMR and PLR, the ratio of lymphocytes to monocytes is the most sensitive and specific. AUC=0.712 (C.I. 95% = 0.638 to 0.779), p value of 0.0029. **Conclusions:** Elevated LMR levels are associated with the occurrence of bowel necrosis in strangled or incarcerated incisional hernias. **Keywords:** INCISIONAL HERNIA, LYMPHOCYTE-TO-MONOCYTE RATIO, PLATELET-TO-LYMPHOCYTE RATIO.

The treatment of incisional hernia is one of the most common pathologies faced by general surgeons. Between 5 and 30 patients out of 100 operated cases will develop an incisional hernia in the first 12 months (1). There are a multitude of surgical techniques, nonperfect, that must be adapted to all patients and their comorbidities. We often encounter obesity, advanced age, chronic diseases like diabetes or chronic obliterative bronchopneumonia (COPD) as associated pathology (2, 3, 4).

An abdominal wall defect, with or without the local presence of a bulging tumor during intra-abdominal pressure increasing maneuvers, clinically or paraclinically detectable in the first year after surgery is a common definition of incisional hernia. Incisional hernias appear as a complication of a laparotomy, especially if the postoperative wound had a septic evolution. The patient presents to the surgeon at the time of clinical manifestations. Because not every incisional hernia has an operative indication, the physician must consider several major elements: pain, bowel movements disorders, manifestations that reduce the quality of life and the risk of complications. Incarceration and strangulation are the events that require emergency surgery, due to the possibility of intestinal necrosis (5).

The current trends are to use positive criteria for systemic inflammatory response syndrome (SIRS) as a potential "alarm signal", both preoperatively and postoperatively. The magnitude of preoperative SIRS shows the urgency of applying treatment and postoperative prolongation is associated with organ dysfunction and the occurrence of postoperative complications (6,7). A simple method of measuring SIRS is to calculate the ratios of serum lymphocytes (LYM), monocytes (MON) and platelets (PLT) - LMR and PLR (8). The current study aims to identify the role of LMR and PLR reports as a predictive factor for the occurrence of intestinal necrosis in patients with incisional hernias and the cutoff values for LMR and PLR.

MATERIAL AND METHODS

We performed a 4-year retrospective study in the General Surgery Department of the Onești "Saint Hierarch Dr. Luca" Municipal Hospital. Thus, between January 1, 2016 and December 31, 2019, we analyzed the clinical records and the hospital's own computer system - InfoWorld, from where we selected 168 patients who underwent surgery for incisional hernia. We excluded from the study patients with incisional hernias who have other conditions that may lead to systemic inflammatory syndrome, as well as those who needed to perform a colon resection.

When admitting patients to the hospital in emergency conditions or by appointment, they were collected usual laboratory analyzes (serum glucose, complete blood count, exploration of liver function, exploration of renal function, coagulation, blood compatibility). We turned our attention to the complete blood count (CBC), from where we tracked the serum values of LYM, MON, neutrophils (NEU), PLT and white blood cells - WBC. With these values we calculated the ratios between lymphocytes and monocytes (LMR), as well as between platelets and lymphocytes (PLR).

RESULTS

From the analysis of the collected data, we observed a uniform distribution of the number of patients for the years included in the study (tab. I). Regarding the age of the patients in our observational study, it ranged from 19 to 89 years.

From the descriptive analysis of the data, we noticed that the gender division of the cases counted 38 men (approximately 23%) and 130 women (78%). Regarding the type of hospitalization, we obtained a percentage of emergency admission of 57%, which represents 96 patients. The number of those admitted to the surgery department by appointment was lower, of about 43%.

TABLE I

Distribution by year of the number of patients

		Frequency	Percent	Valid Percent
Valid	2016	46	27.38	27.38
	2017	39	23.21	23.21
	2018	56	33.33	33.33
	2019	27	16.07	16.07
	Total	168	100.0	100.0

At the time of admission, all patients underwent an initial surgical consultation to determine whether surgery was required or could be delayed. The physician's decision was the result of a combination of local clinical examination, medical history, and the patient's biological condition. Thus, we observed that the surgery was performed in emergency in 55 cases, and in 113 cases, the surgery was performed under scheduled conditions (tab. II).

TABLE II

Time of surgery

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Acute	55	32.74	32.74	32.74
	Chronic	113	67.26	67.26	100.0
	Total	168	100.0	100.0	

In 16.07% of cases, during the surgery due to intestinal viability disorders, it was necessary to practice a small bowel resection (tab. III). All surgeries were performed in the classic manner with lateral-to-lateral anastomoses as well as intra-abdominal and subcutaneous drainage. Also, in 41% of cases, for the restoration of the abdominal wall it was necessary to use prosthetic materials - polypropylene mesh (tab. IV).

TABLE III

The need for intestinal resections

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	27	16.07	16.07	16.07
	NO	141	83.93	83.93	100.0
	Total	168	100.0	100.0	

TABLE IV

The use of prosthetic materials in abdominal wall reconstruction

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	69	41.07	41.07	41.07
	NO	99	58.93	58.93	100.0
	Total	168	100.0	100.0	

The descriptive statistics of the two LMR and PLR reports depending on the time of surgery show that the variables do not have a Gaussian distribution, and the median of LMR has the value of 4.57 (tab. V).

TABLE V

LMR and PLT analysis depending on the time of surgery

Acute / Chronic INTERVENTION		LYM/MON	PLT/LYM
Acute	Mean	4.261418	154.904105
	Std. Deviation	3.2908009	123.4327013
	Median	3.407800	128.571400
	Minimum	.3842	23.6907
	Maximum	20.0000	905.6818
Chronic	Mean	5.136754	129.837650
	Std. Deviation	2.3282385	59.4598913
	Median	4.848400	119.433100
	Minimum	.6233	38.7417
	Maximum	20.7272	510.4166
Total	Mean	4.843259	138.242285
	Std. Deviation	2.7115718	86.7893866
	Median	4.575400	122.861100
	Minimum	.3842	23.6907
	Maximum	20.7272	905.6818

From the analysis of inferential statistics, by applying the Mann-Whitney test, we noticed that the LMR value was statistically correlated with the need to perform an intestinal resection, with a p value below 0.05 (tab. VI).

TABLE VI

Statistical significance of LMR

Intestinal Resection	LYM/MON	PLT/LYM
Mann-Whitney U	1008.000	1143.000
Wilcoxon W	1308.000	11874.000
Z	-3.330	-2.725
P value	0.001	0.006

In the case of the LMR report, in patients with intestinal resection, the established cutoff level is 2.716, below this threshold the sensitivity decreases and the specificity increases (fig. 1) and for PLR the established cutoff is 124 (fig. 2).

The values of LMR report above 2.716 correlate with the existence of intestinal necrosis in patients with incisional hernias. Comparing LMR and PLR reports, the ratio of lymphocytes to monocytes is the most sensitive and specific (tab. VII).

In the case of PLR report, we established the existence of a cutoff value of 124 which correlates with the existence of intestinal necrosis in the case of the studied patients (fig. 2).

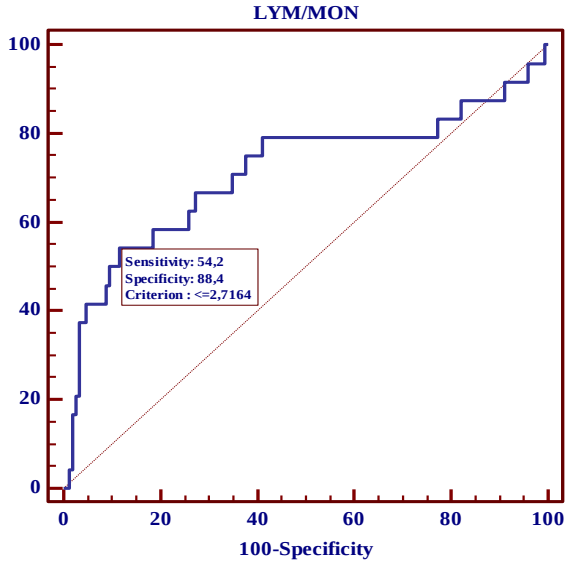

Fig. 1. Sensitivity and specificity of LMR in patients undergoing bowel resections

TABLE VII

Comparison of LMR and PLR reports

	AUC	SE [a]	95% CI [b]
LYM_MON	0.712	0.0713	0.638 to 0.779
PLT_LYM	0.674	0.0601	0.598 to 0.744

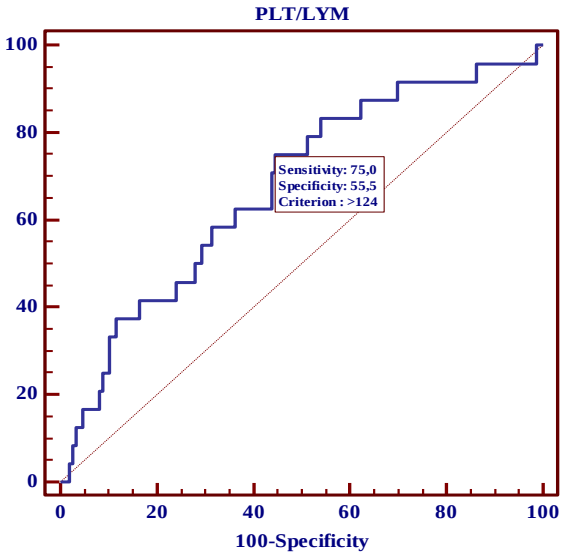

Fig. 2. Sensitivity and specificity in the case of PLR

DISCUSSION

Incisional hernias are a topical public health issue. Currently there is an upward trend to use the endoscopic or laparoscopic approach in the treatment of abdominal pathologies, which reduces in number and difficulty the surgical operations dedicated to the abdominal wall defects. As a result, incisional hernias remain the prerogative of patients debilitated by severe, long-lasting comorbidities, elderly, in whom the emergency surgical indication becomes the rule. The main risk factors associated with surgery are diabetes and chronic obstructive pulmonary disease (9, 10).

Complications of incisional hernias - incarceration and strangulation, determine the initiation of the systemic inflammatory process. The quantification of the patient's inflammatory status is based on the criteria of the systemic inflammatory response syndrome (11-13). It is well known that the migration of NEU into the affected tissues is the first response to the onset of the cytokine storm. The local inflammatory process involves the participation of many serum cells: WBC, NEU, LYM, MON, PLT. Thus, the variation of the number of serum cells may indicate the progression of the local inflammatory syndrome, as well as the appearance of local and general complications (14).

In some patients with incisional hernia, the intestinal loops may suffer irreversible damage leading to their necrosis. Local examination of patients with complicated incisional hernias is not sufficient to determine the viability of bowel loops. Necrosis of the intestinal segment causes peritonitis and septic shock. In this situation the patient's life depends on the emergency surgery (15, 16).

Many studies have sought to establish a correlation between changes in the patient's serum cellularity and inflammatory status secondary to intestinal necrosis. In this sense, the importance of the ratios between the serum values of NEU, LYM, MON and PLT – neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) was investigated (17-20). It has been observed that LMR and PLT can be used as biological markers with predictive potential in determining the extent of the inflammatory response, and therefore in the occurrence of intestinal necrosis (21,22).

In our study from the data analysis, we note that the median of LMR in patients with intestinal resection is 3.956, and in the group without resection is 4.989. From the statistical analysis we noticed that the cut-off value for LMR as a predictive factor in the case of intestinal resections is 2.716 (sensitivity of 54.2% and specificity of 88.4%). For PLT the cutoff level is 124, below this threshold the specificity decreases and the sensitivity increases. We consider that monitoring the serum levels of WBC, LYM, NEU, PLT and MON is easy to do and does not require additional special equipment.

One of the main limitations of our study is the small number of patients included in the study. Although the results are promising, there is still a need for studies on larger groups of patients, randomized and prospective.

CONCLUSIONS

Elevated Lymphocyte-To-Monocyte ratio levels are associated with the occurrence of bowel loop necrosis in incarcerated and strangled incisional hernias.

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NEWS

ANSWERING KEY QUESTIONS ABOUT COVID-19 VACCINES

In the US, 4 vaccine candidates are in phase 3 clinical trials with initial results expected soon. If studies succeed, 1 or more vaccines may become available within a few months. The article focuses on common questions people are likely to ask about COVID-19 vaccines. ***How Much Does a Vaccine Reduce the Risk of COVID-19 and Its Complications?*** The US Food and Drug Administration (FDA) guidance set as an expectation for licensure that a COVID-19 vaccine would prevent disease or decrease its severity in at least 50% of people who are vaccinated. The study showed a 50% reduction in illness, but the confidence interval for the efficacy estimate might be 30% to 80%, which means efficacy may be as low as 30% or as high as 80%. Also, a vaccine reduces not only mild but also more severe disease, as well as hospitalizations and deaths. ***Will the Vaccine Be Effective for All Patients?*** COVID-19 is more common and severe among individuals often underrepresented in clinical trials, including older individuals, people with chronic illnesses, and persons in racial/ethnic minority populations. When results become available, it will be important to evaluate the characteristics of people included in the trial and determine whether they are similar to patients seen in the practice setting because a given vaccine may be more appropriate for some patients than others. ***Is a Vaccine Licensed or Provided Under an Emergency Use Authorization?*** FDA has a long track record of licensing vaccines that have protected individuals against diseases like measles, polio, and pneumonia and has stated it will apply its usual high standards to COVID-19 vaccines. ***Will All COVID- 19 Vaccines Be the Same?*** Different vaccines are likely to perform and be used differently and clinicians will need to be aware of any differences between vaccines including dose numbers and schedules, as well as safety and efficacy because some vaccines may be preferred for certain populations. ***Can Vaccinated People Stop Worrying About COVID-19?*** While a vaccine will help protect individual patients and those around them, a large proportion of the population must be immunized and protected before transmission is substantially reduced. No vaccine will be 100% effective and a vaccine that protects against developing clinical illness may not prevent transmission to others. Even after vaccines become available, SARS-CoV-2 will be a continuing concern. Effective public health measures, such as social distancing, and wearing masks, will be needed for at least several more months, and potentially longer. (Goodman JL, Grabenstein JD, Braun MM. Answering Key Questions About COVID-19 Vaccines. *JAMA*. Published online October 16, 2020. doi:10.1001/jama.2020.20590).

OPHTHALMIC PATHOLOGY TREATED DURING COVID-19 AT THE OPHTHALMOLOGY CLINIC OF "SF. SPIRIDON" COUNTY CLINICAL EMERGENCY HOSPITAL IN IAŞI, ROMANIA

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OPHTHALMIC PATHOLOGY TREATED DURING COVID-19 AT THE OPHTHALMOLOGY CLINIC OF "SF. SPIRIDON" COUNTY CLINICAL EMERGENCY HOSPITAL IN IAŞI, ROMANIA (Abstract): Within the current epidemiological context and due to the increased risk of infectivity, this article focuses on the practical activity of "Sf. Spiridon" Ophthalmology Clinic in Iaşi, during the emergency period (March 16th -May 16th, 2020), the evaluated ocular pathology and the approached strategy from the point of view of medical and surgical vision. **Material and methods:** As an Emergency Hospital and Tertiary Center of Moldova, Romania "Sf. Spiridon" Hospital in Iaşi was the only hospital in the region that dealt with all the urgent ophthalmological pathology during COVID-19. The clinic worked due to the established protocol, the patient's circuit was changed, with the buffer zone for the suspicious COVID-19 patient, where the patient was hospitalized until the negative PCR result. All patients having a negative PCR test benefited from both medical and / or surgical treatment depending on the type of ocular pathology. **Results:** 619 patients were examined in the emergency room, of which 61 patients were hospitalized in Clinic I Ophthalmology, "Sf. Spiridon" Hospital in Iaşi, between March 16 and May 16, 2020. Most patients (59%) came from the neighboring counties (Suceava, Vaslui, Piatra Neamţ, Bacău) the rest being from Iaşi (41%). The age varied from 2 to 87 years, the average age being 51.72 ± 22.16. Most frequently, patients were hospitalized with retinal detachment (29.5%), trauma (24.6%) and corneal sclerosis (13.1%), most cases being surgical. The average length of stay was 6.3 days. The average time of surgery stages was 45 minutes. The condition at discharge was in 60% of cases improved and 30% cured. All patients tested negative for COVID-19 PCR. Visual acuity and IOP showed slightly higher / lower changes at discharge than at the time of admission. Depending on the diagnosis, the average stay for the patients with retinal detachment was about 4 days, while for patients with glaucoma (13 days) or ulcer corneal (12 days), the long-term hospitalization exceeded 12 days. The multivariate analysis showed that about 10% of the number of days spent in hospital (dependent variable) can be explained by the diagnosis of hospitalization and the type of intervention. Correlated with the status of discharge, the number of days spent in hospital did not differ significantly. **Conclusions:** Most of the ophthalmological activity during the pandemic was surgical, due to the large number of trauma cases from neighboring counties, being the only non-COVID-19 hospital in the North-eastern region of Romania. Complying with the protocol suggested by

the American Academy of Ophthalmology for protection in the case of ophthalmic surgery allows standard eye care and safe surgery to maintain a sustainable application. **Keywords**: COVID-19, SARS-CoV-2, SURGICAL MANAGEMENT, MEDICAL MANAGEMENT.

SARS-CoV-2, which causes COVID-19, is a virus discovered in December 2019 in Wuhan, China. In the immediate aftermath of the rapid spread, the World Health Organization (WHO) declares it a public health emergency, of international concern on 30 January 2020 and a pandemic on 11 March 2020. The initial cases of zoonotic transmission from bats (1) have evolved into a human-to-human transmission through drops, vomiting (2), feces (3), and tears since then. Studies show that the detection of SARS-CoV-2 in tears and conjunctival secretions shows an increased risk of transmission in the field of ophthalmology, which determines the creation of special measures for medical and surgical procedures. Within this epidemiological context, the AAO (American Academy of Ophthalmology) on March 18 provides a series of recommendations for ophthalmologists in solving urgent cases during the COVID-19 emergency period (4). The emergency period was established in Romania in March, the ophthalmology clinic operating on the basis of a protocol involving patient reception and testing circuits in the buffer zone, until the COVID-19 test.

MATERIAL AND METHODS

The ophthalmology clinic within St. Spiridon Hospital in Iaşi is the tertiary center in Moldova, Romania, being the only hospital in the region was the only hospital in the region that dealt with all the urgent ophthalmological pathology during COVID-19. The clinic worked due to the established protocol, the patient's circuit was changed, with the buffer zone for the suspicious COVID-19 patient, where the patient was hospitalized until the negative PCR result. Subsequently, the patient with negative PCR test received both medical and / or surgical treatment depending on the eye pathology. Strict infection control measures have been put in place to minimize the spread of COVID-19 infection. These include personal protective equipment (PPE) for healthcare workers and routine cleaning of "high-touch" surfaces. During the emergency period, both the outpatient activity of non-urgent cases or controls was restricted. The patients were monitored as follows: their temperature was taken at the tent in the Hospital Triage, and they filled in a questionnaire on the main symptoms of COVID-19 infection. Certain surgical cases that underwent postponement (elective, scheduled cases): cataract surgery, strabismus, oculoplastic, orbital surgery were postponed for safety reasons. Urgent cases coming from the emergency unit were accepted at the hospital: corneal wounds, strong contusive traumas, recent retinal detachment, intraocular foreign bodies, advanced drug-decompensate glaucoma, and various severe eye injuries in children. All cases presented in the outpatient clinic that were not urgent were rescheduled, and all patients who were scheduled in the emergency period were called and will be rescheduled outside this period. Both medical staff and nurses were equipped according to the protocol suggested by the WHO (World Health Organization), the Ministry of

Health and the AAO (American Academy of Ophthalmology). It consisted of a surgical face mask, FFP2, FFP3, disposable gloves, goggles, a visor, and a special protective gown. The COVID-19 + suspicious cases from the emergency reception unit were evaluated in the special room and with complete special protection equipment (coveralls, mask, gloves, boots, visor, glasses). There were particular cases when in addition to the evaluation the fully equipped doctor performed a series of maneuvers and surgeries in the special room: removal of foreign bodies from the cornea, suturing of eyelid and corneal wounds due to the increased risk of exposure depending on the type of intervention and its duration.

Statistical processing. The database was built in *EXCEL* and was statistically processed with the *SPSS 18.0.* These represent software for statistical and graphical data analysis, with an interface that helps the user to solve any need for processing experimental data. The statistical analysis of the data was performed with *SPSS 18.0* and consisted in applying the following tests: *ANOVA, Student t-test, F-test* etc.

RESULTS

619 patients were examined in the emergency room, of which 61 patients were hospitalized in Clinic I Ophthalmology, St. Spiridon Hospital in Iaşi, between March 16th and May 16th, 2020. Most patients (59%) came from the neighboring counties the rest being from Iaşi (41%) (fig. 1).

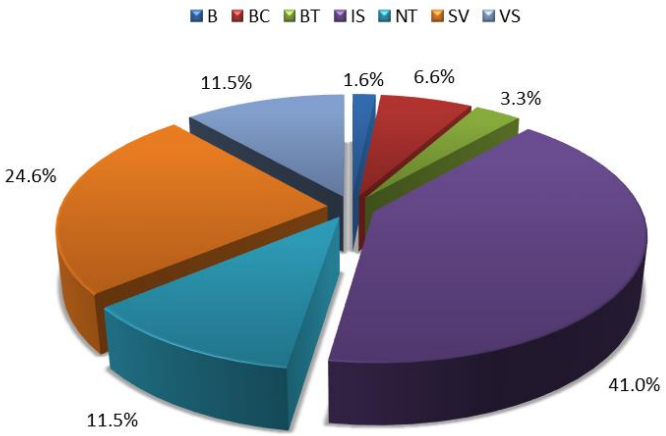

Fig. 1. Distribution of subjects according to the county of origin [of Iași (41%), Suceava (24.6%), Neamț (11.5%) and Vaslui (11.5%) counties]

Age ranged from 2 to 87 years; the mean level 51.72 ± 22.16 years close to the median value (55 years). Most frequently, patients were hospitalized with retinal detachment (29.5%), trauma (24.6%) and corneal sclerosis (13.1%); most cases being surgical (fig. 2). The lowest mean age level was observed in the patient with preseptal cellulitis and in patients with corneal burns, while patients with dislocated crystalline mass or shingles were over 76 years (p = 0.02). The

distribution by sex highlighted the predominance of male cases, except for glaucoma where all cases were female. The average length of stay was 6.15 days (fig. 3). The average time of surgery stages was 45 minutes. The condition at discharge was in 60% of cases improved and 30% cured Postoperative complications were identified in 7 of 61 patients (11.5%) especially the increase in IOP in 3 of 7 cases.

Of the total study group, COVID-19 testing was performed in 65.6% of patients, the results being negative in all cases. The test was not performed in all patients, because, on the one hand, they came with the test performed in another diagnostic center and, on the other hand, they received emergency surgical treatment, stayed in the buffer zone, and then were discharged. Of the total cases, 82% required surgery.

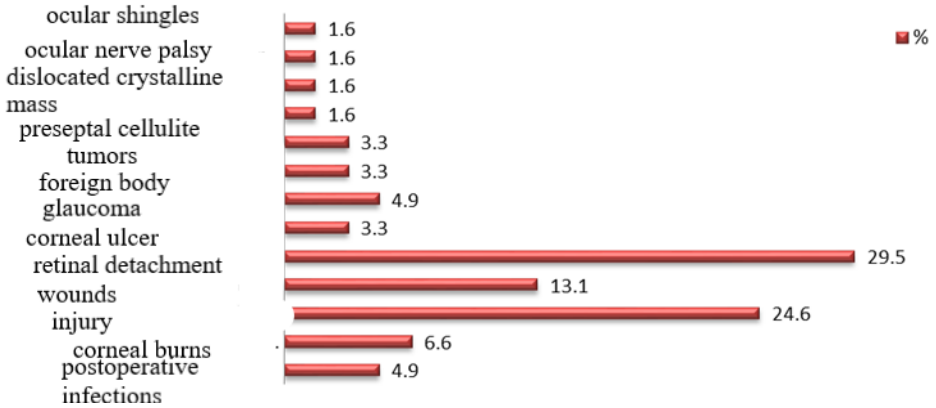

Fig. 2. Distribution of cases according to the diagnosis at admission

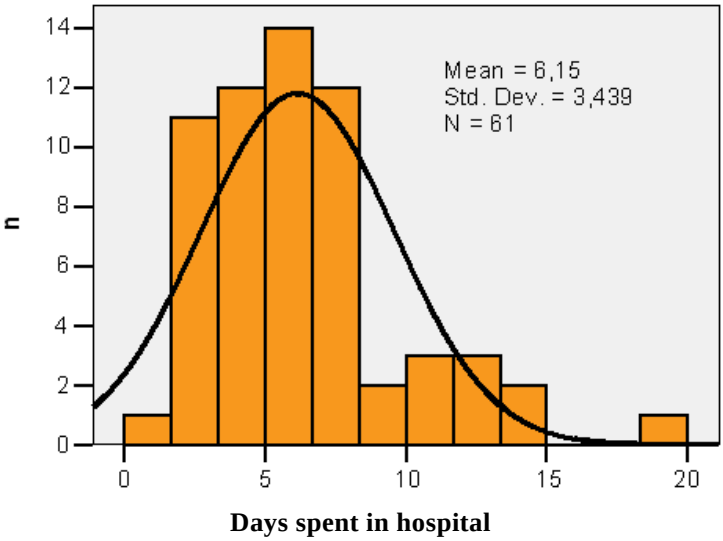

Fig. 3. Histogram of the days spent in hospital

Visual acuity in the affected eye at admission varied within the range of 0-1, with a slightly higher mean level in patients with a negative COVID-19 test result (0.181 *vs.* 0.178; p = 0.969). In patients with a negative COVID-19 test at discharge, the visual acuity slightly increased compared to the time of admission, but the difference was not statistically significant (0.181 ± 0.333 *vs.* 0.256 ± 0.332; p = 0.08) (fig. 4).

IOP in the affected eye at admission varied in the range of 4-56, registering a slightly lower average level in patients with a negative COVID-19 test result (12.90 *vs.* 13.24; p = 0.907). In patients with a negative COVID-19 test at discharge, intraocular pressure ranged from 3 to 31, with a slightly higher mean level than in non-tested patients (12.75 *vs.* 11.10; p = 0.298). In patients with a negative COVID-19 test at discharge, the IOP slightly decreased compared to the time of admission, but the difference was not statistically significant (12.90 ± 9.95 *vs.* 12.75 ± 6.17; p = 0.587) (fig. 5).

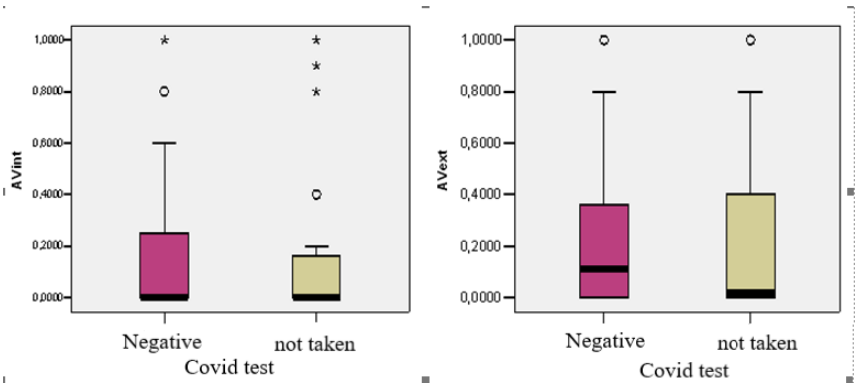

Fig. 4. Average values of visual acuity during hospitalization depending on the COVID-19 test result

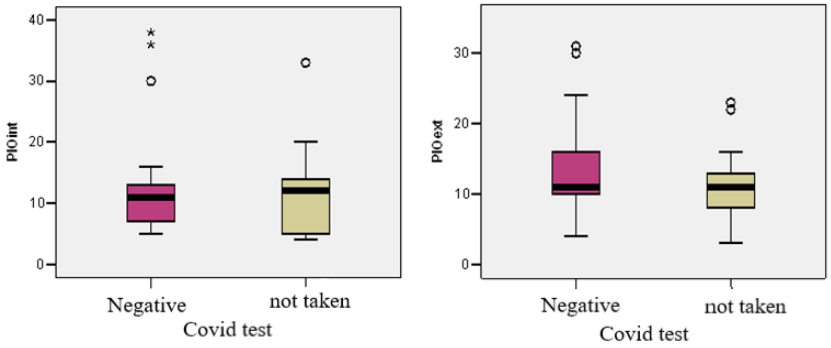

Fig. 5. Average IOP values during hospitalization depending on the COVID-19 test result

The number of days spent in hospital was significantly lower in patients with PPV (pars plana vitrectomy) (4.15 ± 1.09 days) compared to patients without surgery (7.0 ± 3.32) (p = 0.027) (fig. 6).

Depending on the diagnosis, the average

of stay for the patients with retinal detachment was about 4 days, while for patients with glaucoma (13 days) or ulcer corneal (12 days), the long-term hospitalization exceeded 12 days. The multivariate analysis showed that about 10% of the number of days spent in hospital (dependent variable) can be explained by the hospitalization diagnosis of and the type of intervention (R square = 0.102; p = 0.031) (tab. I).

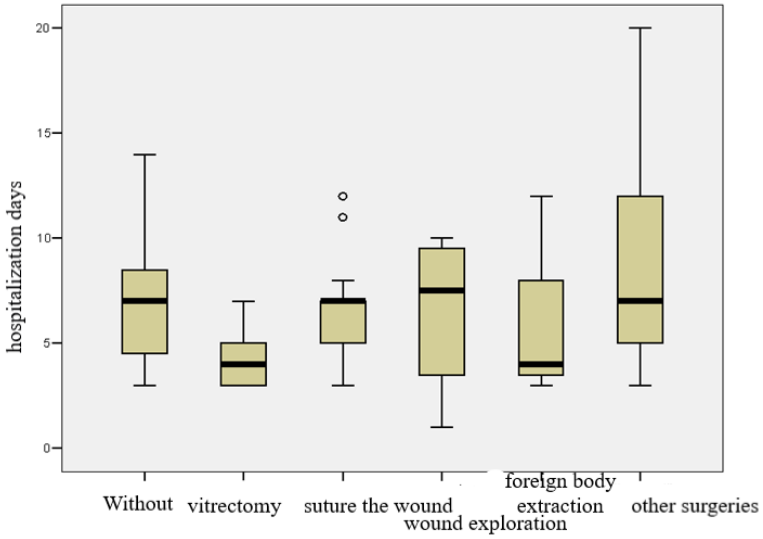

Fig. 6. Number of days spent in hospital related to the type of surgery

TABLE I

Multivariate analysis, variation in the number of days spent in hospital

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	,162[a]	,026	,010	3,422	,026	1,592	1	59	,212
2	,320[b]	,102	,071	3,315	,076	4,897	1	58	,031

a. Predictors: (Constant), DGinternare

b. Predictors: (Constant), DGinternare, Interventie

Correlated with the status of discharge, the number of days spent in hospital did not differ significantly (p = 0.909). The multivariate analysis confirmed that the number of days spent in hospital and the diagnosis after hospitalization are not good predictors in determining the condition at discharge (R square = 0.015; p = 0.425). Of the total study group, the discharge status of patients was 60.7% improved and 34.4% cured.

DISCUSSION

Due to the fact that "Sf. Spiridon" Hospital in Iaşi is the tertiary center in Moldova, Romania, being the only hospital in the region was the only hospital in the region that dealt with all the urgent ophthalmological pathology during COVID-19, the medical staff was exposed to suspected or confirmed COVID-19 patients. Several studies report what the attitude of the ophthalmolo-

gist would be and what the precautions to be taken during the risk of COVID-19 infection 19 would be. Ocular involvement has been reported in studies as being in 30% of cases, especially in those severe. The possibility of transmission in the ocular route is due to the presence of a similar enzyme converting angiotensin 2 (ACE2) receptors on ocular surfaces as well as on the host, the transmission being done through the exposed mucous membrane. Another route of transmission cited by studies is through the nasolacrimal duct, from where the virus migrates to the lower meatus. Ocular symptoms may occur 2 weeks after infection (3, 5). The main involvement being at the conjunctival level, the patient may present acute hemorrhagic conjunctivitis, with watery secretions, with pseudo-membranes that do not bleed by removal, being very important periocular and eyelid hygiene, topical treatments with antibiotics and anti-inflammatory drugs. Other manifestations such as superficial punctate keratitis have been reported as intraocular (3, 5, 6). Basak Bostanci Ceran and Serdar Ozates demonstrated the presence of ocular manifestations in the acute phases of the infection, thus including 93 confirmed COVID-19 patients in their study, who were having ocular symptoms besides systemic manifestations. As eye signs, 20% of them show hyperemia, increased secretion, epiphora, chemosis, follicular conjunctivitis and episcleritis and the most common symptoms were photophobia and itching. All had temperatures above 37 degrees, laboratory changes (neutrophilia, elevated CRP, elevated ESR, lymphopenia), all of which were associated in the study with ocular impairment (6). The authors of the study claim that all patients with conjunctivitis are considered COVID-19 suspects and should be consulted in a special room by an ophthalmologist wearing protective equipment (3).

Preventing the transmission of this infection through the eyes requires a series of recommendations and measures that ophthalmologists from all over the world have faced and followed and that we have also observed in our clinic. Regarding the clinical evaluations in outpatient services, avoiding the congestion of waiting rooms, reprogramming patients who were not urgently seen, disinfecting rooms with special disinfectant at each time interval, placing the UV lamp for 10-15 minutes were among the few useful measures in preventing the transmission of the infection. All patients in the emergency room presented for admission are recommended: epidemiological survey questionnaire, temperature monitoring, chest CT and COVID-19 emergency test (4, 6, 7, 8, 9).

Regarding the rules in the operating room, the studies provide a series of clarifications: banning the use of air conditioning (to reduce aerosol production), oxygen mask (reduction of nasopharyngeal flow), and decreasing operating time (operations performed by professional surgeons), while reducing the number of staff in the operating room. Wearing FFP 2 and 3 masks, FFP2 masks have a filtration rate of at least 94%, while FFP3 masks are the most filtering FFP mask, playing a key role and protecting against very fine particles such as asbestos, because many procedures produce aerosols. In ophthalmology: fragmentation of the nucleus by phacoemulsion and vitrectomy, where ultrasound-induced probe movement occurs at more than 50,000 Hz, with a significant production of aerosols. In addition to masks, ophthalmic operating rooms are usually ventilated with positive pressure systems, changing the air approximately 20 times per hour, with air passing through a high-efficiency particulate air (HEPA) air

filter, where the nanosized particles descend to be removed. Increasing the interval between surgeries to 45 minutes, thus allowing the disinfection and the action time of the used substances (4, 7, 10, 11).

Being the only hospital in the region of Moldova that was open during the pandemic, we faced many cases, mostly surgical. There were 619 patients in the emergency department in two months, of which an average of 10 patients were hospitalized per day, 61 patients (10%) required surgery being hospitalized with contusive trauma, wounds, retinal detachments, etc. The average duration of surgery was 45 minutes, and the average length of hospitalization was 6.3 days. Compared to the similar period in 2019, it was found that the number of general admissions decreased 5.6 times (342 *vs.* 61) and 77.6 times (233 *vs.* 3) for 1 day of hospitalization, due to restrictions on elective admissions (cataracts, strabismus, etc.), practically during the state of emergency, only the pathology that had to be surgically or medically resolved immediately was hospitalized.

Regarding the distribution of the most prevalent diseases that were treated during the emergency period, we have noticed that a large share of the admitted patients had a vitreoretinal emergency: retinal detachment (29.5%), postoperative infection (4.9%) or dislocated crystalline mass (1.6%). It is widely known that in rhegmatogenous retinal detachment, the prognosis is much improved if the surgery is performed in the first 3 days after the detachment of the macula (which is perceived by the patient as a dramatic decrease of visual acuity) (12). Such an acute event probably determined the patients to confront their apprehension about coming to a hospital during a lockdown period. Postoperative complications of cataract surgery are also susceptible to be rapidly diagnosed and emergency therapy is of utmost importance (13, 14,15, 16, 17,18). Special interdisciplinary procedures helped reduce the length of hospital stay (18). Another important percentage of patients had ocular trauma, either open globe (13.1%) or closed globe (24.6%), many of which normally would have been managed in county hospitals.

Conversely, elective surgeries like cataract extractions or macular procedures were practically stopped. While it is widely accepted that these conditions are slowly progressive or may be stable for months or years (16, 17), we are nevertheless wary about the possibility that many patients would present in the future with advanced stages or even complications (such as phacomorphic glaucoma or lens-related uveitis). Having no HEPA filter systems and having difficulties in creating circuits, the waiting time between surgeries increased and their number was drastically reduced. Since only half of beds were available, most cases were carefully selected and resolved in the previously established order. Exactly all the recommendations described above and mentioned in various studies in the literature were observed, as well as those suggested by the American Academy of Ophthalmology and the Romanian Ministry of Health if we compare the number of hospitalized patients.

CONCLUSIONS

Most of the ophthalmological activity during the pandemic was surgical, due to the large number of trauma cases from neighboring counties, being the only non-COVID-19 hospital in the northeastern region of Moldova (the other three public ophthalmology clinics being closed, as well as the entire private clinics-closed by law). Complying with the protocol suggested by the

American Academy of Ophthalmology for protection in ophthalmic surgery allows for standard eye care and safe surgery to maintain a sustainable application. Ultimately, the goal is to maintain patient confidence and secure access to both routine and emergency eye evaluation, while caring balances additional needs during an epidemic.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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FIRST YEAR OF THE COVID-19 PANDEMIC INFLUENCE ON BLADDER CANCER PATIENTS IN ONE SINGLE ACADEMIC UROLOGICAL CENTER FROM NORTH-EASTERN ROMANIA

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FIRST YEAR OF THE COVID-19 PANDEMIC INFLUENCE ON BLADDER CANCER PATIENTS IN ONE SINGLE ACADEMIC UROLOGICAL CENTER FROM NORTH-EASTERN ROMANIA (Abstract): The COVID-19 pandemic created an unprecedented situation who made health systems to reorganize their resources and redeploy staff away from routine diagnostic, treatment and follow-up services. **Material and methods:** have studied retrospective the medical files of bladder cancer patients referred to our clinic between 1st of March 2019- 28 of February 2020 (group A) compared with those referred between 1st of March 2020-1st of March 2021 (group B). A total of 640 patients were included, 486 (75.94%) males and 154 (24.06%) females, aged between 22 and 92 years, with a mean age of 69.12 years (SD+/-9.62). Group A had 408 (63.75%) patients while group B 232 (36.25%). **Results:** We have noticed that anemia was significantly more often in group B (p=0.032), this has led to a higher necessity of preoperative blood transfusion (p=0.039) but with no differences regarding inflammatory syndrome or kidney failure incidence. Also, the hospitalization period (p=0.001) and the time from admission to surgery have been significantly shorter in group B (p=0.05). **Conclusions:** By trying to avoid hospitalization many patients are admitted with a poorer biological status. In addition, many bladder cancer cases will go undetected and untreated and once the pandemic has passed, we will face an excess in cancer-related mortality. **Keywords:** COVID-19, BLADDER CANCER, TURBT, HEMATURIA.

Since December 2019, when first cases of pneumonia of unknown etiology were reported to the World Health Organization (WHO), COVID-19 has spread worldwide causing WHO to declare it officially as a pandemic on 11th March 2020. The disease caused by coronavirus, a large family of single-stranded RNA viruses. They are responsible for severe acute respiratory syndrome coronavirus (SARS), the Middle Eastern respiratory syndrome coronavirus (MERS) (1). The rapid increase in the number of COVID-19 cases has led to fear and panic among health care professionals and the general population. This unprecedented situation has made health systems to reorganize their resources and redeploy staff away from routine diagnostic, treat-

ment and follow-up services.

COVID-19 could have a direct impact on urological/nephrological diseases. According to Hirsch *et al.*, 89.7% of mechanically ventilated patients due to SARS-CoV-2 developed acute kidney injury with 23.2% receiving kidney replacement therapy, in contrast to a 21.7% incidence of acute kidney injury and 0.2% requirement for kidney replacement therapy in non-ventilated patients (2). Also, this disease could have an indirect influence because in addition to wearing a mask, one of the most important recommendation to minimize the chance of viral transmission is social distancing however, this may not be possible for patients who require cancer-related treatments in hospitals.

In March 2020, a "state of emergency" was declared in Romania for a period of 30 days as of the 16th of March. The highest increase on COVID-19 case in first wave of the pandemic was in North-eastern Romania (47.1% of the infected medical staff nationwide, in April 2020). The neighboring Suceava County this area has often been referred to as the "Romanian Lombardy" , a suggestive comparison with the Italy's region which was severely affected by COVID-19 (3). We aimed to highlight the impact of the pandemic on bladder cancer patients on North-Eastern Romania in our region.

MATERIAL AND METHODS

We have studied retrospective the medical files of bladder cancer patients referred to our clinic between 1st of March 2019- 1st of March 2021. We have divided them into two groups: group A - patient admitted in our clinic between 1st of March 2019 and 28th of February 2020 (pre-pandemic) and group B - those treated 1st of March 2020- 1st of March 2021. We compared demographic data, laboratory findings and hospitalization period. Because between 1st of March 2020- 1st of March 2021 no cystectomy was performed in our clinic, we have not included this category of patients in the pre-pandemic group.

Statistical analysis was made using *t-Student* test for 2 Independent Means and *Chi-Square* test with Yates's correction, calculated online on http://www.socscistatistics.com/tests/ Default.aspx, statistical significance was defined as p value ≤ 0.05.

RESULTS

A total of 640 patient were included, 486 (75.94%) males and 154 (24.06%) females, aged between 22 and 92 years, with a mean age of 69.12 years (SD+/- 9.62). The demographic data on each group are shown in first table.

We have noticed that anemia was significantly more often in group B, this has led to a higher necessity of preoperative blood transfusion but with no differences regarding inflammatory syndrome or kidney failure incidence. Also, the hospitalization period and the time from admission to surgery have been significantly shorter in group B, as shown in second table.

TABLE I

The demographic data on each group

	Group A	Group B	p
No. Patients (n)	408 (63.75%)	232 (36.25%)	
Age (years)	42-92 (SD-8.68) mean-69.63	22-92 (SD-10.84) mean-68.61	0.054
Gender Females / Males (n)	99 /309	55/177	0.950
Home Urban / Rural (n)	201/207	131/101	0.094

TABLE II
Characteristics of the two groups

	Group A	Group B	p
First presentation / Relapse	154/254	74/158	0.161
Admission serum hemoglobin (g/dL)	3.9-16.2 (mean-11.98)	2.7-17.4 (mean-12.72)	0.032
Preoperative blood transfusion (n)	10 (2.45%)	13 (5.60%)	0.039
Mean serum hemoglobin at discharge (g/dL)	10.16	11.01	0.043
Admission hematocrit (%)	13.6-50.4 (mean=39.46)	9-49.5 (mean=38.27)	0.017
Discharge hematocrit (%)	17.6-48.4 (mean=30.8)	21.3-51.9 (mean-34,1)	0.046
Admission WBC (× 10^9/L)	2.87-50.3 (mean=8.23)	3.1-27.56 (mean=8.38)	0.31
Admission C-reactive protein (mg/dL)	0.1-244.5 (mean=10.32)	0.1-293.9 (mean-14.04)	0.068
Admission serum creatinine (mg/dL)	0.57-13.32 (mean=1.07)	0.46-13.87(mean=1.18)	0.104
Discharge serum creatinine (mg/dL)	0.62-3.76 (mean=1.19)	0.54-8.53 (mean=1.29)	0.29
Type of tumor NMIBC / MIBC (n)	276/132	46/186	0.0009
Time from admission to surgery (days)	1-17 (mean=2.74)	1-16 (mean=2.5)	0.05
Hospitalization period (days)	2-19 (Mean=6.12)	2-22 (Mean=5.43)	0.001

DISCUSSION

Bladder cancer (BC) is the 10th most common cancer worldwide, according to Cumberbatch *et al.*, it represents 3% of all malignancies, in 2018, 549,000 new cases and 199,000 deaths have been recorded (4). The unprecedented COVID-19 pandemic has placed a huge burden on healthcare systems worldwide with the requirement to treat large influxes of infected patients, many of whom require respiratory support. This led to a rapid reallocation of healthcare resources affecting urologic oncology practices. Moreover, professional associations (e.g.: European Association of Urology-EAU, American Urological Association-AUA) rapidly adapted the practice guidelines resulting in postponement of lots of medical and surgical procedures (5). According to these guidelines, in patients with small papillary recurrence/s or bladder tumors < 1 cm and history of Ta/1 low grade tumor, transurethral resection of the bladder tumor (TURBT) was to be defer up to 6 months, while those with bladder lesion and intermittent macroscopic hematuria or history of high-risk non-muscle invasive BC should be treated within < 6 weeks (5).

In the first pandemic year, almost half of patients we referred to our clinic compared to the previous year. This situation could have several explanations. First of them could be the reorganization of the county emergencies hospitals north-eastern Romania, many of the urological services could not treat patient for a period of time. In some countries, the situation was more dramatic. According to Maccagnano *et al.*, during March 2020 in the region of Lombardy an important number of urologists were reassigned to COVID-19 wards while four (out of 11) urological centers had no operative blocks dedicated to urology (6). The authors also noticed a significant reduction of the number of radical cystectomies and TURBT compared to March 2019. This led to an increased number of

patients evaluated for hematuria and hemostatic TURBT in academic centers northern Italy.

Bladder cancer patients in United Kingdom have been also affected by the COVID-19 impact on healthcare system. In a survey by Spencer-Bowdage *et al.*, between April and July 2020 in 37.5% of patients TURBT was postponed while in 18.8% of cases it was canceled (7). Regarding the cystoscopic surveillance, the authors noted that in 41.2% of cases the procedure was postponed while in 11.3% cancelled. In a survey by Teoh *et al.* on average, 28% of outpatient clinics, 30% of outpatient investigations and procedures, and 31% of urological surgeries had a delay of > 8 weeks (8). In another survey that included 2494 urologists from 76 countries, Gravas *et al.* revealed significant restrictions in outpatient examination and non-emergency surgery, the restriction was worldwide but lower in East/Southeast Asia, in addition elective operations that may require transfusion were performed only if patients were at high risk of disease progression, according to 47.3% of respondents (9).

Patient's fear is another important factor that is leading to delays in the diagnostic algorithm and a subsequent progression of undetected bladder cancers. Considering that according to Wong *et al*., BC occurs mainly in the elderly population with an average age at the time of diagnosis of 73 years, in our group mean age was of 69.12 years, many of them are worried about contracting COVID-19 and fear of its presence in healthcare facilities (10). To this is added the fact that, according to CDC COVID-19 Response Team, up to 80% of deaths due to COVID-19 occurred among adults aged ≥ 60 years (11). In Spencer-Bowdage's survey, a total of 76% of patients expressed some concern about attending hospital for their treatment and follow-up appointments (7). Macroscopic hematuria is a symptom encountered in 10% of patients with bladder cancer according to Sutton et al. and according to Gonzalez *et al*. only 1.2% of patients with microscopic hematuria have bladder cancer (12, 13). Therefore, such symptoms can be minimized and consequently overlooked by patients and doctors, in addition to the fact that, according to Wee *et al.*, most centers have reduced their outpatient capacity and are doing a triage to only select patients that are in most need of a cystoscopy (14). This could explain the higher incidence of anemia in our patients from group B. Although, in another Romanian urological center during spring 2020, hematuria was recorded only in 3.92% of hospitalized patients (15).

Although according to Wee *et al*., in order to accommodate the influx of COVID-19 positive patients, admissions to the urology, oncology or radiotherapy departments are being avoided, according to Méjean *et al.*, even in low-risk cases, urological procedures like TURBT cannot be postponed for more than 3 months (14, 16). More than this, according to Rexer *et al.*, other bladder cancer specific procedures like Bacillus Calmette–Guerin (BCG) instillations, cannot be reduced despite the COVID-19 crisis (17).

The hospitalization period was significantly shorter in group B because we wanted to avoid keep social distancing and avoid unnecessary contact with other patients and hospital staff. Also, the time from admission to surgery was shorter in group B mainly because more patients were referred as urological emergencies (e.g.: severe hematuria, anemia due to hematuria or urinary retention through blood clots)

because at least when "state of emergency" was declared in Romania, elective surgery have been forbidden and outpatient consultations were suspended (18).

CONCLUSIONS

The current COVID-19 pandemic has major impact in urological and especially bladder cancer patients worldwide. By trying to avoid hospitalization many patients are admitted with a poorer biological status. In addition, many bladder cancer cases will go undetected and untreated and once the pandemic has passed, we will face an excess in cancer-related mortality. It is the moral obligation of clinicians to sound the alarm in mass media about the risks to those who do not show up at the first signs of the disease.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

COMBINED LIFESTYLE FACTORS, ALL-CAUSE MORTALITY AND CARDIOVASCULAR DISEASE

The study performed a systematic review and meta-analysis to quantify the relationship of combined lifestyle factors (e.g., cigarette smoking, alcohol consumption, physical activity, diet and overweight/obesity) with the risk of all-cause mortality, cardiovascular mortality and incident cardiovascular disease (CVD). Methods PubMed and EMBASE were searched from inception to April 2019. Cohort studies investigating the association between the combination of at least three lifestyle factors and all-cause mortality, cardiovascular mortality or incidence of CVD were filtered by consensus among reviewers. Pairs of reviewers independently extracted data and evaluated study quality. Random-effects models were used to pool HRs. Heterogeneity and publication bias were tested. In total, 142 studies were included. Compared with the participants with the least-healthy lifestyles, those with the healthiest lifestyles had lower risks of all-cause mortality (HR=0.45, 95% CI 0.41 to 0.48, 74 studies with 2,584,766 participants), cardiovascular mortality (HR=0.42, 95% CI 0.37 to 0.46, 41 studies with 1,743,530 participants), incident CVD (HR=0.38, 95% CI 0.29 to 0.51, 22 studies with 754,894 participants) and multiple subtypes of CVDs (HRs ranging from 0.29 to 0.45). The associations were largely significant and consistent among individuals from different continents, racial groups and socioeconomic backgrounds. Authors concluded that given the great health benefits, comprehensively tackling multiple lifestyle risk factors should be the cornerstone for reducing the global disease burden. (Zhang Y, Pan X, Chen J, *et al.* Combined lifestyle factors, all-cause mortality and cardiovascular disease: a systematic review and meta-analysis of prospective cohort studies. *J Epidemiol Community Health* 2021; 75: 92-99).

VACUUM ASSISTED STABILIZATION OF FLAIL CHEST – A COMPARATIVE CASE CONTROL PILOT STUDY

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VACUUM ASSISTED STABILIZATION OF FLAIL CHEST-A COMPARATIVE CASE CONTROL PILOT STUDY (Abstract): Flail chest is associated with high rates of morbidity and mortality and management of such condition is a multidisciplinary task that should be based on pain control, judicious fluid administration, adequate pulmonary hygiene and management of the paradoxical movements of the thoracic wall. Many methods of treating rib fractures and flail chest have been developed over the years. Our aim was to evaluate a novel method of flail chest stabilization using negative vacuum pressure and compare it to a traditional method. **Material and methods:** 10 patients with flail chest from trauma admitted in Thoracic Surgery Clinic from Clinical County Emergency Hospital of Craiova and County Emergency Hospital of Targoviste were submitted to stabilization using Vacuum Assisted Closure kit. This series of patients were compared with 10 patients in whom a traditional method of stabilization was used, selected from the database of patients from the Thoracic Surgery Clinic of Clinical Emergency Hospital of Craiova, used as control group. **Results:** The 2 groups were comparable as no statistically significant differences were found regarding the gender, mean age, type of trauma, associated trauma, preexistent conditions, number of rib fractures, presence of pulmonary contusion, thoracic deformity. The parameters investigated: number of days with mechanical ventilation, number of days spent in ICU, total hospital stay, mortality didn't reach statistically significant differences. The only significant results found were for the pain scores recorded at 2 weeks and 1 month after discharge, the VAC stabilization being significantly better than traditional method. **Conclusions:** A larger study is required to establish if the advantages of the VAC assisted stabilization of the flail chest are superior to traditional treatment. **Keywords:** VACCUM ASSISTED, FLAIL CHEST.

Flail chest is associated with high rates of morbidity and mortality and management of such condition is a multidisciplinary task that should be based on pain control, judicious fluid administration, adequate pulmonary hygiene and management of the paradoxical movements of the thoracic wall. Also, when dealing with thoracic trauma it is adamant to conduct an accurate evaluation of the entire patient and classify the magnitude and severity of all the sustained injuries (1, 2).

Depending on the localization of the multiple broken ribs, flail chest can be (figs. 1 and 2) (I) anterior, mainly associated with sternum pathology, with fracture foci concerning only the bone component or the bilateral costochondral joints; (II) lateral or anterolateral, with fracture foci regarding both the bone and cartilage matrix of the ribs; (III) posterior or posterolateral (3).

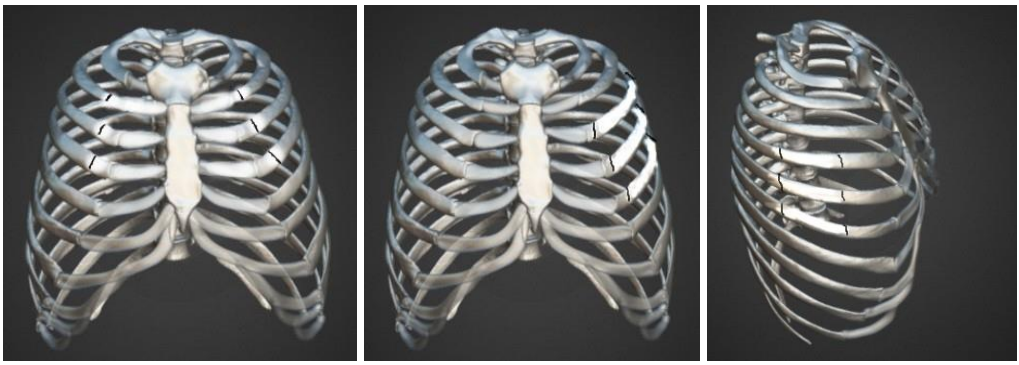

Fig. 1. Antero-lateral, lateral and anterior flail chest

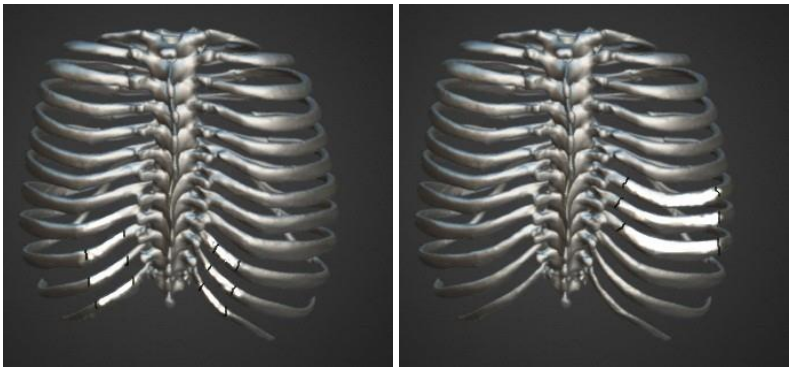

Fig. 2. Posterior flail chest and Postero-lateral flail chest

Flail chest is a common adverse effect of rib fractures, cardiopulmonary resuscitation, sternotomy and chest wall interventions (4). It prolongs hospitalization, pulmonary recovery and rehabilitation. To treat this type of pathology, many surgical techniques based on internal fixation have been described over the years.

From the methods Ambroise Pare described in the 16th century to modern bioresorbable plating systems, many methods of treating rib fractures and flail chest have been developed (3).

The Vacuum Assisted Closure®(14) device had proved significant improvement in the treatment of complications such as mediastinitis and thoracic wall instability in patients that have undergone thoracotomy (4).

VAC assisted therapy may also be used in the stabilization of the flail chest Topical negative pressure would have a stabilizing

effect on the thoracic wall. Expected advantages are stabilizing the paradoxical respiratory movements without surgical intervention, reducing associated complications of flail chest, spending shorter periods in the intensive care unit and reducing financial costs.

There are only 3 case reports that describe their experience using VAC therapy in managing flail chest: one describes thoracic trauma after a motor vehicle accident (5), one describes chest wall injuries sustained from cardiopulmonary resuscitation (6) and the later describes flail chest after surgical resection (7).

Our objective was to investigate if VAC therapy stabilization of flail chest brings better outcome compared to the traditional conservative medical treatment.

MATERIAL AND METHODS

We performed a comparative study of twenty cases, with severe trauma inducing flail chest with stern-costal fractures, in which ten cases received Vacuum Assisted Closure therapy in order to stabilize the paradoxical movements of the thoracic wall, whilst the other ten received standard conservative treatment.

Eligibility criteria

The study fulfilled the following eligibility criteria:

Target population: patients over 14 years who had suffered traumatic fractures of the ribs, with flail chest and paradoxical respiratory movements.

Comparator: Vacuum Assisted Closure therapy used to stabilize flail chest and conservative treatment.

Outcome: patient number of days spent on mechanical ventilation, number of days spent in the intensive care unit, number of days spent in the hospital, presence of pneumonia, rate of mortality and social reintegration.

10 patients with flail chest admitted the Emergency Clinical County Hospital in Craiova and the Emergency County Hospital in Targoviste were included in the series treated with vacuum assisted immobilization.

10 patients, as control, were selected from the database of the Thoracic surgery clinic from the Emergency Clinical County Hospital in Craiova treated by conservative measures.

The method used for VAC assisted immobilization consisted in an open-cell foam, covered the pieces with 3 metal bars, secured them with plastic bands and then covered the entire area with a transparent adhesive membrane, tightly secured to the skin and connected to the vacuum machine (fig. 3). As the device places suction, the system takes the shape of the body, and the space underneath the adhesive membrane becomes inflexible, stabilizing the chest wall and diminishing the paradoxical respiratory movements (5).

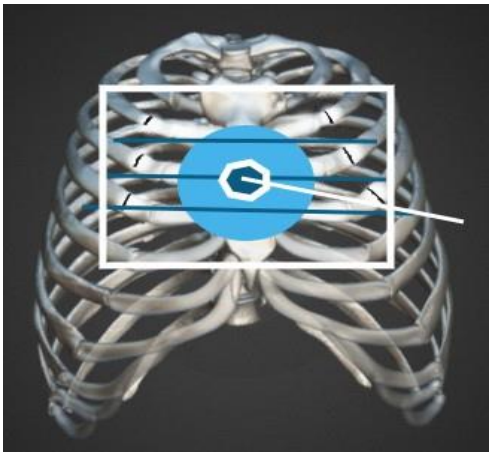

Fig. 3. VAC system on anterior flail chest

All of the patients included in the VAC series were treated with negative pressure for 14 days and were not discharged while wearing this system. The VAC suction pressure that we used was 200 mmHg.

Conservative treatment of flail chest consisted in immobilization of the flail segment by strapping the chest with adhesive tape or internal pneumatic stabilization for the more severe cases. Pain management, oxygen therapy and judicious use of fluid are also part of the medical treatment provided. The following data and parameters were recorded: patient age, sex, type of trauma that produced the lesions, number of fractured ribs, presence of pulmonary contusion, presence of thoracic deformity, number of days spent on mechanical ventilation, number of days spent in the intensive care unit, number of days spent in the hospital, presence of pneumonia, rate of mortality, associated trauma, preexistent patient pathology and social reintegration.

After discharge, patients were scheduled for two follow up visits, the first one was 2 weeks after the discharge and the second one after a month.

The patients were asked to estimate their current level of pain using a Wong Baker pain scale (8). The type of stabilization of the flail chest was decided in a multidisciplinary team consisting of specialists in thoracic surgery, intensive care and radiology.

We used the Mann-Whitney nonparametric test to compare the numerical variables recorded for the patients in our study. In order to compare categorical variables, we used the Chi square test. Level of significance was set for $p<0.05$.

RESULTS

The characteristics and data recorded for the patients submitted to VAC assisted stabilization of flail chest are presented in first table.

The data recorded for the patients who received a conservative and traditional method of flail chest stabilization are presented in table II.

The data regarding the patients, diagnosis, number of days spent in the hospital and in intensive care, mortality rate and treatment are presented in table III.

TABLE I

Data recorded for the patients submitted to VAC

Patient	1	2	3	4	5	6	7	8	9	10
Age	85	58	68	16	18	54	61	73	84	69
Type of trauma	Animal aggression	Fall	Traffic accident	Fall	Traffic accident	Traffic accident	Animal aggression	Fall	Fall	Fall
Sex	F	M	M	F	F	M	M	F	M	F
Nr. of fractured ribs	1	8	7	12	6	6	7	3	6	11
Pulmonary contusion	NO	YES	YES	YES	YES	YES	NO	NO	YES	YES
Thoracic deformity	NO	YES	NO	NO	YES	NO	NO	NO	NO	NO

Patient	1	2	3	4	5	6	7	8	9	10
Days on mechanical ventilation	0	5	0	7	5	0	0	0	0	0
Days in ICU	0	5	3	8	7	2	0	0	0	2
Days in hospital	14	5	20	60	18	15	15	15	15	16
Pneumonia	NO	YES	NO	YES	YES	NO	NO	NO	NO	NO
Death	-	YES	-	-	-	-	-	-	-	-
Associated trauma	0	head, thorax, abdo-men pelvis	abdo-minal, limbs	head	head, spinal cord, thorax, limbs	thorax	thorax	0	thorax	thorax
Preexistent conditions	0	0	Diabetes mellitus type II	Chronic depres-sion	grade II obesity	0	history of TBC	medi-astinal goiter, osteo-porosis, heart failure	heart failure	chronic viral hepatitis with C virus
Pain score 2 weeks	3	No data	6	2	4	3	2	6	5	5
Pain score 4 weeks	2	No data	3	0	2	1	1	3	3	2

TABLE II

Data concerning patients who received a conservative and traditional method of flail chest stabilization

Patient	1	2	3	4	5	6	7	8	9	10
Age	47	73	19	60	64	82	61	51	72	74
Type of trauma	Traffic accident	Fall	Fall	Traffic accident	Traffic accident	Fall	Traffic accident	Fall	Fall	Fall
Sex	M	M	M	M	M	F	M	M	F	M
Nr. of fractured ribs	5	4	8	5	2	3	4	4	5	9
Pulmonary contusion	YES	YES	YES	NO	NO	NO	YES	NO	NO	YES
Thoracic deformity	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES
Days on mechanical ventilation	0	0	7	0	0	0	5	0	0	0

Patient	1	2	3	4	5	6	7	8	9	10
Days in ICU	0	0	7	7	7	2	10	0	0	4
Days in hospital	7	4	7	16	17	10	25	5	5	4
Pneumonia	NO	NO	YES	YES	YES	NO	NO	NO	NO	NO
Death	-	-	YES	-	-	-	-	-	-	YES
Associated trauma	thorax	thorax	head, thorax	thorax	thorax, abdomen, pelvis	thorax	thorax, abdomen, spinal cord	0	thorax	head, thorax, spinal cord
Preexistent conditions	0	0	0	0	high blood pressure, atrial fibrillation, minor thalasse-mia	high blood pressure, history of stroke	0	0	0	atrial fibrilla-tion, high blood pressure, heart failure
Pain score 2 weeks	6	7	No data	5	6	8	4	7	6	No data
Pain score 4 weeks	4	5	No data	3	3	6	2	3	5	No data

TABLE III

Data regarding the patients, diagnosis, number of days spent in the hospital, mortality rate and treatment

Number of patients	VAC treatment for flail chest N= 10 (100%)	Conservative treatment for flail chest N= 10 (100%)	Mann-Whitney test/ chi square
Gender (M/F)	5(50%) 5 (50%)	8 (80%) 2 (20%)	NS NS
Mean age(years) +/- std dev	58.6 +/- dev std	60.3 +/- dev std	NS
Type of trauma	Falls 5 (50%) Traffic accidents 3(30%) Animal aggressions 2 (20%)	Falls 5 (50%) Traffic accidents 4(40%) Animal aggressions 0(0%)	NS NS NS
Associated trauma	8 patients presented associated trauma	9 patients presented associat-ed trauma	NS
Preexistent conditions	7 patients had preexistent conditions	3 patients had preexistent conditions	NS

Number of rib fractures (mean) +/- std dev)	6.70 ± 3.27	4.90 ± 2.13	0.138
Presence of pulmonary contusion (number of patients)	7	5	0.3613
Thoracic deformity (number of patients)	2	1	0.5312
Mechanical Ventilation (days, mean +/- std dev)	1.70 ± 2.79	1.20 ± 2.57	0.690
Intensive care unit stay(mean)	2.70 ± 3.02	3.70 ± 3.80	0.695
Hospitalization (mean +/- std dev)	19.30 ± 14.82	10.00 ± 7.07	0.080
Pneumonia (number of patients)	3	3	1.0000
Mortality	0.20 ± 0.42	0.10 ± 0.32	NS
Pain score 2 weeks (mean +/- std dev)	4.00 ± 1.58	6.13 ± 1.25	**0.014**
Pain score 4 weeks (mean +/- std dev)	1.89 ± 1.05	3.88 ± 1.36	**0.008**

There were no statistically significant differences between the 2 series of patients regarding the gender, mean age, type of trauma, associated trauma, preexistent conditions, number of rib fractures, presence of pulmonary contusion, thoracic deformity, number of days with mechanical ventilation, number of days spent in ICU, total hospital stay, mortality.

The only significant results found were for the pain scores recorded at 2 weeks and 1 month after discharge.

DISCUSSION

In our two groups the patients treated with VAC therapy spend less time on mechanical ventilation and in ICU, but more time in the hospital.

Prolonged hospitalization was determined by the multiple associated traumas that also required medical attention and to the fact that we did not discharge patients while receiving active VAC therapy. In the future we may obtain less hospitalization time if we discharge patients with the VAC system in place and instruct them to come for weekly medical visits.

Social reintegration is challenging after being treated for flail chest. Although both of our series presented with mild pain the after 1 month of being discharged (according to the Wong Baker pain score classification), for the VAC therapy group we obtained statistically significant results for the pain scores recorded at 2 weeks and 1 month after discharge.

In a country such as Romania where recovery programs and access to kinesitherapy workouts are scarce and strictly available in urban areas, using this stabilization method for flail chest may provide better social outcome.

Advantages of using VAC therapy in flail chest treatment include:

- The VAC machine is small, does not require a lot of space, can be easily stored and reused
- The patient can mobilize with the ma-

chine thus avoiding complications linked to excessive bed rest

- The system can be fast applied and can be used during transportation
- In order to install the VAC therapy system for flail chest, no special training is required
- Applying and taking off the system is easy and painless
- No anesthesia is required, avoiding the risks of general anesthesia and the unpleasantness of local anesthesia
- ICU stay is not required (unless other associated pathologies require ICU stay)
- If the patient requires immediate surgery, stabilizing the flail chest with negative pressure does not contradict other surgeries
- The foam dressings, the contact layer and VAC drape are the only single use components of the system. The VAC therapy unit, the Sensa TRACÔ Pad and the metal bars can be sterilized and reused, cutting further costs
- There is no need of different sizes for every patient
- Using the system does not require any type of incision, thus there is no risk of developing an infection linked to osteosynthesis and no aesthetic problems or complications linked to scars.
- The intercostals muscles are not affected because there is no need for a thoracotomy
- The VAC system is easily procured and is available in every country

Disadvantages of using VAC therapy in flail chest treatment include:

- Increased hospitalization
- While turned on the machine produces slight noise
- When applying the device, the patient might feel a slight pulling sensation and after some time they might experience pain or itching
- If one suffers a bilateral flail chest(for example anterior and lateral) 2 VAC systems are required of each side of the thorax
- The patient has to wear the system a considerable amount of time
- Showering while wearing the system may be difficult
- While the patient practices their daily exercises one must carry the therapy unit with them and be careful not to pull the Sensa TRACÔ Pad

After applying vacuum therapy for flail chest a few questions have come to mind. When is the best moment to apply this type of external fixation? As with other osteosynthesis methods, the sooner the fracture is treated, the better; applying the negative pressure device to the flail chest should be performed upon patient admission.

How much should we keep the device in place? The healing process of a fracture includes 3 phases: the inflammatory phase, the repairing phase and the bone remodeling phase. The first phase starts immediately after the lesion is produced and ends in approximately 1 week. In this phase the body immobilizes the fracture. The second phase starts within the first days after the fracture has occurred and lasts between 2-3 weeks. In this phase the human organism produces tissue and cartilage surrounding the fracture site, stabilizing it. The last phase is the bone remodeling phase and consists in removing woven bone and replacing it with lamellar bone and the resorption of excess callus. This process can last up to several months (9, 10, 11). The median healing time of a rib fracture is 2

months. We presume that it is safe to keep the negative pressure device on the flail chest up to 3 weeks in order for the repairing phase to end and that the flail segment no longer presents paradoxical respiratory movements.

If the patient is mechanically ventilated, should there be a relation between the internal continuous positive airway pressure and the external pressure executed by the vacuum device? The pressure that VAC therapy exercises on the chest should be small in order not to interfere with the pressure exercised by mechanical ventilation and avoid creating a situation similar to the physiopathology encountered in chronic obstructive pulmonary disease.

A lot of variables have to be taken into consideration and in order to assemble a clinical protocol regarding this matter; a mechanical thorax model should be the base of an experiment regarding how to balance the pressure in the vacuum therapy system and the mechanical ventilator. Applying a negative pressure device to a flail thoracic segment may provide better results to non-intubated patients. More studies are required in order to provide a more accurate answer on this subject.

Can the patient go home with the system in place and return for weekly check-ups? If the patient does not present any other life-threatening lesions and the respiratory constants are in normal range, we presume the patient can be discharged after one week and return for weekly controls.

In order to avoid skin lesions the pressure in the VAC machine should be adjusted in order to either provide small periods of low pressure or small periods without any pressure exercised on the chest.

Further study is necessary in order to elaborate a standard therapeutic algorithm of VAC external fixation in flail chest.

CONCLUSIONS

External fixation of flail chest using Vacuum Assisted Closure seems to improve respiratory outcome, stabilize the chest wall and reduce paradoxical respiratory movements.

The system is not hard to apply, can be quick and easily procured, and it can be used during transportation. In order to apply this method of fixation, no thoracotomy and no anesthesia are required. It is cost efficient and does not demand special training.

This method of stabilization may be used when other stabilization methods are either not available or not recommended due to the patient's serious condition.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

HEPATIC NEUROENDOCRINE TUMOUR

Primary hepatic neuroendocrine tumor (carcinoid) is an extremely rare type of neuroendocrine tumour (between 60-90 cases are reported in the literature). Metastatic neuroendocrine tumor from the gastrointestinal tract to the liver is far more common. Some patients present with carcinoid syndrome. Elevated 5-HIAA and/or gastrin levels have been reported. Histologically, the tumor has features of classic neuroendocrine tumor (trabecular and pseudo glandular pattern) and dense core granules demonstrated by electron microscopy or by immunohistochemistry (positive staining by chromogranin antibody). There is speculation that the tumor might arise from ectopic pancreatic tissue in the liver or through differentiation from biliary epithelium. There are no specific imaging features, and cases are diagnosed retrospectively. One case report described an imaging appearance on ultrasound and contrast-enhanced CT similar to focal nodular hyperplasia. Somatostatin-receptor nuclear medicine studies (In-111 pentetreotide - Octreoscan) is positive. Given the rarity of the finding, an investigation for an extrahepatic primary carcinoid tumor is necessary. No standard treatment exists. Surgical resection has been performed. (Morgan M, Jones J. *Hepatic neuroendocrine tumour*. https://radiopaedia.org/articles/hepatic-neuroendocrine-tumor?lang=gb).

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AORTIC ENDOTHELIAL CELL PROLIFERATION INDUCED BY EXOSOMES OBTAINED FROM iPSC UNDER THE ACTION OF VARIOUS MODULATORS

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AORTIC ENDOTHELIAL CELL PROLIFERATION INDUCED BY EXOSOMES OBTAINED FROM iPSC UNDER THE ACTION OF VARIOUS MODULATORS (Abstract) **Background**: Extracellular vesicles (also known as EVs), secreted naturally by all cells, are known to act as mediators for intercellular communication. **Aim**: The goal of our study was represented by the quantification of rat aortic endothelial cell proliferation in culture in the presence of pluripotent stem cells (iPSC), chemically-induced from rat aortic smooth muscle cells (in co-culture) or in the presence of exosomes obtained, as a supernatant, from the culture medium of the latter cells, in the presence of 0.5 µM trichostatin A, 1 µM 6-mercaptopurine, 2 µM triamterene and 2 µM metformin. **Material and methods**: We used flow cytometry to quantify cell proliferation, using a membrane-permeable, tetramethylrodamine-based cellular tracking agent, 48 hours after inclusion in the culture medium. **Results**: The best proliferation for aortic endothelial cells, in the presence of exosomes obtained from the culture supernatant of pluripotent stem cells, chemically-induced from vascular smooth muscle cells, was obtained when grew these latest cell types on 3D biosupports and treated with 2 µM metformin. **Conclusions**: It is suggested that epigenetic changes, genome stabilization, and, especially, reprogramming of energy metabolism, result in the release of exosomes from induced pluripotent stem cells that have the ability to significantly amplify aortic endothelial cell proliferation in culture. **Keywords:** iPSC, GENOME STABILIZATION, ENERGY METABOLISM REPROGRAMMING, AORTIC ENDOTHELIAL CELL, AORTIC SMOOTH MUSCLE CELL.

Regenerative medicine has promised, since statutory, to meet two of the most stringent needs of modern organ transplantation, i.e., transplant that does not induce immunosuppression and, at the same time, ensuring an inexhaustible organ source. In absolute ideal way, organs obtained by bioengineering would be manufactured using patient own biomaterials, so both cells and support media, in which cells can be incorporated and allowed for a time to mature to regenerate, finally, the organ in question. While many research groups focus on the feasibility of this type of approach, very few truly target the immunogenicity of supports, which are lately developed for organs bioengineering (1).

Reprogramming of human cells, trans-

formed and functionally and morphologically differentiated into induced pluripotent stem cells (iPSC), already has a multitude of applications in basic biology, but also in drug development and, last but not least, in transplantation. The derivation of human iPS cells initially required a number of vectors to integrate into the genome, but it has been shown that these approaches might create mutations and then limit the usefulness of the obtained cells, in both research and clinical applications. More recently, these techniques have been able to be replaced by ones that have almost completely overcome these drawbacks (2).

The administration of vascular cells derived from pluripotent stem cells, fully immunologically compatible, obtained by vascular bioengineering, in the replacement therapy of autologous cells, associates a huge clinical potential. On the other hand, the industrial-scale production of vascular lines specific to each patient, for inclusion in regenerative cell therapies, is totally dependent on cell multiplication and differentiation under extraordinarily well-defined and reproducible conditions (3).

Multipotent mesenchymal stem cells are also potential candidates for innovative cell-based regenerative therapies. However, the mechanisms underlying the differentiation of this cell type and other important functions for tissue engineering remain far from known. Recently published data suggested a key role for intercellular contacts in mesenchymal stem cell differentiation processes (4).

The goal of our study was represented by the quantification of rat aortic endothelial cell proliferation in culture, in the presence of pluripotent stem cells, induced from rat aortic smooth muscle cells (in co-culture) or in the presence of exosomes obtained, as a supernatant, from the culture medium of the latter cells, in the presence of 0.5 μM trichostatin A, 1 μM 6-mercaptopurine, 2 μM triamterene and 2 μM metformin.

MATERIAL AND METHODS

The experiments began by multiplying young rat aortic smooth muscle cells (meaning a small number of passages in culture), grown in a complete DMEM environment, which stimulates proliferation, being improved in terms of composition with 2 mM L-glutamine, as well as with a series of classic antibiotics, represented by 100 U/ml penicillin and, in addition, 100 μg/ml streptomycin, and a concentration of 10% decomplemented FBS.

To obtain pluripotent stem cells induced from rat aortic smooth muscle line cells, we used a prior described and adapted chemical technique (5), completed with 5-bromo-2'-deoxyuridine, AM580 and "2i" inhibitors (6, 7).

In order to verify the importance of biologically active matrices in the development and functioning of induced pluripotent stem cells, the cells were multiplied, after stabilization, using as attachment factors, on the surface of flasks, collagen type IV, fibronectin, laminin and even a 3D biosupport, i.e., Alvetex type (Reinnervate gift). All of these were used in accordance with the manufacturer's instructions.

On the other hand, the rat aortic endothelial line cells were grown and multiplied in approximately the same way as vascular smooth muscle cells, except that we used DMEM/Ham F12 medium in a ratio of 1:1 and a factor for cell attachment to the surface of flasks (collagen type I).

For exosome separation, the culture medium of pluripotent stem cells induced from rat aortic smooth muscle cells was centrifuged up to 100,000 xg (8).

The active substances we used for the var-

ious experiments and protocols were: 0.5 µM trichostatin A, 1 µM 6-mercaptopurine, 2 µM triamterene and 2 µM metformin.

Methodologically, we used flow cytometry to quantify cell proliferation. Proliferation of pluripotent stem cells induced from aortic smooth muscle cells and endothelial cells was evidenced by a membrane-permeable, tetramethylrodamine-based cellular tracking agent, 48 hours after inclusion in the culture medium.

All the experimental data we obtained during the research were statistically extensively processed by the One-Way *ANOVA* test, completed only in conditions of absolute necessity with the Student-Newman-Keuls test.

RESULTS

Proliferation of pluripotent stem cells induced from aortic smooth muscle cells was evidenced by a cell-tracking agent, permeable from the outside to the inside, based on tetramethylrodamine, after 48 hours from inclusion in the culture medium, and flow cytometry. This cell tracking agent is very well retained intracellularly and detectable (due to the intensity of the fluorescence signal) for up to 72 hours. It has an extremely low cytotoxicity and does not affect cell viability or proliferation. The maximum excitation / emission is 541/565 nm (orange).

In a series of experiments, a number of aortic endothelial cells were treated with 0.01% exosomes obtained from the supernatant of pluripotent stem cells induced from aortic smooth muscle cells, which had previously been treated with 0.5 µM trichostatin A, an inhibitor relatively nonspecific of HDAC (histone deacetylase) type 1, 2, 3, 6, 7 and 9.

The best proliferation for aortic endothelial cells, in the presence of exosomes obtained from the culture supernatant of pluripotent stem cells derived from vascular smooth muscle cells and treated with 0.5 µM trichostatin A was obtained when grew these latest cell types on 3D biosupports (fig. 1).

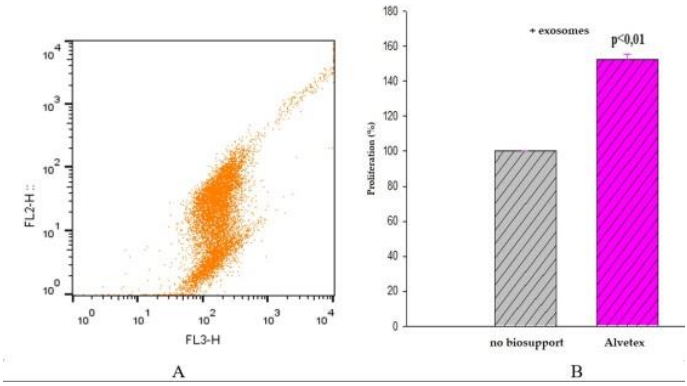

Fig. 1. Aortic endothelial cell proliferation in monoculture and in the presence of exosomes at a concentration of 0.01%, obtained from pluripotent stem cells induced from aortic smooth muscle cells, multiplied on 3D support and treated with 0.5 µM trichostatin A, evidenced by flow cytometry (representative image for this type of experiment) (A). The analysis of the flow cytometry results (n = 5) shows that the proliferation was 152.14 ± 2.79% as compared to that obtained without biosupport and considered to be in this case 100% (B). p <0.01 as compared to the lack of specific biosupport.

In other experiments, a number of aortic endothelial cells were treated with 0.01% exosomes obtained from the supernatant of pluripotent stem cells induced from aortic smooth muscle cells, which had previously been treated with 1 µM 6-mercaptopurine, a stimulant/major inducer of HDAC type 1 (fig. 2).

Furthermore, the derived pluripotent stem cells were treated with 2 µM triamterene, a pharmacologically active substance, considered a stabilizer of the genome, reducing its instability and mutations (fig. 3), and 2 µM metformin, considered a reprogrammer of energy metabolism (fig. 4).

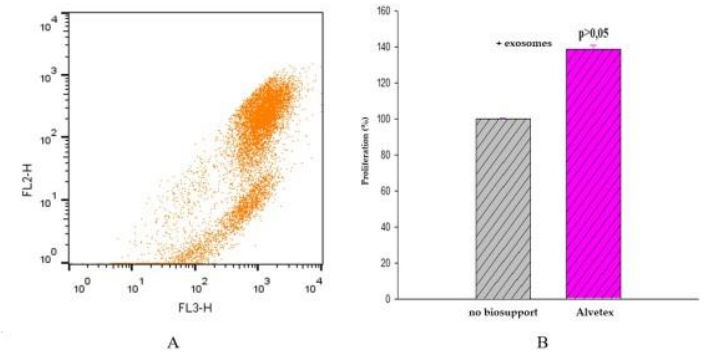

Fig. 2. Proliferation of aortic endothelial cells in monoculture and in the presence of exosomes at a concentration of 0.01%, obtained from pluripotent stem cells, multiplied on 3D support and treated with 1 µM 6-mercaptopurine (A). The analysis of the flow cytometry results (n = 5) shows that the proliferation was 138.29 ± 2.81% as compared to that obtained without biosupport (B). p <0.01 as compared to the lack of specific biosupport. p> 0.05 as compared to the absence of 6-mercaptopurine.

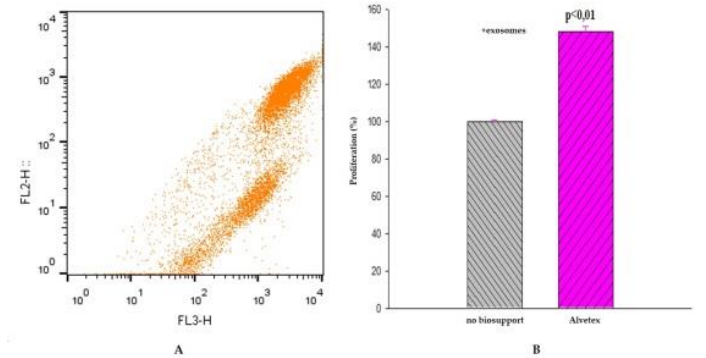

Fig. 3. Aortic endothelial cell proliferation in monoculture and in the presence of exosomes at a concentration of 0.01%, obtained from pluripotent stem cells, grown on 3D support and treated with 2 µM triamterem (A). The analysis of the flow cytometry results (n = 5) shows that the proliferation was 148.72 ± 2.99% as compared to that obtained without biosupport (B). p <0.01 as compared to the lack of specific biosupport. p> 0.05 as compared to the lack of triamterene.

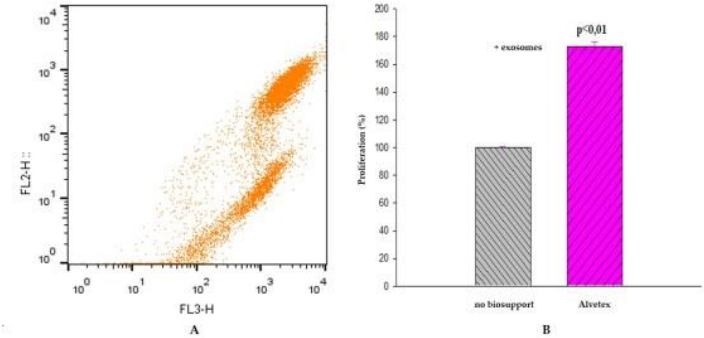

Fig. 4. Proliferation of aortic endothelial cells in monoculture and in the presence of exosomes at a concentration of 0.01%, obtained from pluripotent stem cells, grown on 3D support and treated with 2 μM metformin (A). The analysis of the flow cytometry results (n = 5) shows that the proliferation was 172.54 ± 4.22% as compared to that obtained without bio support (B). $p < 0.01$ as compared to the lack of specific biosupport. $p > 0.05$ as compared to metformin absence.

DISCUSSION

Given their ability to grow and differentiate, human-induced pluripotent stem cells represent a well-characterized cellular source that can almost unlimited, and can be used for the processes of mass generation of vascular cells in everything is patient-specific, even without any ethical concerns. Vascular line cells, derived from human pluripotent stem cells, are perfect for vascular disease modeling studies, given that these cells retain the disease-causing mutation unaltered, which could be crucial for the complete expression of the disease phenotype (3).

Acute limb ischemia is a condition in which tissue necrosis occurs as a result of arterial occlusion, which in many cases can lead to limb amputations. Both endothelial cells and vascular smooth muscle cells are absolutely necessary for the regeneration of peripheral arterial tissues in the post-ischemic stage. However, it is difficult to isolate and multiply the primary endothelial cells and, at the same time, the vascular smooth muscle cells, from the same patient, in order to initiate therapeutic angiogenesis. Induced pluripotent stem cells have been and are considered to be particularly useful stem cells, given the potential for multicellular differentiation. A series of studies have looked at the therapeutic efficacy of endothelial cells and smooth muscle cells, both derived from induced pluripotent stem cells, in the murine peripheral artery disease model. Simultaneous transplantation of these two derived cell types significantly improved blood flow and increased the rate of recovery of the affected limbs. This compares with single endothelial cell transplantation or vascular smooth muscle cells (9, 10).

Extracellular vesicles, secreted naturally by all cells, are known to act as mediators for intercellular communication. They can carry important loads to the recipient cells, such as nucleic acids, a series of proteins and lipids, which basically reflect the changes that occur in the cells of origin. Therefore, extracellular vesicles have been recognized as potential and important theranostic agents that could be used suc-

cessfully for diagnosis, treatment, and prognosis (11).

Extracellular vesicles are known to be important mediators of cell-cell communication. Thus, they are involved in many types of cellular, physiological and pathological processes, including immune regulation, but also cell proliferation and differentiation, in cell death, but also in angiogenesis. It is important to note that EVs can be used extensively as biomarkers of various pathophysiological conditions and/or as therapeutic agents, both as drug carriers or even as drugs themselves (12, 13).

Mesenchymal or stromal stem cells (also called MSCs) include progenitor cells of various origins (e.g., adipose tissue or umbilical cord - or Wharton jelly, respectively), which have a potential for multiple differentiation, and are currently the most studied stem cell population, even if we are talking about pre-clinical or clinical studies. Most of the effects of mesenchymal stem cells are due implicitly to their paracrine activity, which includes the massive release of extracellular vesicles. Extracellular vesicles are thought to have more health benefits in the process of tissue repair and regeneration as compared to their parental cells. There is a significant amount of research conducted both *in vitro* and *in vivo* on animal models, but also in clinical trials, in connection with a number of neurological diseases, cardiovascular diseases, but also liver, kidney and skin diseases. These studies can thus provide encouraging evidence for the pro-regenerative capacity of EVs derived from mesenchymal stem cells in various disease models, mediated by a multitude of mechanisms. Although the direct molecular mechanisms of action of these types of extracellular vesicles are still being investigated (14,15).

Within the modern and emerging concept of allogeneic benefit, regarding stem cell therapies, the use of extracellular vesicles obtained from allogeneic sources becomes the truly pragmatic choice, although a universal source of allogeneic cells still seems necessary. For example, the human placenta, basically a single temporary organ that can ensure the mutual coexistence of two allogeneic organisms, namely the mother and the fetus, could be a persuasive source of allogeneic stem cells, absolutely necessary for the development of therapeutic extracellular vesicles. The promotion of cell-free therapy offers outstanding prospective for the development of regenerative and/or reparative medicine (16).

Mesenchymal stem cells are being extensively researched for their potential application in tissue engineering and, at the same time, in regenerative medicine. Considering all the experimental data, it has recently been suggested that most of the beneficial effects of mesenchymal stem cells could undoubtedly be attributed to released extracellular vesicles and exosomes, respectively. Thus, the direct use/administration of mesenchymal stem cells does not appear to be necessary. Thus, *in vitro* and *in vivo* preclinical studies have suggested that exosomes derived from mesenchymal stem cells may exhibit a number of substantial therapeutic properties under many pathophysiological conditions, effectively restoring the functionality of a wide range of damaged or diseased tissues and organs. These beneficial effects of mesenchymal stem cell-derived exosomes can be demonstrated independently of the fluid or bodily anatomical origin of the mesenchymal stem cells and at the same time independent of cellular origin (e.g., bone marrow, or adipose tissue, or

umbilical cord, or urine, etc.). Furthermore, it is suggested that the effects of exosomes of this type could be greatly enhanced by changing the way donor mesenchymal stem cells are grown (e.g., multiplication under hypoxic conditions as compared to normoxic conditions, under 3D conditions as compared to culture in 2D formats), and/or by bio design (e.g., inclusion or association with specific loads) (17).

CONCLUSIONS

The best proliferation for aortic endothelial cells, in the presence of exosomes obtained from the culture supernatant of pluripotent stem cells induced from vascular smooth muscle cells, was obtained when we grew these last cell types on 3D biosupports.

The data obtained demonstrate first of all that the exosomes released into the environment by the induced pluripotent stem cells have a composition dependent on the intracellular pathways activated under various conditions.

Thus, it is suggested that generalized inhibition of histone deacetylases (HDAC), i.e. epigenetic changes, genome stabilization, and, especially reprogramming of energy metabolism, result in the release of exosomes from induced pluripotent stem cells that have the ability to significantly amplify aortic endothelial cell proliferation in culture.

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CONFLICT OF INTEREST AND FUNDING

Authors declare that there is no conflict of interest, and they received no funding for this study.

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NEWS

UTILIZATION AND OUTCOMES OF EXTRACORPOREAL CO_2 REMOVAL ($ECCO_2R$): SYSTEMATIC REVIEW AND META-ANALYSIS OF ARTERIO-VENOUS AND VENO-VENOUS $ECCO_2R$ APPROACHES

Extracorporeal carbon dioxide removal ($ECCO_2R$) provides respiratory support to patients suffering from hypercapnic respiratory failure by utilizing an extracorporeal shunt and gas exchange membrane to remove CO_2 from either the venous (VV-$ECCO_2R$) or arterial (AV-$ECCO_2R$) system before return into the venous site. AV-$ECCO_2R$ relies on the patients native cardiac function to generate pressures needed to deliver blood through the extracorporeal circuit. VV-$ECCO_2R$ utilizes a mechanical pump and can be used to treat with inadequate native cardiac function. Existing evidence was sought by comparing the subgroups of patients supported on VV and AV-$ECCO_2R$ devices. Twenty-five studies including 826 patients were reviewed. 60% of patients (497/826) were supported on VV-$ECCO_2R$. The most frequent indication were acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disease (COPD). ICU length of stay was significantly shorter in patients supported on VV-$ECCO_2R$ compared to AV-ECCO2R (15 vs 42 days, p=0,05). In-hospital mortality was not significantly different (27% vs 36%, p=0,26). Both VV and AV-$ECCO_2R$ provided clinically meaningful CO_2 removal with comparable mortality (Yu T, Tatum R, Saxena A, *et al.* Utilization and outcomes of extracorporeal CO 2 removal (ECCO 2 R): Systematic review and meta-analysis of arterio-venous and veno-venous ECCO 2 R approaches. *Artif Organs* 2021; 00: 1-12 / doi: 10.1111/aor.14130).

CLINICAL AND LABORATORY FEATURES OF ACUTE BACTERIAL MENINGITIS IN NORTH-EASTERN ROMANIA – PREDICTORS OF SEVERE PROGNOSIS

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CLINICAL AND LABORATORY FEATURES OF ACUTE BACTERIAL MENINGITIS IN NORTH-EASTERN ROMANIA - PREDICTORS OF SEVERE PROGNOSIS (Abstract): Bacterial meningitis is a medical emergency; therefore, it is necessary to study the risk factors associated with poor outcome. **Material and methods:** We conducted a retrospective observational study at "Sf. Parascheva" Infectious Diseases Clinical Hospital from Iasi, between January 2015 and January 2018. The study included 145 patients diagnosed with bacterial meningitis who were divided into two groups: group I - surviving patients and group II deceased patients. Appropriate statistical methods were applied to the collected data. **Results:** Risk factors associated with an unfavorable development were age over 50, altered mental status, triad fever-headache-neck stiffness, as well as systemic damage, low Glasgow score upon admission or late presentation to the hospital. Factors associated with favorable prognosis in meningitis were the presence of headache, vertigo, and vomiting. **Conclusions:** The obtained results outline the recent clinical-evolutionary peculiarities of bacterial meningitis in the North-Eastern region of Romania in a group of demographically heterogeneous patients. **Keywords:** BACTERIAL MENINGITIS, RISK FACTORS, PREDICTORS.

Bacterial meningitis remains a neurological emergency and current epidemiological changes require a periodic adaptation of the method of early therapeutic approach even in developed countries. From this point of view, it is necessary to know as well as possible the profile of this disease as patients require immediate evaluation and treatment. The incidence of bacterial meningitis is almost 5 cases/100,000 adults in developed countries and may be 10 times higher in less developed countries. The most common etiological agents are *Streptococcus pneumoniae* (*S. pneumoniae)* and *Neisseria meningitidis (N. meningitidis)* which are responsible for almost 80% of all cases (1, 2).

Meningitis is an inflammatory condition involving the meninges and is classically manifested by fever, altered mental status, headache and neck stiffness. The disease is diagnosed by examining the cerebrospinal

fluid (3, 4).

The incidence, morbidity and mortality rate of bacterial meningitis varies and depends on several factors, such as age, geographical region, etiological agent, and immune status (4, 5). The epidemiology of bacterial meningitis has undergone changes with the introduction of vaccines (4). In underdeveloped countries, due to low vaccination coverage, the disease continues to strike, affecting high number of children (5). Therefore, the purpose of this research was to study the profile of the disease as well as to determine the risk factors associated with an unfavorable evolution in the North-Eastern region of Romania, characterized by substantial changes in the patient's behavior as well as the approach of this disease by different medical services.

MATERIAL AND METHODS

Study Design and Settings

We conducted a retrospective observational study at "St. Parascheva" Infectious Diseases Clinical Hospital, Iasi. The study included patients diagnosed with bacterial meningitis between January 2015 and January 2018 and was carried out with the approval of the Ethics Committee of "Gr. T. Popa" University of Medicine and Pharmacy from Iasi (No.8322/2.05.2018). The data was anonymized before it was used. To ensure the quality of the study we used STROBE (6).

Study Population

We analyzed the medical records of patients diagnosed with bacterial meningitis. The study group included 145 patients, aged over 18, divided into two study groups: group I – 105 patients diagnosed with bacterial meningitis, survivors and group II – 40 patients diagnosed with bacterial meningitis, deceased. Patients for whom, following lumbar puncture, the diagnosis of bacterial meningitis was not confirmed on the basis of laboratory criteria (7), as well as patients aged under 18 and patients with viral meningitis were excluded.

Definition and Data Collection

Bacterial meningitis diagnosis was based on the patient's history, the presence of clinical symptoms (such as headache, neck stiffness, altered mental status, fever) and analysis of laboratory parameters in the cerebrospinal fluid (CSF) and blood (7). The data collected also included information about the therapeutic approach of patients.

Outcome Measures. Results were reported according to the two groups considered. Demographic, clinical, imaging and laboratory parameters were evaluated in both groups and statistical significance was subsequently established.

Statistical Analysis

Data were systematized and centralized in an SPSS 18.0 database and processed using the appropriate statistical functions. A 95% confidence interval was used in data presentation. Indicators like minimum, maximum, mean, standard deviation, standard error were used for descriptive statistical analysis. Qualitative significance tests, such as the chi^2 test were used for comparing the distributions of frequencies and statistic power was establish depending on p-value (<0.005). Odds Ratio (OR) were used to measure the association between the exposure and the outcome. The *t-Student* test / *ANOVA* was used for comparing the means of any two normally distributed variables. The diagnostic accuracy was determined using receiver operating characteristic curves (ROC) and calculating the area under the ROC curve (AUC).

RESULTS

Demographic and clinical data

Among demographic characteristics, the factors which have correlated with the mortality risk were age over 50 (p=0.002, OR=3.07, 95%CI: 1.643-8.343) and behavioral factors such as smoking (p=0.001). Immunosuppression has also been associated with high mortality risk (p=0.001, OR=7.07, 95%CI: 2.435-20.534) (tab. I).

Alteration of mental status was observed in 36 of the patients who ultimately died and in 50 of the patients with favorable evolution (90% *vs.* 47.6%) and it was correlated with mortality risk (p=0.001, OR=9.90, 95%CI: 3.290-29.792). The classic triad: fever – headache – neck stiffness was present in a significant number of patients (p=0.001) - 27 cases in deceased group and 14 cases in survivors group (tab. I).

Analyzing the AUC, we concluded that only age ≥ 50 years old, immunosuppression, Glasgow Coma Sale (GCS) and altered mental status could be considered good predictors of mortality (tab. II).

TABLE I.

Risk of lethality in patients with bacterial meningitis according to demographical data, signs and symptoms

	Total n=145 (%)	Deaths n=40(%)	Survivors n=105(%)	p-value	OR	95% C.I.
Demographic data						
>50 years old (n,%)	73 (50.3)	30 (75)	47 (44.8)	**0.001**	**3.70**	1.643-8.343
Male (n,%)	79 (54.5)	21 (52.5)	58 (55.2)	0.767	0.89	0.432-1.859
Rural (n,%)	87 (60.0)	20 (50.0)	67 (63.8)	0.129	**1.76**	0.844-3.683
Smoking (n,%)	25 (17.2)	0 (0.0)	25 (23.9)	**0.001**	**-**	**-**
Alcoholism (n,%)	43 (29.7)	10 (25.0)	33 (31,4)	0.449	0.72	0.318-1.661
Immunosuppression (n,%)	18 (12.4)	12 (30.0)	6 (5.7)	**0.001**	**7.07**	2.435-20.534
Clinical Findings						
Altered mental status (n,%)	86 (59.3)	36 (90)	50 (47.6)	**0.001**	**9.90**	3.290-29.792
Fever (n,%)	113 (77.9)	28 (70.0)	85 (81.0)	0.164	0.55	0.241-1.279
Headache (n,%)	93 (64.1)	12 (30.0)	81 (77.1)	**0.001**	0.12	0.056-0.287
Vomiting (n,%)	40 (27.6)	3 (7.5)	37 (35.2)	**0.001**	0.14	0.043-0.516
Vertigo (n,%)	37 (25.5)	3 (7.5)	34 (32.4)	**0.002**	0.16	0.049-0.588
Neck stiffness (n,%)	90 (62.1)	22 (55.0)	68 (64.8)	0.279	0.66	0.317-1.395
Triad (n,%) (fever, headache, neck stiffness)	41 (28.3)	27(25.7)	14 (35.0)	**0.001**	**4.12**	1.675-10.158

TABLE II.
AUC for signs and symptoms of meningitis.

Sign	AUC	Standard error	C.I. 95%	
			-95% C.I.	+95% C.I.
Age ≥ 50 years old	**0.649**	0.050	0.551-0.747	
Male	0.488	0.054	0.383-0.594	
Rural	0.431	0.054	0326-0.536	
Smoking	0.375	0.047	0.282-0.468	
Alcohol	0.466	0.053	0.362-0.570	
Immunosuppression	**0.621**	0.054	0.370-0.583	
GCS	**0.769**	0.042	0.687-0.852	
Altered mental status	**0.710**	0.045	0.622-0.797	
Fever	0.446	0.055	0.339-0.554	
Headache	0.265	0.048	0.170-0.360	
Vomiting	0.360	0.048	0.266-0.453	
Vertigo	0.374	0.048	0.279-0.469	
Neck stiffness	0.453	0.054	0.347-0.559	

Imaging and Laboratory findings

Cranial computed-tomography scan (Cranial CT scan) was performed in 35 deceased patients and 66 survivors (87.5% *vs.*62.9%). Cranial CT scan identified abnormalities of the central nervous system in 25 patients with unfavorable outcome and in 29 survivors (62.5% *vs.* 27.6%, p=0.001) (tab. III).

In deceased patients, the mean values of some hematological and biochemical laboratory parameters showed significantly higher differences between deceased and survivors patients: white blood cell count (WBC), neutrophils cells (PMN), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), urea, creatinine, glycemia, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and bilirubin (tab. III).

The CSF examination in deceased patients revealed mean levels of white blood cells and albumin significantly higher than those recorded in survivors, while the mean level of lymphocytes was significantly lower (tab. III).

After analyzing the AUC (fig. 1), some hematological parameters like white blood cells (AUC=0.628), PMN (AUC=0.668), urea (AUC=0.791), ESR (AUC=0.658), CRP (AUC=0.939), creatinine (AUC=0.654), fibrinogen (AUC=0.534), PMN (AUC=0.750; C.I. 95%: 0.326-1.000), ALT (AUC=0.666), AST (AUC=659), glycemia (AUC=0.631), AP (AUC=0.623), bilirubin (AUC=0.644) counted as good predictors of mortality for bacterial meningitis.

From the CSF, only the albumin level (AUC=0.647) correlated with increased mortality rate and could be considered a good predictor of mortality (fig. 2).

The length of hospitalization was significantly higher in older patients (r= +0.163; p=0.05). In both deceased and surviving patients, the GCS at hospital

admission ranged from 3 to 15. The average score was significantly lower in the group of deceased patients (6.75 ± 4.47 *vs.* 13.63 ± 2.88; p=0.001). In the group of patients with poor outcome, hospital admission was generally late, the average level of days after the onset of the disease being significantly higher in this category (5.05 ± 1.05 *vs.* 3.75 ± 1.28; p=0.046) (fig. 3).

TABLE III.

Mean values of laboratory parameters in blood and CSF according to outcome.

	Total n=145	**Deceased n=40(%)**	**Survivors n=105(%)**	**p-value**
Imaging				
CT (n,%)	101 (69.7)	35 (87.5)	66 (62.9)	
CT lesions (n,%)	54 (37.2)	25 (62.5)	29 (27.6)	**0.001**
Blood parameters				
WBC (mean±SD)	13,913.06±7,381.19	16,611±8,963	12,910±6,466.47	0.366
PMN (%)	79.66±14.54	84.39±14.84	77.91±14.09	**0.017**
Lymphocytes (%)	12.30±11.10	7.52±5.45	14.08±12.12	**0.002**
CRP	99.64±106.93	320±110.85	84.61±89.67	**0.001**
ESR	54.47±41.30	76.21±49.44	49.98±38.20	**0.011**
Fibrinogen	5.34±6.40	5.02±2.49	5.43±7.16	0.806
Hemoglobin	13.34±7.03	12.56±2.50	13.62±8.09	0.425
Platelets	238,562±107,784	211,153±114,210	248,742±104,026	**0.050**
Urea	50.80±40.73	84.26±56.98	38.26±22.50	**0.001**
Creatinine	1.11±0.82	1.52±1.39	0.96±0.36	**0.001**
Glycemia	137.41±70.69	159.21±95.94	129.23±56.97	**0.023**
ALT	67.40±83.18	98.24±116.11	56.13±64.37	**0.007**
AST	49.89±77.08	94.70±157.38	39.48±34.57	**0.002**
AP	371.34±500.79	252.86±121.04	578.69±787.71	**0.036**
Bilirubin	0.88±0.46	0.82±0.42	1.05±0.55	**0.008**
CSF parameters				
White blood cells	2,798±7,121	4,292±9,585	2,243±6,066	0.130
PMN (%)	72.50±29.78	60.88±39.70	76.74±24.11	**0.007**
PMN- absent	2,298±569	1,836±587	3,513±1,393	**0.019**
Albumin	3.26±0.40	5.49±7.29	8.03±30.80	0.636
Glycemia	51.33±38.64	54.24±51.66	50.09±31.79	0.582
Known Etiology	28 (19.3)	14 (50)	14 (50)	0.003
Streptococcus pneumoniae	20 (13.79)	11(55)	9 (45)	0.061
Other etiology (*S. epidermidis, Enterococcus, S. xylosus, S. aureus, E. coli, L. monocytogenes, B. cepacia)*	8 (5.51)	3 (37.5)	5 (62.5)	0.023

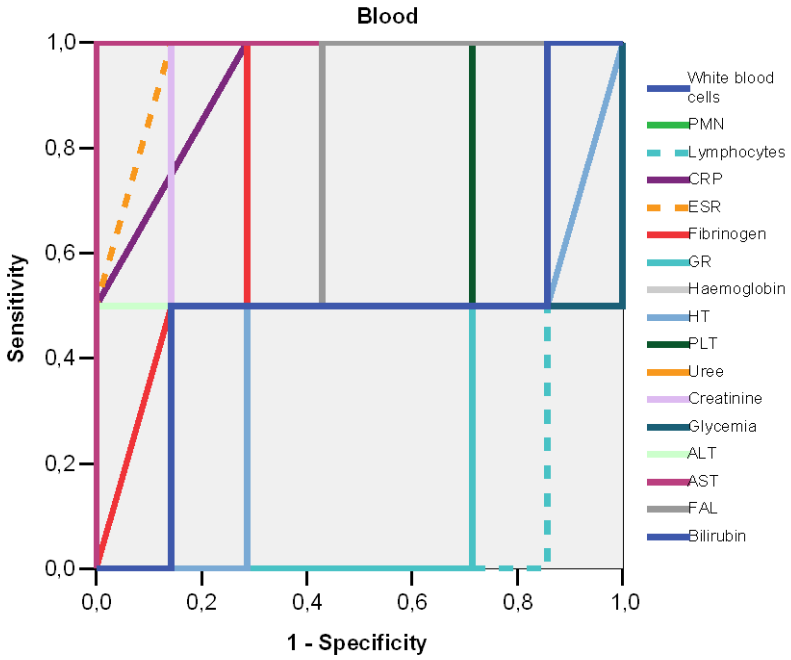


Fig. 1. AUC for blood parameters

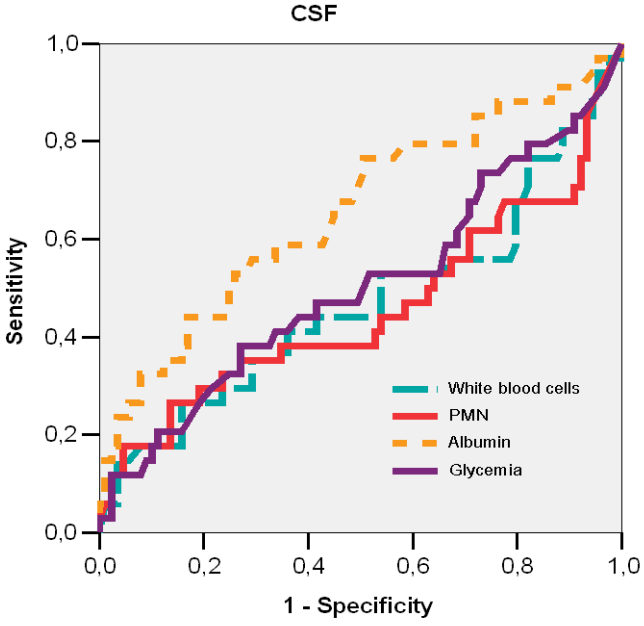
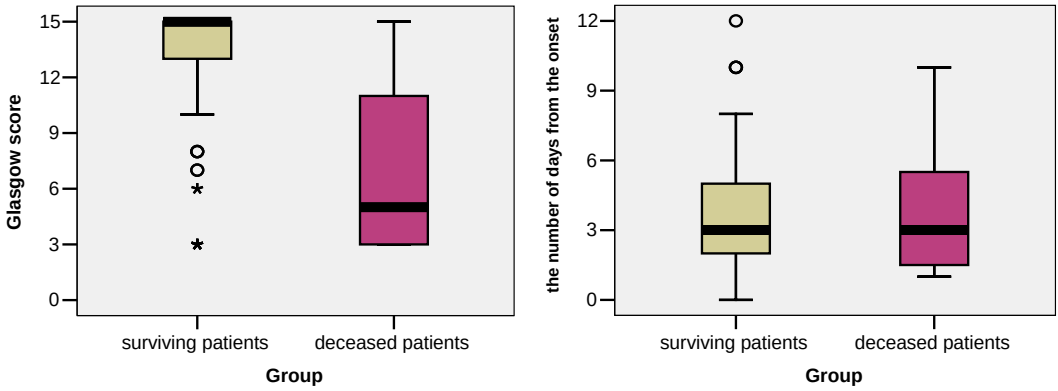


Fig. 2. AUC for cerebrospinal fluid parameters



(a) (b)

Fig. 3. (**a**) Comparison of mean values of GCS at onset; (**b**) Comparison of mean values of number of days from the onset

DISCUSSION

In our study, age over 50 years old and immunosuppression presented a 3- and 7-times higher mortality risk. Among comorbidities, diabetes mellitus was the most common (15%), as reported by literature (4-25%) (8). Other studies have also found older age to be a risk factor for poor outcome (9). Van de Beek reported immunosuppression as risk factor for meningitis with *S. pneumoniae*, *L. monocytogenes*, while immunosuppressed HIV-infected patients may present a risk of developing bacterial meningitis 8 times higher (2). Behavioral risk factors such as alcohol consumption and smoking were significantly correlated with the mortality risk in our study. By comparison, Larry *et al.* (4) described smoking as a risk factor for unfavorable evolution in adults and elderly. In the same study, they reported immunosuppression as a risk factor for meningitis (4).

Regarding the clinical symptoms presented by patients at admission, we found similar results as those in literature. The most common combination of symptoms found in our study was represented by fever, headache, neck stiffness and altered mental status (77.9%, 64.1%, 62.1% and 59.3%), in accordance with new data reports (2, 3, 9, 10).

In our study, 28.3% of patients presented the triad of fever - altered mental status - neck stiffness compared to data reports of 44% (9). In deceased patients, the association of fever with altered mental status and/or neck stiffness was significant, the risk of mortality being higher in those who presented this triad of symptoms in our study (OR=4.04).

Vomiting, vertigo and headache have been shown to be protective factors in bacterial meningitis. This is explained by the rapid hospital addressability of patients presenting these symptoms and therefore, an early diagnosis. So far, in literature no protective factors have been reported.

Among the meningeal signs represented by neck stiffness, Kernig 1-2 and Brudzinski 1-2, the most common was neck

stiffness, which was, also, suggested by the literature. The other signs were reported in 10-30% of patients. Their sensitivity and specificity are restricted in our study as well as in other studies (2).

The most common etiological agent in our study was *S. pneumoniae* (13.79%). These findings confirm previous data reports stating that pneumococcus is the most common cause of bacterial meningitis in adults, with rates between 35-50% (9, 12, 13). The low isolation rate of the etiological agent can also be explained by self-administration of antibiotherapy at home (21.01%). *S. pneumoniae* is also reported as the most common etiological agent (29.77%) isolated from the cases who died (14).

The analysis of laboratory results showed that cases with unfavorable evolution presented a higher inflammatory syndrome, thrombocytopenia, nitrogen retention syndrome, hepatocytolysis syndrome and elevated blood sugar. These parameters correlated significantly with the risk of death. As a result, systemic impairment may be an element of poor outcome, this aspect being also described in literature, as a risk factor for unfavorable evolution in patients with bacterial meningitis (9).

The CSF examination of patients with unfavorable outcome revealed that the mean levels of white blood cells and albumin were significantly higher compared to those recorded in survivors, which also confirmed the literature data (11).

Our study group reports a 27.6% mortality rate, different from the one revealed by Miftode *et al.* in a 10-year study conducted in the same North-Eastern area of Romania, which was 13.1% (15). The reported mortality rate by literature was 21-25% (9, 16). Other authors reported a relatively stationary mortality rate between 1998 and 2007 22.2%, and 24.5% between 2006 and 2015 (9, 16). The number of inpatient days was significantly higher in older patients, generating an additional risk of adverse outcome. Similarly with other reports, the average of GCS at the admission was significantly lower in the group of deceased patients (8). Patients with unfavorable outcome generally presented late for admission and the median time from onset of illness to hospital admission was significantly higher in the group of deceased patients.

CONCLUSIONS

Despite vaccination programmes lead to a decrease in the development rate of meningitis, this disease continues to be a challenge. In the present research, risk factors associated with unfavorable evolution were age over 50 years old, altered mental status, headache, fever - altered mental status - cervical stiffness triad, as well as systemic impairment, a low GCS upon admission or late presentation to the hospital. Factors that can be considered "protective" during meningitis were the presence of headache, vertigo and vomiting. In patients presenting these symptoms, the suspicion of meningitis was raised early on, and the diagnosis was confirmed without delay.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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AEROBIC EXERCISE IMPROVES WORKING MEMORY IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

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AEROBIC EXERCISE IMPROVES WORKING MEMORY IN AN ANIMAL MODEL OF PARKINSON'S DISEASE (Abstract): Regular physical activity is known to have extensive health benefits. Physical activity increases overall survival and reduces the risk of various chronic diseases such as cardiovascular disorders, cancer, hypertension, morbid obesity, depression, diabetes, and osteoporosis. Various molecular mechanisms may be responsible for the observed positive effect associated with physical activity. The most important one refers to the capability of physical exercise to influence the brain's chemistry and plasticity. **Material and methods:** we created 2 experimental groups of 6 rats each. First group received a physical exercise intervention, consisting of running on a treadmill adapted for small rodents, for a period of 2 weeks with training sessions composed of 3 sets of 5 minutes per day. The second group was used as control group. All the 12 rats received the pharmaceutical treatment with the substance was N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) for the Parkinson disease model (20 mg / kg). **Results:** One-Way *ANOVA* showed that there was a statistically significant difference ($p<0.001$) between the rats which were randomly assigned to the control group and the rats assigned in the second group, which received the aerobic exercise intervention in regard to the spontaneous alternations' percent $F(1, 10) = 41.096$. These results show that aerobic exercise significantly increased spontaneous alternation percent by enhancing neurogenesis in our animal model of Parkinson's disease. Physical exercise improved memory acquisition, memory retention and reversal learning in the Y-maze task. **Conclusions:** aerobic exercise increases neurogenesis, which influences both learning and memory. The interactions between the aerobic physical exercise, neurogenesis, learning, and memory are very complex and remarkably dynamic. **Keywords:** AEROBIC EXERCISE, PARKINSON'S DISEASE, WORKING MEMORY, ANIMAL MODEL.

Regular physical activity is known to have extensive health benefits. Physical activity also increases overall survival and reduces the risk of chronic diseases such as cardiovascular disorders, cancer, hypertension, morbid obesity, depression, diabetes, and osteoporosis. Various molecular mechanisms may be responsible for the observed positive effect associated with physical activity (1). In addition, physical activity has also been discovered to have an influence on the brain's chemistry and plasticity

(2). The available data from the literature reports that the potentially critical mediators of the beneficial effects corelated with physical activity may be connected to the upregulation of neurotrophins and several nerve growths factors (3). Furthermore, other factors that contribute to the exercise induced neuroprotection may be related to the reduction of oxidative stress, enhancement of energy production, and mitochondrial function (4). Moreover, the central nervous system through the adaptive neuroplasticity may benefit from the physical activity (5). In animal models, the reported data shows that regular physical exercise triggers changes in rodents' central nervous system plasticity, these changes include synaptogenesis, increased glucose usage, angiogenesis, and neurogenesis (6). In human studies, scientists using MRI scans have demonstrated the positive effect of physical exercise in healthy individuals. For example, in a study on a healthy population, physical activity caused a statistically significant increase in the volume of gray and white matter (7).

Parkinson's disease is an age-related neurodegenerative disorder determined by an acute loss of the nigrostriatal dopaminergic neurons (3). Given that the number of people who suffer from Parkinson's disease is projected to significantly increase over the coming decades, interventions that might reduce morbidity and increase the quality of life are important. Currently the dopamine replacement therapy is considered to be the golden standard in treating symptoms of Parkinson's disease. Although this therapy has been proven to be effective for relieving the motor deficits, Parkinson's disease has a progressive course. Therefore, at this moment there is no entirely proven pharmacological therapy that can alter or slow down the disorder progression. Therefore, finding alternative, non-pharmaceutical therapies, which are easy to implement and have reduced costs, is vital in order to delay and slow down the dopamine neuron degeneration. Thus, physical activity can be an important complementary intervention to the pharmacological therapy to reduce the inherent decline corelated with the disease (8). Available evidence from the literature suggests that individuals who suffer from Parkinson's disease might benefit from physical activity in various ways. Physical activity may contribute to disorder-modifying effects in addition to general improvements in general health and quality of life of Parkinson's disease patients (9).

However, it is well documented that only a part of patients with the neurodegenerative disorder-Parkinson's disease, progress to developing major cognitive disturbances (10). Yet, minor cognitive deficits caused by this neurodegenerative disorder are common (11, 12). These minor cognitive disturbances can occur before motor symptoms develop, as demonstrated in siblings of patients with familial Parkinson's disease (13). The early minor cognitive deficits can be used to predict the development of dementia in the later stages of the disease (14, 15). It is these early minor cognitive deficits that do not respond well to dopamine therapies, and significantly alter the quality of life (16, 17). The before mentioned minor cognitive impairments include deficits in set-shifting (18), in switching tasks (19), in probabilistic reversal learning (20), in a delayed win-stay task connected to disfunctions in striatal and the prefrontal cortex (21), in long term memory (22, 23), and short-term memory (24, 25).

In human studies, results reported demonstrate that an active lifestyle and cardiovascular fitness are corelated with better cognitive function during aging (26, 27). Likewise, in animal models, increased physical activity in laboratory rodents has been reported to positively influence memory acquisition and retention in different behavioral tasks, which measure distinct forms of learning. For example, the results reported by some authors demonstrate that after one month of cardiovascular exercise, mice master the Morris water maze task faster when compared to the sedentary control mice (28, 29). In another study, rats that had a running wheel in their housing for 4-8 weeks presented a higher frequency in the freezing behavior in a fear conditioning task (30). In addition, in the same study, aerobic exercise also statistically significant increased memory retention in the passive shock avoidance task in mice (31). However, how exactly various aspects of the learning and memory processes are specifically influenced by exercise remains unclear.

Thus, in our experiment we examine the influence of physical activity, aerobic exercise on a treadmill, on learning and memory in more detail. The learning task that we used is the Y maze, a spatial reference memory task in which the rats have to memorize in which of two arms is the food reward. We differentiated between the possible effects of aerobic exercise on memory acquisition and on long-term memory retention. We also measured the consequences of cardiovascular physical exercise on reversal learning. Reversal learning is defined as the ability to use the acquired information to a new situation and to inhibit the previously used response (behavioral inhibition).

Therefore, in the present study, we analyzed the potential relationship between exercise, learning and memory, and Parkinson's disease. We hypothesized that the runner rats would master the Y-maze task better than the sedentary controls.

MATERIAL AND METHODS

Animals. Our experimental design was simple. We created 2 experimental groups of 6 rats each. First group received the physical exercise intervention, the second one was used as control group. All the 12 rats received the pharmaceutical treatment. We used adult male rats (8-10 weeks) Wistar breed with weight 30 g ± 2g. Animals were housed in cages in a controlled temperature room (22°C), a light / dark cycle of 12 : 12hrs. (starting at 08:00), with food and water *ad libitum*. Experiments took place within the *"Advanced Research and Development Center in Experimental Medicine"(CEMEX).*

Animals were brought into the laboratory at least 30 minutes before the start of the experiment for acclimatization (with temperature and background noise).

Pharmaceutical injection. The administration of the pharmaceutical was made with peritoneal injection. The substance was N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) for the Parkinson disease model (20 mg / kg).

Aerobic exercise intervention. Intervention through physical exercise aerobic physical exercise was performed on a treadmill adapted for the small rodents, for a period of 2 weeks with training sessions consisted of 3 sets of 5 minutes per day.

Y Maze .Our model of the Y Maze was based on the previous validated model (32, 33). Alterations in working memory-related behaviors were measured in the Y-maze

test using a cardboard apparatus that consisted of 3 enclosed arms with measures of 30 × 5 × 15 cm, which converged on a triangular center area with measures of 5 × 5 × 5 cm. Spontaneous alteration was defined as the number of successive triple entry performances into each of the three arms without any repeated entries (32, 33). In addition, total number of entries into arms were also evaluated. The rate of spontaneous alteration performance, calculated as a percentage, was calculated as a parameter for the working memory-related behaviors. The total number of entries into arms was examined as a measurement of locomotor activity (32, 33). At the beginning of each experimental session, each rat was placed in the center of the maze, facing all three arms exactly before the session.

RESULTS

Our One-Way *ANOVA* showed that there was a statistically significant difference ($p<0.001$) between the rats which were randomly assigned to the control group and the rats assigned in the second group, which received the aerobic exercise intervention in regard to the spontaneous alternations percent $F(1, 10) = 41.096$.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises presented a higher percent of spontaneous alternations ($x=69.29$, $s^2=4.09$) compared to the control rats ($x=52.18$, $s^2=5.06$) (fig. 1).

In addition, the maximum percent of spontaneous alternations was 76.47 in the aerobic exercise group and 58.33 in the control group. The minimum percent of spontaneous alternations in the aerobic exercise group was 64.71 and 46.15 in the control group of rats.

The second One-way *ANOVA* we performed indicated that the difference between the rats in the control group and the rats in the aerobic exercise group was also statistically significant ($p<0.001$) in regard to the number of total entries $F(1, 38)= 83.674$.

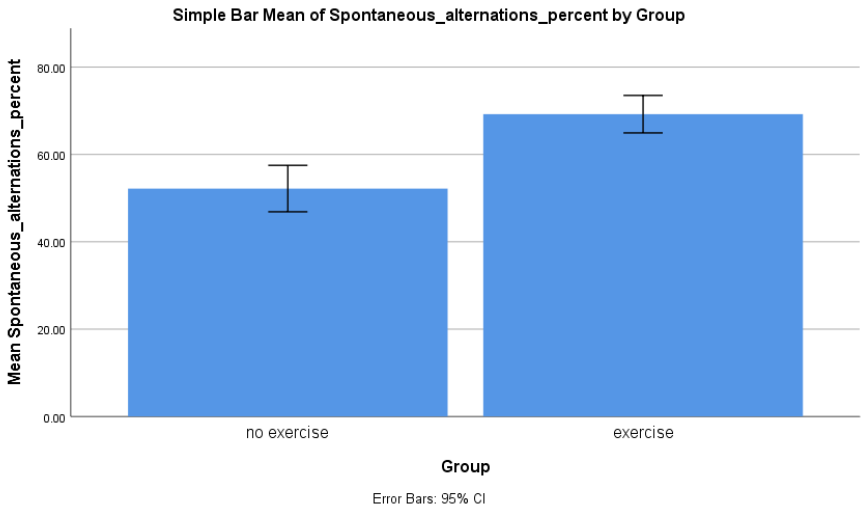

Fig. 1. Spontaneous alternations percent comparison between the two experimental groups.

The discovered significantly differences between the two groups of animals were in the sense that the rats which performed the aerobic exercise protocol had a higher number of entries (x=18.67, s^2= 2.58) compared to the control rats (x=14.17, s^2 = 1.16) (fig. 2).

Furthermore, the maximum number of entries was 22 in the aerobic exercise group and 15 in the control group. The minimum number of entries in the aerobic exercise group was 14 and 12 in our control group.

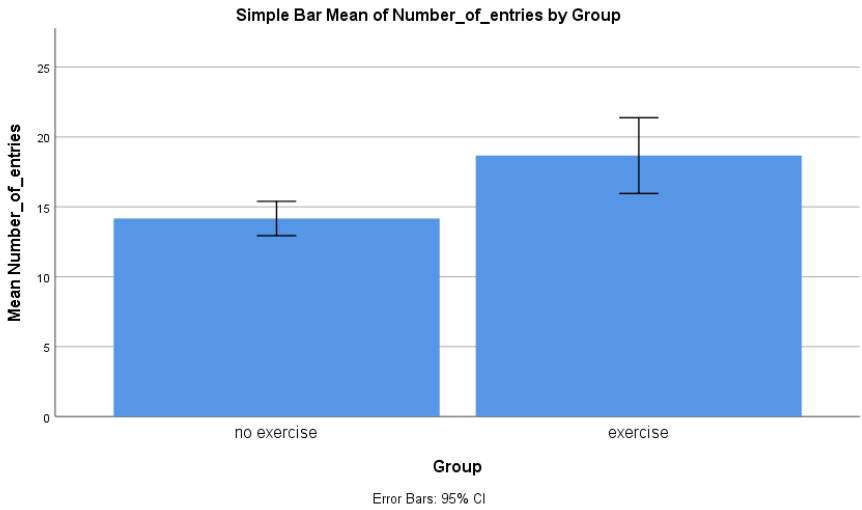

Fig. 2. Number of entries comparison between the two experimental groups.

DISCUSSION

In our animal model of the neurodegenerative-disorder Parkinson's disease, that we used in the present study, the experimental intervention consisting of sessions of aerobic exercise significantly increased both the spontaneous alternation percent and the total number of entries of Wistar rats. These uniform beneficial effects reported above are consistent with most of those reported in the literature. As noted in several previous studies, exercise has tended to increase the spontaneous alternation percent, the number of entries in these arms during the 5-minute test period (effects that reached statistical significance).

The deficits observed in our study in the rats which were sedentary, in the Y-maze, accompanied by those reported in other studies related object-place recognition and novel object recognition tests may be connected to an altered cholinergic system, as these tasks are impaired by administration of scopolamine, a muscarinic antagonist (34-36). The connection with our experimental design consists in the impairment observed in the cholinergic system in Parkinson' s disease. Patients suffering from this neurodegenerative disorder report cholinergic cell loss in the basal nucleus of Meynert (37). Furthermore, Lewy body pathology has been reported in this region as early as stage 3 of pre-manifest of Par-

kinson's disease (38). In addition, ChAT activity is also found to be reduced in the prefrontal cortex of patients who suffer from Parkinson's disease (39). Furthermore, individuals with Parkinson's disease present a decrease level of nicotinic ACh receptor binding in many cortical regions (40). Moreover, reported results from a study reported a significant accumulation of human a-synuclein in the cholinergic neurons located in the basal nucleus of Meynert (40). In the same study it was documented a 30% decrease in ACh levels in the cerebral cortex of mice from an animal model of Parkinson's disease. We can speculate that the same mechanisms revealed the presence of cognitive deficits in the present study. It is plausible to assume that the a-synuclein overexpression in the neurons, as the cholinergic neurons in the basal nucleus project to the cerebral cortex (41), has determined the decrease of cortical ACh, as it is documented to alter structures such as synthesis, packing into vesicles, and delivery of various of neurotransmitters (42).

The results from our experiment demonstrate that 14 days of aerobic exercise elevated the rate of acquisition in the Y maze, improved retention of already acquired memories, and positively influenced reversal learning. The fact that the aerobic exercise intervention had a positive effect on Y-maze's spontaneous alternations and number of entries is in concordance with other studies, using various learning animal models (28, 42, 43).

Therefore, we demonstrate in the present study that physical exercise not only increases the acquisition of a spatial learning function but also is helpful for the retrieval of spatial reference memory. Other author also reported results that demonstrated beneficial effects of aerobic exercise on memory retention (30, 31). Nevertheless, in these animal models, the rodents had access to a running wheel before the acquisition of the function. Some authors have speculated that, in these cases, the observed increased memory retention could be explained by the aerobic exercise-induced improvement of memory formation or an increase in memory retrieval. Therefore, in our experimental design, a difference between the effects of aerobic exercise on memory formation and memory retrieval could be made by providing the aerobic exercise intervention before the acquisition of the Y-maze task. In this way, we demonstrated that an improvement in physical activity can significantly increase memory retention by aiding memory retrieval.

Aerobic exercise intervention through running on a treadmill also facilitated reversal learning. During reversal learning, the rat has to adapt previously collected memory to a new situation. Because the majority of the features of the assignment were learned during training are still valid, the former memory trace does not need to be forgotten. As an alternative, the rodent modifies the current memory trace, which is necessary for the correct recovery of the memory and the adaptability to unlearn various details of the process, or to restrict the acquired response and to relearn new tasks.

Here we demonstrate that Wistar rats that had been exposed to a running treadmill for 14 days were significantly faster in learning and memorizing the new position

of the food in the Y maze than sedentary rats. These results suggest that physical exercise has a positive effect on the behavioral flexibility of a Wistar rat. Therefore, the performance in the Y maze regarding the memory acquisition assignment, memory retention or reversal learning was positively influenced by the aerobic exercise intervention.

There are various probable molecular mechanisms that can explain the observed exercise-induced improvements in learning and memory. The positive effects of treadmill running on Y-maze performance observed in our sample of rats, could also be due to elevated transcription or phosphorylation of genes that promote long-term memory acquisition (44-46). Therefore, an elevation in pCREB-positive cell number after 14 days of exercise could also probably occur in our experiment, exceeding the elevation in the number of DCX-positive cells, implying that aerobic exercise also caused CREB phosphorylation in other cells, not only in the recently built granule neurons.

In concordance, other available studies presented results according to which aerobic exercise has been shown to increase levels of different growth and neurotrophic factors (47-49). In addition, aerobic exercise has also been shown to promote cerebral blood circulation (50), angiogenesis (51) and cholinergic synaptic communication (43). All of these before mentioned positive effects on these various processes and markers may improve learning and memory. As the rats from the control group did not engage in any other activity, the positive effects of aerobic exercise could also partially be influenced by the enrichment of having a new daily activity and not by the physical exercise itself (52). It could therefore be mentioned, as a direction for future studies, the need to use a locked treadmill for the control rodents.

Further, some studies have reported reduced neurogenesis after various bouts of aerobic exercise in different animal models that measure different type of memories. A potential explanation for this reported decrease neurogenesis, is that it might assist in blocking interference between current memory traces and new ones. Therefore, 14 days after the rats have memorized the Y-maze model, the memory is probably stored in the cortex and retrieval is independent of the hippocampus (53, 54). Oxidative stress, inflammation and various vascular factors could be also implicated in this context (55-57). Nevertheless, being exposed again to the same model may revive the hippocampal memory trace and to reactivate it to an easier to alter state, may prove to be sensitive to disruption (58). Thus, it might be essential for the optimal retrieval of memories to partially decrease neurogenesis when memories are in this labile condition. Because new formed neurons may produce a vital substrate for the construction of new memory traces. Therefore, some author hypothesized that active suppression of neurogenesis during reexposure to a familiar context may help avoiding the formation of redundant memories and decrease possible meddling between the existing and the newly acquired memory trace.

CONCLUSIONS

To conclude, our results show that aer-

obic exercise significantly increased spontaneous alternation percent by enhancing neurogenesis in our animal model of Parkinson's disease. Physical exercise improved memory acquisition, memory retention and reversal learning in the Y-maze task. Taken together, these results imply that aerobic exercise increases neurogenesis, which influences both learning and memory. However, the interactions between the aerobic physical exercise, neurogenesis, learning, and memory are very complex and remarkably dynamic.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

VITAMIN D AND OPHTHALMOPATHIAS A REVIEW

The importance of hydroxycholecalciferol as one of the liposoluble vitamins is known in the prevention and treatment of metabolic bone diseases (rickets, osteomalacia, osteoporosis) In recent years, however, information has increased on the importance of vitamin D3 in numerous organ systems and in the pathogenesis of various diseases, e g ophthalmopathies The immunological functions of vitamin D3 are the subject of studies dealing with autoimmune optic nerve disorders and their results appear to have a positive effect on demyelinating diseases It also plays an important role in maintaining the thickness of the retinal nerve fiber layer, but its additional administration has not been successful It appears that sufficient serum vitamin D3 levels may protect patients from deterioration in the form of a further attack of demyelination The course of diabetic retinopathy is probably also influenced by vitamin D3, by correlating the fact that its receptor and the enzymes of its metabolism are expressed on the retina Low serum levels of vitamin D3 may even trigger age-related macular degeneration Conversely, higher dietary intake of vitamin D3 may positively affect neovascularization The optimal level of hydroxycholecalciferol is between 60 and 200 nmol /l, the severe deficit represents a decrease below 25 nmol/l However, the demonstration of its protective character in connection with the mentioned diseases of the retina and optic nerve will require a sufficient number of studies to confirm the facts found so far about this rediscovered vitamin (Hribová, P, and Š Sotak "VITAMIN D AND OPHTHALMOPATHIAS A REVIEW " "VITAMÍN D A OFTALMOPATIE PREHĽAD" Ceska a slovenska oftalmologie : casopis Ceske oftalmologicke spolecnosti a Slovenske oftalmologicke spolecnosti vol. 2, Ahead of Print (2022): 1001-1004)

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THE EFFECT OF AEROBIC EXERCISE ON MEMORY, DEPRESSION, AND ANXIETY LIKE SYMPTOMS IN AN ANIMAL MODEL OF AUTISM

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THE EFFECT OF AEROBIC EXERCISE ON MEMORY, DEPRESSION, AND ANXIETY LIKE SYMPTOMS IN AN ANIMAL MODEL OF AUTISM (Abstract): Given that individuals with autism frequently display various psychiatric symptoms, such as depression and anxiety, we decided to measure depression and anxiety like symptoms in an animal model of autism, and to test aerobic exercise as an experimental intervention for reducing these symptoms. **Material and methods**: Our experimental design consisted of 2 experimental groups of 6 rats each. The first group of rats received a physical exercise intervention, consisting of running on an adapted treadmill, for a period of 2 weeks with training sessions of 3 sets / 5 minutes per day. The second group was used as control group. All the 12 rats received the pharmaceutical treatment with the substance valproic acid (500 mg/kg). **Results:** Our One-Way ANOVA demonstrated that there was a statistically significant difference ($p<0.001$) between the rats which were randomly assigned to the control group and the rats assigned in the second group, which received the aerobic exercise intervention in regard to the spontaneous alternation percent in the Y maze, number of entries and time spent in the open arms in the Elevated Plus Maze, swimming and floating time in the Forced Swim Test and number of working and reference errors in the Radial Maze. Our results show that aerobic exercise significantly decreased anxiety and depression-like symptoms and also increased spontaneous alternation percent in the Y Maze in our animal model of autism spectrum disorder. **Conclusions:** Taken together, our findings imply that aerobic exercise might be considered a feasible intervention for patients with autism spectrum disorder in order to increase both learning and memory and to decrease anxiety and depression. **Keywords:** AEROBIC EXERCISE, AUTISM SPECTRUM DISORDER, ANXIETY, DEPRESSION, WORKING MEMORY, ANIMAL MODEL.

Despite the fact that the etiology of autism is still a matter of considerable controversy, it is well demonstrated that being exposed before birth to drugs such as thalidomide (1, 2) and valproic acid (3, 4) is partly responsible to the development of autism. Therefore, based on these evidence, animal models of autism have been constructed by prenatally exposing animals to valproic acid for a better measurement of the pathophysiological, morphological, and behavioral aspects of autism (5, 6).

The studies that examined thoroughly these animal models (7) have reported

results according to which prenatally exposed to valproic acid rats presented higher serotonin levels in the hippocampus and elevated levels of dopamine in the frontal cortex. Several clinical studies have observed a hyperactivation of the dopamine system in people with autism. For example, risperidone, which is known to block postsynaptic serotonin and dopamine receptors, have been reported in various studies to reduce tantrums, aggression, or self-injurious behavior in children with autism (8). In addition, in another study (9) the same risperidone has been demonstrated to significantly improve the repetitive, restricted, and stereotyped behavior in children with autism. All this evidence may suggest that people with autism could exhibit a hyperactivated dopamine system.

Furthermore, given that individuals with autism frequently display various psychiatric symptoms, such as depression and anxiety (10), we decided to measure depression and anxiety like symptoms in an animal model of autism, and to test aerobic exercise as an experimental intervention for reducing these symptoms.

We chose the forced swim test because it has been established to measure depressive behaviors in rats. When rats are positioned in a cylinder of water without any possibility for escape, they become immobile. This immobility behavior is believed to indicate an occurrence of depression-like behavior that prevents the rat from exhibiting active forms of coping with stressful stimuli (11).

On the other hand, the elevated plus maze measures anxiety like behaviors. This conflict test is based on a natural tendency of rats to actively explore a new environment, *versus* the aversive properties of an elevated open runway (12-14).

In addition, we also used Y-maze alternation which is a test of working memory also based on the natural tendency of rats to explore new territory whenever possible. The number of arm entries and the number of triads is recorded in order to calculate the percentage of spontaneous alternation. The spontaneous alternation percent is considered to measure short term spatial working memory (15).

Furthermore, rats also present various survival habits, such as instinctively locating and retrieving food (16). Spatial learning and memory are essential for recording information regarding spatial orientation and the environment. Therefore, we also used the radial arm maze for measuring spatial learning and memory in rodents. This commonly used maze allows for the measurement of spatial working and reference memory. A radial maze usually has a center area with eight arms radiating outward. At the end of each arm are located food sites. These food sites are not visible from the central area. Four arms have food at the distal end, and the other four arms do not have any. Throughout the trial part of the radial maze experiment, the rat must acquire food from all four arms of the maze. For each of the following trial, the same arrangement is used so that rats can memorize to gather food from all four arms without accessing the arms that do not have food in them. In this manner, two types of memories are examined during the maze task, working and reference. Examination of working memory implies rats re-enter in the same trial in previously entered arms. While the measurement of reference memory involves rats entering in each trial in the arms with no food.

Although the beneficial effect of physical exercise on children with autism spec-

trum disorder has been demonstrated in the literature and is presently recommended for reducing various symptoms by the National Center for Physical Activity and Disability, children with autism spectrum disorder rarely engage in physical activities (17-20). One explanation may be that these decreased levels of physical activities and exercises may be a result of the limited opportunities available for children with autism spectrum disorder (17). The social and behavioral deficits that are commonly present in children with autism spectrum disorder that make participation in activities with peers difficult, may explain these restricted opportunities (21). Therefore, social constraints, more than existing impairments, could be the limiting factor determining the low levels of physical activities observed in children with autism spectrum disorder (22).

In regard to the positive effect of physical exercise and physical activities, it has been studied the possible positive effect of physical exercise on stereotypic behaviors in children with autism spectrum disorder. In a small study on 3 children with autism spectrum disorder, with ages between 7 and 11, Kern *et al* investigated the effect of exercise on various symptoms of autism spectrum disorder (23). The results reported by the authors demonstrated that stereotypic behaviors diminished following vigorous jogging. Interestingly, no reduction in the frequency of the stereotypic behaviors was observed following mild intensity exercise (23). Therefore, a certain intensity might be necessary in order to promote optimal beneficial effects. In addition, in a similar article, Watters and Watters reported similar results in a sample 5 boys with autism spectrum disorder with ages between 9 and 11 years old (24). They discovered that the maximum decrease in the frequency of stereotypic behavior was observed following physical activity (jogging for 8-10 minutes) in comparison with academic work or television watching. Likewise, a study investigating a sample of three children with an age of 11 years old, with autism spectrum disorder, found that 15 minutes of continuous jogging significantly decreased stereotypic behaviors (25). The authors of this study also investigated the carryover of the observed decrease in stereotypic behaviors and reported that the behavior carryover was restricted to 90 minutes after the running (25). Therefore, the frequency of the physical activity might also be important when implementing such an intervention in order to observe positive effects.

Another group of authors conducted a systematic review evaluating the effects of physical exercise on the frequency of stereotypic behaviors of children with autistic spectrum disorder (17). This systematic review analyzed 7 studies which included a total of 25 children. From this total sample, twenty-two were children with ages between 6-15 years old and 3 were younger children with ages from 3 to 5 years old. In the studies that contained younger children in the sample, the physical activity intervention involved walking compared to jogging, only jogging, and jogging compared to ball playing (23, 26, 27). The results reported demonstrated that only jogging led to an observed decrease in stereotypic behaviors (27).

From the same systematic review, three studies had an experimental design that measured the possible positive effect that physical exercise might have on academic performance (28). Two studies out of three reported results that showed improved

academic performance following jogging (27, 29), while Watters and Watters found no significant effect of physical exercise on academic performance (24). In addition, another study found an additional positive effect of exercise, documenting a significant increase in on-task behavior (28).

Another interesting study that measured the effect of exercise on academic performance of children with autistic spectrum disorder found that jogging, at an intensity that elevated the individuals' breathing rate and caused flushed faces, lowered stereotypic behavior, and increased both on-task time and academic performance (27). In a similar study, but on a sample of 6 adults with autistic spectrum disorder, identical results were found (29). These results demonstrated that aerobic activity elevated the frequency of appropriate behavior and reduced off-task time in this sample of adults with autistic spectrum disorder.

In addition, in another study which used roller skating for 10 minutes as the aerobic exercise intervention, similar results were found in an 8-year-old child with autistic spectrum disorder. Their results showed a reduced level of stereotypic behavior and an increase level of on-task performance (30).

Therefore, the available data shows that aerobic exercise improves correct responding, increase on-task time, and decrease the frequency of stereotypic behaviors in older children (with ages from 6 to 15 years old) with autistic spectrum disorder (17). Regarding young children (3 to 5 years old), although few studies have explored such an effect their results are similar (30).

The purpose of this study was to determine whether participation in aerobic exercise improves memory and reduces anxiety and depression like behavior in an animal model of autism.

MATERIAL AND METHODS

Samples of adult male rats (8-10 weeks) Wistar weighing 30 g ± 2 g were formed. The animals were housed in cages in a temperature-controlled room (22 ° C) and a light / dark cycle. 12: 12h (starting at 08:00), with food and water ad libitum. The experiments were carried out within the "Advanced Center for Research and Development in Experimental Medicine – CEMEX". The animals were brought to the laboratory at least 30 minutes before the start of the experiment, to acclimatize it (with temperature and background noise).

Pharmaceutical injection - The administration was made with an intraperitoneal injection. The substance was valproic acid for the animal model of autism (500 mg/kg).

Intervention through aerobic exercise - Aerobic exercise was performed on an adapted treadmill for 2 weeks with training sessions consisting of 3 sets of 5 minutes each day.

Forced swim test. The forced swimming test consisted of rats individually being placed in a glass cylinder (45 cm high, 25 cm in diameter) containing 30 cm of water, maintained at room temperature during the test (25 ± 1.0°C). Subsequently, the rats were removed and returned to their cages after 15 minutes in water. They were placed in the cylinder again 24 hours later. The total duration of immobility in rats was measured during a 5-minute test. Rats were considered immobile when they remained floating passively in the water.

Y Maze. At the beginning of each experimental session, each rat was placed in the center of the maze, facing all three arms exactly before the session. Spontaneous altera-

tions in working memory-related behaviors were measured in the Y-maze test using an apparatus that consisted of 3 enclosed arms with measures of 30×5×15 cm, which converged on a triangular center area with measures of 5×5×5 cm. Spontaneous alteration was defined as the number of successive triple entry performances into each of the three arms without any repeated entries.

The Elevated Plus Maze. The elevated plus-maze, a standard paradigm to assess anxiety-related behavior in rodents, consisted of an apparatus with four arms, 49 cm long and 10 cm wide, elevated 50 cm above the ground, with two arms enclosed by walls of 30 cm high and the other two exposed. After accommodation to the test room for half an hour, each rat was placed at the junction of the four arms, facing an open arm and allowed to freely explore the maze for 5 min. The entries and time duration in each arm were recorded as indicators for anxiety.

Radial Maze. Our radial maze was an apparatus consisting of a circular center compartment from which 8 equally spaced arms draw out. Each arm was a stretched column of equal distance, the far end of which contained (or not) a food reward not visible from the center compartment.

RESULTS

Y maze

Our One-Way ANOVA showed that there was a statistically significant difference (p=0.001) between the rats in the control group and the rats which received the aerobic exercise intervention in regard to the spontaneous alternations' percent F (1, 10) = 22.011.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises presented a higher percent of spontaneous alternations (x=88.72, SD=10) compared to the control rats (x=56.05, SD =13.7) (fig. 1).

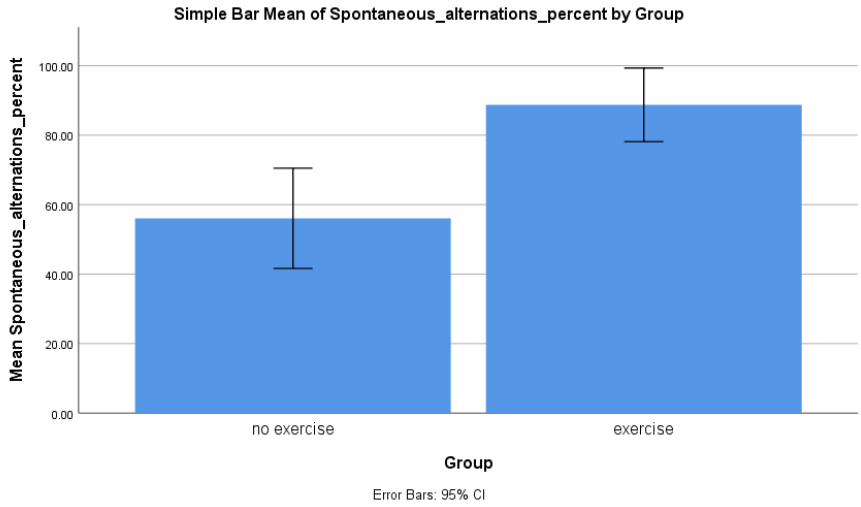

Fig. 1. Comparison of spontaneous alternations in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.011)

In addition, there was a statistically significant difference (p=0.004) between the rats in the control group and the rats assigned in the second group, which received the aerobic exercise intervention in regard to the number of entries F (1, 10) = 13.857.

The reported significantly differences among the two samples of rats were in the sense that the rats in the aerobic exercise group presented a higher number of entries (x=21.5, SD=4) compared to the control rats (x=10.83, SD =5.7) (fig. 2).

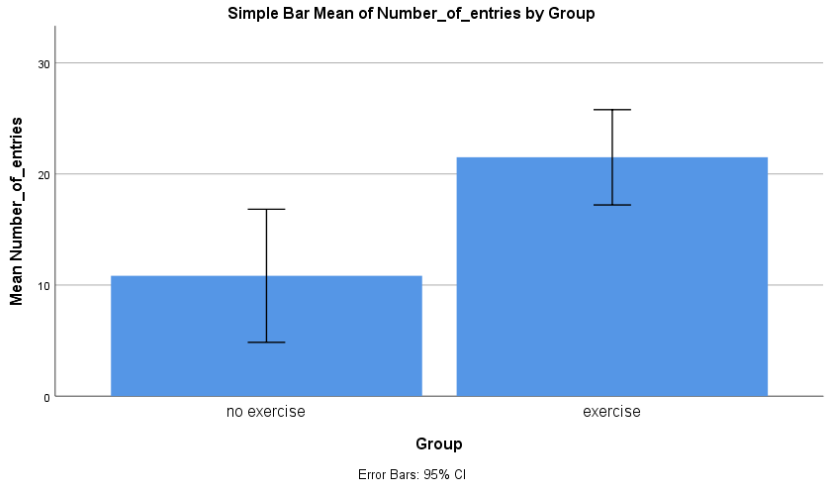

Fig. 2. Comparison of number of entries in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.004)

Elevated plus maze

In the elevated plus maze, there was a statistically significant difference (p=0.033) between the rats assigned to the control group and the rats which received the aerobic exercise intervention in regard to the number of entries in the open arms F (1, 10) = 6.154.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises displayed a higher number of entries in the open arms (x=1.67, SD=0.5) compared to the control rats (x=0.33, SD =1.2) (fig. 3).

In addition, there was also a statistically significant difference (p=0.028) between the rats from the control group and the rats from the aerobic exercise intervention group regarding the time spent in open arms F (1, 10) = 6.644.

These significant differences were in the sense that the rats which performed the aerobic exercises spent more time in the open arms of the maze (x=8.33, SD=5.9) compared to the control rats (x=1.5, SD =2.5) (fig. 4).

However, there was a non-statistically significant difference (p=0.157) between the rats which were randomly assigned to the control group and the rats assigned in the second group, which received the aerobic exercise intervention regarding the number of entries in the closed arms F (1, 10) = 2.341.

Although the rats which performed the aerobic exercises presented a lower number

of entries in the closed arms (x=4.67, SD=1.8) compared to the control rats (x=6.83, SD =2.9) this difference was not statistically significant (fig. 5).

However, a statistically significant difference (p=0.028) was found regarding the time spent in closed arms (F (1, 10) = 6.644) between the rats in the control group and the rats assigned in the second group, which received the aerobic exercise intervention.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises spent less time in closed arms (x=291.67, SD=5.9) compared to the control rats (x=298.5, SD =2.5) (fig. 6).

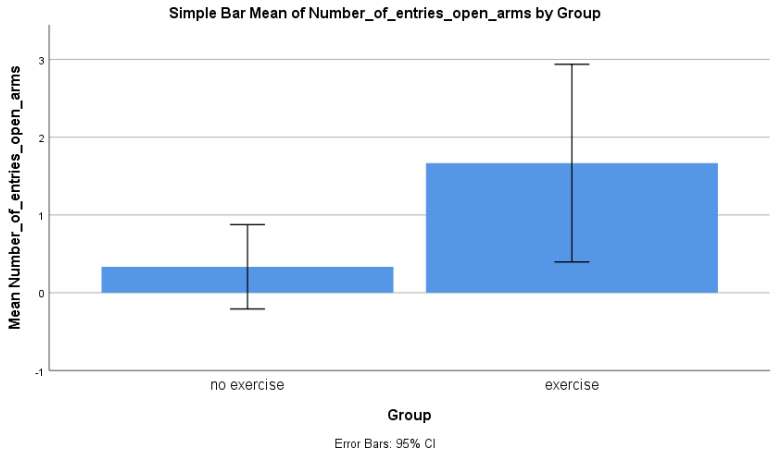

Fig. 3. Comparison of number of entries in open arms in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.033)

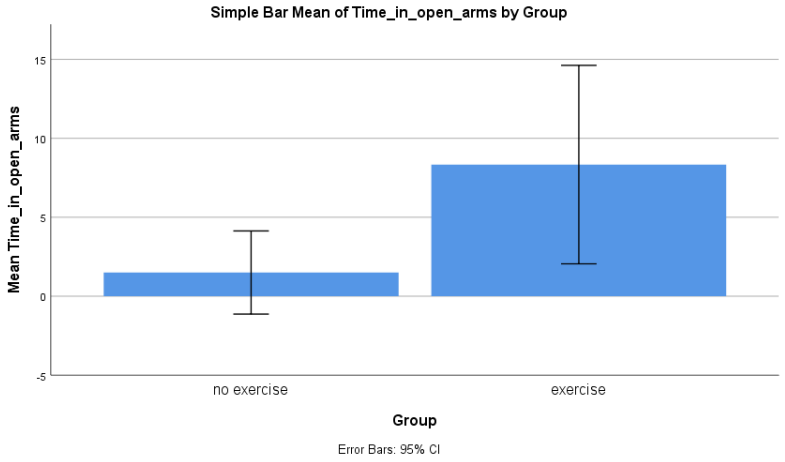

Fig. 4. Comparison of time spent in open arms in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.028).

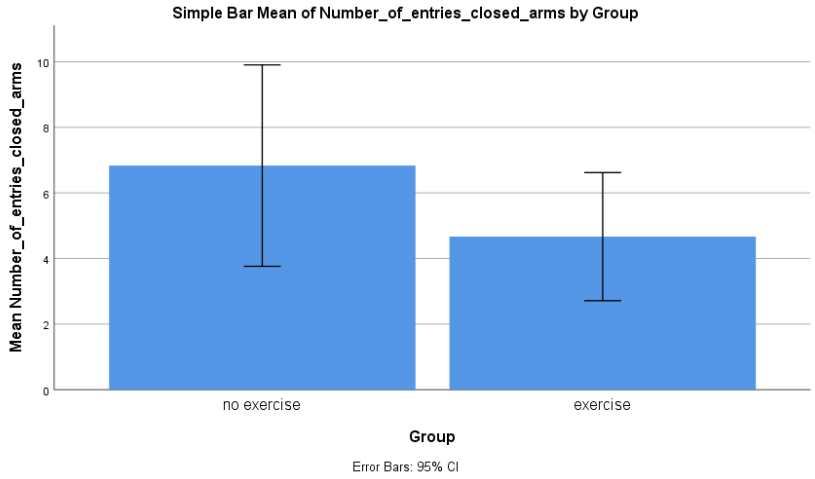

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Fig. 5. Comparison of number of entries in closed arms in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.157)

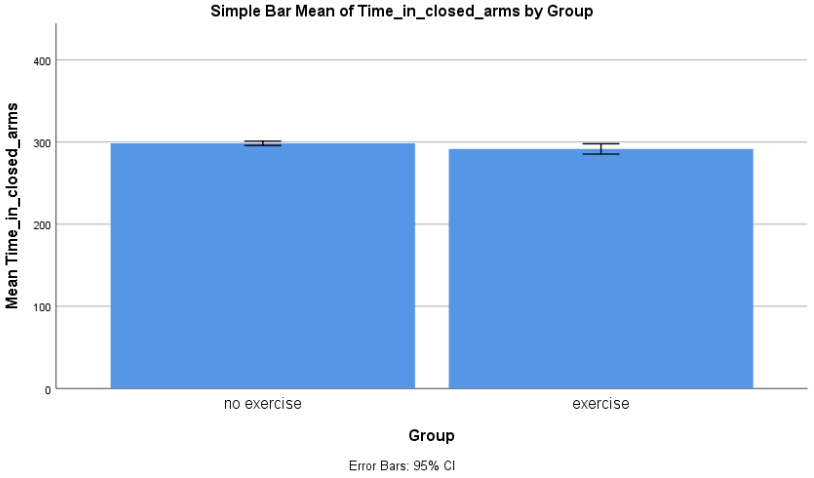

Fig. 6. Comparison of time spent in closed arms in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.028)

Forced swim model

In the forced swim model, we found a statistically significant difference (p<0.001) between the rats assigned to the control group and the rats assigned in the second group, which received the aerobic exercise intervention in regard to the swim time F (1, 10) = 9.653.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises spent more time swimming (x=275.33, SD=19.7) compared to the control rats (x=226.67, SD=32.8) (fig. 7).

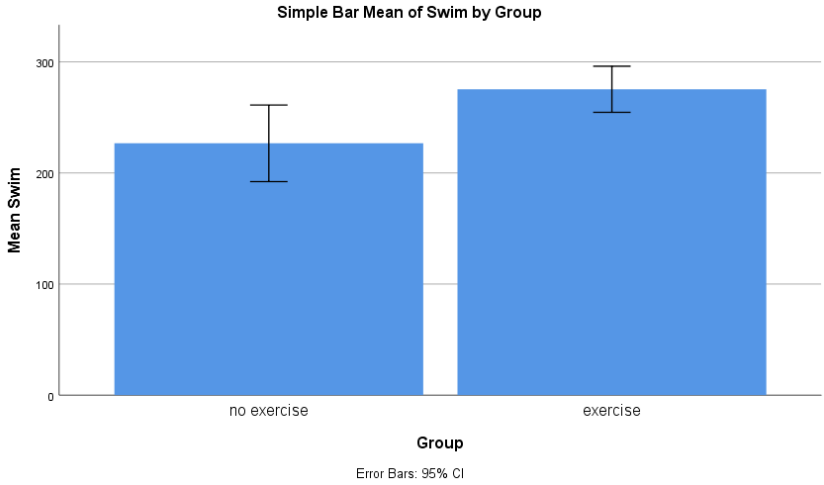

Fig. 7. Comparison of time spent swimming in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.011)

There was also a statistically significant difference (p=0.023) between the rats which were randomly assigned to the control group and the rats which received the aerobic exercise intervention regarding the floating time F (1, 10) = 7.215.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises spent less time floating (x=21.67, SD=17.9) compared to the control rats (x=48.33, SD=16.4) (fig. 8).

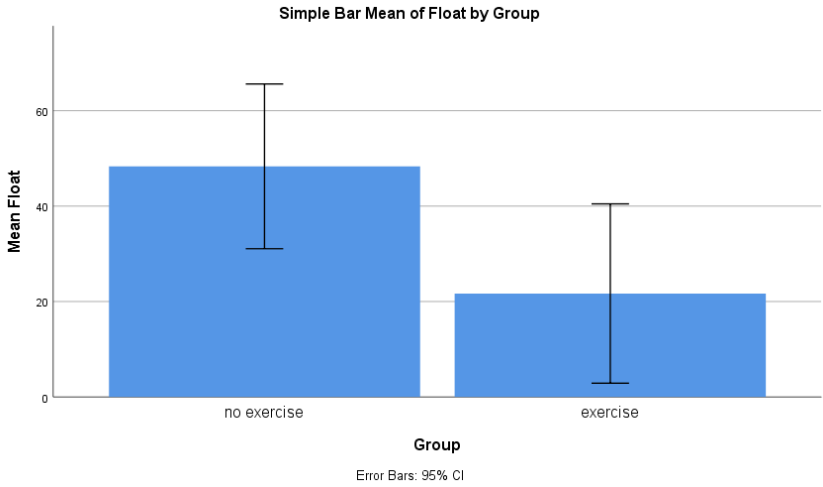

Fig. 8. Comparison of time spent floating in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.023)

In addition, there was also a statistically significant difference (p=0.023) between the control rats and the rats assigned in the second group, which received the aerobic exercise intervention regarding the mobility time F (1, 10) = 41.096.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises presented a higher mobility time (x=278.33, SD=17.9) compared to the control rats (x=251.67, SD =16.4) (fig. 9).

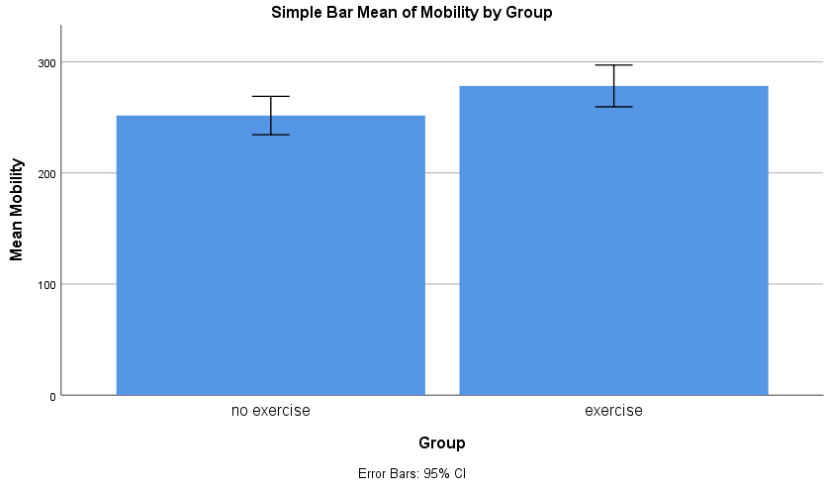

Fig. 9. Comparison of mobility time in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.023)

Radial maze

The results regarding the radial maze showed that there was a non- significant difference (p=0.139) between the rats which were distributed in the control group and the rats distributed in the second group, which received the aerobic exercise intervention in regard to the total number of entries F (1, 10) = 2.581.

Although the rats which performed the aerobic exercises presented a lower number of total entries (x=6.67, SD=1.2) compared to the control rats (x=9.33, SD=3.8) this difference was statistically non-significant (fig. 10).

However, also in the Radial Maze, there was a statistically significant difference (p=0.041) between the rats in the control group and the rats in the aerobic exercise intervention group regarding the number of working errors F (1, 10) = 5.517.

This significant difference among the two samples of rats was in the sense that the rats which performed the aerobic exercises made fewer working memory errors (x=0.83, SD=0.9) compared to the control rats (x=2.17, SD =0.9) (fig. 11).

In addition, the next One-Way ANOVA demonstrated that there was a statistically significant difference (p=0.04) between the rats which were distributed in the control group and the rats distributed

in the second group, which received the aerobic exercise intervention in regard to the number reference errors F (1, 10) = 5.538.

The reported significantly differences among the two samples of rats were in the sense that the rats which performed the aerobic exercises made fever reference errors (x=1.17, SD=0.4) compared to the control rats (x=3.17, SD =2) (fig. 12).

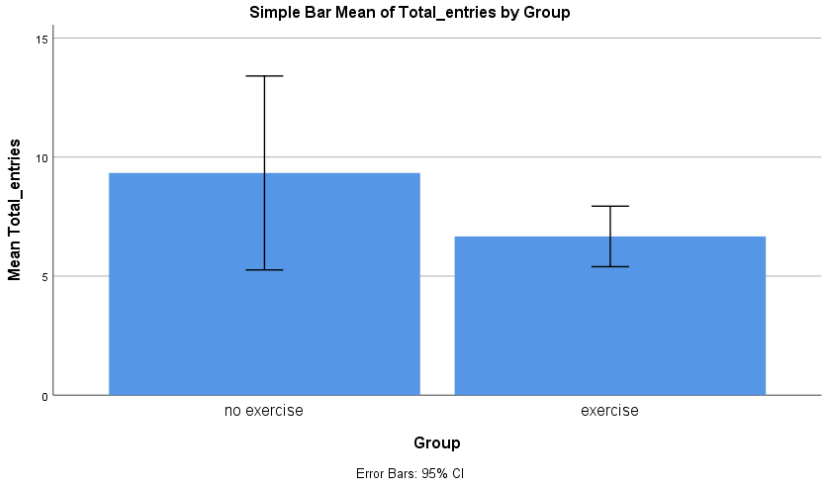

Fig. 10. Comparison of mobility time in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.139)

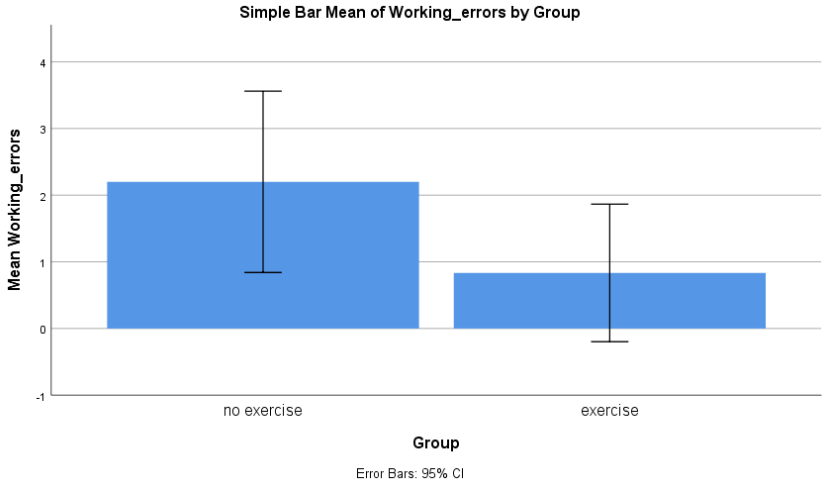

Fig. 11. Comparison of number of working memory errors in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.041)

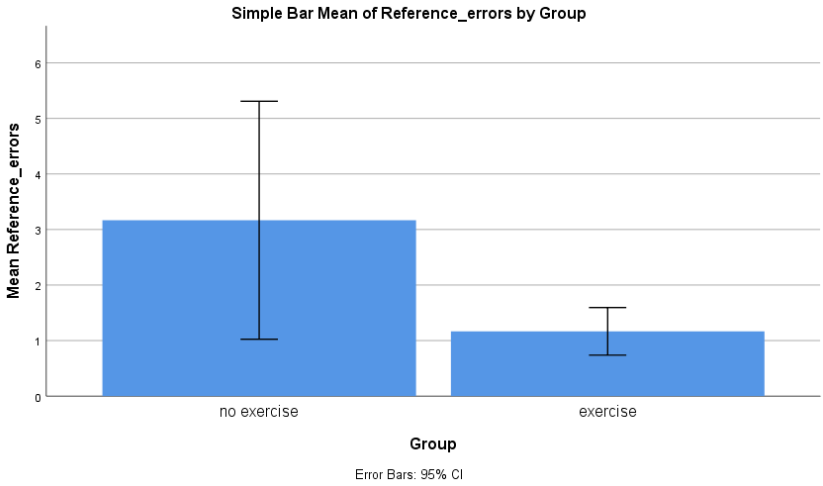

Fig. 12. Comparison of number of reference errors in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.04)

Nevertheless, there was a statistically non-significant difference (p=0.241) between the rats in the control group and the rats distributed in the aerobic exercise intervention group regarding the total time to complete the session F (1, 10) = 1.552.

Although the rats which performed the aerobic exercises presented a lower total time to complete the session (x=183.17, SD=90.6) compared to the control rats (x=213.42, SD =77), this difference was not statistically significant (fig. 13).

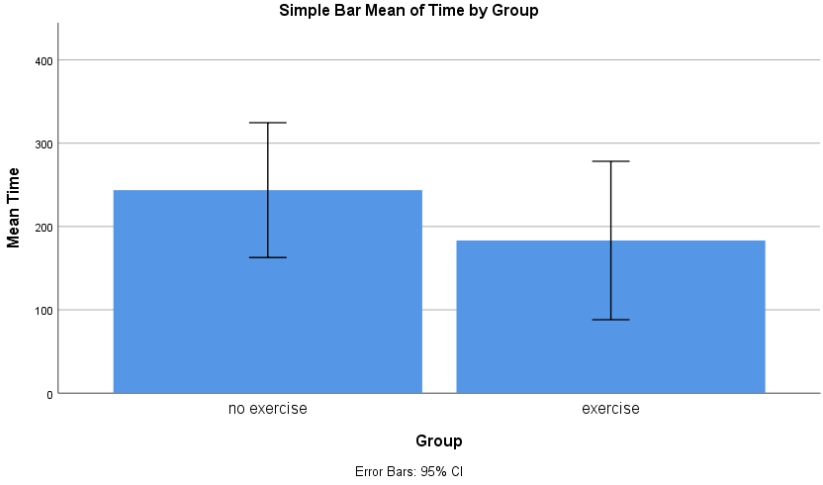

Fig. 13. Comparison of total time to complete the session in the control group *versus* the group receiving the exercise intervention in the animal model of autism induced by valproic acid injection (p=0.241)

DISCUSSION

In our animal model of autism spectrum disorder induced by valproic acid, experimental intervention with aerobic exercise significantly reduced the symptoms of this condition in Wistar rats. These uniform beneficial effects reported above are consistent with most of those reported in the literature. In the forced swim test, as is evident in several previous studies (31, 32), exercise tended to increase the total time of swimming, struggling and mobility and to decrease the time of immobility during the 5-minute test period (effects that have reached statistical significance). One possible explanation would be that although physical activity, as an antidepressant treatment, increases hippocampal BDNF (brain-derived neurotrophic factor) and provides protection against the harmful effects of stress on the hippocampus, the exercise in the current study produced behavioral effects in the test of forced swimming. are comparable to those associated with pharmaceutical antidepressants. Therefore, it appears to be a correlation between hippocampal BDNF levels and motor activity in the forced swimming model after aerobic exercise.

However, some of the previous studies on exercise and the forced swimming test also had mixed results. Some groups of researchers have reported an increase in swimming time during forced swimming after performing aerobic exercise (33-35), and some have not found any significant change or even reported a decrease in the swimming time (36, 37). Therefore, unlike this study, exercise decreased swimming time in other studies, and physical activity also decreased the observed duration of struggle and mobility. It is possible that these contradictory results are explained by the experimental conditions specific to each design, such as the strain of the animals, but especially the duration, type, or the intensity of exercise (38). For example, a longer duration of exercise (two weeks or more) may be required to produce behavioral activation in the forced swim test. Therefore, the significant results of our experiment could be explained by the duration of the intervention through aerobic exercise. In our experimental design, the aerobic exercise was performed on an adapted treadmill for 2 weeks with training sessions consisting of 3 sets of 5 minutes each day.

It is also important to note that the forced swim test in our experiment was performed with a water depth of 30 cm, which is significantly higher than the water depth used by some other studies, 20 cm (32, 38). The depth of the swimming pool could be important because the ability of the animals to feel the bottom of the cylinder with their tails can significantly affect their behavior in the forced swimming test (39).

In addition, the time of day can also be a very important factor contributing to the final results, as our animals were tested at the beginning of the day and it was observed that rats participate in a significantly lower escape-oriented activity towards the end of the day, a result that may be associated with low anxiety and differential hormone levels (40). In addition, it is very important to note that exercise has been reported to produce significant anti-anxiety effects (41-43), and the significant increase in swimming time, fighting and mobility observed in our study may reflect high anxiety during the forced swimming test. Therefore, it has been questioned in recent years whether the decrease in swimming time during the forced swimming test reflects behavioral despair or may denote something completely different (44-46), such as a more relaxed mental state, or a better physical condition that may led to an easier maintaining of the floating posture.

For example, some research groups have explained the decrease in swimming time observed in forced swim test as an indicator of a reduced stress response (40). Decreased anxiety after exercise may also explain the lack of correlation between hippocampal BDNF levels and motor activity in the forced swim test. Increased motor activity, although a specific response to antidepressant stimuli (exercise in our case), may not correlate with hippocampal neuro-protection.

Also, in this study, aerobic exercise intervention had significant effects on our model of autism spectrum disorder obtained by injecting valproic acid. Valproic acid combined with exercise decreased immobility behaviors and also produced a tendency to increase swimming time. Other investigators have shown that selective noradrenaline reuptake inhibitors increase the duration of swimming during the forced swim test and significantly less of this specific behavior is observed in animals treated with serotonin selective agents (47, 48). Because exercise is known to enhance the central release of both norepinephrine and serotonin (49-51), physical activity may have contracted the selective behavioral effects of valproic acid used to induce patterns of autism spectrum disorder. In addition, the serotonin component released by exercise may be responsible for a calming effect on animal behavior.

Our results indicate that exercise interventions may decrease depression-like behaviors in the forced swimming test to a level comparable to regular antidepressants. In addition, previous research has shown that not all antidepressants increase swimming time as well as exercise in our experiment. SSRIs (serotonin reuptake inhibitors), in particular, have been reported to produce a significant positive response in the tail suspension test, but often fail to do so in the forced swimming test (39). However, serotonin reuptake inhibitors are now among the most effective and prescribed antidepressants (39). Yet, some research groups have made changes to the forced swim test that still allow the measurement of antidepressant-like behaviors induced by serotonin reuptake inhibitors, such as increasing the duration of swimming (47, 52).

Regarding the observed anxiolytic effect of physical exercise on our autism animal model, it has been proposed as an explanation an implication of dopamine in fearful emotional responses in the rats (53-55). The mesocortical dopamine system which is projecting from the midbrain to the frontal cortex, is demonstrated to be sensitive to physical and psychological stressors (56). Therefore, physical exercise might reduce the anxiety like symptoms in rats by reducing stress markers in our rats' autism model.

Regarding another possible positive effect that physical exercise might have, studies that measured serotonin levels in the frontal cortex, could not find any significant changes in frontal cortex serotonin levels during and after the physical exercise intervention. Therefore, the observed positive effects of physical exercise in the present study might be explained by the changes in frontal cortex dopamine levels during and especially after the physical exercise intervention.

As previously demonstrated in other studies (57) we found significant differences between the control rats and the rats which received the aerobic exercise intervention in a measure of baseline anxiety as revealed by a 5-min trial on the elevated plus maze. Therefore, in the elevated plus maze control rats showed a significant higher frequency in anxiety-like behaviors compared to rats from the aerobic exercise

intervention. Specifically, the rats which performed the aerobic exercise before being tested in the elevated plus maze presented a higher number of open arms entries and spent significantly more time in the open arms. These findings are important for our autism model given that previous studies have revealed differential effects in stressor reactive and stressor resilient mouse strains to various psychogenic, neurogenic, and systemic stressors (58). This increased anxiety in BTBR mice (BTBR mouse is an inbred mouse strain with face validity as a preclinical model of the core autism symptom domains) was demonstrated in the literature by a decreased amount of time spent in the open arms of the apparatus and an increase in the time spent in closed arms of the apparatus (59). The inverse relationship between the amount of time spent in the open arms and the level of anxiety is of course well demonstrated (60), while several other studies have also shown that an increase in the time spent in the closed arms in the elevated plus maze is a sign of anxiety (57, 61). Therefore, the elevated time spent in open arms observed in the rats which received the aerobic exercise intervention might demonstrate the importance of physical exercise intervention in reducing several autism spectrum disorder symptoms.

The large number of closed arms entries is also considered a sign of high anxiety in rats (62). Interestingly, we were unable to find significant differences between our two experimental groups regarding the number of entries in the closed arms in the elevated plus maze, although the rats from the aerobic exercise group spent significantly less time in the closed arms. Although the entry in the closed arms of the maze is not a typical measure of abnormal social behavior, it has been suggested that it may prevent their engagement in reciprocal social interactions (57, 63, 64). Perhaps it was the energy expenditure from the aerobic effort which prevented us from observing significant differences in the time spent in the closed arms of the elevated plus maze.

Therefore**,** in our animal model of autism spectrum disorder, the rats that received the exercise intervention spent significantly more time swimming compared to the control rats. Moreover, control rats had a longer duration of immobility (measured in seconds) compared to the rats in the exercise group. Therefore, the intervention through aerobic exercise has statistically significantly decreased the level of symptoms similar to depression in the model of forced swimming.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE PROLONGATION OF THE ANTINOCICEPTIVE EFFECTS OF NOVEL NANOSYSTEMS ENTRAPPING DICLOFENAC SODIUM IN SOMATIC PAIN IN RATS

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THE PROLONGATION OF THE ANTINOCICEPTIVE EFFECTS OF NOVEL NANOSYSTEMS ENTRAPPING DICLOFENAC SODIUM IN SOMATIC PAIN IN RATS (Abstract): The prolongation of the antinociceptive effects of novel nanosystems entrapping diclofenac sodium in somatic pain in rats (Abstract): The **aim** of this study was the investigation effects of nanosystems incorporating diclofenac sodium on the nociceptive reactivity in rats. **Material and methods**: In the experimental study were used 24 white healthy, non-genetically modified Wister rats, weighing 200-250 g, with a uniform distribution by sex, purchased from the National Medical-Military Institute "Cantacuzino" for Research and Development, Băneasa, Bucharest. The substances were given orally as follows: Group I (control): distilled water 0.3 mL/100 g body weight, Group 2 (DCF): 5 mg/kg body weight (kbw) diclofenac sodium; Group 3 (DCF-ves): lipid vesicles entrapping diclofenac sodium 5 mg/kbw. The experimental protocol was approved by the University Committee for Research and Ethical Issues, in compliance with the international ethical guidelines regarding the handling of laboratory animals. Data were statistically processed using *SPSS software for Windows 17.0* version and ANOVA one-way method. **Results**: Lipid vesicles containing diclofenac, stabilized with chitosan were designed. The measurements indicated a mean size of 562 nm and a mean Zeta potential of +45 mV, thus suggest a reasonable stability of the colloidal suspension. The use of DCF resulted in a prolongation of the latency period response to thermal noxious tail stimulation, with a statistical significance during 90 minutes in the experiment. The treatment with DCF-ves was accompanied by a substantial increase of the latency time reaction in the interval between 2 hours and 8 hours after the administration. **Conclusions:** Both entrapped and non-entrapped DCF proved to have an important analgesic effect in tail flick test in rats. The administration of lipid vesicles as carriers for DCF demonstrated a prolonged antinociceptive activity, compared to the non-entrapped drug in this cutaneous pain model in rats. **Keywords:** LIPID VESICLES, DICLOFENAC SODIUM, TAIL FLICK, NOCICEPTION, RATS.

Obtaining and studying various nanoformulations incorporating pharmacologically active substances, represents a modern direction of research and is an attractive starting point for the identification of new drug delivery systems with therapeutic

benefits in various pathological conditions. The study is in line with modern trends in scientific research, which aims to assess the effects of drugs or drug delivery systems in various pathological conditions (accompanied by pain and inflammation), which can be found in medical practice. Experimental investigations will be able to highlight the advantages of using original nanoformulations containing diclofenac on the cutaneous nociceptive reactivity in rats.

Among all nanosystems developed, liposomes have proven to be some of the most established drug delivery systems due to their small size, good biocompatibility, increased biodegradability, hydrophobic and hydrophilic character, low toxicity and immunogenicity (1, 2). A vast literature describes the feasibility of encapsulating a wide range of drugs, including anticancer drugs, antimicrobial agents, analgesics, peptide hormones, enzymes and genetic materials, in the aqueous or lipid phases of liposomes that have shown an enhanced therapeutic activity and/or reduced toxicity compared to the non-liposomal formulations, in various preclinical models or in clinical trials (3, 4).

One of the most interesting and, at the same time, with great clinical utility is the use of nanosystems that incorporate non-steroidal anti-inflammatory drugs (NSAIDs), with the idea of prolonging the pharmacological effects and at the same time, of reducing the adverse effects, especially the gastrointestinal irritation (5).

Diclofenac is a phenylacetic acid derivative, structurally related and with pharmaco-logical properties similar to mefenamic acid and sodium meclofenamate. It blocks the both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), being used to counteract the acute and chronic pain associated with inflammatory pathology. There are evidence that diclofenac inhibits lipoxygenases, as well as phospholipase A2, and activates the antinociceptive nitric oxide-guanzine monophosphate (GMP) pathway. These additional actions may explain the high potency of diclofenac, which is considered one of the most powerful NSAIDs (6).

Diclofenac is an active drug belonging to class II (according to the biopharmaceutical classification system) with a high degree of permeability, but with a low solubility (7). Characteristically, it has an acidity constant of level 4, (considered to be a weak acid) and a partition coefficient of 13.4, which signifies a partial solubility in both aqueous and hydrophobic media. Other structural characteristics of the molecule, respectively, the presence of the phenylacetic acid group and a phenyl ring that contain two chlorine atoms, facilitate the maximum torsion of the phenyl ring, a fact that ensures the proper binding coupling to cyclooxygenases (8, 9). It has a reduced oral bioavailability due to its poor solubility in water and in the gastric acid environment. It is eliminated 65% through the urine, has an enterohepatic circuit and is concentrated 35% in the bile. It has a relatively long duration of action (6-8 hours), but a half-life of 1.5-2 hours, which requires its administration in 3-4 daily doses (10).

Nanoparticles incorporating diclofenac sodium could represent innovative delivery systems with potential for prolonged action and lower side effects in rats, with advantages over use compared to the unincorporated drug.

MATERIAL AND METHODS

Substances

The test substance (diclofenac sodium) and those required for the preparation of

nanoparticles (chitosan, phosphatidylcholine, cholesterol, chloroform) were procured from Sigma-Aldrich Chemical Co., Germany (www.sigma-aldrich.com). The chitosan used had a degree of N-deacetylation of 79.7%, the average molecular weight was Mw = 310,000 g/mol and 3.26 polydispersity index. The 0.5% (w/w) chitosan solution was prepared in a 0.5% (v/v) acetic acid solution. The lipid used was L- α -phosphatidylcholine, type XVI-E, approximately 99% pure. Distilled water was obtained from Sicomed, Romania.

The preparation of lipid vesicles containing diclofenac

The lipid vesicles were prepared by dissolving the lipid in chloroform and removing the solvent by evaporation (11). The obtained dry lipid film was hydrated using a diclofenac solution in alcohol and the resulted solution was sonicated for 20 minutes. The lipid vesicles were mixed with the chitosan solution, which was thereafter dialyzed for 10 hours, to increase the pH to values close to the physiological ones.

To characterize the obtained particles by dimension and Zeta potential a Malvern Zetasizer Nano ZS ZEN-3500 apparatus (Worchestershire, UK) was used.

In vivo Experiments

For the experimental researches were used, 24 healthy, non-genetically modified white Wistar rats (3-month-old), weighing 200-250 g, with a uniform distribution by sex (half males, half females), which were purchased from the National Medical-Military Institute "Cantacuzino" for Research and Development, Băneasa Resort, Bucharest, Romania and brought to the CEMEX laboratory ("Advanced Center for Research and Development in Experimental Medicine - CEMEX") biobase, of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi.

The animals were kept under standard laboratory environment (relative humidity 55-65%, constant air temperature 23.0±2.0°C and 12 hours light: dark regimen). Before the experiment, rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

The rats were fed fully with standardized solid specific food (pellets). The amount of food ingested by each animal was measured daily. Drinking water was administered *ad libitum*. In the days of the experiments, the animals were deprived of food and fluids. To prevent chronobiological influences, the tests were performed between 8-12 a.m. each day.

The laboratory animals were assigned into 3 groups of 5 rats each, which received the substances directly in the stomach (using an eso-gastric device), according to the following scheme:

- **Group 1 (Control):** distilled water 0.3 mL/100 g body - control;
- **Group 2 (DCF):** 5 mg/kg body weight (kbw) diclofenac sodium;
- **Group 3 (DCF-ves):** chitosan-based nanoparticles entrapping diclofenac sodium 5 mg/kbw;

The tail flick test (tail withdrawal test) is a thermoalgesic test that evaluates the reactivity to pain, by measuring the reaction time of animals whose tail is exposed to an infrared source of radiant energy, at a temperature of 50˚C (Tail flick Apparatus, UgoBasile, Pennsylvania, United States) which causes the tail withdrawal reaction. The response latency period is measured before (time zero) of the experiment, and 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 4 hours, 6 hours, 8 hours, 10 hours and 12 hours after the ad-

ministration of the testing substance. The maximum allowed time (cut-off time) for each test is 12 seconds, to prevent damage to the biologic tissues (12, 13).

The results of the response to the tail flick test are expressed as mean values ± standard deviation of the mean (D.S.) of the latency time of the response to the painful thermal stimulus, at each time point and for each studied substance. To quantify the antinociception intensity, latencies are converted to percentages of the maximum possible effect (%EMP) according to the following formula:

%MPE = (observed latency – baseline latency) x100 / (cut of time – baseline latency) (14)

Changes in the pain threshold, obtained after the administration of the substances investigated, were considered relevant above 60% of EMP, because values below 60%, although may have statistical significance, the biological one is debatable.

The prolongation of the reaction time compared to the values obtained before and after the administration of the tested compounds or of the control group, corresponds to an antinociceptive effect. Shortening the latency of the response signify a hyperalgesic activity of the investigated substance (13).

The experimental protocol was approved (Certificate No. 24 / 21.12.2020) by the University Committee for Research and Ethical Issues, in compliance with the international ethical guidelines regarding the handling of laboratory animals (15, 16).

RESULTS

Soft matter lipid vesicles containing DCF, based on phosphatidylcholine and stabilized with chitosan were designed. The prepared DCF-ves were found to have a mean dimension of 562 nm and +45 mV mean Zeta potential, thus correspond to a relatively good stability of the solution.

The influence of DCF and the microvesicles containing DCF was evaluated in tail flick test, a standard somatic pain model in rodents.

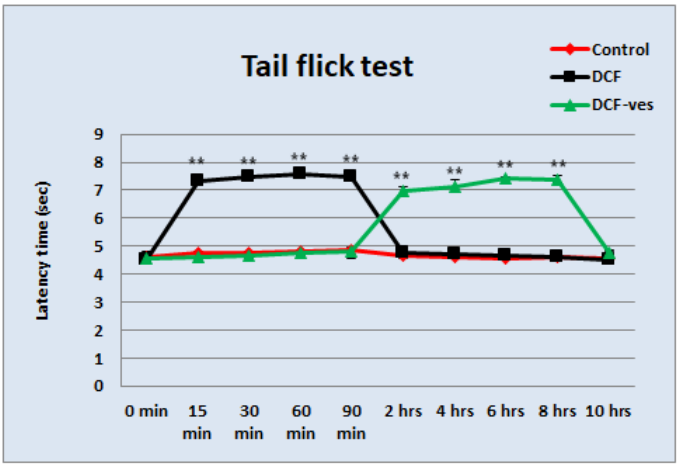

Fig. 1. The effects of DCF and DCF-ves in tail flick test in rats. Each point expresses the arithmetic mean ± SD of mean values for five animals in a group. The values of *p* coefficient (probability) **p<0.01 are considered statistically significant *versus* control.

Statistical analysis of data revealed that the probability associated with the calculated value of the Fisher test is higher (Sig. = 0.227) than the risk α=0.05, which means that there are no obvious differences between the three studied groups of animals at moment zero, respectively after 10 hours in tail flick test. No substantial variation of the latency time response to noxious thermal tail stimulation, at baseline, as well as at 10 hours, between the control, DCF and DCF-n groups.

The treatment with DCF was associated by a rapid and progressive prolongation of response latency period, statistically significant (**p<0.01) compared to the control group from 15 minutes (7.35±0.16 seconds) to 90 minutes (7.48±0.12 seconds) in this somatic pain model in rats. The longer latency time was observed at 60 minutes (7.58±0.12 seconds) after the beginning of the experiment. The obtained results are in accordance with the communicated data about the analgesic effect of this NSAID in tail flick test in rodents (17, 18).

The use of DCF-ves was associated with the lengthening of the latency period of the response to painful thermal tail stimulation, statistically significant (**p<0.01) compared to the control, in the interval between 2 hours (6.95±0.16 seconds) and 8 hours (7.40±0.11 seconds), in tail flick test in rat. The peak increase in the latency time was noted after 6 hours (7.45±0.05 seconds) in the experiment, after that, the nociceptive reactivity was gradually decreased to 8 hours.

Supplementary, %MPE, was used to assess the strength of antinociception, which represents an essential parameter to estimate the biological significance of the effects. The quantification of the intensity of the analgesic effect highlighted that the treatment with DCF resulted in an antinociceptive effect which was manifested immediately after administration of the substance, had a peak at 60 minutes (40.8±0.2 %EMP), and was maintained for 90 minutes (40.8±1.3 %EMP) in this cutaneous pain model in rats.

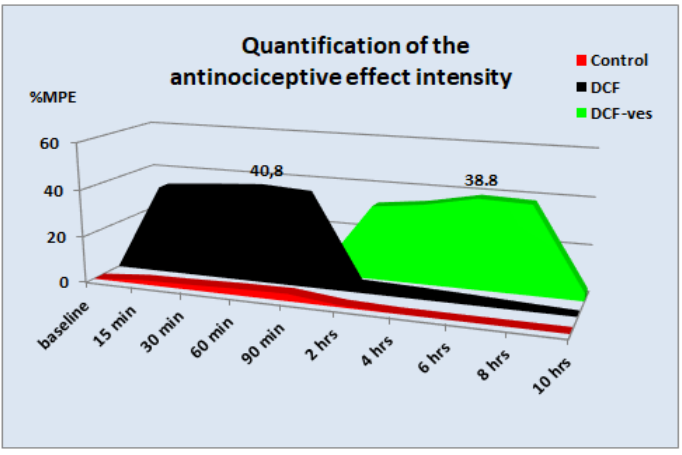

Fig. 2. Maximum possible effect (%MPE) produced by DCF and DCF-n in tail flick test in rats. Each point expresses the arithmetic mean ± S.D. of mean values for five animals in a group.

The use of DCF-ves resulted in a significant antinociceptive action, which started after a latency period of 120 minutes (32.0±1.7 %EMP) and lasted up to 8 hours (38.1±0.9 %EMP) in the experiment. The treatment with DCF-ves induced a %MPE (38.8±0.12) of analgesia at 6 hours in the experiment. The %EMP produced by DCF and DCF-ves were below 60% at any time of the determinations.

DISCUSSION

In the case of diclofenac sodium, it is a drug from the pharmacological class called non-steroidal anti-inflammatory drugs (NSAIDs) (19). Diclofenac sodium was developed to synthesize an NSAID with a high level of activity and good tolerability, having a percentage of hepatic metabolism of 50-52%. From an analgesic point of view, it is 6 times stronger than indomethacin, sulindac or codeine, 15 times stronger than naproxen and more than 40 times stronger than acetylsalicylic acid. It has low oral bioavailability due to its low solubility in water and in the gastric acid environment. It is eliminated in the proportion of 65% through the urine, has an enterohepatic circuit and is concentrated 35% in the bile. It has a relatively long duration of action (6-8 hours), but a half-life of 1.5-2 hours, which is why it needs to be administered in 3-4 daily doses (6).

In order to improve the pharmacokinetic profile, the pharmacodynamic effects and to reduce adverse effects, especially gastrointestinal, several types of nanoparticles with diclofenac were designed, using various materials including poly acid (lactide-co-glycolide); chitosan/poly methacrylic acid or polypyrrole/menthol; polyvinyl alcohol or didodecyldimethylammonium bromide (20-22).

Lipid-based formulations have been shown to significantly improve the bioavailability of hydrophobic drugs such as diclofenac compared to conventional dosage forms (23). The mucus covering the surface epithelium of the gastrointestinal tract possesses an adsorbent layer of phospholipids, which creates a hydrophobic zone between the epithelium and the luminal contents. At this level there are a number of lipid species reminiscent of surface-active phospholipids, of which phosphatidylcholine is suitable to incorporate diclofenac. It has been shown that in the presence of the ionic surfactant shield, the complex remains lipophilic (24).

Among all nanoparticulate systems, liposomes have a number of advantages, represented by the low number of excipients required for their formulation, the simple preparation procedure, a high physical stability and the possibility of sustained drug release, which could be used in the treatment of various pathological conditions (25).

Particulate systems such as micro- and nanoparticles contain an active principle in the core of the particle, which is called encapsulation. The purpose of encapsulation is to protect the unstable active ingredients, to achieve a modified, most often prolonged release, and to target the action of the active ingredients (24, 26).

Liposomes that efficiently incorporate diclofenac, based on chitosan of different molecular weights, were obtained using an ionotropic gelation method, formulations that were shown to have significant antibacterial activity (27).

We investigated the influence on the somatic cutaneous nociceptive reactivity of DCF and micro-particles containing DCF in tail withdrawal experimental model in rats. We proved that the use of both entrapped and non-entrapped DCF was accompanied by a considerable analgesic effect. The antinociceptive action of the non-entrapped

NSAID was evident in the first 90 minute, while the analgesic influence of micro-vesicles with DCF was manifested between 2 hours and 8 hours in this somatic pain model. The delayed appearance of the DCF-ves's pharmacodynamics effects can be attributed to the way in which the active substance is released from lipid micro-vesicles stabilized with chitosan.

Different studies have shown that the use of diclofenac sodium microsphere systems, based on sodium alginate, obtained by ionotropic gelation methods, improved the release profile of diclofenac, *in vitro* but also *in vivo* in the case of oral administration in healthy volunteers (28), while another study demonstrated a high degree of absorption of this anti-inflammatory incorporated into sodium alginate/ nanocrystal cellulose/polyvinyl alcohol microspheres functionalized with polyethyleneimine (29). Other researchers have prepared by a thin film hydration technique, wide lamellar vesicles of diclofenac sodium using soy lecithin, cholesterol and as solvents ethanol or a mixture of chloroform: methanol, but the effects in the laboratory animal have not been investigated (30-32). Another nanoparticle design consisted of encapsulating diclofenac sodium in phosphatidylcholine, cholesterol, stearyl amine and dicetylphosphate, through a lipid hydration technique (28). These liposomes have been well characterized, in vitro drug release has been described, but there are no data on the effects of this formulation system in laboratory animals.

CONCLUSIONS

Original micro-particles entrapping diclofenac with a mean size of 562 nm and a reasonable stability in suspension were prepared. The treatment with diclofenac incorporated and not incorporated in micro-vesicles produces significant analgesic effects in the laboratory animal in the tail flick test. The use of soft matter vesicles stabilized with chitosan as carriers for diclofenac presented the advantage of drug's sustained release, and a prolongation of the antinociceptive effects, comparing with non-entrapped substance in this somatic pain model in rats.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding the scientific research.

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WHY ARE TEACHERS NOT ALWAYS WILLING TO FIGHT AGAINST CYBERBULLYING? EXPLORING THE BELIEFS, ATTITUDES AND COPING STRATEGIES OF TEACHERS IN A MULTI-COUNTRY CROSS-SECTIONAL STUDY

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WHY ARE TEACHERS NOT ALWAYS WILLING TO FIGHT AGAINST CYBER-BULLYING? EXPLORING THE BELIEFS, ATTITUDES AND COPING STRATEGIES OF TEACHERS IN A MULTI-COUNTRY CROSS-SECTIONAL STUDY (Abstract): The increasing phenomenon of cyberbullying among adolescents in all countries needs preventive, educational, legal, and psycho-interventional measures. The present study **aimed** to identify the opinions of teachers about cyberbullying and their coping strategies. **Material and methods:** The study included 798 teachers from six countries (Italy, Portugal, Greece, Türkiye, Lithuania, and Romania). An online questionnaire was especially constructed for this research, gathering socio-demographic and job-related data along with information about teachers' opinions about the cyberbullying phenomenon, about victims and perpetrators, and their most frequent behaviors and coping strategies used to deal with the problem. The questionnaire also included 3 psychometric instruments. **Results:** The analysis of questionnaires revealed that even if only 38.5% of the teachers took a course on cyberbullying, more than 70% of them answered that they knew how to deal with cyberbullying. In teachers' opinions, cyberbullying occurs equally in boys and girls. The age 11-14 is the most exposed to bullying, while 15-18 is the most exposed to cyberbullying. More than 60% considered that students were responsible for bullying and cyberbullying. Age, gender, length of experience in the field of education, type of institution, and marital status influence cyberbullying perceptions and coping strategies. **Conclusions:** Personal beliefs and the occurrence of observed and reported incidents determine teachers' willingness to intervene in cyberbullying. The results are important for teachers and trainers to consider the teachers' role in analyzing both duties and barriers in fighting the cyberbullying phenomenon. **Keywords:** CYBERBULLYING, ADOLESCENT, VICTIM, PERPETRATOR, BYSTANDER, TEACHER, SCHOOL, COPING STRATEGIES.

Teaching is a noble profession that needs a powerful determination for life-long learning about science, skills, and communication techniques in order not only to transfer knowledge or to develop skills and capabilities but also to create emotion, build self-confidence, and stimulate curiosity in all kinds of students. Teachers enrich minds and souls, develop behaviors, form characters, and shape personalities. Involvement in the transformation and progress of students is inevitably accompanied by their own development.

The student–teacher trust relationship uniquely contributes to students' performance through school adjustment and academic motivation (1). Scholars discovered that a lack of trust between students and teachers, as well as feeling unsafe in school, increase the risk of mental health problems, traditional bullying, cyberbullying, various health problems, a low level of well-being and quality of life, and learning difficulties (2, 3). According to Wang and Degol (4) a safe school environment consists of three components: physical safety (reduced violence and aggression), emotional safety (lack of traditional bullying and psychological support and counseling), and order and discipline (school rules, conflict resolution, clarity, and fairness in decision making).

As a result, some studies have linked a deteriorating school climate to increased aggressive behavior among adolescents (5). Bullying, in all its forms, is an increasing phenomenon in all countries, especially in developed ones (6, 7, 8). Due to the increased use of the internet for academic tasks but also for communication, fun, social networking, or entertainment, the online bullying, also known as *cyberbullying*, has become a systemic public health issue (9) with a great impact on the physical, psychological, and social health of all those involved (victims, perpetrators, and bystanders). Cyberbullying rates worldwide vary from 2% to 56% and the "EU KIDS ONLINE" study of over 25,000 children between the ages of 9 and 16 reported a prevalence of cybervictimization ranging between 2 and 14%, the highest rates being found in Estonia and Romania and the lowest in Italy and Portugal (10) while Ortega and colleagues found a rate between 4 and 6.2% in Spain, Italy and UK (6, 7, 8).

Cyberbullying (CB) has been defined as an aggressive and deliberate behavior that is frequently repeated over time, carried out by a group or an individual using electronics and aimed at a victim who cannot defend himself/herself (11). The impact of cybervictimization has both short-term and long-term consequences, such as depression, anxiety, sleep-troubles, drop-off, suicide thoughts, self-isolation, and inconsistency in behavior (12-15). Due to its consequences on students' psychological, physical and social lives, and considering the negative impact on their academic performance, teachers are the key to solving bullying problems.

Reporting bullying behaviors to adults is crucial. According to Blomqvist *et al.* (16), being told about victimization was the best single predictor of teachers' intervention, frequently exceeding the effect of direct teachers' observations. Reports by victims are often teachers' only source of information about aggressive behaviors that take place in and outside the school environment. Bokkel *et al.* (17) mentioned that teachers are the only people to whom victimized students turn because of their proximity to all students (especially those hav-

ing difficult relationships with other adults, including parents) and their responsibility to create a safe classroom climate.

Data showed that when victimized students decide to tell someone about being bullied, they tend to tell friends rather than adults (16), and they tell parents more frequently than teachers (18,19). In fact, looking for help in school environments is low, and only 3-18% of the victims report the incidents to teachers. The decision to disclose to a teacher is a complex trade-off between potential costs and losses. First, they may be anxious that their situation will worsen when they tell their teacher (20). Further, students may be ashamed, convinced they will not be taken seriously or even be blamed by teachers for provoking or not standing up to victimization (21). In turn, teachers may misinterpret non-disclosure by a victimized student as the absence of a need for support, while the student might have that need. This begs the question to what extent teachers can facilitate disclosure. Cortes & Kochenderfer-Ladd (22) showed that punishing bullies can be associated with a lower likelihood of reporting to a teacher.

Teachers and other adults at school seemed to fail to recognize bullies, and the lack of identifying the victims led to a lack of appropriate intervention and encouraged bullying acts to continue. Blomqvist *et al.* (16) identified that the factors related to increased likelihood of telling an adult about cybervictimization were female gender, age, the chronicity of victimization, perceived negative teacher attitude towards bullying (teacher not tolerating bullying), and perceived peer support for victims (classmates' tendency to defend students who are victimized). Shaw (23) highlighted that talking about victimization was more likely among students who had been bullied for longer periods of time, with the result suggesting that when teachers found out about cyberbullying incidents after a considerable period of time, the students failed to stop the acts of aggression. When teachers do not intervene immediately, bullies may perceive an implicit consent to keep up their behavior (24). On the other hand, bystanders may feel less motivated to intervene with victims and report or blame the perpetrators (25). Therefore, a vicious circle is constructed and maintained.

All these aspects identified by a plethora of studies proved that dealing with cyberaggression and cybervictimization is a challenging task for teachers. The prevalence of cyberbullying involvement is largely inconsistent. Recent studies and meta-analyses indicated that bully prevention programs produce minimal change in student aggressive behavior and that there was a need for increased training for teachers on how to identify and combat cyberbullying (16, 23, 25). Despite the progress in cyberbullying research in recent years and the programs that were implemented in diverse countries, cyberbullying is still a challenging task for teachers. To cover the nowadays space, the aims of the study were: a) to identify the personal beliefs about this phenomenon among teachers and b) to identify the attitudes and strategies used to cope with cyberbullying among pre-university teachers in six countries and factors that influence them. Based on these, we assessed if there will be differences in what concern cyberbullying perception and strategies used to cope with cyberbullying considering the variables gender, number of children, marital status, length of experience, type of institution and experience with cyberbullying.

MATERIAL AND METHODS

Participants, eligibility criteria and informed consent

The study was approved by the Institutional Review Board No. 192/30.04.2022 of EuroEd School, Iasi, Romania. The questionnaire was distributed online simultaneously among teachers from six countries (Romania, Greece, Lithuania, Portugal, Italy, and Türkiye) in twenty-two middle and high schools from rural and urban areas between May 1st to June 30th, 2022.

To facilitate the approach of the group of teachers, invitations to complete the survey were sent by individual schools. The researchers first informed the principals of the schools regarding the study and the questionnaire was distributed to the teaching staff. Participants were informed online about the purpose of the study and ethical principles of the research (voluntary participation, protecting the confidentiality of data, the right to withdraw from the research at any time, being an *ERASMUS+* research and principal investigator contact). No incentive was given to the participants. After providing digital informed consent, participants were asked to complete the questionnaire.

The inclusion criteria were questionnaires filled in by teachers enrolled in private, public, or special schools, submitting fully filled in questionnaires before the deadline. The criteria for excluding questionnaires from the research were questionnaires not fully completed or submitted after the deadline. A total of 853 surveys were filled in. After excluding a number of 55, finally 798 questionnaires were included in the research. Figure 1 provides details on the response rate.

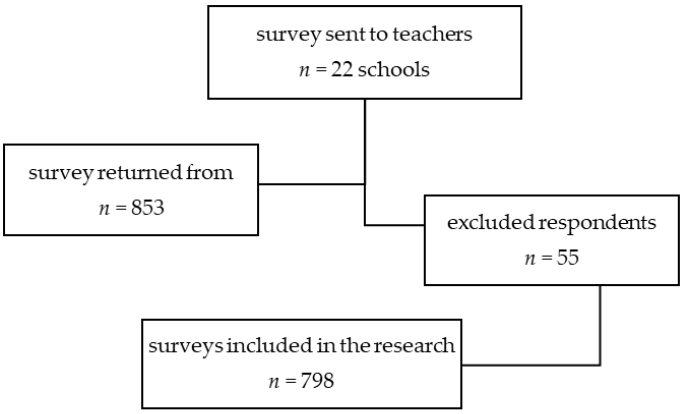

Fig. 1. Study profile.

Data collection and instruments

The questionnaire was constructed using the Google Forms application (Alphabet, Mountain View, CA, USA), was translated from English into all six national languages, and was developed to address the prevention, recognition, and intervention of online harassment against its cruel social, psychological, medical, and educational impact on adolescents.

a) The first section of the questionnaire collected socio-demographic information

(such as country, age, gender, marital status, number of kids, profession, length of experience, total number of years in teaching at their current school, and type of school where they teach).

b) The second part contained items about the teachers' opinions regarding the knowledge of bullying and cyberbullying behaviors, including their skills in recognizing the signs of bullying, preventing, and acting in such incidents among their students. Answers were registered with YES/NO or TRUE/FALSE about if they have taken anti-bullying courses; if they think that bullying is more common than cyberbullying; if they think that bullying and cyberbullying occur more frequently in the case of girls; if they teach students how to deal with bullying behaviors; and what they consider to be the age range at which bullying and cyberbullying occur most frequently.

c) The third part examines teachers' perceptions about cyberbullying by using a questionnaire developed by Li in 2009 (26). The instrument contains 22 items regarding perceptions about cyberbullying and about teachers' educational experiences in relation to cyberbullying. Responses to each item were indicated using a five-point Likert scale, with responses ranging from strongly disagree to strongly agree.

d) The fourth part of the survey collected information about teachers' views on the actions that teachers, parents, school principals, or even the Ministry of Education should take in order to prevent cyberbullying in schools. Participants also answered questions about those responsible for bullying or cyberbullying (students, parents, teachers, or the school) as well as the school's clear position on bullying, compliance or non-compliance against bullying, the impact of cyberbullying on students, and how teachers or the school principal intervene in cases of observing such behaviors. In addition, teachers were asked whether they agreed that students were exaggerating about cyberbullying behaviors, whether students should manage bullying and cyberbullying on their own, and whether they were confident that they could recognize and manage bullying and cyberbullying situations. Self-assessed items were constructed, and responses were assessed on a 5-point Likert scale.

e) In the next section, respondents were asked to tick the number that best matches the following statements (on a Likert scale from 1 to 5, where 1 = never and 5 = always): requesting a discussion with parents when it comes to issues related to cyberbullying among students, sending students to the principal when it comes to cyberbullying, or not involving teachers in these issues. Respondents continued to answer YES/NO to questions about rumors that students were harassing each other during or outside class; if cyberbullying intensified among teenagers; if some students even complained to teachers about the situations of cyberbullying in which they had taken part, if the teachers knew of students who were harassing or had been harassed online; if parents, the school principal, or the school counsellor were informed; if teachers were told or even witnessed bullying behavior; and not least if teachers considered that cyberbullying could be reduced by prevention or intervention.

f) The sixth section includes questions about the frequency of bullying and cyberbullying behaviors observed or reported by others among students, and how often teachers intervened to discipline or

counsel students during this behavior. Answers range on a Likert scale from 1 (several times a day) to 7 (never). In addition, some questions were raised about the risks that some children had (obese, mentally ill, with physical syndromes, those with a single parent, those with a precarious economic situation, introverts, those with a high or low level of intelligence, or those with poor school results) could be subjected to cyberbullying. Answers range on a Likert scale from 1 (very low risk) to 5 (very high risk).

g) The final part of the questionnaire addressed two psychometric instruments used to identify the ways teachers succeeded in perceiving cyberbullying problems and to identify what kinds of strategies they usually used in order to deal with this phenomenon among their students:

The *Teacher Cyberbullying Perception Scale* (TCPS) is a 14-item scale which was created by Ayas and Horzum in 2011 (27) to determine how well teachers perceived the problems of cyberbullying among their students. A 5-point Likert-type rating was used to express the level of agreement with the items on the scale. This rating is: always (5), often (4), sometimes (3), rarely (2), and never (1).

The *Strategies for Coping with Cyberbullying Scale for Teachers* (SSBCS) was created in 2018 by Altundag and Ayas (28). It is a measurement tool for the coping strategies of teachers often witnessing cyberbullying events experienced by adolescents. The measurement tool consists of two sub-domains, the 'knowledge area' and the 'application area'. The scale was prepared in a five-point Likert type, with responses ranging from 1 (strongly disagree) to 5 (strongly agree). High scores on both the knowledge and application areas of the scale indicated the high level of teachers' perceptions of cyberbullying coping strategies.

Statistical analysis

All analyses for this research were performed using IBM *Statistical Package for Social Sciences* (*SPSS*) Statistics for Windows, version 29 (SPSS Inc., Chicago, IL, USA). Results for descriptive statistics were expressed as means and standard deviations (SD).

The normality of data distribution was assessed by using the Kolmogorov-Smirnoff test. Given the fact that all data points are not normally distributed, bivariate analysis will be performed and non-parametric tests will be applied.

To assess comparative results considering gender, profession, and taking anti-bullying classes, the Mann-Whitney test was performed. Similarly, comparative results considering country, marital status, the number of children from a household, and the type of school where the respondents teach were assessed using the Kruskal-Wallis H test to determine if there were statistically significant differences between more of two groups of an independent variable on a continuous or ordinal dependent variable.

The Spearman correlation was used to assess the relationship between variables. A p-value < 0.05 was considered statistically significant.

RESULTS

Socio-demographic data, family characteristics, professional details

Teachers included in the research were from six different countries: Romania (n = 343, 43.0%), Türkiye (n = 197, 24.7%), Portugal (n = 94, 11.8%), Greece (n = 59,

7.4%), Lithuania (n = 59, 7.4%), and Italy (n = 46, 5.77%). More than half of the participants were married (n = 556, 69.7%) and had an average of M = 1.35 ± 0.97 children.

The mean age of the teachers participating in the study was M = 46.90 ± 9.66 with a minimum of 20 and a maximum of 68 years old, more than 3/4 being women (76.47%). The results of the study showed that the participants had practiced their profession for an average of 21.29 ± 10.76 years. In the school where the respondents now teach, they had been within the department for M = 11.19 ± 9.46 years.

Most of the respondents included in the study were teachers (n = 753, 94.4%) and very few were school counselors (n = 45, 5.6%). Moreover, most teachers worked in public schools (n = 718, 90.0%).

Teachers' opinion about the age and gender of students who are more prone to experiencing cyberbullying.

More than half of the teachers had never taken anti-bullying courses (n = 491, 61.5%). However, more than half of the teachers considered that they knew how to deal with cyberbullying (n = 560, 70.2%).

More than half of the teachers believed that bullying occurred more often than cyberbullying (n = 509, 63.8%), and a similar percentage believed that bullying (n = 499, 62.5%) and cyberbullying (n = 502, 62.9%) did not occur more frequently in girls.

Moreover, more than half of the teachers pointed out that the age most prone to bullying is between 11-14 years old (n = 489, 61.3%), followed by the categories of 15-18 (n = 252, 31.6%) and 6-10 years old (n = 57, 7.1%), while in terms of cyberbullying, the most exposed age categories are approximately equal: 15-18 (n = 405, 50.8%) and 11-14 (n = 367, 46.0%) years old**.**

Teachers' perceptions of cyberbullying

The third part of the research focused on teachers' perceptions of cyberbullying and their educational experiences in relation to cyberbullying. Answers for each perception item were indicated using a five-point Likert scale, with responses ranging from 1 (strong disagreement) to 5 (strong agreement). The Alpha coefficient of the internal reliability of the instrument was 0.90.

The majority of the teachers also agreed that they were concerned about cyberbullying. Data analysis showed that only a quarter of the teachers thought that cyberbullying was a problem in schools, while less than half held neutral positions. When asked if the children were affected by cyberbullying, the pattern changed. Approximately 65% of the teachers agreed that cyberbullying affected children. Regarding teachers' confidence in approaching cyberbullying, it was found that about half of them had confidence in both identifying and managing cyberbullying issues with a gender difference (teachers' confidence in approaching cyber-bullying.

Teachers' beliefs about the importance of school engagement were examined and were broadly defined to include aspects from school policy and classroom strategies to school activities. Thus, the results showed that more than 75% of the participants agreed that all these strategies should be addressed to prevent or deal with the problem of cyber harassment.

Finally, the questionnaire aimed to find out how prepared the teachers were to

manage bullying and cyberbullying incidents. The responses showed that less than half of the teachers felt that their current university education did not prepare them to deal with cyberbullying, and more than half of them wanted to spend more time studying to learn more about cyberbullying. Detailed results regarding the answers to the mentioned items are presented in first table.

TABLE I.
Teachers' perceptions about cyberbullying[1]

ITEMS	Disagree or strongly disagree	Neutral	Agree or strongly agree
Problems in schools	255 (32.0)	334 (41.9)	209 (26.2)
Children are affected	112 (14.1)	166 (20.8)	520 (65.2)
Teachers are concerned	49 (6.1)	144 (18)	605 (75.8)
Identifying cyberbullying	93 (11.7)	216 (27.1)	487 (61)
Managing cyberbullying	13, (17.5)	281 (35.2)	378 (47.4)
Schools' policies	30, (3.8)	97 (12.2)	670 (84)
Training teachers	34 (4.3)	69 (8.6)	695 (87.1)
Curriculum	54 (6.8)	134 (16.8)	609 (76.3)
Classroom activities	4 (5.3)	113 (14.2)	643 (80.6)
School-wide activities	31 (3.9)	79 (9.9)	688 (86.2)
Discussing with parents	29 (3.7)	87 (10.9)	681 (85.3)
University prepares teachers	350 (43.9)	183 (22.9)	263 (32.9)
Teachers want to learn more	110 (13.8)	189 (23.7)	498 (62.4)

*[1]Number (n) and percentages (%).

Scale results for teachers' perceptions of cyberbullying and their educational experiences in relation to cyberbullying ranged from 27 to 109 ($M = 84.05 \pm 10.47$).

The Mann-Whitney U test revealed significant gender differences ($U = 47726.50$, $Z = -2.975$, $p = 0.003$), with men ($Mdn = 83.00$) scoring lower on the scale that measures teachers' educational experiences in relation to cyberbullying than women ($Mdn = 85.00$) but higher regarding the confidence in approaching cyberbullying ($U = 48590{,}000$, $Z = -3.222$, $p = 0.001$), showing that male teachers ($Md = 4$) were more confident than women ($Md = 3$).

Significant differences were found regarding the type of institution in which teachers work ($U = 19710.00$, $Z = -3.837$, $p < 0.001$) in the sense that teachers from public schools ($Mdn = 85.00$) had higher scores on this scale compared to teachers from private schools ($Mdn = 81.00$). Additionally, a Mann-Whitney U test revealed significant differences in terms of participants' marital status ($U = 3572.50$, $Z = -2.630$, $p = 0.009$) in the sense that married teachers ($Mdn = 84.00$) had higher scores on the scale which measured teachers' perceptions and educational experiences in

relation to cyberbullying than single teachers (Mdn = 78.00).

A Kruskal-Wallis test was conducted to determine if there were differences in these scale scores among teachers between countries of origin. As assessed by visual inspection of a boxplot, the distributions of these scale scores were not similar for all groups. Median scores were statistically significantly different between the countries, $\chi^2(5) = 94.528$, $p < 0.001$. A pairwise comparison method was performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted *p*-values are presented. This post hoc analysis revealed statistically significant differences in median scores of this scale between: Romania (85.00) and Lithuania (81.00) ($p = 0.002$), Romania (85.00) and Greece (70.00) ($p < 0.001$), Romania (85.00) and Portugal (89.00) ($p = 0.003$), Greece (70.00) and Portugal (89.00) ($p < 0.001$), Greece (70.00) and Italy (86.00) ($p < 0.001$), Portugal (89.00) and Lithuania (81.00) ($p < 0.001$), Portugal (89.00) and Türkiye (83.00) ($p < 0.001$), Italy (86.00) and Türkiye (81.00) (p = 0.015), Lithuania (81.00) and Italy (30.00) (p = 0.012), Türkiye (81.00) and Greece (70.00) ($p < 0.001$), Türkiye (81.00) and Romania (85.00) ($p < 0.001$).

The opinions of teachers Regarding the institutions or responsible that should take attitude in preventing cyberbullying

More than half of the participants agreed that teachers (56.9%, n = 454), parents (53.1%, n = 424), school principals (54.3%, n = 433), and the Ministry of Education (50.9%, n = 406) should do more to prevent cyberbullying. Furthermore, more than half of the teachers (60.3%, n = 481) agreed and strongly agreed that students were responsible for bullying and cyberbullying. Additionally, more than half of the teachers agreed that parents and the school were responsible for tackling cyberbullying inside the school. Detailed results are presented in second table.

TABLE II.
Responsibility of cyberbullying[1]

The most responsible for cyberbullying phenomenon	Disagree or strongly disagree	Neutral	Agree or strongly agree
Teachers	266 (33.3)	242 (30.3)	290 (36.4)
Parents	208 (26)	242 (30.3)	347 (65.2)
School	162 (20.3)	219 (27.4)	416 (52.1)

*[1]Number (n) and percentages (%).

Teachers were asked to express their opinion regarding the schools' rules established against bullying and cyberbullying, and most of them considered that these rules were followed. When it comes to the impact of cyberbullying on students, most teachers believed that there was a negative impact of cyberbullying such as depression and poor academic performance.

Most teachers stated that they agreed (51.8%, n = 413) and totally agreed (36.7%, n = 293) to intervene if they noticed aggression among students. They declared that the school principal approved of the teacher's decision to act in cases of cyberbullying of students (agree: 53.0%, n = 423; totally agree: 17.5%, n = 140). Most of them stated that they agreed (50.6%, n = 404) and totally agreed (21.9%, n = 175) that it was their duty to intervene in any

incidents of cyber aggression among their students. The analysis of answers revealed that more than half of the teachers did not agree that these conflicts prepared students for life, or that victims should manage these situations on their own. Table III presents the detailed results regarding the teachers' answers to the mentioned items.

TABLE III.
Results regarding the teachers' answers

ITEMS	Disagree or strongly disagree	Neutral	Agree or strongly agree	M ± SD
Non-compliance with school rules regarding bullying	376 (47.2)	244 (30.6)	177 (22.1)	2.64 ± 1.04
Non-compliance with school rules regarding cyberbullying	46 (57.7)	243 (30.5)	93 (11.7)	3.67 ± 0.85
Negative impact of cyberbullying	134 (16.8)	85 (10.7)	576 (72.2)	3.88 ± 1.12
Cyberbullying causes depression in students	20 (2.5)	82 (10.3)	694 (87)	4.16 ± 0.72
Cyberbullying causes poor academic results in students	28 (3.5)	127 (15.9)	64 (80.5)	4.03 ± 0.77
Students exaggerate cyber aggression	292 (36.6)	321 (40.2)	185 (23.2)	2.80 ± 1.09
Cyberbullying prepares students for life	63 (79.4)	97 (12.2)	68 (9.5)	1.75 ± 1.09
Students should handle bullying situations on their own	594 (74.5)	136 (17)	68 (8.5)	2.10 ± 0.90
Students should handle cyberbullying situations on their own	513 (64.3)	152 (19)	132 (16.5)	2.32 ± 1.03

[1]Number (n), percentages (%), and means and standard deviations (M ± SD).

Self-evaluation about the attitudes and behaviors in case of bullying and cyberbullying incidents

In general, most teachers believed that the phenomenon of cyberbullying was intensifying among adolescents and that this phenomenon could be eradicated by both intervention and prevention. A series of items were used to evaluate the attitude of teachers in the event of being told about an incident. The analysis of answers revealed that more than a third of the teachers (37.6%, n = 300) said they sent students to the principal for cyberbullying problems only occasionally. Also, in more than a third of cases (38.2%, n = 305), teachers always asked for a discussion with their parents when it came to cyberbullying among students. In similar percentages (35.2%, n = 281), respondents said they never agreed with the non-involvement of students' cyberbullying.

The data gathered showed that few students reported bullying events to teachers as victims (whether they had been the subject of malicious gossip, received malicious messages, their identity had been stolen etc.). Instead, most teachers reported any events they attended or heard about to parents (tab. IV).

TABLE IV.

Self-rated items regarding teachers' behavior in case of bullying events[1].

ITEMS	YES	NO
I was told that students harass each other online during class	161 (20.2)	636 (79.7)
I was told that students harass each other online after class	271 (34)	527 (66)
I think that the phenomenon of cyberbullying is intensifying among teenagers	69 (**86.6**)	106 (13.3)
The students complained directly to me that they had received malicious text messages on the phone about themselves	240 (30.1)	552 (69.2)
The students directly complained to me that they had been the subject of online rumors.	224 (28.1)	573 (71.8)
My students complained directly about receiving malicious/threatening emails/messages.	167 (20.9)	630 (**78.9**)
My students complained directly that someone had hijacked their identity online	116 (14.5)	682 (**85.5**)
I know students who are cyber-harassed by others	237 (29.7)	561 (70.3)
I know students who cyberbully other students in my school	152 (19)	645 (**80.8**)
I always inform parents about an incident of aggression that I witness	634 (**79.4**)	162 (20.3)
I always inform parents about an aggression incident of which I am informed	629 (**78.8**)	168 (21.1)
I believe that the phenomenon of cyberbullying can be mitigated by prevention	720 (**90.2**)	78 (9.8)
I believe that the phenomenon of cyber harassment can be mitigated by intervention	721(**90.4**)	74 (9.3)

*[1]Number of answers (N) and percentage (%).

Frequency of bullying behaviors and typology of victims

Teachers were asked what their views were on how exposed certain children were to becoming victims of cyberbullying. The results showed that children with mental illness (34.2%, n = 273), obesity (30.6%, n = 244), physical syndromes (31.2%, n = 249), and introverted children (31.3%, n = 250) were at a very high risk of becoming victims. On a Likert scale from 1 to 7, where: 1 = several times a day, 2 = once a day, 3 = once a week, 4 = once a month, 5 = once every few months, 6 = once a year or once every few years 7 = never, the analysis of the responses revealed that teachers did not see or report bullying behaviors in school very frequently. In addition, in very few cases, teachers did not intervene to discipline or counsel during the bullying or cyberbullying process. The distribution of the answers is presented in table V.

TABLE V.
Self-rated items regarding teachers' behavior in case of bullying events[1.]

ITEMS	1	2	3	4	5	6	7	M ± SD
How often have you directly noticed aggressive behavior in your school?	57 (7.1)	58 (7.3)	139 (17.4)	101 (12.7)	172 (21.6)	130 (16.3)	141 (17.7)	4.54 ± 1.81
How often have you been told about aggressive behavior in your school?	42 (5.3)	49 (6.1)	140 (17.5)	142 (17.8)	176 (22.1)	147 (18.4)	102 (12.8)	4.52 ± 1.65
To what extent did you personally intervene to discipline, send, or counsel during the assault process?	21 (2.6)	49 (4.9)	86 (10.8)	97 (12.2)	176 (22.1)	186 (23)	192 (24.1)	5.13 ± 1.61
To what extent did you personally intervene to discipline, support, or counsel during the cyberbullying process?	9 (1.1)	21 (2.6)	75 (9)	71 (8.9)	149 (18.7)	151 (18.9)	320 (40.1)	5.59 ± 1.52

*[1]Number of answers (N) and percentage (%).

Teacher Cyberbullying Perception Scale (TCPS)

The global cyberbullying perception scores of teachers as measured with the TCPS ranged from 15 up to 67 (M = 28.09 ± 7.14). For the present study, the Cronbach Alpha score was 0.822. There are significant differences on this scale in terms of participants' gender (U = 45357,500, Z = -4.203, $p < 0.001$) in the sense that women had a lower score on cyberbullying perception (Mdn = 26.00) compared to men (Mdn = 30.00).

A Kruskal-Wallis test was conducted to determine if there were differences in *TCPS* scores among teachers between countries of origin: Italy (N = 46), Greece (N = 59), Lithuania (N = 59), Portugal (N = 94), Romania (N = 343), and Türkiye (N = 197). As assessed by visual inspection of a boxplot, the distributions of these scale scores were not similar for all groups. Median Cyberbullying Perception scores were statistically significantly different between the countries, $\chi2(5) = 151.254$, $p < 0.001$. Subsequently, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented. This post hoc analysis revealed statistically significant differences in median TCPS scores between: Romania (25.00) and Lithuania (28.00) ($p < 0.001$), Romania (25.00) and Greece (37.00) ($p < 0.001$), Greece (37.00) and Italy (27.00) ($p < 0.001$), Greece (37.00) and Lithuania (28.00) ($p < 0.001$), Portugal (25.00) and Greece (37.00) ($p < 0.001$), Portugal (25.00) and Türkiye (30.00) ($p < 0.001$), Italy (27.00) and Türkiye (30.00) ($p = 0.006$), Lithuania (28.00) and Türkiye (30.00) ($p = 0.008$), Türkiye (30.00) and Greece (37.00) ($p < 0.001$), and Türkiye (30.00) and Romania (25.00) ($p < 0.001$).

A Kruskal-Wallis test was conducted to determine if there were differences in *Cyberbullying Perception* scores among teachers between the numbers of children: none (n = 179), one kid (n = 252), two kids (n = 296), three kids (n = 57), four kids (n = 11) or more than four children (n = 3). As assessed by visual inspection of a boxplot, the distributions of these scale scores were not similar for all groups. Median *TCPS*

scores were statistically significantly different between the mentioned categories, $\chi 2(5) = 15.339$, $p = 0.009$. Post hoc Mann Whitney analysis showed that teachers who had no children ($U = 3933.50$, $Z = -2.605$, $p = 0.009$, $Mdn = 26.00$) or only one child ($U = 4959.50$, $Z = -3.654$, $p < 0.001$, $Mdn = 26.00$) had a lower score on the *Perception of Cyberbullying Scale* compared to teachers who had three children ($Mdn = 30.00$).

A Kruskal-Wallis test was conducted to determine if there were differences in the *Teacher Cyberbullying Perception* scores between types of institutions in which teachers work: public schools (n = 718), private schools (n = 76), and special schools (n = 3). As assessed by visual inspection of a boxplot, the distributions of these scale scores were not similar for all groups. Median Cyberbullying Perception scores were statistically significantly different between the mentioned categories, $\chi 2(2) = 32.213$, $p < 0.001$.

Post hoc Mann-Whitney analyses showed that teachers who worked in a public school ($U = 17240.50$, $Z = -5.288$, $p < 0.001$, $Mdn = 27.00$) had a lower score on the scale of perception of cyberbullying compared to those who worked in a private school ($Mdn = 32.50$), while teachers in both public ($U = 23.500$, $Z = -2.930$, $p = 0.003$) and private schools ($U = 16.000$, $Z = -2.516$, $p = 0.005$) had a lower score on the scale of perception of cyberbullying, compared to teachers working in special schools ($Mdn = 49.00$).

Strategies for Coping with the Cyber bullying Scale for Teachers (SSBCS)

The total score for the SSBCS scale was on average $M = 144.47 \pm 18.68$, scores ranging from 36 (0.1%, N = 1) to 180 (0.8%, N = 6). The Cronbach Alpha score was 0.944.

There are significant differences on this scale in terms of participants' gender ($U = 46782.50$, $Z = -3.682$, $p < 0.001$), in the sense that women had a higher score on the SSBCS scale ($Mdn = 147.00$) compared to men ($Mdn = 143.00$). Additionally, the Mann-Whitney U test ($U = 15339.00$, $Z = -6.284$, $p < 0.001$) showed that there was a significant difference on this scale between the types of institutions in which teachers worked, meaning that teachers from private institutions ($Mdn = 81.00$) had lower scores on this scale than teachers from public schools ($Mdn = 85.00$).

A Kruskal-Wallis test was conducted to determine if there were differences in these scale scores among teachers' marital status: unmarried (n = 146), in a relationship (n = 76), married (n = 556), or widowed (n = 20). As assessed by visual inspection of a boxplot, the distributions of these scale scores were not similar for all groups. Median scores were statistically significantly different between these categories, $\chi^2(3) = 13.124$, $p = 0.004$. A Mann-Whitney U post hoc test ($U = 16861.50$, $Z = -2.858$, $p = 0.004$) revealed that married teachers ($Mdn = 147.00$) had higher scores than teachers who were in a relationship ($Mdn = 143.00$) on the scale which measured teachers' coping strategies for perceptions cyberbullying. Furthermore, another Mann-Whitney U post hoc test ($U = 3779.50$, $Z = -2.436$, $p = 0.015$) showed that married teachers ($Mdn = 147.00$) had higher scores on this scale compared to single teachers ($Mdn = 136.50$).

A Kruskal-Wallis test was also conducted to determine if there were differences in SSBCS scores among teachers between countries of origin. As assessed by visual inspection of a boxplot, the distributions of these scale scores were not similar for all groups. The Median SSBCS scores

were statistically significantly different between countries, $\chi^2(5) = 162.178$, $p < 0.001$. Subsequently, results of post hoc analysis revealed statistically significant differences in median SSBCS scores between: Romania (149.00) and Greece (108.00) ($p < 0.001$), Romania (149.00) and Italy (143.00) ($p < 0.001$), Romania (149.00) and Lithuania (136.00) ($p < 0.001$), Greece (108.00) and Portugal (147.50) ($p < 0.001$), Greece (108.00) and Lithuania (136.00) ($p < 0.001$), Portugal (147.50) and Lithuania (136.00) ($p < 0.001$), Portugal (147.50) and Romania (149.00) ($p = 0.020$), Italy (143.00) and Greece (108.00) ($p < 0.001$), Italy (143.00) and Lithuania (136.00) ($p = 0.022$), Lithuania (136.00) and Türkiye (149.00) ($p < 0.001$), Türkiye (149.00) and Greece (108.00) ($p < 0.001$), Türkiye (149.00) and Italy (143.00) ($p < 0.001$).

Correlation analysis

The results of the Spearman correlation analysis showed that there were negative correlations between the total number of years the teachers had been practicing, as well as their beliefs that the phenomenon of cyberbullying was on the rise among adolescents ($r = -0.579$, $p < 0.001$) and their perception of cyberbullying ($r = -0.092$, $p = 0.010$), in the sense that the higher the number of years in education and the higher the teachers' beliefs that cyberbullying was rising, the lower the teachers' perception regarding cyberbullying. There are also negative correlations between the TCPS scale and the SSCBS scale ($r = -0.442$, $p < 0.001$) in the sense that as teachers' perceptions of cyberbullying increase, the teachers' coping strategies regarding cyberbullying decrease.

The total number of years that teachers worked correlates positively with the scale of teachers' coping strategies in terms of cyberbullying ($r = 0.074$, $p = 0.036$), in the sense that the higher the number of years worked, the higher the score on the SSCBS scale. Furthermore, there is a negative correlation between teachers' beliefs that the phenomenon of cyberbullying is on the rise among adolescents and the SSCBS scale ($r = -0.159$, $p < 0.001$), in the sense that the higher the teachers' beliefs that cyberbullying is rising, the lower the teachers' coping strategies regarding cyberbullying.

Both positive and negative correlations were identified between teachers' views of those responsible for cyberbullying, the main factors that could prevent cyberbullying, its negative effects, and the number of children in the teachers' families, the number of years they had been practicing, their faith in the fact that cyberbullying is becoming more prevalent among teenagers and the TCPS and SSCBS scales (tab. VI).

Strong positive correlations were identified between age ($r = 0{,}144$, $p < 0.001$), the total number of years of experience in the education field ($r = 0{,}135$, $p < 0.001$), teachers' perceptions of cyberbullying ($r = 0.122$, $p = 0.001$), and the frequency with which teachers had observed aggressive behavior in their school, in the sense that the older the teachers, the more experience they had and the more increased perception of cyberbullying, the more often they observed aggressive behaviors in school, and the more sensitive and empathic they were.

Some other variables, such as age ($r = 0.094$, $p = 0.008$), the total number of years teachers had been working ($r = 0.110$, $p = 0.002$), and the scale of coping strategies for cyberbullying ($r = 0.158$, $p < 0.001$) correlated positively with the frequency of observing aggressive online behaviors. Other significant and relevant correlations are presented in table VII.

TABLE VI.

Correlational results of teachers' views on the actors that could prevent cyberbullying[1]

ITEMS	Parental status (no. of kids)	Length of experience in the field	Cyber-bullying is a growing phenomenon	TCPS scale	SSCBS scale
I think I should do more to prevent cyber-bullying	no correlation	no correlation	r = -0.192 p < 0.001	r = -0.332 p < 0.001	r = 0.296 p < 0.001
I believe that parents should do more to prevent cyberbullying	r = -0.211 p < 0.001	no correlation	r = -0.185 p < 0.001	r = -0.381 p < 0.001	r = 0.231 p < 0.001
I believe that principals should do more to prevent cyberbullying	r = 0.130 p < 0.001	no correlation	r = -0.158 p < 0.001	r = -0.289 p < 0.001	r = 0.309 p < 0.001
I believe that the Ministry of Education should do more to prevent cyberbullying	r = -0.097 p = 0.006	no correlation	r = -0.173 p < 0.001	r = -0.423 p < 0.001	r = 0.266 p < 0.001
I think students are responsible for bullying and cyberbullying	r = 0.134 p < 0.001	r = 0.077 p = 0.029	no correlation	no correlation	r = 0.101 p = 0.004
I think teachers are responsible for tackling cyberbullying inside the school	r = -0.093 p = 0.008	r = -0.139 p < 0.001	no correlation	r = -0.124 p < 0.001	r = 0.077 p = 0.029
I think parents are responsible for tackling cyberbullying inside the school	r = -0.214 p < 0.001	r = -0.123 p = 0.001	r = -0.105 p = 0.003	r = -0.186 p < 0.001	r = 0.081 p = 0.023
I think the school is responsible for tackling cyberbullying inside the school	no correlation	r = -0.073 p = 0.039	r = -0.130 p < 0.001	r = -0.176 p < 0.001	r = 0.131 p < 0.001
I believe that cyberbullying causes de-pression among the victims	no correlation	no correlation	r = -0.201 p < 0.001	r = -0.409 p < 0.001	r = 0.353 p < 0.001
I believe that cyberbullying causes poor academic results in performance among victims	no correlation	no correlation	r = -0.194 p < 0.001	r = -0.361 p < 0.001	r = 0.316 p < 0.001

*p < 0.05; **p < 0.001.

TABLE VII.

Correlation analysis considering the type of vulnerable child for cyberbullying as a variable[1]

ITEMS	Cyberbullying is a growing phenomenon	TCPS scale	SSCBS scale
Obese children are at risk of being cyberbullied	r = -0.201 p < 0.001	r = -0.337 p < 0.001	r = 0.165 p < 0.001
Children with mental illness are at risk of being cyberbullied	r = -0.181 p < 0.001	r = -0.316 p < 0.001	r = 0.203 p < 0.001
Children with physical syndromes are at risk of being cyberbullied	r = -0.193 p < 0.001	r = -0.324 p < 0.001	r = 0.208 p < 0.001

ITEMS	Cyberbullying is a growing phenomenon	TCPS scale	SSCBS scale
Children in precarious economic situations are at risk of being cyberbullied	r = -0.117 p = 0.001	r = -0.270 p < 0.001	r = 0.158 p < 0.001
Children with one parent are at risk of being cyberbullied	r = -0.096 p = 0.006	r = -0.222 p < 0.001	r = 0.137 p < 0.001
Introverted children are at risk of being cyberbullied	r = -0.171 p < 0.001	r = -0.331 p < 0.001	r = 0.242 p < 0.001
Children with a high level of intelligence are at risk of being cyberbullied	r = -0.086 p = 0.015	r = -0.215 p < 0.001	no correlation
Children with a low level of intelligence are at risk of being cyberbullied	r = -0.149 p < 0.001	r = -0.287 p < 0.001	r = 0.241 p < 0.001
Children with poor school results are at risk of being cyberbullied	r = -0.163 p < 0.001	r = -0.268 p < 0.001	r = 0.245 p < 0.001

*p < 0.05; ** p < 0.001

DISCUSSION

Most of the teachers included in the research (86.6%) sustained that cyberbullying was an increasing phenomenon among adolescents. Even if there was a worrying perception about this problem, only 38.5% of the teachers had taken a course on cyberbullying. Less than three quarters of the teachers answered that they knew how to deal with cyberbullying, but it is evident from the analyze of the answers that even if one third of them had taken a course about cyberbullying, twice as many teachers rated themselves as capable of handling it. Less than half of the teachers felt that their current university education had not prepared them to deal with cyberbullying, and more than half of them wanted to spend more time studying more about cyberbullying. Our data are consistent with those presented by scientific literature. For example, the Dutch teachers included in a study conducted by Oldenburg *et al*. (29) gave incomplete definitions of bullying. The authors identified that respondents had limited strategies to find out about bullying, and many of them never recognized the self-reported victims in their classroom. These extremely important results suggested by some research on teachers revealed that, even if teachers have a central role in tackling bullying, they are not always fully trained for this task. Similar results were found in a study conducted by Khanolainen *et al.* (30). The research identified that the majority of the teachers indicated either seeing no bullying or only seeing bullying rarely as a justifiable reaction to provocation, while their students reported that bullying and cyberbullying incidents were quite frequent in their school and sustained that "teachers are seeing nothing".

It is important to separate the skill of identifying bullying and cyberbullying incidents in general and the willingness to do it. Rigby and his team (31, 32) found that a large proportion of adolescent girls (43.0%) and boys (51.4%) were not confident in teachers' ability to respond effectively. Shaw *et al* (23) found that if students perceived that teachers were unlikely to intervene or that the school climate was tolerant of bullying, they would be less likely to disclose that they were being vic-

timized.

A sequence of items investigated teachers' sensitivity to the phenomenon. In total, 20% of the teachers sustained that they were informed about cyberbullying in school and 34% of them about cyberbullying incidents outside of school. Considering the gender of their students and cyberbullying victimization, respondents appreciated that cyberbullying occurred equally in boys and girls, and more than 60% considered that students were the main ones responsible for bullying and cyberbullying incidents. In their opinion, the age 11-14 was the most exposed to bullying, while 15-18 was the most exposed to cyberbullying. Age (more frequent, the older teachers were more prone to being sensitive to victimization), gender (more women than men), length of experience in the field of education (experienced teachers were more prone to identify victimization), type of institution (teachers from public schools were more sensitive to cyberbullying than teachers from private schools), and marital status (married teachers and those who had children) were influencing cyberbullying perceptions and the coping strategies that they used in order to solve cyberbullying incidents. Similar findings were identified by Yilmaz (20) Yildirim *et al*. (33) who suggested that married teachers had a higher level of cyberbullying sensitivity.

The key to success in the elimination of cyberbullying and its negative effects lies in school policies, parental support, and cyberbullying sensitivity, the last one being the most important tool for teachers. Our study identified that age, length of experience, and a high level of perception of cyberbullying were related to a high level of identifying aggressive behavior in school. Furthermore, women obtained higher scores on the scale that measured teachers' educational experience than men. We noticed that married teachers had higher scores on the scale which measured teachers' coping strategies for perceptions of cyberbullying than teachers who were not married yet, and the same when compared to single teachers. This variable was mentioned in some studies focusing on teachers' perception of cyberbullying. For example, Yildirim *et al.* (33) sustained that it can be suggested that married teachers had a higher level of cyberbullying sensitivity, and that analyzing teachers' cyberbullying, and cyber victimization experiences should be related to cyber sensitivity. The authors identified that teachers who had an undergraduate degree had a higher level of cyberbullying sensitivity. As Eden *et al.* also identified, female teachers were found to express more concern than male teachers, as did teachers of younger children.

Our study identified that teachers appreciated that children with mental illness, obesity, diverse physical syndromes, and introverted kids are at a very high risk of becoming victims. These results are congruent with data obtained by Zhu, (34) Olenik-Shemesh *et al.* (14), Bokkel (17) and Fossum (33). Evaluating all the risky variables identified by the scientific literature, the most common are, of course, the variables that are easily identified by direct observation. But some other characteristics of the students must not be neglected, and preconceptions should guide the interventions in the case of cyberbullying with precociousness. For example, some authors (30-32) maintain that even popular students or students with high academic grades are victims of cyberbullying, but usually they do not share this in order not to be seen as week or to lose their status in school or class. Moreover, mentally ill students and

lonely ones usually do not share in order not to be more severely aggressed by the persecutor. Considering the small percentage of teachers that are being told about, we can consider that these answers are the results of their experience with reporting incidents or of their preconceptions (opinions) regarding the type of the victims. The students who were identified as being more vulnerable to victimization need more attentive care and permanent supervision, in general, due to their mental, physical, or emotional condition.

The current research also highlights some dilemmatic results. We identified that the higher the number of years in education and the higher the teachers' beliefs that cyberbullying is rising, the lower the teachers' perception regarding cyberbullying. There were also negative correlations between the TCPS scale and the SSCBS scale, meaning that as teachers' perceptions of cyberbullying increased, the teachers' coping strategies regarding cyberbullying decreased. Additionally, the length of experience in the field positively correlated with the score on the Scale of Teachers' Coping Strategies, in the sense that the higher the number of years worked, the higher the score on the SSCBS scale. Furthermore, the higher the teachers' beliefs that cyberbullying is rising, the lower the teachers' coping strategies regarding cyberbullying. These results could be sustained by the fact that respondents realized that cyberbullying is increasing extremely rapidly, and there is no possibility to fight by themselves, or the phenomenon that is spreading becomes normality in the school environment.

The results identified by this study and that can also be seen in some other published research led to a general, common idea that teachers did not invest time and effort in countering the phenomenon of bullying and cyberbullying and were not always willing to get involved in mediation between the victim-aggressor-witnesses (30-32).

Many of the teachers declared that bullying, in all its forms, prepared students for adult life, although a large part of them admitted that there were important negative consequences on the physical and mental health, as well as the academic results of the victim, with a long-term impact even in adult life.

The first explanation would be the lack of training programs. Teachers must be trained in how to identify, analyze, behave, intervene, and constructively manage the bullying phenomenon in their classes, and how to help the victim, perpetrator, and bystander. Studies have shown that students lose confidence if the first reported incident is not resolved conveniently. Additionally, the literature presents studies that explain the way in which the victim reaches the aggressor's turn in order to defend themselves, and the active spot witnesses are also considered aggressors (30-32). Passivity in the case of bullying and cyberbullying incidents only induces the aggressor to believe that he/she is right and powerful, and the victim will have a high risk of self-isolation, helplessness, depression, anxiety, and suicidal thoughts. In this sequence of changing roles, the importance of the teacher's intervention is crucial in reducing the incidence of aggressive behaviors.

The second explanation would be the focus on the didactic activity rather than the counseling activity - the teachers believe that the time should be invested in teaching, and the class time should be dedicated to science, for the benefit of all stu-

dents.

A third explanation can be related to the fact that some teachers consider fighting bullying at school useless because some of them are themselves victims of bullying by the same students who bully their colleagues.

Fourthly, the recognition of a problem must come together with the structuring of intentions to intervene over a long period, to find solutions together with students, parents, school management, school psychologists, and other stakeholders, which means that the teacher must invest time, effort, and resistance to stress, denial, and frustration from others.

Fifthly, the recognition of a behavior problem and some incidents that take place in the school space may induce the opinion that the other colleagues or the members of the school committee consider them unskilled in solving such cases, the explanation being, therefore, the desire to shield themselves from a negative evaluation from the teaching staff.

The sixth explanation could be due to the students' disclosure. A lot of studies show that students are not willing to report bullying incidents to their teachers, and most of them talk about an aggressive issue to a friend, a colleague, or a parent. Bullying incidents are deliberately hidden from teachers; in general, teachers' responses were perceived as ineffective, insensitive, or excessive, and even peers discourage 'telling tales' to adults. For example, some researchers (35-38) identified that pupils reported a reluctance to tell adults, particularly teachers, about their experiences of bullying, and this tendency increased with age. The implications of the present findings, also revealed by some scarce literature, are important for the advancement in anti-bullying policy and practice that received support from schoolteachers.

The seventh explanation is related to the personal aspects such as personality, life experience, familial-socio-professional status, and their own representation of bullying and cyberbullying and aggressive behavior in general. Some studies revealed that teachers with victimization antecedents were more prone to identify and interfere in cyberbullying incidents (32-25).

An eighth explanation could be related to the high level of knowledge about traditional bullying and a good package of skills applied in the classroom to stop bullying behaviors, a situation that makes teachers feel powerless in cyberbullying situations. For example, a study by Elledge *et al.* (39) and Paez (40) showed that when the students evaluated their teacher as highly capable of intervening in a traditional bullying incident, the other type of aggression-cyberbullying - increased. So, the perpetrators will try to find ways to aggress others by engaging in more covert instances of bullying (online) because traditional bullying incidents are more likely to be halted by their high-intervening teachers.

Taking into account the aspects mentioned above (which, of course, do not cover the entire range of explanations because they do not refer to others, such as cultural, religious, or ethnic variables as well as the legislative aspects specific to each country), the intervention and prevention programs that take place in schools, with the help of teachers, must be built in such a way that they are adapted to the needs of students but also to the possibilities of teaching staff to get involved in the management of bullying and cyberbullying incidents. (41-44) As we have sustained by the results of the present study, school

rules, peer-cultural acceptance of aggressive behaviors, the student-teacher relationship, but also the perception and previous experience of students regarding the ability of teachers to resolve aggressive incidents are important in the fight against cyberbullying. The involvement of teachers depending on the way in which they manage to overcome their own personal barriers in the face of repetitive aggressive behaviors should also not to be neglected.

Strengths and limitations of the study

The results of the present study are important because they mirror the current opinion of teachers regarding cyberbullying. Studies have shown an increasing use of the internet in the last decade. Apart from that, we must consider the enormous use of the internet and digital technologies in school activity, imposed by the COVID-19 pandemic lockdown all over the world. The research points out the big gap between the reality of cyberbullying and the lack of preparedness of teachers to deal with this impactful phenomenon.

This research has some limitations. The first limitation is related to the unbalanced gender distribution of respondents that is imposed by the specifics of the teaching profession. More women are embracing this job compared to men. The second limitation is due to the unbalanced distribution of respondents by country, and that is why comparative analysis must be treated with precociousness. But this limitation should also be seen as a productive one, because studies showed that cyberbullying is a widespread phenomenon, with no geographically related cause. The adverse effects caused by cyberbullying, including reduced safety, lower educational attainment, poorer mental health, and greater unhappiness among students in all countries, led UNICEF to state in 2017, that *"no child is absolutely safe in the digital world".* (45) The third limitation is determined by the restrictions imposed by the questionnaire. It did not consider variables that could influence teachers' opinions regarding cyberbullying, such as psychological troubles (depression, burnout), job-related factors (dissatisfaction with their job or with the relationships with students/colleagues/managers), the personal experience as a victim of cyberbullying or having a member in the family (husband, child) with such an experience, negative antecedents concerning solving cyberbullying problems among their students or being the victim of cyberbullying by one of the students in the school.

CONCLUSIONS

This research confirms the major role that teachers have in fighting the increasing phenomenon of cyberbullying and the fact that this role must be constantly prepared by awareness, training, and collaborative relationships with students, parents, and colleagues. The results are important for students, teachers, parents, and school staff in order to realize the tremendous need for good communication, for training and developing skills in order to manage cyberbullying by encouraging the disclosure of incidents, being able to identify cyberbullying in the school environment, and offering help to all those involved (victims, perpetrators and bystanders). The results showed that policy makers should consider teachers' experiences and needs in designing policies aimed at preventing and managing the online deviant behaviors among children and adolescence immediately that the aggressive acts are reported.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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THE SIGNIFICANCE OF LIFESTYLE, ASSOCIATED DISEASES, AND ANATOMICAL VARIANTS OF THE CIRCLE OF WILLIS IN THE FORMATION OF ANTERIOR COMMUNICATING ARTERY ANEURYSMS

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THE SIGNIFICANCES OF LYFESTYLE, ASSOCIATED DISEASES AND ANATOMICAL VARIANTS OF CIRCLE OF WILLIS IN THE FORMATION OF ANTERIOR COMMUNICATING ARTERY ANEURYSMS (Abstract): Intracranial aneurysm represent a cerebrovascular disease with a relatively low incidence, but among these the most common are aneurysms developed in the anterior part of the circle of Willis (CoW). **Material and methods:** We collected the demographic and morphological data of the patients admitted in "Prof. Dr. N. Oblu" Emergency Clinical Hospital, Iași, Romania, with ruptured AcomA aneurysms, who later deceased and were autopsied in the Pathology Department of the same hospital, over a period of 6 years (January 2014 to December 2019), and analyzed data related to: age, gender, lifestyle (heavy smoking and alcoholism), type of anatomical variant of circle of Willis, and presence of any associated diseases. **Results:** We identified 7 patients with ruptured AComA aneurysm. 57.14% were male patients. The average age was 60.85 (range 34 to 69). Left posterior communicating artery (PcomA) was hypoplastic in 100% of all deceased patients. Right PComA presented hypoplasia (71.42%) or fetal type variant (28.57%). 71.42% of all cases showed anatomical variants both in the anterior and the posterior part of CoW. 66% of the female patients with AComA aneurysm presented obesity, type 2 diabetes, atherosclerotic disease and hypertension. 100% of male patients presented hypertension and atherosclerotic disease, 75% of them were chronic alcohol consumers and 50% were heavy smokers. **Conclusions:** The lifestyle, associated diseases, and anatomical variants of the component arteries of the circle of Willis, both from its anterior and posterior parts, could be risk factors for the formation of anterior communicating artery aneurysm. **Keywords:** CIRCLE OF WILLIS, ANTERIOR COMMUNICATING ARTERY ANEURYSM, ANATOMICAL VARIANTS, LIFESTYLE.

Intracranial aneurysm represents a cerebrovascular disease with a relatively low incidence, as it ranges between 3.6 - 9% in the general population (1, 2).

Aneurysms located at the circle of Willis (CoW) level are defined as a localized dilatation of a component artery, being situated at weak points along this anatomical structure. Approximately 85% of aneurysms develop in the anterior part of CoW, predominately at its bifurcations (3). Aneurysms of anterior communicating artery (AComA) are the most common, representing 23% - 43.5% of all intracranial aneurysms (4-6).

Over time, numerous theories have emerged about the development of an intracranial aneurysm. At first it was considered that the embolic and inflammatory reactions were the significant factors in this process, but later it was found that the presence of some defects in the development of the vascular media at the bifurcation of an artery are necessary. In addition, some degenerative changes of muscle of the vascular media, as well as of internal elastic membrane were associated (7).

More recently, Stojanović *et al.* (2) concluded that the asymmetric configuration of the circle of Willis causes increased perfusion requirements in certain areas and thus initiates the process of blood vessel remodeling in the area of permanently increased perfusion flow. Some of the risk factors that contribute to the occurrence of an intracranial aneurysm have been identified so far: familial history of intracranial aneurysm, high blood pressure, cigarette smoking, alcohol consumption, and female gender (8). However, these factors do not seem to explain the entire way of aneurysm formation, especially in the case of AComA, because in 1991 some Japanese researchers published an article about an AComA aneurysm diagnosed in a 13-month-old child (9).

During its evolution, the AComA aneurysm ruptures and leads to the occurrence of subarachnoid hemorrhage, often with ventricular involvement, leading in 30-40% of cases to death or severe neurologic dysfunction and disability in survivors (8).

The hypothesis that anatomical variants of the component arteries of CoW can influence the formation of intracranial aneurysms was first reported by Padget *et al.* in 1944 (10). Considering AComA aneurysms, hypoplasia of one or both Anterior Cerebral Arteries (ACAs) play a defining role in its development by disturbing local hemodynamics (11-14). There are only few studies identifying the presence of a fetal-type of Posterior Communicating Artery (PComA) in patients with AComA aneurysms (15-17).

The first aim of our study was to analyze the anatomical variants of the anterior and posterior parts of CoW in a series of autopsy specimens in order to identify the relationship between any of its component arteries and AcomA aneurysm formation and rupture. Our second aim was to identify the risk factors that intervene in the formation and rupture of an AComA aneurysm by analyzing associated diseases and lifestyle of the patients included in our series.

MATERIAL AND METHODS

We collected the demographic and morphological data of the patients admitted in the main neurological and neurosurgical regional centre in North-Eastern Romania ("Prof. Dr. N. Oblu" Emergency Clinical Hospital of Iasi) with ruptured AComA aneurysms, who later deceased and were autopsied in the Pathology Department of the same hospital, over a period of 6 years (January 2014 to December 2019), analyzing both medical records and protocols of the autopsies.

Based on the resulting list, we created a database with the following data:

1. Demographic data: age, sex, lifestyle (heavy smoking and alcoholism)

2. Morphological data: the presence of any type of anatomical variant for each component artery of CoW, taking into account the literature definitions, as well as the presence of any associated diseases (obesity, hypertension, type 2 diabetes).

According to literature, we considered that:

- "hypoplasia" of a component artery of CoW is defined by an external diameter of less than 1 mm, except for communicating arteries, which are considered hypoplastic at an external diameter of less than 0.5 mm (18).
- the posterior communicating artery (PComA) is defined as complete fetal (fPComP) when it originates entirely from the internal carotid artery (ICA), with no connection to the Basilar Artery (BA). We defined the posterior communicating artery as partially fetal (pfPComA) when it originates from ICA and retains a small atresic connection with BA (19).

The obtained results were collected in a database using the *Microsoft Excel* 2007 program and were used to calculate the mean, number and percentage, as well as to make the corresponding graphs. At the end, we compared our results with the data from the specialized literature.

RESULTS

We identified 7 deceased patients with ruptured AComA aneurysm and fatal outcome. Among them, 57.14% were male patients (fig. 1), with a M:F ratio of 1.33. The average age of the patients from our series was 60.85 (range 34 to 69). Mean age of female patient was 66 (range 60 to 69), while for male patients was 57 (range 34 to 71).

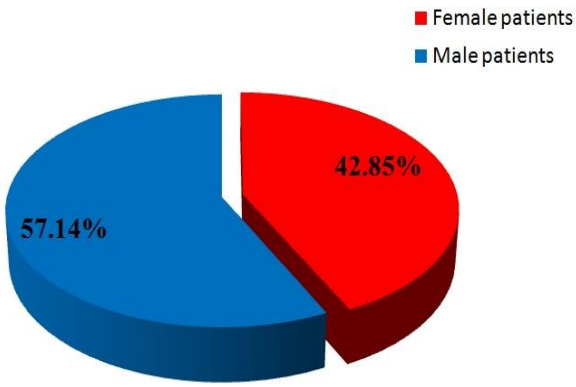


Fig. 1. Chart showing the gender ratio

Macroscopic examination showed that none of our patients presented a symmetrical circle of Willis. AComA presented a normal conformation in 100% of all cases but left PComA was hypoplastic in 100% of all deceased patients. Right PComA presented hypoplasia in 71.42% of cases and partial fetal type in 28.57% of cases.

All other vessels presented only hypoplasia. Right ACA presented hypoplasia in 57.14% of all cases, but left ACA showed hypoplasia only in 14.28% of the analyzed specimens. Right Posterior Cerebral Artery (PCA) was hypoplastic în 28.57% of the deceased patients and left PCA in 14.28% of cases (fig. 2).

57.14% of all circles of Willis presented three anatomical variants, 28.57% had four variants, and 14.28% exhibited only two variants (fig. 3).

In our series, 71.42% of all cases presented anatomical variants both in the anterior and the posterior part of CoW. 28.57% presented variants only in the anterior part. None of the analyzed cases had only the anterior part affected (0%) (fig. 4).

The analyzed AComA aneurysms varied in diameter from 4 mm to 13 mm, with a mean diameter of 8.42 mm (figs. 5-7).

66% of the female patients who died with AComA aneurysm were previously diagnosed with obesity, type 2 diabetes, atherosclerotic disease and arterial hypertension, but none were heavy smoker or alcohol drinker. 100% of male patients with AComA aneurysm were previously diagnosed with arterial hypertension and atherosclerotic disease, and 75% of them were chronic alcohol consumers and 50% were heavy smokers (fig. 8).

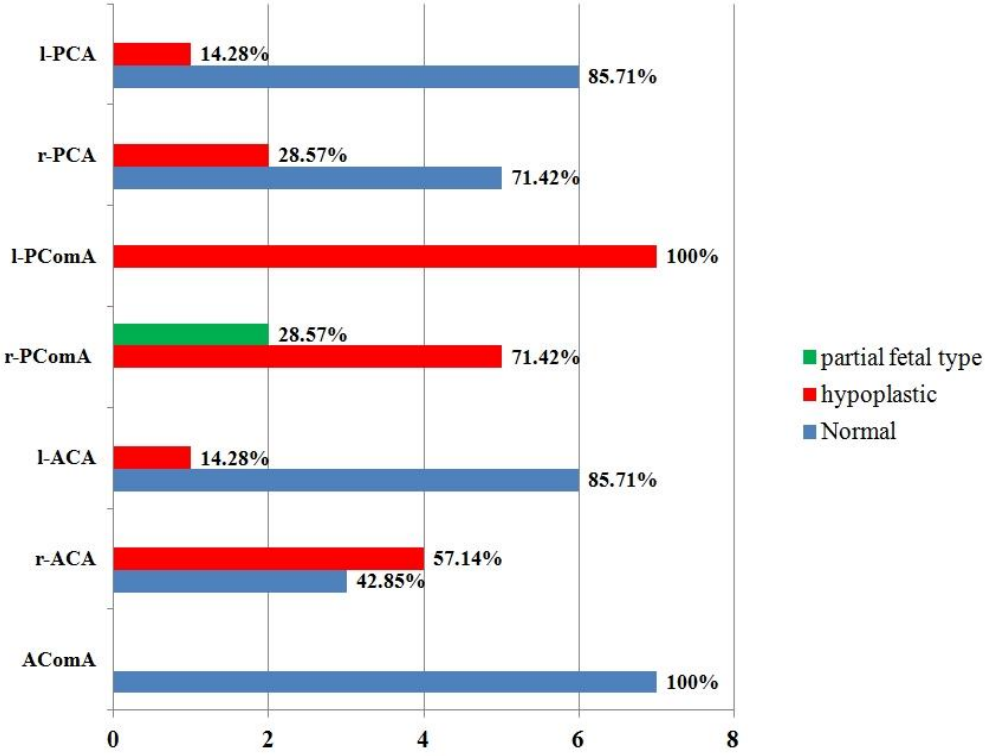


Fig. 2. Chart showing the relation between each artery of the circle of Willis and the anatomical variant it expressed in our case series. Abbreviations: r =right; l =left

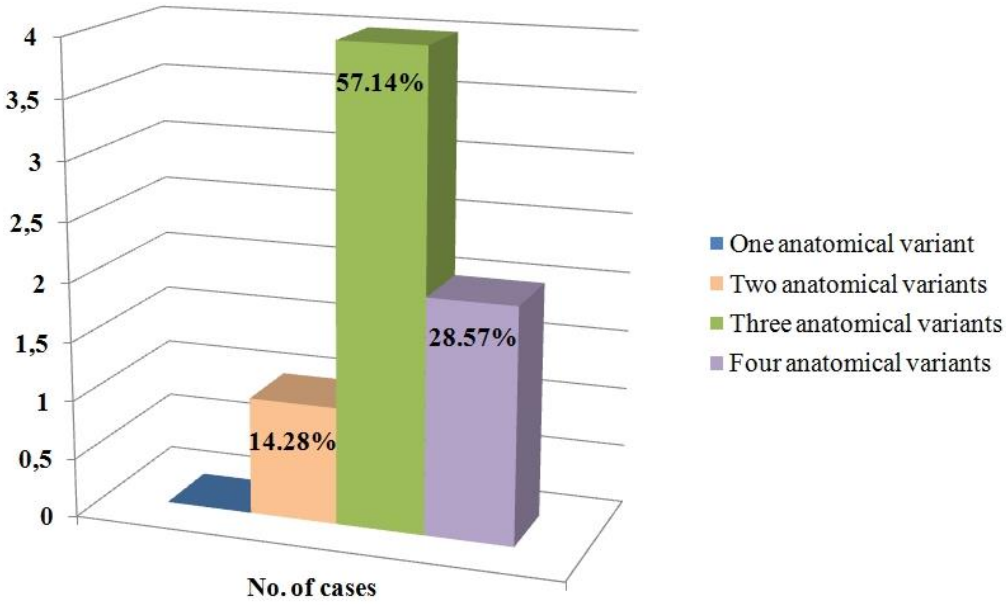


Fig. 3. Chart showing the number of anatomical variants expressed by the component arteries of the circles of Willis in our case series.

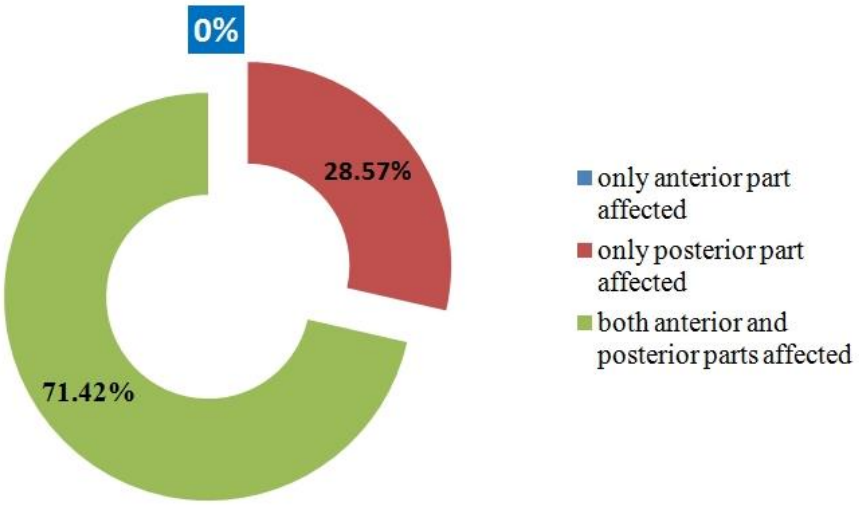
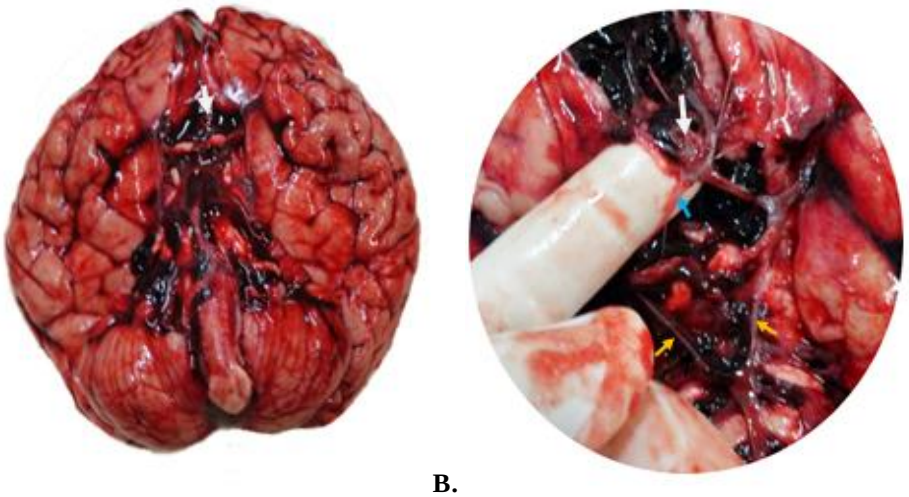


Fig. 4. Chart showing the percentage of the affected part of the analyzed CoW by anatomical variants.



A. **B.**

Fig. 5. M, 34 years-old. A) AComA ruptured aneurysm (white arrow), having a diameter of 9 mm, with subarachnoid hemorrhage and massive intraventricular hemorrhage. B). Aneurysm is associated with right ACA hypoplasia (blue arrow), and bilateral PComAs hypoplasia (orange arrows).

Fig. 6. M, 50 years old. AComA ruptured aneurysm (white arrow), with a diameter of 4mm) with subarachnoid hemorrhage associated with left ACA hypoplasia (blue arrow), right PComA hypoplasia (yellow arrow), partial fetal type left PcomA (green arrow), and left PCA hypoplasia (purple arrow).

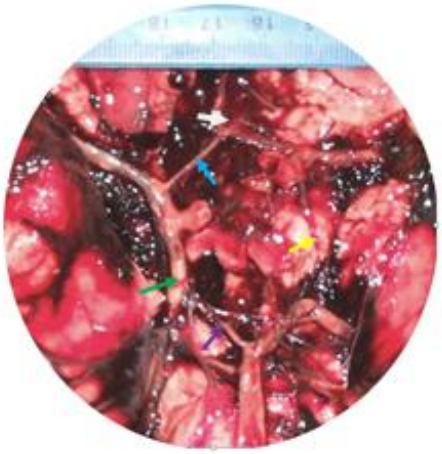


Fig. 7. F, 60 years old. AComA ruptured aneurysm (white arrow), with a diameter of 8mm, with subarachnoid hemorrhage and massive intraventricular hemorrhage associated with right ACA hypoplasia (blue arrow), partial fetal-type right PcomA (green arrow), right PCA hypoplasia (purple arrow), and left PComA hypoplasia (yellow arrow).

DISCUSSION

Regarding the circle of Willis, aneurysms developed mostly in the anterior part (20, 21). Literature unequivocally states that ruptured intracranial aneurysms are associated with the female sex (8), but, in the case of AComA aneurysms, male patients outnumbered female patients (22, 23). Ghods *et al.* also found that male patients with ruptured AComA aneurysm were diagnosed at a significantly younger age than female patients (23). Even though our series included a small number of cases, we identified the same gender differences and found out that male patients were diagnosed with ruptured AComA aneurysms 9 years earlier than female patients.

Previous angiographic studies demonstrated that abnormalities of the AComA complex, most frequently hypoplasia of A1 segment of ACA, are associated with AComA aneurysm in more than two thirds of cases (11, 12, 24, 25). Our study demonstrated on an autopsy series the same association in almost three quarters of the cases.

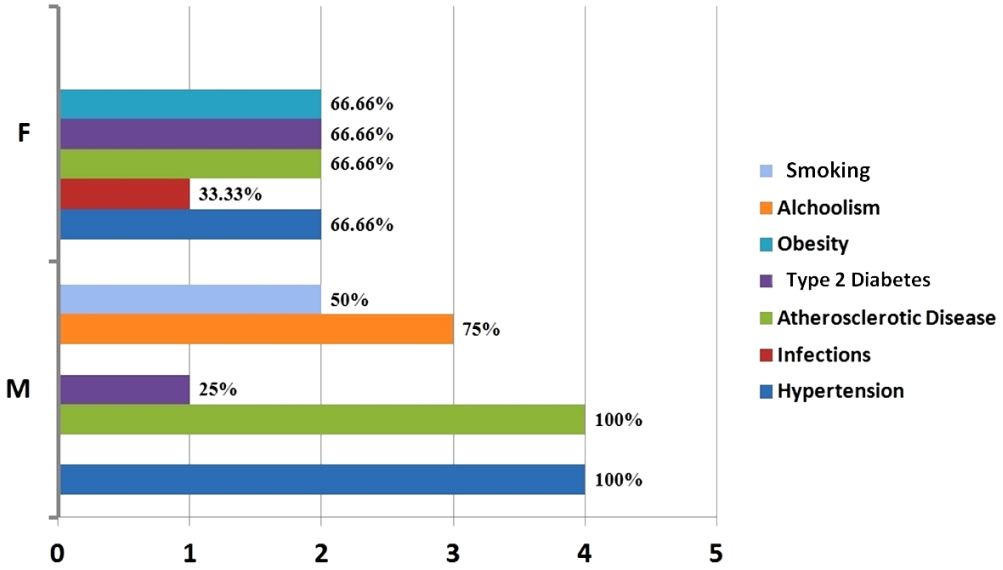


Fig. 8. Chart showing the relation between gender and associated diseases to the AComA aneurysm. Abbreviations: M=male patients; F=female patients

Tarulli and Fox, in 2010, showed that the presence of an asymmetric configuration of the A1 segment of ACA facilitates the development of AComA aneurysms by flow stresses (13). More recently, in 2017, Rinaldo *et al.* analyzed digital subtraction angiography images of 204 patients with AComA aneurysm. Of their patients, only 1/6 were found to have a hypoplastic A1 segment, even if they had no history of smoking (14), but they also took into consideration the unruptured aneurysms.

The relative lack of traditional risk factors for aneurysm formation (such as smoking) in patients with A1 segment hypoplasia should direct researchers' attention to investigating some other hemodynamic factors with a role in AComA aneurysm

formation. Therefore, it may be of interest to search for associations between AComA aneurysm and some other types of anatomical variants along with A1 segment hypoplasia. We already found in our series that patients with AComA aneurysm presented an association between hypoplasia of A1 segment and hypoplasia or partial fetal type of at least one of the two PComAs.

Rinaldo *et al.* reported that AComA aneurysms associated with hypoplastic A1 segment have a mean diameter of 7.7 mm (14), a value similar to that identified in our study.

A group of Turkish researchers analyzed the intraoperative anatomic vascular variations associated with AComA aneurysms in 120 patients. They found that more than half of them had anatomical variants in the vicinity of the AComA aneurysm, such as: marked hypoplasia of the A1 segment in more than a quarter of cases, but less often the median artery of the corpus callosum, duplication of AComA, duplication of A1 segment and azygos artery (26). Another intraoperative study conducted on 130 cases with AComA aneurysms by Bazowski *et al.* identified anatomical variants in more than one third of them. The most common abnormality reported was hypoplasia of the A1 segment of ACA (27). More than one third of Polish patients and one-third of Egyptian patients with AComA aneurysms were reported to present hypoplasia/aplasia of A1 segment of ACA (28-30). Thus, aneurysms of AComA were most often associated with an asymmetry of A1 segment of ACAs.

As far as we know, only recently were published three articles that angiographically confirmed the role of partial fetal type AComP associated with hypoplasia of A1 segment of ACA in the formation and rupture of the AComA aneurysm as a result of hemodynamic stress in susceptible patients (16-17). So, our article brings more knowledge to this matter.

The hypothesis that anomalies of the posterior part of CoW could play a role in the development of AComA aneurysm seems to be possible because in cases with hypoplasia of PCA, the blood from the ICA is no longer directed to the ipsilateral PCA and thus the blood pressure is directed to the ipsilateral ACA and implicitly to the AComA, favoring the development of aneurysms at this location by increasing the flow pressure, even in the absence of an anatomical variant of any of the two ACAs.

Considering the formation of aneurysms along CoW, Permiakov *et al.* emphasized the fact that the etiopathogenetic element underlying the appearance of such an aneurysm is the existence of congenital dysplasia of the arterial wall, which is also associated with certain risk factors such as essential or transient arterial hypertension and atherosclerosis (21). Other authors have considered the following as risk factors for the occurrence of intracranial aneurysms: smoking, hypertension, excessive alcohol consumption, one or more relatives affected with subarachnoid hemorrhage, autosomal dominant polycystic kidney disease, and a previous episode of subarachnoid hemorrhage (31).

Recent evidence shows that type 2 diabetes and atherosclerosis share common mechanisms for arterial wall alteration, such as lipid regulation, apoptosis, endothelial activation and inflammation, mitochondrial oxidative stress, changes in extracellular matrix components, and disruption of cellular defense systems (32).

On the other hand, in arterial hypertension all layers of the vascular wall are af-

fected due to aberrant vascular signaling, oxidative and inflammatory responses. Subsequently, hypertensive vasculopathy predisposes to atherosclerosis as a result of increased transmural pressure, blood flow and shear stress, as well as endothelial dysfunction and vascular stiffness (33). On the other hand, obesity directly contributes to cardiovascular risk factors, being itself associated with obvious atherosclerotic lesions (34). Therefore, it seems reasonable to state that the presence of hypertension, type 2 diabetes and obesity may be risk factors for the occurrence of AComA aneurysm as we found in our series.

Literature reveals that smoking plays a role both in aneurysm formation, its growth, its rupture, as well as in its recurrence after endovascular repair, probably due to the effects that the cigarette substances have on the artery walls during a multi-step process, i.e. initial vascular inflammation and hemodynamic stress, then endothelial dysfunction, and, finally, wall weakening and rupture (35, 36).

CONCLUSIONS

Our study brings new information regarding the association between patients' gender and certain risk factors for the formation, progression, and rupture of Anterior Communicating Artery aneurysm. We can conclude that the lifestyle (alcohol consumption, and heavy smoking) of our patients as well as their associated diseases (obesity, type 2 diabetes, atherosclerotic disease, arterial hypertension) could represent risk factors in the formation and rupture of Anterior Communicating Artery aneurysm. Also, the hemodynamic factor must also be taken into consideration because all our patients with Anterior Communicating Artery aneurysm presented at least two anatomical variants of the component arteries of the circle of Willis, both from its anterior and posterior parts. We emphasize the fact that neuroradiologists and neurosurgeons must keep in mind that, in cases with an unruptured Anterior Communicating Artery aneurysm, not only the anatomical variants of Anterior Cerebral Arteries should be searched on computed tomography angiographies or Magnetic Resonance Angiography, but also the anomalies of the posterior part of the circle of Willis in order to prevent the rupture of that aneurysm.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest regarding this scientific research. This article was co-funded from the European Social Fund - the Human Capital Operational Programme, Project/ Grant No: POCU/993/6/13/154722.

ETHICAL APPROVAL

The current study was approved by the Ethics Committee of "Prof. Dr. N. Oblu" Emergency Clinical Hospital Iasi, Romania.

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RENAL FUNCTION DYNAMICS IN PATIENTS WITH RADICAL PROSTATECTOMY FOR PROSTATIC ADENOCARCINOMA

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RENAL FUNCTION DYNAMICS IN PATIENTS WITH RADICAL PROSTATECTOMY FOR PROSTATIC ADENOCARCINOMA (Abstract): Renal function parameters, readily assessed through routine blood tests, have been largely unexplored in relation to prostate cancer. This study aims to investigate the associations between renal function parameters (creatinine, urea, estimated glomerular filtration rate-eGFR), both pre- and post-prostatectomy, and histopathological characteristics of prostate adenocarcinomas. Additionally, it evaluates potential correlations with prostate biochemical recurrence, offering insights into kidney function dynamics post-prostatectomy. **Material and methods:** We analyzed data from 72 patients with prostate adenocarcinoma who underwent radical prostatectomy. Histopathological reevaluation was conducted, including Gleason score, pT stage, prognostic grade groups, and biochemical recurrence assessment. Preoperative and postoperative renal parameters were recorded, and eGFR was calculated using the CKD-EPI formula. Statistical analysis was performed to assess associations. **Results:** Preoperative eGFR showed a significant association with Gleason score and prognostic grade groups ($p<0.05$). However, there were no significant correlations between renal parameters and biochemical recurrence or pT stage. **Conclusions:** This study emphasizes the potential utility of eGFR as a marker for identifying prostate adenocarcinomas with higher Gleason scores and prognostic grade groups. While the complexity of renal function dynamics in prostate cancer warrants further investigation, our findings contribute to understanding the intricate relationship between kidney function and prostate tumor behavior. **Keywords:** RENAL FUNCTION, PROSTATE CANCER, CREATININE, UREA, eGFR.

Prostate cancer (PCa) is the most commonly diagnosed urologic male malignancy worldwide (1), with more than 1.4 million new cases in 2020 and a global mortality rate of 7.7/100,000 (2). From a histopathological perspective, conventional acinar adenocarcinomas (ADKs) and their less common histological variations,

account for over 95% of prostate cancers, while less prevalent types include non-acinar carcinomas, such as sarcomas and neuroendocrine tumors (3).

Prostate-specific antigen (PSA) remains the predominant biomarker used in prostate cancer screening (4) and also plays a crucial role in treatment strategy selection and predicting biochemical recurrence (5, 6).

While histopathological characteristics for prostate cancer continue to be refined and augmented by innovative molecular classifications (7), the quest for novel biochemical parameters that can provide insights into the broader systemic and organ-level implications of this neoplasia remains ongoing. In the context of increasing longevity among the population, there is a noticeable overlapping between conditions such as chronic kidney disease and PCa (8). Parameters related to renal function, which are readily assessable through routine blood tests, may be influenced by neoplastic conditions. However, there is a paucity of research examining the importance of these parameters in prostate cancer and their potential associations with the histopathological features of this malignancy (9, 10).

Therefore, the aim of our study is to investigate the relationships between renal function parameters (creatinine, urea, estimated glomerular filtration rate), both pre- and post- prostatectomy, and the histopathological characteristics of prostate adenocarcinomas. Furthermore, it is the first study to evaluate the possible associations between these nephrological parameters and prostate biochemical recurrence, hence assessing their potential prognostic significance. These potential correlations may help identify new insights into the complex dynamics of kidney function in patients who have undergone prostatectomy for prostate cancer. The analysis of renal function could be integrated as a potential indicator of aggressiveness level of prostate acinar adenocarcinoma, facilitating patient stratification.

MATERIAL AND METHODS

Patients, histopathological assessment and nephrological parameters

The study cohort comprised 72 patients diagnosed with prostate adenocarcinoma and who underwent radical prostatectomy at the "Dr. C. I. Parhon" University Hospital in Iaşi, Romania, between 2010 and 2018. This research obtained ethical approval from the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, (No. 292/2023).

Each case underwent a microscopic reevaluation following the guidelines provided in the most recent edition of the World Health Organization (WHO) classification for tumors of the urinary and male genital systems, released in 2022 (11). During this reevaluation, we reassessed various aspects, including the Gleason score, the dominant pattern, and aggressiveness parameters such as capsular, perineural, and lymphovascular invasion, as well as the pathological tumor-node-metastasis (pT) stage, and subsequently attributed prognostic grade groups.

Biochemical recurrence was defined as serum PSA values $\geq$ 0.2 ng/mL following radical prostatectomy.

Preoperative and postoperative paraclinical biochemical parameters (postoperative PSA levels, serum creatinine and serum urea) were recorded from the patients' medical charts.

Using the 2021 calculation formula from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (12), we computed estimated preoperative and postoperative glomerular filtration rates by taking into account serum creatinine levels and age.

For each renal function-associated parameter we evaluated the distribution in our study lot and calculated a cutoff value based on the median, used for grouping the cases in order to apply the statistical Chi-square test for assessing the relation to other parameters.

Statistical analysis

Associations between the histopathological features (Gleason score, pT stage, prognostic grade groups and biochemical recurrence) and the preoperative and post operative renal parameters (serum creatinine, serum urea and eGFR) were evaluated using Chi-square test at the critical significance level p=0.05.

Statistical analysis was conducted using *Statistica version 7* (Tibco, Palo Alto, CA, USA) and *Excel 2016 version 16.0* (Microsoft, Redmond, WA, USA).

RESULTS

Pathological features of the study lot

The defining histopathological characteristics of each case in our study lot, reconsidered in accordance with the updated 2022 WHO Classification for tumors of the urinary and male genital systems (11), are detailed below (tab. I).

TABLE I.

General histopathological characteristics of the study lot

Case no.	histological variant / associated variant	Gleason Score	pT	Prognostic grade group	Biochemical recurrence	Invasion		
						Cp	Pn	Lv
1	conv. acinar/mucinous	6=3+3	T1	1	present	present	present	absent
2	conv. acinar/foamy cell	7=3+4	T2	2	present	present	present	absent
3	conv. acinar	6=3+3	T2	1	present	present	present	absent
4	conv. acinar/mucinous	7=4+3	T2	3	absent	present	present	absent
5	conv. acinar	7=3+4	T2	2	present	present	present	absent
6	conv. acinar	6=3+3	T2	1	absent	present	absent	absent
7	conv. acinar	7=3+4	T2	2	present	present	present	absent
8	conv. acinar	6=3+3	T2	1	absent	present	present	absent
9	conv. acinar/foamy cell	6=3+3	T2	1	absent	absent	absent	absent
10	conv. acinar	7=3+4	T2	2	present	present	present	absent
11	conv. acinar	7=3+4	T2	2	absent	present	present	absent
12	conv. acinar	7=4+3	T2	3	present	present	present	absent
13	conv. acinar	7=3+4	T2	2	absent	present	absent	absent
14	conv. acinar	6=3+3	T2	1	present	present	absent	absent
15	conv. acinar	7=3+4	T2	2	present	present	present	absent
16	conv. acinar	6=3+3	T2	1	absent	absent	absent	absent
17	conv. acinar/foamy cell	6=3+3	T2	1	present	present	present	present
18	conv. acinar	7=3+4	T2	2	present	present	present	present
19	conv. acinar	7=3+4	T2	2	present	present	present	absent
20	conv. acinar	6=3+3	T2	1	absent	present	present	absent
21	conv. acinar/foamy cell	7=3+4	T2	2	absent	present	present	absent
22	conv. acinar/foamy cell	7=3+4	T2	2	absent	present	present	absent
23	conv. acinar	7=3+4	T2	2	absent	present	present	absent
24	conv. acinar	7=3+4	T2	2	present	present	present	absent

Case no.	histological variant / associated variant	Gleason Score	pT	Prognostic grade group	Biochemical recurrence	Invasion		
						Cp	Pn	Lv
25	conv. acinar	7=4+3	T2	3	absent	present	present	present
26	conv. acinar	7=3+4	T2	2	absent	present	absent	absent
27	conv. acinar	7=3+4	T2	2	absent	present	present	absent
28	conv. acinar	7=3+4	T2	2	absent	present	present	absent
29	conv. acinar	7=3+4	T2	2	absent	present	absent	absent
30	conv. acinar	7=3+4	T2	2	present	present	present	absent
31	conv. acinar/foamy cell	7=4+3	T2	3	present	present	present	absent
32	conv. acinar	7=3+4	T2	2	absent	present	present	present
33	conv. acinar	7=3+4	T2	2	absent	present	present	absent
34	conv. acinar	9=4+5	T2	5	present	present	present	present
35	conv. acinar	7=3+4	T2	2	absent	present	present	absent
36	conv. acinar	7=3+4	T2	2	absent	present	present	present
37	conv. acinar	7=3+4	T2	2	present	present	present	absent
38	conv. acinar	7=3+4	T2	2	present	present	present	present
39	conv. acinar	7=3+4	T2	2	present	present	present	absent
40	conv. acinar/microcystic	6=3+3	T2	1	present	present	present	absent
41	conv. acinar	6=3+3	T2	1	present	present	present	absent
42	conv. acinar	7=3+4	T2	2	absent	present	present	present
43	conv. acinar	6=3+3	T2	1	present	present	present	absent
44	conv. acinar	6=3+3	T2	1	absent	absent	absent	absent
45	conv. acinar	7=3+4	T2	2	present	present	present	absent
46	conv. acinar	7=3+4	T2	2	absent	present	present	absent
47	conv. acinar	7=3+4	T2	2	absent	absent	absent	absent
48	conv. acinar	7=4+3	T2	3	present	present	present	present
49	conv. acinar	6=3+3	T2	1	absent	present	present	absent
50	conv. acinar/foamy cell	7=3+4	T2	2	present	present	present	present
51	conv. acinar	6=3+3	T2	1	absent	present	present	absent
52	conv. acinar	7=3+4	T2	2	absent	present	present	present
53	conv. acinar/mucinous	6=3+3	T2	1	present	present	present	absent
54	conv. acinar	7=3+4	T3	2	present	present	present	absent
55	conv. acinar	8=4+4	T3	4	present	present	present	absent
56	conv. acinar/mucinous	7=3+4	T3	2	present	present	present	absent
57	conv. acinar	8=4+4	T3	4	absent	present	present	absent
58	conv. acinar	7=3+4	T3	2	absent	present	present	absent
59	conv. acinar	6=3+3	T3	1	absent	absent	absent	absent
60	conv. acinar/atrophic	8=4+4	T3	4	absent	absent	absent	absent
61	conv. acinar	8=4+4	T3	4	absent	present	present	absent
62	conv. acinar	8=5+3	T3	4	present	present	present	absent
63	conv. acinar	9=4+5	T3	5	present	present	present	absent
64	conv. acinar/foamy cell	7=3+4	T3	2	absent	present	present	absent
65	conv. acinar	9=4+5	T3	5	absent	present	present	present
66	conv. acinar	7=3+4	T3	2	present	present	present	absent
67	conv. acinar	9=4+5	T3	5	present	present	present	absent
68	conv. acinar/foamy cell	9=4+5	T3	5	present	present	present	present
69	conv. acinar	7=3+4	T3	2	present	present	present	present
70	conv. acinar	9=4+5	T3	5	absent	present	present	absent
71	conv. acinar/mucinous	8=4+4	T3	4	absent	present	present	present
72	conv. acinar	7=3+4	T3	2	absent	present	present	absent

Conv=conventional, Cp=capsular, Pn=perineural, Lv=lymphovascular.

All reevaluated cases were conventional acinar adenocarcinomas, with certain cases showing associated histopathological variants: foamy cell (12.5%), mucinous (6.94%), atrophic (0.72%) and microcystic (0.72%). The predominant Gleason score was 6 (52.77% of cases) and the main Gleason pattern was 3 (76.38% of cases). The majority of cases presented neoplasic involvement of the prostate capsule (99.66%), with perineural spread of the tumor (84.72%). Lymphovascular invasion was observed in only 20.83% of cases. In 19 cases (26.38%), the tumor had extended beyond the confines of the prostate, resulting in a pT3 stage and biochemical recurrence was confirmed in 48.61% of cases.

Renal function parameters

The identified values for preoperative and postoperative serum creatine, serum urea and eGFR led to the median levels comprised in the table below (tab. II).

TABLE II.

Preoperative and postoperative values of renal function parameters

Renal function parameter	Range	Median Value
Preoperative Creatinine (mg/dL)	0.6-2.2	0.885
Preoperative Urea (mg/dL)	11-62	30
Preoperative eGFR (mL/min/1.73m^2)	33-111	94
Postoperative Creatinine (mg/dL)	0.67-1.7	0.9
Postoperative Urea (mg/dL)	17-66	33
Postoperative eGFR (mL/min/1.73m^2)	44-110	95

Correlations between renal function parameters and histopathological features

There were no statistically significant correlations between the biochemical recurrence and the preoperative and postoperative renal parameters. Also, there were no significant associations between pT stage and renal parameters, both preoperative and postoperative.

The only notable and statistically significant association was registered between the preoperative eGFR and the Gleason score and prognostic grade groups, respectively (tab. III).

TABLE III.

Correlations of renal function parameters and histopathological features of PCa

Renal function parameter	Biochemical recurrence			Gleason score			Prognostic grade group			pT stage		
	P	A	p	≤7	>7	p	1-2	3-4	p	1-2	3-4	p
preoperative creatinine (< 0.88 mg/dL)	16	16	0.832	26	6	0.671	22	10	0.172	24	8	0.810
preoperative creatinine (≥ 0.88 mg/dL)	19	21		34	6		33	7		29	11	
preoperative urea (<30 mg/dL)	18	15	0.354	28	5	0.750	25	8	0.907	24	9	0.875
preoperative urea (≥30 mg/dL)	17	22		32	7		30	9		29	10	
preoperative eGFR (< 94)	16	18	0.803	32	2	0.02	21	3	0.03	28	6	0.111

Renal function parameter	Biochemical recurrence			Gleason score			Prognostic grade group			pT stage		
	P	A	p	≤7	>7	p	1-2	3-4	p	1-2	3-4	p
preoperative eGFR (≥ 94)	19	19		28	10		24	14		25	13	
postoperative creatinine (< 0.9 mg/dL)	15	20	0.342	30	5	0.598	26	9	0.682	27	8	0.505
postoperative creatinine (≥ 0.9 mg/dL)	20	17		30	7		29	8		26	11	
postoperative urea (< 33 mg/dL)	17	18	0.994	29	6	0.916	26	9	0.682	27	9	0.899
postoperative urea (≥ 33 mg/dL)	18	19		31	6		29	8		26	10	
postoperative eGFR (< 95)	19	15	0.242	27	7	0.398	26	9	0.682	22	12	0.104
postoperative eGFR (≥ 95)	16	22		33	5		29	8		31	7	

There were no significant correlations between the aggressiveness indicators (capsular, perineural and lymphovascular invasion) and renal function parameters, both preoperative and postoperative (p value 0.05 for t-test).

DISCUSSION

In the context of evaluating the overall health of individuals with prostate adenocarcinomas, the assessment of kidney functionality is an integral aspect of their perioperative management, with a crucial role especially in anti-androgen therapy (13). Several studies have been conducted in this direction, demonstrating that renal dysfunction can occur as early as one month after the institution of hormone deprivation therapy, persisting throughout the duration of treatment (14). This dysfunction can be attributed to various mechanisms, such as hyperglycemia and dyslipidemia, which can disrupt glomerular function by affecting interstitial tubule membranes. Additionally, hormone castration leads to testosterone depletion, which can counteract the vasodilatory effects of testosterone on renal blood vessels. Furthermore, this hormonal imbalance can result in estrogen deficiency, which has negative implications for renal tubular function (15).

Although these studies collectively highlight the complex relationship between hormone therapy, renal dysfunction, and the underlying physiological mechanisms involved, there is limited data on assessing renal function indicators with actual clinical or histopathological parameters of prostatic carcinoma. Only two recent studies focused on the relationship between total PSA levels and uric acid levels (16), and several renal parameters (creatinine, urea, uric acid and cystatin C) respectively (17).

In light of this data, our study stands out as the first effort to assess how various kidney function indicators (including creatinine, urea and eGFR), both pre- and post- radical prostatectomy, relate to the histopathological parameters that define the clinical behavior of prostatic adeno-carcinomas, ultimately influencing treatment outcomes and responses. Our results revealing a statistically significant correlation between preoperative eGFR and

Gleason score, and consequently with the prognostic groups. These findings are concordant with a recent study, supporting, through a multivariable analysis, after controlling for age, BMI, prostate-specific antigen doubling time (PSA), Gleason score, and clinical stage, the role of a statistically significant predictor of overall mortality after radical prostatectomy (18).

Surprisingly, we did not observe any noteworthy correlations between serum creatinine and urea levels and the histopathological characteristics of prostate adenocarcinomas, including capsular, perineural, and lymphovascular involvement. One potential explanation for these findings may be linked to the relatively small size of our study lot. Additionally, it's important to note that the majority of cases in our study comprised localized prostate adenocarcinomas limited to the prostate gland, thus lacking aggressive characteristics related to extensive tumor spread, which could contribute to the absence of significant associations with kidney function indicators.

CONCLUSIONS

While the underlying pathogenetic mechanisms of renal damage in prostatic neoplasia remain incompletely understood, our study has shed light on the potential role of eGFR as a valuable marker for identifying prostate adenocarcinomas associated with higher Gleason scores and prognostic grade groups. This finding opens up perspectives for a more comprehensive understanding of kidney function dynamics in the context of prostatic malignancy.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflict of interest.

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THERAPEUTIC OBSTINACY AT THE END OF LIFE - CREATING A TOOL FOR RESEARCHING THE PERCEPTION OF HEALTHCARE PROFESSIONALS

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THERAPEUTIC OBSTINACY AT THE END OF LIFE - CREATING A TOOL FOR RESEARCHING THE PERCEPTION OF HEALTHCARE PROFESSIONALS (Abstract): Introduction: The delivery of end-of-life care presents ethical quandaries regarding the delineation between therapeutic efficacy and the potentially ambiguous advantages of subjecting individuals with terminal illnesses to medical interventions. Therapeutic obstinacy at the end of life represents medical interventions that are inappropriate for the patient's clinical condition and circumstances, considering their potential outcomes, expectations, and burdens on the patient and family. These procedures try to prolong life rather than help it progress naturally. End-of-life care workers often have different views on therapeutic obstinacy and futile therapies, which affects their overall view of this type of care. The **aim** of this study is to create a questionnaire to collect data on health care professionals' perceptions of therapeutic obstinacy at the end of life. **Materials and methods**: To fulfill the objective of this work, a qualitative investigation was conducted, comprising the formation of three focus groups and the implementation of two pilot surveys. The aforementioned steps were taken to ensure that the questionnaire devised would effectively address the research objectives by acquiring information that is valid, accurate, and dependable. **Results:** The questionnaire created collects data on the perception of health care staff about interventions considered to be excessive and futile at the end of life, about the causes and pressure factors in these situations, as well as possible solutions to reduce this phenomenon. **Conclusions:** The perspectives obtained from the survey conducted in this study hold potential significance for professionals working in the fields of palliative care, oncology, critical care in ICU, emergency medicine, as well as individuals aspiring to pursue training and careers in these domains. Furthermore, the findings derived from our comprehensive study will serve as a significant contribution to the current discourse on ethical considerations within the realm of end-of-life care. **Keywords:** THERAPEUTIC OBSTINACY, FUTILE TREATMENT, END OF LIFE, HEALTH-CARE PROFESSIONALS.

Death is a natural, inevitable part of life. However, death is often treated as a disease (1) and people's attitudes to it vary according to culture, religion, tradition or personal

conscience (2. Individual perspectives on death can influence attitudes in end-of-life care and are essential given that one of the most important principles of good medical care is conforming to the patient's wishes and values (1-4). Medicine has always tried to go beyond the limits of life, often through technological and scientific progress. For example, modern intensive care techniques now make it possible to keep alive patients with pathologies that were considered lethal in the past by compensating for organ failure (2). In the latter part of the 20th century, advances in biomedical technology have led us to consider a "good death" as one involving disease control. Partly in response to this view, the modern palliative care movement has emerged, redefining a "good death" as one that includes acceptance of the end of life (5).

Medical practice and the approach to the end of life have changed drastically due to constant scientific and technological development (6). Thus, health professionals are faced with situations that promote not only life-prolonging care but also a culture of death denial, which can create significant difficulties in determining the prognosis of critically ill patients (7) and complex ethical concerns such as the utility of known therapies and treatments (8). From the perspective of patient demand and supply in the health care business, moral hazard makes mortality expensive (9-11). The last weeks of life receive a disproportionate share of healthcare expenditures, promoting inequity (12-13). To live to the end is the "right to die with dignity"- not to die. Medical practitioners and society need to comply with dying ethics (14). Palliative care addresses these ethical standards. Thus, most believe that artificially prolonged anguish degrades the death (4, 14).

Palliative care relieves suffering, improves QoL, and lowers healthcare costs for persons with significant chronic illnesses and their families from diagnosis through death (15-16, 17-18). It is a type of care provided by trained clinicians (specialist palliative care, - SPC) and/or by primary care, community, or hospital employees with basic palliative care training (19).

Defining the end of life

End of life is the final common pathway of many progressive diseases with irreversible decline of the body's functional state before death (20). In the literature, there are different assessments of the length of time that characterize the concept of the end of life. Certain research pertaining to end-of-life care focuses on the final stages of an individual's life, namely the concluding days, hours, or minutes (21). One of the most common definitions of the end of life is that developed by the National Hospice and Palliative Care Organization (NHPCO), which specifies the temporal nature of a period of less than six months of expected life. NHPCO states that end-of-life care or hospice care begins when a person has a diagnosis of terminal illness with less than six months to live and curative treatments have been exhausted. (20-21).

Therapeutic obstinacy and therapeutic futility

End-of-life care, since its early history, has been recognized as raising significant ethical concerns for medicine (22). Interventions such as cardiopulmonary resuscitation, mechanical ventilation, mechanical circulatory support, artificial nutrition and hydration, terminal sedation, non-initiation

and discontinuation of treatment, euthanasia, and physician-assisted suicide are issues that correspond to circumstances in which end-of-life decision-making difficulties ap-pear to be of greatest importance (23-25).

According to the *Pallipedia Medical Dictionary*, therapeutic obstinacy is "the initiation or continuation of medical actions that have no other aim but to prolong the patient's life when the patient is facing irreversible death" (26). Therapeutic obstinacy describes medical interventions that only delay death and do not allow life to unfold, which are inappropriate to the patient's clinical reality and situation in relation to the results, hopes, and burden they cause to the patient and family. It is an overzealous, unjustifiable therapeutic urge that pro-longs death without benefiting the patient, causing artificial anguish (27). It creates the illusion of a long life for patients who have no chance of a cure or of minimally preserving quality of life, thus becoming a useless and upsetting treatment (28).

The dividing line between therapeutic obstinacy and timely therapy is one of ethical and clinical reasoning, which considers the complex reality of the advanced and/or terminally ill patient. The criteria that characterize therapeutic obstinacy in end-of-life care relate to the ineffectiveness of care interventions, the pain or burden on the patient, and the exceptionality of the interventions proposed. Proportionality or disproportionality of care are judgements that derive from the relationship between the therapeutic means used and the clinical reality of the patient to whom it is applied. When the clinical situation requires it, it is more useful for the patient to intervene through palliative care than by insisting on treatments with illusory curative aims (27-28). The "prognostic" factor is therefore important in assessing the appropriateness of certain medical interventions at the end of life in relation to the benefits obtained (29). In clinical practice, however, it is very difficult to decide what therapeutic obstinacy truly is, requiring great professional competence, clinical experience, a sense of proportion, as well as the continuous updating of therapeutic guidelines and the ongoing progress of clinical research in the field (24).

Personalist bioethics assesses end-of-life interventions as therapeutic obstinacy, the use of futile therapies that increase patient suffering and are dis-proportionate to the risk-benefit binomial, actually prolonging the dying process without providing healing or increasing the patient's quality of life; they are characterized by futility, suffering caused, and disproportionality. Disproportionality is judged by the patient, the physician, the patient's family, society, and the Bioethics Committee of the medical institution where the patient is ad-mitted (30).

Although therapeutic obstinacy and futile therapies are concepts with different meanings, they are often considered synonymous. Thus, according to some authors, "therapeutic futility" can be defined as treatments that do not provide immediate or long-term benefit to the patient (31). A medical procedure/treatment is considered "futile" when it meets one or more of the following five criteria: 1) the burden outweighs the benefits; 2) the patient has no chance of survival outside the intensive care unit; 3) the patient is in an irreversible coma; 4) the procedure does not meet the patient's expectations; and 5) death is imminent (32). Despite years of debate, futile therapy can delay or pro-long death (33)

and create moral distress for patients, families, and healthcare providers (34). For healthcare professionals, it can be a difficult ethical dilemma-ma to decide when to stop or not initiate futile treatments. Conflicts can arise when physicians and patients have different perspectives on treatment goals. Tensions can also arise when patients or their families request futile therapies (31-34).

In our opinion, the distinction between "therapeutic futility" and "therapeutic obstinacy" is related to questions about the third element of human action, i.e. circumstances. We consider that some therapies may be futile at the end of life (e.g. vitamin therapy for prophylactic purposes), (35) but they do not also fulfil the condition of disproportionality and prolongation of the dying process. In this context, we consider that "therapeutic obstinacy" is the most appropriate term for this concept. According to the results of a literature review published in 2016 (36), therapeutic futility in the Intensive Care Unit (ICU) can have a negative impact on professional satisfaction and induce emotional exhaustion in ICU physicians and nurses, with related consequences for patients and their families (37).

The aim of our study is to create a research tool to investigate the views of health care professionals on therapeutic futility in end-of-life care. The questionnaire is designed to collect data on the opinions of healthcare professionals about end-of-life care interventions, which they rate as disproportionate in relation with the benefits, about the causes of therapeutic obstinacy and pressure factors in such situations, and about the solutions to reduce therapeutic obstinacy in end-of-life care. No instrument has been identified in the Romanian literature that explores the perception of health care professionals regarding therapeutic obstinacy at the end of life. It is interesting to collect data on the opinion of medical staff about interventions considered disproportionate and futile at the end of life, the causes and pressure factors in these situations, and possible solutions to reduce this phenomenon. Such data are of interest to palliative care, oncology, and critical care practitioners in ICU and emergency medicine, to those who intend to train and practice in these fields, and will provide input to debates on ethical issues in end-of-life care.

MATERIALS AND METHODS

In order to achieve the proposed aim, qualitative research was carried out, which included three focus groups and two pretests (pilot surveys), so that the questionnaire would allow valid, accurate, and reliable data to be obtained. The starting point in developing the questionnaire was a literature review that high-lighted a concern for research on ethical issues related to good practice in end-of-life care, namely therapeutic obstinacy and futile treatment at the end of life.

The participants in the first two focus groups and the two pilot surveys were nurses with experience in end-of-life care: surgery, ICU, oncology, radiotherapy, and palliative care departments in two hospitals, one public-the Regional Institute of Oncology of Iași and the Palliative Care Hospital in Vaslui. The participants of the third focus group were physicians from different specialties in the above-mentioned hospitals and medical scholars caring for patients at the end of life: ICU, radiotherapy, oncology, surgery, palliative care, and forensic/bioethics.

The data from the literature were cor-

roborated with the suggestions of the participants in the three focus groups and the two pretests, resulting in the final version of the research questionnaire. All three focus groups were conducted face-to-face, audio recorded, and later transcribed verbatim. This was followed by the management, organization, and thematic analysis of the data, followed by the rereading of the texts, the thematic separation of the data, the identification of themes and sub-themes, and coding. The questions in the questionnaire resulted from the grouping of initial themes and sub-themes and from collaboration between the researcher and research participants. The quasi-final form of the questionnaire was then sent to the participants in the last focus group to harmonize views and obtain agreement on the final form of the questionnaire.

Research participants were informed about the purpose, objectives, and procedures of the study and the manner in which the confidentiality of the data collected was ensured. Participants signed the informed consent form prior to enrollment and were given the opportunity to withdraw from the study at any time. This study was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași by Approval No. 103 of July 12, 2021.

RESULTS

In the first stage of the questionnaire design, data from the literature was used to develop the first version of the research questionnaire addressed to health care professionals caring for patients with a short prognosis.

The questionnaire was structured into three parts. The first part collects demographic data on the study participants; the second part explores the level of awareness of the concept of therapeutic obstinacy at the end of life and assesses the frequency of encountering such situations in daily practice; and the third part assesses the participants' opinions on some end-of-life interventions that may be considered disproportionate to the benefits obtained and the patients' quality of life. It also collects data on the perception of specialists regarding possible causes that may lead to situations of therapeutic obstinacy at the end of life and possible solutions to improve the situation.

The first version of the questionnaire was pretested in a pilot survey. The participants in the pilot survey were 14 nurses working in palliative care in two hospitals, one public and one private, in two different counties (Iași and Vaslui). Participants were invited to define their perceptions of the concept of end-of-life therapeutic obstinacy. Their responses were diverse and extremely interesting. "The refusal of the patient or his or her family to accept the situation, the hope of prolonging life at any cost, even at the expense of quality of life.", "Not knowing the prognosis of the disease can lead to demands from the family that bring no benefit to the patient (transfusions, infusions)", "The therapy prescribed is without effect and useless to the patient in the last days of life.", "By this concept, one can understand the refusal of the relatives to come to terms with the idea that nothing can be done about the patient's illness.", "The refusal of the patient or family to accept the fact that there comes a time when drug therapy no longer helps." "Refusal to accept the end of life".

The results obtained led to the organization of the first focus group interview, in which eight nurses working in the pallia-

tive care service of the Regional Institute of Oncology of Iași participated. Following this interview, it was considered necessary to reformulate some questions and introduce new ones considered to be relevant to the research in question. Thus, questions were introduced that explore the perception of specialists regarding the importance of psychological and spiritual preparation of patients in the process of accepting their prognosis, as well as the importance of psychological and spiritual preparation of medical staff as well as the patient's family for accepting the patient's death. It was also considered relevant to introduce a question on the most common wishes of patients at the end of life.

This new version of the questionnaire was pretested in a new pilot survey. Six nurses working in the palliative care service of the Regional Institute of Oncology of Iași participated in the second pilot survey, other than those in the previous pretest. The results of the pretest and this new version of the questionnaire were analyzed in the succeeding two focus groups. The second focus group was attended by 9 nurses (5 senior nurses and 4 nurses with more than 5 years' professional experience in the field) working in departments that also care for patients at the end of life: surgery, radiotherapy, oncology, and palliative care. Suggestions and comments from participants led to the following changes and additions:

• The system for quantifying respondents' level of agreement or disagreement according to the Likert scale was reformulated.

• The wording of the question on the assessment of the respondent's religiousness was clarified.

• The importance of responders expressing personal experiences (with relatives or close people) that might be classified as "therapeutic obstinacy at the end of life" was mentioned.

• The question highlighting possible pressure factors that lead to therapeutic obstinacy at the end of life was answered in several different ways: patient or family background (urban, rural, or diaspora; patient's religiosity; patient's ethnicity; pressure factors in society, including pressure from the patient's social status; non-recognition of the patient's terminal condition by medical staff; non-acceptance of the patient's terminal condition by medical staff.

• When asked about possible end-of-life medical interventions that may be considered disproportionate and futile in relation to the benefits obtained and the patient's quality of life, participants suggested supplementing the answer options with interventions such as: complex diagnostic investigations (CT/MRI/PET-CT); nasogastric intubation (NGS) for feeding; nasogastric (NG) tube insertion for hydration; use of existing NG tube for hydration and feeding; use of PEG/jejunostomy tube in the last days of life; feeding on previously inserted PEG tube; hydration on previously inserted PEG tube.

• Participants have emphasized the importance for the patient and family of choosing the place of end-of-life care for the patient.

The third focus group, organized as part of the research, comprised 11 physicians from different specialties (ICU, palliative care, oncology, radiotherapy, surgery, bioethics) with different professional ranks (university professor, university lecturer, senior lecturer, consultant physician). The role of this focus group was to analyze the

wording of the questions, their sequence, the link between the questions, and the content of the questions in relation to the research objectives. This led to the refinement of the research questionnaire and its final form. The suggestions offered in this focus group were:

• The demographic data collected was to be completed with the specialty of the responding physician. Thus, when analyzing the collected data, variations in response would also be observed based on medical specialty.

• The definition of "end of life" was clarified, suggesting the replacement of the phrase "last 72 hours of life" with "72-hour prognosis". Participants emphasized the relevance of the "72 hour" period for assessing interventions that may be considered disproportionate at the end of life in relation to the benefits and considered that a longer patient life span would create a non-homogeneous study group.

• When it comes to the question on the respondent's religiosity, participants suggested to also include the answer option "I do not wish to answer".

• The question about the respondent's personal experience with therapeutic obstinacy involving relatives, friends, or close persons was rephrased and clarified, and the connection to the question exploring the frequency of encountering such situations in professional work was created. It was considered appropriate and relevant to separate these two situations: personal experiences and professional experiences.

• When asked about the "first impression" of interventions that could be considered therapeutic end-of-life obstinacy, some non-aggressive interventions (e.g. oxygen therapy), and "traps" were also suggested to capture the degree of understanding the concept of therapeutic end-of-life obstinacy.

• When specifying pressure factors in situations of therapeutic obstinacy, a clearer division of the "patient age" factor was recommended, with a separate specification of age over 75 years, an age considered vulnerable in relation to certain medical interventions that may be considered aggressive in relation to the benefits.

• With regard to the pressure factor "patient's religiosity", it was decided to divide the situations into "religious patients" and "non-religious patients".

• Concerning possible causes of therapeutic obstinacy, it was suggested that the situation of "conspiracy of silence" be included, in which the patient or family does not know the diagnosis and/or prognosis of the disease due to the express wish of one of the parties involved (patient or family) in the process of communicating medical information.

• Fear of death was suggested as a possible cause of obstinacy, and the physician's fear of death was considered in addition to that of the patient and the patient's family.

DISCUSSION

End-of-life care raises ethical issues and could be a source of medical, ethical, and legal conflicts for physicians, lawyers, and ethics professionals. Physicians have no ethical or legal obligations to provide unnecessary treatment to the patient (34, 37-38). Despite the recognition among numerous medical experts of the phenomenon known as therapeutic obstinacy and its associated issues, it remains prevalent in routine clinical settings where patients are often subjected to such treatment, even when the therapy holds little possibility for

success (34-39).Given that the end of life is a unique phase in the course of life, previous studies have found that perceptions of therapeutic obstinacy highlight the difficult and challenging situation of providing end-of-life care (38-39). From our perspective, divergent opinions have the potential to foster diverse dialogues within a constructive setting that considers various viewpoints, personal experiences, and belief systems, ultimately culminating in a well-deliberated conclusion.

The focus groups organized to refine the questions in the research questionnaire collecting the perceptions of health care staff regarding therapeutic obstinacy at the end of life revealed that both nurses and physicians face this phenomenon, each reporting various causes and pressure factors leading to futile treatment at the end of life. Nurses stressed the importance for the patient and family of choosing the place of end-of-life care for the patient. The choice of place of care can be a pressure factor in end-of-life care. While home care was preferred in the past, today there is an increasing choice of care at this stage of life in medical facilities (40). The place of death is considered an indicator of the quality of care, with the decision on the choice of the place for end-of-life care influenced by economic, cultural, ethnic factors, medical literacy, patient values and beliefs. There is also evidence that the desire to die at home diminishes with age and poor health, the "hospitalization" of the end of life being now preferred (41).

Ivrin D. Yalom, a specialist in existential psychology, inspired by a serious study in the area of Greek philosophy and beyond and following a rich experience in psychotherapy, distinguishes several types of end-of-life and death reporting. The perspective of death is assumed differently according to gender, age, religiosity, culture, education, and profession (42). These variables express not only distinct perspectives but also life, spiritual, and professional experiences that give different grids of perception to the end of life. People who fall into various socio-professional categories experience anguish and fear of death in various ways. However, the same author shows that, in fact, it is not death itself that frightens us, but the meanings we attribute to it. Anguish in the face of death, therapeutic entanglements, the conspiracy of silence in the face of a grim diagnosis, and old age are all expressions of an assumed perspective on death and implicitly on life. Irvin D. Yalom shows that at play is a form of human narcissism that clings to us, to social and professional roles, and to those around us (42). Undoubtedly, this narcissism is greatly enhanced by the modern world and the technologies it makes available to us. Jean Twenge and Keith Campbell speak in this sense of a narcissism pandemic in the contemporary era that is based on the increase of self-esteem, the cult of celebrity, and technology development. Viewed from a broader, interdisciplinary perspective, the answers received reflect the new social and cultural context in which new meanings of death are developing. This, because the collective imagination is focused only on a certain perspective on existence, a perspective in which death is hidden, becomes an out-of-date topic, dispatched from our daily concerns. Moreover, developments in medicine and new technologies promise the extension of life and bring into question concepts such as the death of death (43).

The nurses participating in the focus groups considered it relevant to the research if the respondents to the question-

naire indicated their degree of religiosity or faith. The literature mentions that the nurse's religious beliefs can enhance the nurse's clinical experience, obviously without the nurse trying to impose his or her own beliefs on the patient. End-of-life care evokes personal religious values for both nurse and patient (44). The physicians participating in the focus group also considered the questioning on religiosity relevant, both with regard to the questionnaire respondent and the patient at the end of life, the latter being considered a possible pressure factor regarding therapeutic obstinacy at the end of life (45). With regard to medical interventions that may be considered disproportionate and futile at the end of life, both nurses and physicians proposed a number of interventions that respondents to the questionnaire could rate according to the extent to which they could be assessed as therapeutic obstinacy at the end of life. These types of interventions are in line with the literature, which most frequently subjects to ethical debate the appropriateness of initiating or not initiating artificial nutrition and hydration, ventilation, antibiotherapy, chemotherapy, any surgical intervention, dialysis, diagnostic and therapeutic interventions, cardiopulmonary resuscitation, mechanical ventilation, mechanical circulatory support, discontinuation, and withdrawal of treatment at the end of life (23-24, 46-47).

Physicians' fear of death was proposed by physicians in the third focus group to be assessed by study participants as to what extent it constituted a pressure factor for therapeutic obstinacy at the end of life. Although medicine has long ignored this fear, the literature notes that fear of death prevents health professionals from providing good and compassionate care to patients at the end of life (48). Lack of knowledge of the wishes and preferences of the patient and their family members was proposed for assessment as a possible cause of therapeutic obstinacy at the end of life. Aghabarary *et al*. highlighted that lack of knowledge of patient wishes by medical staff and the patient's family were key pressure factors in the provision of futile treatments at the end of life (39).

The pressure put on caregivers by family members who, for a variety of reasons, demand futile care was another potential therapeutic obstinacy at the end of life that both nurses and doctors participating in the focus groups considered crucial to include in the research. Chamberlin *et al*. showed that futile or potentially inappropriate care is associated with physicians avoiding family members, which is the behavior most strongly associated with burnout (49).

When it comes to assessing the frequency of facing the pressure of end-of-life therapeutic obstinacy in everyday practice, the medical staff decided to include this assessment in the questionnaire. Leuter *et al*. identified an internal struggle among healthcare professionals who felt compelled to perform treatments they believed to be ineffective (50). Results showed that nurses and physicians had different perspectives on morally sensitive circumstances in healthcare activities, which was likely related to differences in training, roles, and accountability to patients and healthcare institutions.

Psycho-emotional and spiritual support for the patient at the end of life, for their family, as well as for the healthcare staff involved in their care, are considered important in end-of-life care. Palliative care is one that provides holistic care for the patient and his or her family, adding psychological, social, and spiritual dimensions to

the physical one as recommended by palliative care guidelines. Spirituality is considered a priority for patients with life-threatening illnesses because of their hopelessness in the face of death and fear of the unknown. Psycho-emotional and spiritual support at the end of life gives a sense of comfort and security to patients and their families and helps to defuse some religious or non-religious attitudes, with healthcare professionals playing an important role in this supportive care (45).

The questionnaire items also identified in the literature reflecting the causes and pressure factors of therapeutic obstinacy at the end of life were supplemented with items reflecting the social, cultural, and medical particularities of our country. Both nurses and physicians identified the attitudes and demands of some patients and caregivers considered to be important members of society as a pressure factor in these situations. It was also proposed to evaluate the differences in attitudes between patients and caregivers from rural and urban areas from this point of view. The question at the end of the questionnaire gives the researcher the opportunity to explore the most common wishes expressed by patients at the end of life, information that may provide new ideas for further research in this area.

The resulting questionnaire will be self-administered, without the need for the researcher to be physically present, and will be sent both online via the internet and in hard copy on paper, along with instructions for filling out the questionnaire and consenting to participate in the research.

Strengths and limitations of this study

Strengths: the questionnaire created collects the views of health care staff on end-of-life therapeutic obstinacy, being the first questionnaire of this type created in Romania and appropriate to the cultural factors in this country. It explores the degree of knowledge and perception of medical staff of the concept of therapeutic obstinacy at the end of life, assesses the frequency of encountering such situations in everyday medical practice, and evaluates the opinion on some end-of-life interventions that may be considered disproportionate to the benefits obtained and the quality of life of patients. It also collects data on specialists' perceptions of possible causes that may lead to situations of therapeutic obstinacy at the end of life and possible solutions to improve such situations.

Limitations of the study: although the questionnaire explores many dimensions of therapeutic obstinacy at the end of life, it does not capture the effects of the phenomenon on healthcare professionals or the impact on end-of-life care.

CONCLUSIONS

Treatments that improve the patient's well-being and minimize the suffering of the patient and family are just as important as those designed to save the patient's life. Even though therapeutic obstinacy and medical futility have been recognized in medical practice for at least two decades, they continue today, with a detrimental impact on patients and their families, healthcare professionals, and the healthcare system. The reasons for discouraging or reducing therapeutic obstinacy at the end of life are simultaneously clinical, ethical, and economic. However, this discouragement has proved difficult because of public expectations of what medicine can achieve as well as because of professionals' percep-

tions of ethical and legal requirements.

The questionnaire created is innovative and the first of its kind in Romania. The structure of the questionnaire includes items resulted from the literature as well as new items identified by the medical professionals participating in the research, which allow the questionnaire to be adapted to the social, cultural, and medical conditions in this country. The data collected by means of this tool can provide important information and arguments for debating ethical, medical, legal, and economic issues related to end-of-life care, for assessing the behavioral and organizational trends of the actors involved in this type of care, and for capitalizing on the ideas of professionals in balancing the management of these patients.

Directions for future research

The present questionnaire does not encompass the collection of data pertaining to the impact of therapeutic obstinacy on healthcare personnel, the healthcare system, or the patient and their family. The findings obtained from this questionnaire can serve as a valuable foundation for conducting further study in this area. Finally, it is critical to explore the perspectives of patients and their families on medical interventions at the end of life, as well as the factors influencing decision-making in medical care in the final days of life. Our findings pave the way for a multidisciplinary approach to a variety of end-of-life treatments and therapies. This would imply the creation of complementary therapies that combine medical, psychological, and spiritual therapies.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflict of interest. This research received no external funding.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iași, by approval no. 103 of 12th of July 2021. The final form of the administered questionnaire is available by request addressed to the authors.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

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PRELIMINARY FINDINGS ON THE EFFECT OF VITAMIN B_6 ON ZEBRAFISH SOCIAL BEHAVIOR

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PRELIMINARY FINDINGS ON THE EFFECT OF VITAMIN B_6 ON ZEBRAFISH SOCIAL BEHAVIOR (Abstract): Vitamin B_6 (vit. B_6) is one of the most important vitamins involved in the maintenance and promotion of a good state of health and body function. Needed in multiple processes, vit. B6 is a water-soluble vitamin that actively participates in hemoglobin formation, glycogenolysis, gluconeogenesis, and the immune system. Its deficiency was many times addressed and linked with serious health issues. Some studies claim that vit. B_6 could be considered an adequate treatment for autism spectrum disorder (ASD). Usually seen as a complex neurodevelopmental disorder, ASD is a long-life condition described by persistent challenges in social communication and interaction, aside from restricted interests and repetitive behavior. It is widely recognized that genetic predisposition is one of the primary risk factors for ASD; however, environmental factors should also be explored due to their particular and complex mechanisms. **Materials and methods:** The main goal of the present study was to determine the effect of vit. B_6 on zebrafish behavior as an animal model for ASD using valproic acid (VPA), an anticonvulsant drug. For a period of five days, fish were exposed to 100 µM vit. B_6, 300 µM VPA, and a mixture of 100 µM vit. B_6 and 300 µM VPA. All the behavioral parameters were quantified through the EthoVision XT 11.5 software and performed using the adapted T-maze. The time spent in left arm, where the social stimulus was added, reflected the zebrafish's sociability level. Besides, data from locomotor activity was gathered. **Results:** No significant effect on social behavior was revealed after five days of exposure to vit. B_6. Despite this, behavioral improvements were observed in groups where fish received vit. B_6. **Conclusions:** Overall, this study provided limited evidence regarding the vitamin's capacity, suggesting the necessity of additional studies to promote the efficiency of vitamin usage in ASD. **Keywords:** VITAMIN B_6, ZEBRAFISH, SOCIAL INTERACTION TEST.

Described for the first time in 1932, vitamin B_6 (vit. B_6) comes from the wide group of B vitamins (1). Over time, its role and implications in various metabolic, physiological, and developmental processes were discovered. Due to its high solubility in

water and the incapacity of humans or other mammals to synthesize it, vit. B_6 can't be stored in the body, and inadequate diet can lead to important health issues. In general, vit. B_6 presents six vitamers, such as: pyridoxine, pyridoxal, pyridoxamine, and its bioactive 5'-phosphate forms: pyridoxine-5'-phosphate, pyridoxal-5'-phosphate, and pyridoxamine-5'-phosphate (2-4). Known to be part of more than one hundred body processes, vit. B_6 has been reported to act on protein folding, the biosynthesis of amino acids, glycogen mobilization, and neurotransmitter biosynthesis (5). Moreover, it was shown that vitamers of vit. B_6 can actively participate in the stress response through the antioxidant capacity proven against endogenous and exogenous sources (4,5). As a consequence of the stress presence, at cellular level, various metabolic products with negative impacts can appear, usually named free radicals (6, 7). Another important implication of vit. B_6 is in the process of erythropoiesis by participating in the addition of iron to the protoporphyrin that is related to theme synthesis (8).

Regarding the nutritional status of vit. B_6, this requires attention in assessing the optimal intakes. For instance, serious health problems were mentioned for its deficiency or even for elevated vitamin levels. Due to the metabolic roles played by the active form of vit. B_6, namely the pyridoxal-5'-phosphate (PLP), it can intervene in the mechanisms of different diseases and symptoms as: microcytic anemia, convulsions, dermatitis, diabetes, immunity, inflammation, or cardiovascular diseases are some of it (9, 10). In addition, connections between neurological conditions and vitamin's deficiency were highlighted in several works. One of the common conditions is represented by peripheral neuropathy, a disease characterized by nerve damage situated outside the brain and spinal cord (11). Insufficient levels of vit. B_6 were also linked to impaired production of neurotransmitters like serotonin, dopamine, γ-aminobutyric acid (GABA), noradrenalin, histamine, glycine, and d-serine (12, 13). Being involved in the synthesis of GABA, vitamin's deficiency can result in impaired neurotransmitter production that lowers the seizure threshold due to the GABA-mediated inhibition. A work from 2018 reported low levels of vitamins and high levels of GABA in a clinical study about depression (14). Similar data was shown for the autism spectrum disorder (ASD) where increased GABA levels were recorded (15-17).

The current description of ASD refers to it as a neurodevelopmental disorder diagnosed based on clinical observations regarding social deficits and repetitive behaviors (18-20). With no cure discovered and with multiple behavioral interventions, ASD is defined as a multi-factorial and complex disorder that can be identified since early childhood (20-22). Accumulated findings suggest that there is a connection between vit. B_6 and autism. The latest work published (2024) indicates that there is no causality between vit. B_6 and ASD risk after applying the Mendelian randomization (23). Despite this, a bunch of works are pointing out a possible implication of vit. B_6 deficiency in the sustaining and promotion of ASD-like symptoms in humans, but also in animal models (24,25). On the other hand, supplementation of the diet with vit. B_6 or combined doses of vit. B_6 with magnesium indicated a favorable response in several clinical studies (26-30).

In order to investigate the possible influence of vit. B_6 in an ASD context, the present work used zebrafish as an animal model. Zebrafish or *Danio rerio,* is a tropical fish brought for the first time to the

research world by George Streisinger in 1981 (31). This is a well-known organism that has been strongly utilized in the last few decades due to its advantages for biomedical and translational research (32-35). Its genome similarity and complex repertoire of behaviors make zebrafish a suitable model for investigating human diseases. It has been demonstrated that zebrafish can display specific-ASD symptoms that are directly related to social domain and repetitive behavior (36-39). Genetically or chemically-induced, autistic-like features could be generated in zebrafish models and further investigated with promising results for mechanisms and treatment discoveries (40-43). For the current work, valproic acid (VPA) was used to generate the ASD model in zebrafish; model which was several times mentioned in literature reports (44-46). This compound is prescribed for the treatment of epilepsy, bipolar disorder, and other types of brain seizures (47).

Nevertheless, the present experimental study aimed to investigate the short-term effects of the vit. B_6 and VPA on zebrafish from a behavioral perspective. The social interaction test brought few findings that required extra exploration of the current hypothesis due to obvious limitations that could be discussed in future works.

MATERIALS AND METHODS

Test organism

Zebrafish (*Danio rerio*) of the WT line (± 0.23 g weight, 26 mm body length) were purchased in a number of 150 animals and acclimatized for three weeks to the laboratory environment. The housing tank was filled with dechlorinated tap water that was maintained at a temperature of ± 26°C, a pH of 7.5, 90.6% dissolved oxygen, and a salinity of 0.25. The circadian rhythm was kept by an automatic timer set to 14: 10 hours (light: dark). Similar conditions were assured for the experimental tanks. Fish were fed twice a day with TetraMin flakes (5% of body weight in food per day, of which vit. B_6 accounted for approximately 12 ppm).

Substances

Valproic acid (P4543-100g) was bought from Sigma-Aldrich as a powder with a purity of 97.5%. For this study, 300 µM was chosen as the test dose, which was obtained by dissolving a certain amount of the stock solution into the medium. Regarding vit. B_6, this was provided by a local pharmacy as a diet supplement (the product brand will remain anonymous due to ethics and conflicting interests). Beside pyridoxine hydrochloride, the liquid product also contained purified water, glycerol, and potassium sorbate. For this study, 100 µM vit. B_6 was selected based on previous data (unpublished).

Experimental design

The first step in the present study consisted of the acclimatization of fish to laboratory conditions for a period of three weeks. After this time, ten zebrafish were randomly selected (a sex ratio of 1:1), and transferred to the experimental tanks, where animals were permitted to adapt to the new space. Four groups were formed, such as: control group, vit. B_6 group, VPA group, and the last group exposed to both chemicals (vitamin and VPA). Each tank (30 cm long × 16.5 cm wide × 20 cm height) had a capacity of 5 L and was equipped with an air pump. Secondly, the initial behavior of each group was assessed by social interaction test that were again performed on the first and fifth days of exposure. Fish from the VPA and mixture groups were constantly exposed to the anticonvulsant drug, while the exposure to vitamin lasted only one hour. This

method was adapted after the protocol of Pena *et al*. (48). Fish were moved to plastic vials filled with vitamin solution and kept for one hour. The control and VPA groups simulated vitamin exposure; its vials were filled with clean water. Behavioral testing started at 10 a.m. after finishing the vitamin's exposure. In the end of the experiment, fish were killed by immersion in cold water (< 4°C), and deposited in the freezer at - 80°C.

Social interaction test

The social interaction test aimed to quantify the changes that can appear in the sociability of zebrafish. For this test, the Noldus maze was adapted and transformed into a T-maze (three arms and a start point). A transparent wall was placed in the left arm to divide an area that will be dedicated to the social stimulus (fig. 1). Usually, this stimulus is represented by a group of 2-3 conspecifics. Each fish was placed at the start point for 30 seconds and recorded for the following 4 minutes. The total distance moved, the average velocity, the active time, and the time spent in arms were measured.

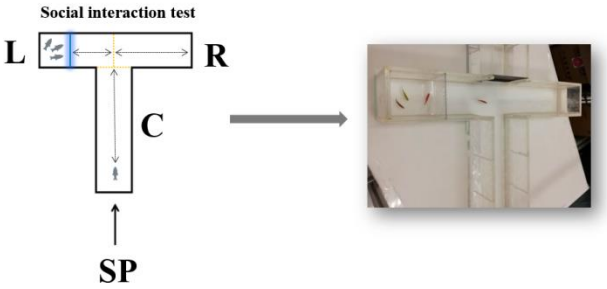

Fig. 1. The design of the social interaction test adapted for the Noldus maze transformed into a T-maze with three arms: left (L), right (R), and center (C) and an established start point (SP) situated in the center arm.

Statistical analysis

The behavioral parameters analyzed in this study were recorded through the EthoVision XT 11.5 software and sorted using the Excel files from the Microsoft Package (Microsoft Office Professional Plus 2021). All the statistical analysis was performed using the OriginPro v.9.8 software (OriginLab Corporation, Northampton, MA, USA, 2021). Data was tested for normality with the Shapiro-Wilk test (α was set at 0.05). A one-way ANOVA followed by a Tukey's multiple comparisons test was applied when the data checked the normal distribution. The results shown are presented as mean ± standard error of the mean (SEM), with $p \leq 0.05$ regarded as statistically significant. The graphical illustration of the results was made using the same statistical software.

Ethical note

This study was approved by the Ethics Committee of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, with registration number 342/09.02.2023. Also, the recommendations from Directive 2010/63/EU of the European Parliament and those of the Council of 22 September 2010 regarding housing, utilization, and protection of animals used for scientific purposes were followed.

RESULTS

No significant changes were recorded for the locomotor parameters evaluated during the social interaction test ($p > 0.05$ ANOVA, Tukey). The group exposed only to 100 µM of vit. B_6 did not exhibit any important improvements after one day (965.5 ± 87.9) or even in the last day of the exposure (860.9 ± 71.1) when compared to the initial behavior (962.2 ± 87.3). Moreover, combined exposure to 100 µM of vit. B_6 and 300 µM of VPA presented a similar trend, but on the last day, the distance moved maintained an appropriate value as recorded on the first day of exposure (863.1 ± 62.7 vs. 862.2 ± 79.1). Also, a slight decrease in the distance was recorded for the VPA group on the last day (868.3 ± 94.6) compared to the first day (1004.5 ± 86.2) and initial behavior (1009.9 ± 101.7) (fig. 2 left). Similar trends were observed for the average velocity parameter (Fig. 2, middle). Regarding the active time spent by the fish moving, its activity decreased on the last day of the experiment, while interestingly, the mixture group demonstrated almost the same activity seen in the initial behavior stage and first day of exposure (fig. 3, right).

In regards to the hypothesis of the present study, it could be seen from Fig. 4 that vitamin generated a short-term effect on the experimental groups, especially on the social behavior. Time spent in the left arm, where the social stimulus was added, presented the most significant activity compared to other arms. The group exposed only to vit. B_6 showed an increase in the time spent (65.2 ± 8.9 %), data similar to the observed behavior in the initial phase (68.6 ± 8.2 %), even if in the first day of exposure, time decreased (55.6 ± 8.1%). When vitamin was added in the VPA group, time spent in the area of the social stimulus increased, recording on the last day a value higher than that from pretreatment (59.9 ± 6.7 %) and the first day (50.7 ± 7.1 %) versus 66.5 ± 5.8 % (Fig. 4 left). After one day exposure to VPA, fish tended to spend more time in the left arm (61.3 ± 6.5 %) in comparison to initial behavior (50.3 ± 7.3 %) and the last day (42.7 ± 9.3 %). No trends were seen in the case of the control group.

Compared to the left arm, the other two arms did not record as much activity that was observed in the area dedicated to the social stimulus (Fig. 4 and Fig. 5). The second preference expressed by the experimental groups was different for each day; center arm for the initial behavior and the last day of the experiment, while the right arm was chosen for the first day by all the groups.

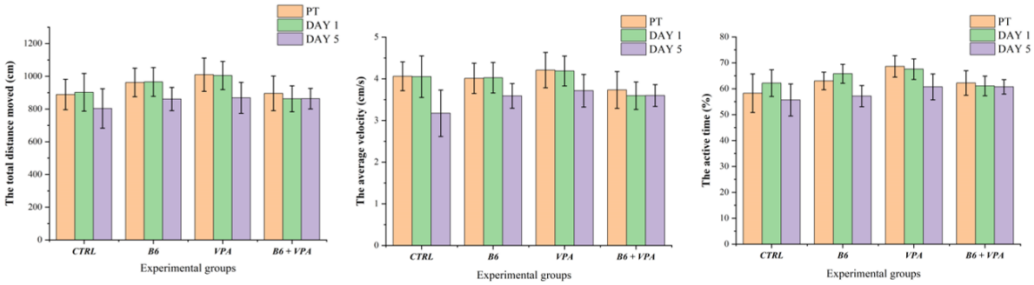

Fig. 2. The locomotor activity parameters recorded during the social interaction test of the experimental groups (n= 10): the total distance moved (left), the average velocity (middle), and the active time (right) during five days of exposure to vit. B_6 and VPA. PT stands for the pretreatment period. Data are expressed as mean ± SEM.

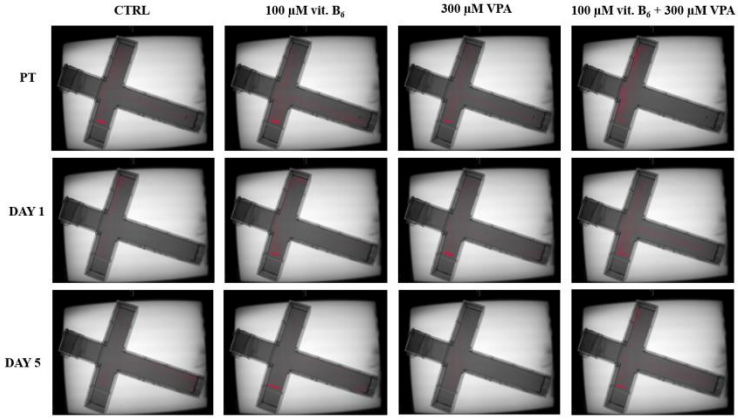

Fig. 3. The track visualization recorded during the social interaction test of the experimental groups where the left arm had the social stimulus added and was the most visited compared to the right and center arms.

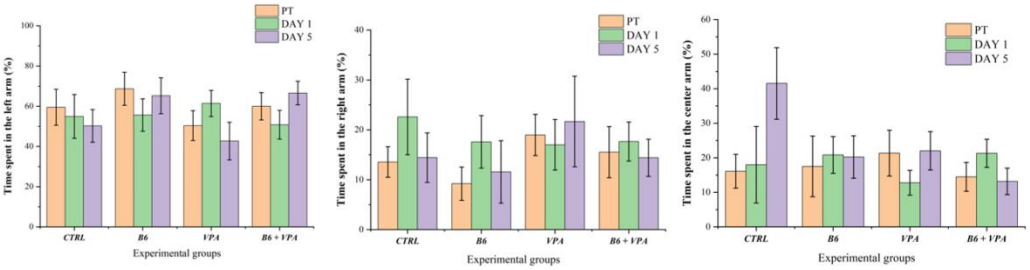

Fig. 4. The time spent in the T-maze arms recorded during the social interaction test of the experimental groups (n= 10): left arm (left), right arm (middle), and center arm (right) during five days of exposure to vit. B_6 and VPA. PT stands for pretreatment period. Data are expressed as mean ± SEM.

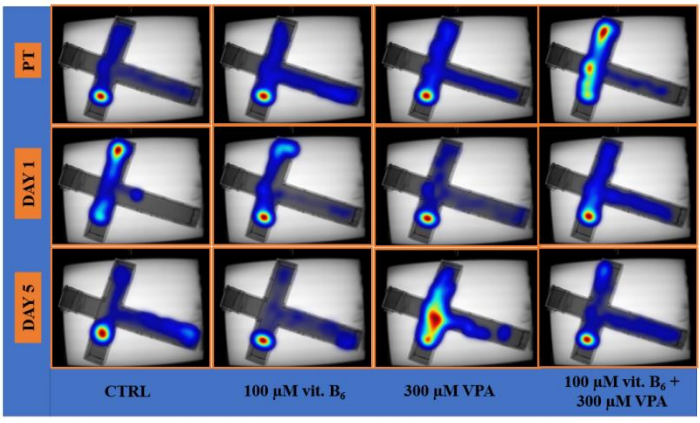

Fig. 5. The time spent in the T-maze arms recorded during the social interaction test of the experimental groups was presented through heatmaps for all testing days.

DISCUSSION

The current experimental study focused on analyzing the influence of vit. B_6 on social behavior in an ASD animal model using VPA as an inductor agent in a short-time exposure. As was expected, no significant data was obtained after five days of exposure to vitamin and VPA. Despite this fact, certain trends were perceived for all the exposed groups, mainly the time spent in the left arm next to the stimulus. In this way, the preliminary findings revealed the tendency of fish exposed to 100 µM vit. B_6 to return to the initial behavior in an established stress condition, while single exposure to 300 µM VPA triggered a lower sociability after five days. This experimental period, the sub-chronic type, could represent one of the main limitations of this study. In general, the effectiveness of vitamin supplementation requires a longer period, as was shown by Adams *et al*. in a research survey regarding vitamin and mineral supplementation for autistic people. A period of 5-8 weeks was necessary to observe improvements in autistic symptoms, and more than 90 % reported good evolution after 9-12 weeks of treatment, as stated by the NSTEA scale (49).

Diagnosed based on two main criteria, ASD is one of the neurodevelopmental disorders that expresses high variability and complexity due to its multifactorial character (50). It is known that genetic predisposition plays a major part in the occurrence of ASD, but the dynamic interactions with certain environmental risk factors leads to a multitude of combinations, highlighting the uniqueness of this disorder (20). With no cure discovered, research on this subject is abundant but still requires knowledge. Although there are several therapeutic tools that have proven its efficacy, vitamin supplementation is still uncertain due to the lack of clinical studies. For example, Adams *et al*. showed that autistic children who were not on a vit. B_6 supplementation revealed high plasma levels of vitamin and it was suggested that pyridoxal kinase responsible for producing the active form, PLP, was impaired and, consequently, had higher levels of PLP (5,51). Several years later, this hypothesis was disproved by the study of Chelban *et al*. which demonstrated high responsiveness from autistic people who received PLP as a supplement (52).

Another limitation of the present work is the absence of GABA measurement, especially of the direct correlation that might be suggested between it. This comes as a "consequence" of the involvement of vit. B_6 in the neurotransmitter system by its participation in some molecules biosynthesis (13). In ASD cases, based on magnetic resonance spectroscopy, neuroimaging, enzyme-linked immunosorbent assay, and postmortem brain analysis, it is often reported that there are disturbances of the GABA neurotransmitter, such as altered GABA metabolites, a lower ratio of GABA/ glutamate, and lower GABA levels (17, 53-55). Acting as co-factor, vit. B_6 regulates the GABA's activity, and it was indicated that supplementation with vit. B_6 led to increases in GABA levels in a clinical trial for anxiety and depression. The Screen for Adult Anxiety Related Disorders (SCAARED) and the Mood and Feelings Questionnaire (MFQ) tests concluded that vitamin intake could reduce anxiety and improve people's mental state (56).

It can be suggested that VPA might act on the GABA neurotransmitter, but reports are underlying the capacity of VPA to stimulate neurotransmitter production,

which contradicts the existence of a negative impact in some cases (57, 58). Thus, administration of VPA might lead to Parkinson's disease by stopping the voltage-gated ion channels and inhibiting histone deacetylases, for instance (59). Prenatal and neonatal exposure to VPA proved to be an autistic-like inductor in humans and in different animal models (40, 46, 60, 61). The results of this study did not point out significant changes in the total distance moved, average velocity, and active time. Despite this, in the last day of the experiment, it was observed that the fish had the tendency to lower the activity of all the parameters mentioned above compared to the group that also received vit. B_6. In zebrafish studies, early exposure to VPA was linked with hyperactivity, ASD-like symptoms, and impaired sleep behavior (60, 62-64).

Regarding the deficiency of vit. B_6 in zebrafish, the literature lacks in offering too much information. A study from 2019 that investigated neonatal epileptic encephalopathy in zebrafish proved the vitamin's effectiveness in alleviating the morphological and behavioral alterations (65). Pyridox(am)ine 5′-phosphate oxidase (Pnpo) deficiency was evaluated in Pnpo deficient zebrafish, known to be able of inducing serious seizures and neurodevelopmental issues. Early administration of PLP to zebrafish larvae that were Pnpo deficient led to an increase in the survival rate and PLP, glutamate, glycine, and GABA levels after 20 days (66). In a recent study on rats it was shown that vit. B_6 deficiency was able to lead to the appearance of autism-like behaviors in rats through autophagy; a process that was mediated by the inhibition of the mammalian target of rapamycin (mTOR). Moreover, the number of social interactions decreased, along with self-grooming time and bowel frequency, in the rats with insufficient vitamin compared to the control or supplemented group (25). This alteration occurred in the mTOR pathway and was often linked with autism pathology, being the one involved in synaptogenesis regulation, corticogenesis, and other neuronal functions (67-70). Additionally, this perturbation can lead to various negative outcomes, such as: abnormal synapse formation and brain structure, neuroinflammation and oxidative stress, or even mitochondrial dysfunction in different brain areas (70). All these processes mentioned above were associated with significant changes in autistic people that were summarized in impairments in social domain, communication, and behavior when, concomitantly, mTOR was hyperactivated (70, 71).

Beside the common symptoms reported in ASD, disruptions of the gastrointestinal tract and microbiota composition were highlighted several times (72-74). Interestingly, the work of Li *et al*., demonstrated that deletion of EphB6 (a pseudo kinase) can lead to autistic-like features alongside microbial disturbance in mice as a model animal for ASD (24). Improvements in social behavior were observed when vit. B_6 was intraperitoneally injected into EphB6-deficient mice. Moreover, transplantation of the fecal microbiota from wild type mice resulted in an increase of vit. B_6 in the plasma (24). The latest data published that focused on the gut-brain axis showed a powerful connection between different ASD phenotypes and temporal changes in the microbial composition (75). Although there is no direct or indirect established link for gut microbiota as risk factor in generating ASD, evidence serves both di-

rections. So, disturbances of microbial composition and of its metabolite levels were noticed and brought out not only for the gastrointestinal system but also for ASD (24, 74, 76).

As a final direction of this work could be to mention the vitamin's role as antioxidant. Restoring the social behavior of zebrafish after exposure to VPA might be assigned to vit. B_6 influence in a developed stress environment. One of the proposed mechanisms by which VPA induces toxicity is through the presence of oxidative stress (77-79). Autistic symptoms and cellular changes appeared as a consequence of VPA administration in rats, but it were improved after exposure to a combined extract of purple rice and silkworm pupae (78). Another study on rats looked at the VPA effect and found increased anxiety, hyperactivity, elevated activity for acetylcholinesterase, and decreased activities for superoxide dismutase and glutathione, besides displaying autistic-like features (80). The potential of vit. B_6 against the effects generated by VPA was revealed after a seven days treatment in male rats. The vitamin acted in a positive way on the oxidative stress-specific markers by decreasing the activities of glutathione-S-transferase, glutathione peroxidase, and glutathione reductase and increasing superoxide dismutase (81, 82). The direct correlation between VPA and oxidative stress, along with the effectiveness of vit. B_6 in fighting against free radicals through antioxidant sustenance, could be further explored since the involvement of oxidative stress in ASD appearance is certain.

CONCLUSIONS

This experimental study shows that exposure to vit. B_6 could have a positive impact on the social behavior of zebrafish based on the preliminary data obtained. Although no significant changes were seen in locomotor activity, vitamin supplementation in a stressful condition was helpful, highlighting the vitamin's capacity to act as an antioxidant. Furthermore, due to its limitations (time of exposure, the used vitamin supplement, or the way of exposure), the noted findings could draw future pathways of investigation by exploring the vitamin's influence in an ASD context.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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EVALUATION OF DEMENTIA-TYPE COGNITIVE DECLINE THROUGH 18F-FDG PET-CT QUANTIFICATION AND CORRELATIONS OF THE MMSE TEST WITH FUNCTIONAL IMAGING BIOMARKERS

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EVALUATION OF DEMENTIA-TYPE COGNITIVE DECLINE THROUGH 18F-FDG PET-CT QUANTIFICATION AND CORRELATIONS OF THE MMSE TEST WITH FUNCTIONAL IMAGING BIOMARKERS (Abstract): Recognition of the given moment when a mild cognitive impairment (MCI) converts to Alzheimer's Disease (AD) represents a turning point in the assessment of the mental status rate of decline, but is still insufficiently systematically studied. Our aim was to contribute to the identification of the dementia irreversible starting point by cognitive assessment test Mini Mental State Examination (MMSE) and correlating it with the hybrid imaging technique positron emission tomography (PET) with fluor-18 fluorodeoxyglucose (^{18}F-FDG) combined with localization computed tomography imaging (PET/CT), emphasizing that the method of quantification is crucial for patient evaluation and regression analysis can be helpful. **Materials and methods:** We studied 30 patients aged between 22 and 76 years presented during the assessment of their oncological pathologies. We evaluated their cognitive status by a MMSE test. All patients underwent a ^{18}F-FDG PET/CT examination, the image acquisition including the brain region. After specific image processing, characteristic regions of interest (ROIs) were identified, maximum and mean standardized values (SUVmax and SUVmean) were extracted. We computed correlations between 17 variables including: age, MMSE score value, glycemia, Administered Radiopharmaceutical Dose, Uptake Period, SUVmax and SUVmean for numerous ROIs as cerebral (SUVmaxCC) and cerebellar cortex (SUVmaxCc), posterior cingulate gyrus (SUVmaxPCG) and precuneus (SUVmaxP), as well as two SUV ratios: SUVRPCG and SUVRP. Calculation of mean, minimum, maximum and standard deviation have been made for each variable, and calculation of 4 regression functions (linear, exponential, power and logarithmic) and unique correlation analysis have been made for each pair of variables. **Results:** MMSE tests identified values of over 24, with an average of 28.07 ± 1.5DS, all patients examined having a normal cognitive status. SUVmaxPCG had values between 5.1 and 21.3 g/mL, with an average of 13.36 ± 3.77DS, SUVmeanPCG between 4 and 13.2 g/mL, average 8.68±2.35DS, and SUVmaxP between 4.8 and 21 g/mL with an average of 13.06 ± 3.81DS,

SUVmeanP between 4.1 and 14.1 g/mL with an average of 9.93±2.73DS. From the 34 strong and very strong linear and exponential correlations identified between SUVs of the studied ROIs, 7 correlations have "extremely strong" values (R>0.925) including SUVs of AD cerebral specific ROIs. Also, there is a mean linear correlation between Age and MMSE score (R=0.57). **Conclusions:** Quantitative evaluation of ^{18}F-FDG PET-CT images for patients with neoplasia, found in a stage when an MCI can evolve simultaneously, could bring useful information for a possible early AD diagnostic and would be advisable in the precision medicine of the brain cognitive status evaluation. **Keywords**: COGNITIVE DECLINE, MILD COGNITIVE IMPAIRMENT (MCI), MMSE, PET/CT, IMAGING BIOMARKERS.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, describing 60-70% of cases and affecting approximately 50 million of people worldwide, with an increasing incidence that could triple in the next 30 years, according to the World Health Organization in Guidelines for Risk Reduction of Cognitive Decline and Dementia (1). The adult process of neurogenesis is altered in the earliest stages of the disease, by progressive and irreversible loss of memory, of higher cerebral functions and later, of the self-help skill (2). Consistent with current guidelines, the diagnosis of AD involves clinical evidence of cognitive impairment, imagistic changes and accumulation of intracerebral biomarkers (3).

The brain has the highest avidity for glucose, consuming about 20% of the energy derived from its metabolism. But, in amnestic mild cognitive impairment (aMCI) and in AD, glucose metabolism is dramatically decreased, partly due to oxidative damage of enzymes involved in glycolysis, in tricarboxylic acid cycle and in adenosine triphosphate (ATP) biosynthesis, resulting synaptic dysfunction and neuronal death and thus the thinning of important areas of the cerebral cortex (4, 5, 6).

Neuroimaging, in particular hybrid functional and structural imaging, Single-photon Emission Computed Tomography (SPECT) or Positron Emission Tomography (PET) with various radiotracers currently available, in combination with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), are complementary medical imaging techniques and essential tools for in vivo studies of neurocognitive disorders and especially of AD. While CT and specifically MRI provide cerebral structural information with a higher resolution (hippocampal volume reduction and loss of other structures from the temporal lobes, with neurodegeneration and tissue loss), PET imaging can provide metabolic and molecular information of the brain, with high sensitivity, by mapping the distribution of glucidic metabolism (decreased glucose avidity and neurodegeneration in the temporoparietal cortex, posterior cingulate gyrus and precuneus), of NFTs and βA deposits in the cerebral cortex, from the presymptomatic to the dementia stage (7, 8, 9, 10). The brain areas strictly involved in cognitive decline, the posterior cingulate cortex and the precuneus, are known to represent the first areas of metabolic alterations, that precede cognitive symptoms for patients with dementia and especially with AD.

aMCI is a clinical precursor of AD but only some patients at the respective stage will progress to irreversible AD. Recent

studies have shown that PET examination with the analog radiotracer of fluorine-18 fluorodeoxyglucose (18F-FDG PET) can detect prodromal AD and can highlight the progressive conversion of aMCI to AD with an accuracy of 89%, in a follow-up time of minimum 5 years. (11).

Histopathological changes such as amyloid β plaques (βA), neurofibrillary tangles of tau proteins (NFTs) and neuritic plaques precede symptomatology by more than a decade, during which treatment may be effective in slowing or even stopping the progression of the disease (12). Thus, in addition to serum biochemical markers (cerebral acetylcholinesterase, superoxide dismutase, glutathione peroxidase, catalase, total content of reduced glutathione, carbonyl protein and malondialdehyde and lactate dehydrogenase) and from cerebrospinal fluid (low or inadequate concentrations of βA fractions and increases in the concentration of hyperphosphorylated tau proteins) (6, 13), as well as the imaging markers mentioned above, other accessible and very effective methods, such as those of mathematical statistics, could help in the early diagnosis of the disease.

Assessing mental status and its rate of decline for a given population are still insufficiently studied issues. Cognitive assessment tests such as Mini-Mental State Examination (MMSE), involving the rapid assessment of time and space orientation skills, attention and calculation, comprehension, naming, repetition, memorization and reminder, reading, writing and drawing, are widely used methods for clinical monitoring of cognitive abilities in the elderly, but insufficient to assess the progression of dementia (14).

Although data from literature emphasize the importance of quantitative evaluation of brain metabolism (4, 5, 6, 11, 12, 19, 20, 21, 22), the optimal method and the relationship between different methods that can be used in this regard have not yet been established and studies are still needed, each one bringing different information about the pathophysiology of AD development. In this context, this paper proposes a detailed study of data obtained for the cognitive and metabolic status of patients with potential cognitive decline to AD by the most used hybrid imaging technique PET/CT, qualitative and quantitative processing of functional metabolic images using specific software tools, their correlation with classic MMSE tests and statistical processing of obtained data. The novelty of the study is the combined analysis by specific methods and statistics of data obtained by MMSE and by 18F-FDG PET/CT, allowing correlations of special significance for patients with aMCI.

MATERIALS AND METHODS

For this pilot study, we prospectively evaluated 30 patients from the Regional Institute of Oncology Iasi, Romania, known with various oncological pathologies and undergoing a PET/CT examination. The inclusion criteria included: adult patients hospitalized in the Regional Oncology Institute, Iasi, Romania, in the period June-July 2021, which have a recommendation for PET/CT scan, for the evaluation of the oncological disease. The exclusion criteria included: the patient does not want to participate and did not sign the informed consent; the patient has brain neoplasia (primary tumor or brain metastasis); the patient has a blood sugar level over 160 mg/dL even after a correction attempt, represented by the intravenous administration of 500 mL physiological serum.

All imaging protocols were approved by an internal review board. Prior to participation in this study, signed informed consent for PET/CT examination and for data processing was obtained from all participant patients. The study was approved by the Ethical Committee of "Grigore T. Popa" U.M.Ph. Iasi Doctoral School, being part of the doctoral studies of the first author.

After performing an anamnesis specific to the basic pathology of each patient, we performed a standardized cognitive impairment assessment by applying the MMSE test, to evaluate the skills of orientation, attention and calculation, understanding and memory.

Each patient underwent a rigorous and specific preparation for PET/CT examination with an analog glucose radiotracer. Patients with glycemic levels above 160 mg/dL received a 500 mL saline infusion before injecting the radiotracer, normalizing glycemia according to the EANM recommendations for clinical trials (16).

Acquisition of imaging data and reconstruction of images

In the context of pre-existing oncological pathologies evaluation, all patients needed to perform a ^{18}F-FDG PET/CT examination, the acquisition of images including the brain region. The examination was performed using a fully integrated 3D PET/CT Discovery 710 scanner from General Electric and consisted in a full body acquisition CT scout and in two vertex-thigh (10 beds) or vertex-hallux (17 beds) acquisitions, with an acquisition speed of 2 minutes per bed, depending on the existing oncological pathology: one low-dose CT for localization and attenuation correction and another PET.

The dose of radiotracer administered and the uptake period from the time of administration to image acquisition have met the European Association of Nuclear Medicine required standards, the dose ranging from 235 to 592 MBq, with an average of 374.27 ± 55.7MBq, depending on individual body weight, with the uptake period of 44 to 76 minutes, and an average of 55.7 ± 9.5 minutes (3, 15, 16).

The specific processing consisted in the use of basic reconstruction algorithms of OSEM type (ordered-subsets expectation-maximization algorithm) and secondary algorithms of NAC type (non-attenuation correction) and TOF AC type (time-of-flight attenuation correction). The study of images in two dimensions (2D), in three different incidences (axial, coronal and sagittal), as well as in three dimensions (3D, maximum intensity projections or MIP) allowed the analysis of four sets of images per patient (axial, coronal, sagittal and MIP), i.e. the analysis of a total of 120 sets of images.

After specific processing of the acquired images, characteristic regions of interest (ROIs) were identified and plotted on surfaces of 20 mm diameter, both the maximum and the mean standardized uptake values (SUVmax and SUVmean) being subsequently extracted from the following general regions: descending aorta (Ao) corresponding to circulating metabolic activity and bladder or the most intense area of extracerebral glucidic uptake (B), as well as specific regions for functional imaging assessment of dementia: cerebral cortex (CC), cerebellar cortex (Cc), posterior cingulate gyrus (PCG) and precuneus (P). SUVmax and SUVmean values of general and specific ROIs plotted on the two-dimensional images were collected for each patient and statistically analyzed (fig. 1).

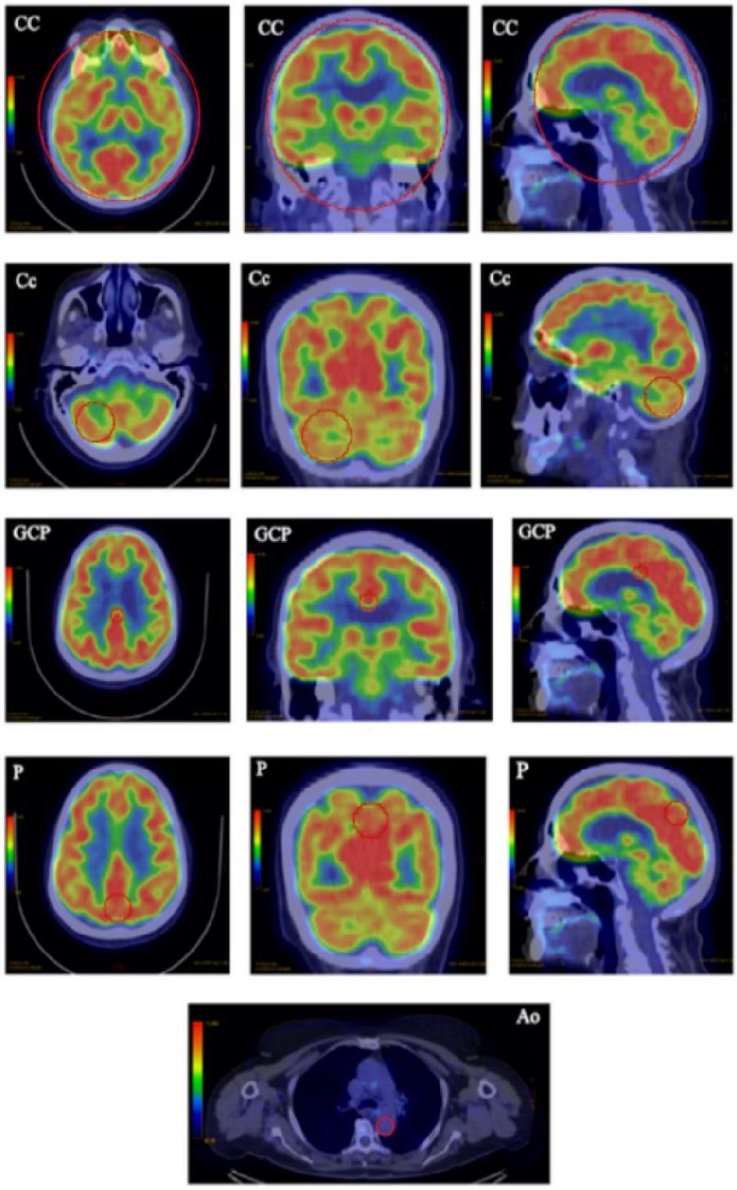

Fig. 1. ^{18}F-FDG PET/CT images axial, coronal and sagittal sections. UV values were collected by delimiting ROIs (dark red circles) in the (CC) Cerebral Cortex, (Cc) Cerebellar cortex, (PCG) Posterior Cingulate Gyrus, (P) Precuneus and (Ao) Descending Aorta

Numerical data analysis

All numerical data used, 17 variables marked V1-V17, are listed in the first table.

Statistical processing included the classical calculation of the following values: mean, minimum, maximum and standard deviation *(SD)*. After that, 4 regression functions are calculated (linear, exponen-

tial, power and logarithmic) for each pair of variables, as described in previous articles (17, 18). The formulas can be seen in the second table**.** For each type of correlation, we calculated *the correlation coefficient (R)* or the ratio between the variable's covariance and the product of their *SDs*

For the variables V1 - V7, V8 or V14, the unique correlation analysis allows the value identification of one variable, knowing only the value of another variable and applying the unique correlation formula.

TABLE I.

List of variables studied for each patient

V1	V2	V3	V4	V5	V6	V7	V8	V9
Age (years old)	MMSE Score	Glycemia (mg/dL)	Administered Dose (MBq)	Uptake Period (minutes)	SUVmaxAo (g/mL)	SUVmaxB (g/mL)	SUVmaxCC (g/mL)	SUVmaxCc (g/mL)

V10	V11	V12	V13	V14	V15	V16	V17
SUVmaxPCG (g/mL)	SUV maxP (g/mL)	SUVRPCG	SUVRP	SUVmeanCC (g/mL)	SUVmeanCc (g/mL)	SUVmeanPCG (g/mL)	SUVmeanP (g/mL)

Age, MMSE Score, Glycemia, Administered Radiopharmaceutical Dose, Uptake Period from injection to examination moment, SUVmax and SUVmean values in (CC) Cerebral Cortex, (Cc) Cerebellar cortex, (PCG) Posterior Cingulate Gyrus, (P) Precuneus, (Ao) Descending Aorta and (B) Bladder, as well as the SUVR for Posterior Cingulate Gyrus represented by SUVRPCG=SUVmaxPCG/SUVmaxCc and for Precuneus represented by SUVRP=SUVmaxP/ SUVmaxCc.

TABLE II.

Variables and correlations calculated

Operation	Formula	Explanations
Linear Correlation	$Y = a \cdot x + b$	x - the independent variable, Y - the calculated variable, a, b – known, constant numbers in the formula, e - the mathematical constant of the exponential ~ 2,71828
Exponential Correlation	$Y = e^{a \cdot x + b}$	
Logarithmic Correlation	$Y = a \cdot ln\, x + b$	
Power Correlation	Y = b · x (weak for all correlations)	
Correlation coefficient (R)	$R = \frac{\sum_{i=1}^{n}(x_i-\overline{x})(y_i-\overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i-\overline{x})^2}\sqrt{\sum_{i=1}^{n}(y_i-\overline{y})^2}}$	$x = x_1, x_2, \ldots x_i, \ldots x_n$ and $y = y_1, y_2, \ldots y_i, \ldots y_n$- the values of the x and y variables, $\overline{x}$ and $\overline{y}$- the averages of the respective variables, R -a number between -1 and 1, indicates the strength of the connection between the two variables (close to 1 -strong bond, 0.5 - average correlation, 0.2 - weak correlation, ≤ 0.1 - lack of correlation).

RESULTS

The 30 studied patients had between 22 and 76 years old, with a balanced male / female ratio of 1.14 (16 men and 14 women). The mean age was 54.03 ± 16.76 SD, divided into age groups: 6 young adults between 20-40 years old, 10 adults between 41-60 years old, 13 middle-old people between 61-75 years old, 1 old man over 76 years old. None of the subjects had an associated cerebral malignant pathology.

The patients group studied had a mean glycemia (V3) of 99.03 mg/dL ± 21.96 SD, with a minimum of 64 mg/dL and a maximum of 179.82 mg/dL.

MMSE tests identified values between 24.4 and 30 and an average of 28.07 ± 1.5DS, all patients examined having a normal cognitive status. The distribution of MMSE evaluation results by age groups is presented in the second figure.

From the acquired imaging data, *$SUVmax_{PCG}$(V10)* had values between 5.1 and 21.3 g/mL, with an average of 13.36 ± 3.77 *SD*, *$SUVmean_{PCG}$(V16)* between 4 and 14 g/mL, an average of 8.68 ± 2.35 *SD*, and *$SUVmax_{P}$(V11)* between 4.8 and 21 g/mL with an average of 13.06 ± 3.81 *SD*, respectively *$SUVmean_{P}$(V17)* between 4.1 and 14.1 g/mL, with an average of 9.93 ± 2.73 *SD*.

The mean, minimum, maximum, and *SD* values for all variables are listed in table III.

Linear, exponential, power and logarithmic regression functions were calculated and the strongest was recorded. As example, for the correlation SUVmeanP–SUVmean PCG (V17-V16) we calculated the 4 regression functions, the strongest being the linear one (R=0.900), where x is the independent variable V16 and Y(x) is the calculated variable V17: Y_((x)) =1.044·x+0.860.

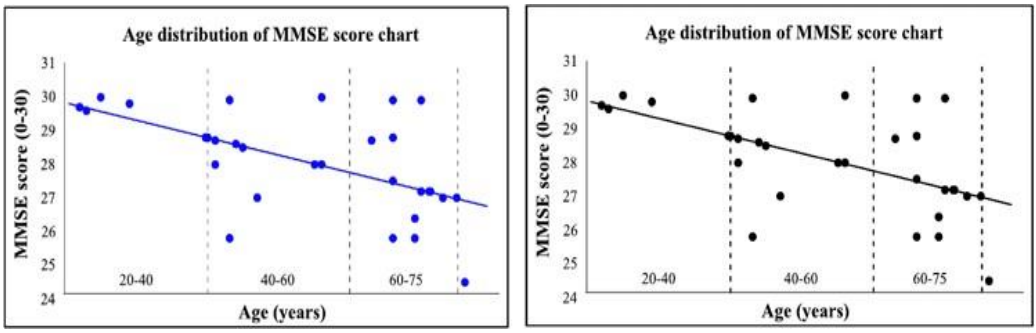

Fig. 2. Age distribution of MMSE score chart.

TABLE III.

Statistical calculation of mean, minimum, maximum and standard deviation values

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17
Mean	54.03	28.07	99.03	374.3	55.70	2.64	31.98	15.31	11.40	13.36	13.06	1.16	1.13	5.52	7.46	8.68	9.93
SD	16.76	1.50	21.96	72.37	9.51	0.56	22.67	4.49	2.45	3.77	3.81	0.14	0.18	1.44	1.95	2.35	2.73
Min	22.00	24.50	64.0	235.8	44.0	1.90	5.90	6.20	5.60	5.10	4.80	0.91	0.81	2.7	3.8	4.0	4.1
Max	76.00	30.00	179.8	592.2	76.00	4.40	103.9	27.20	16.70	21.30	21.00	1.58	1.54	8.3	12.5	14.0	14.1

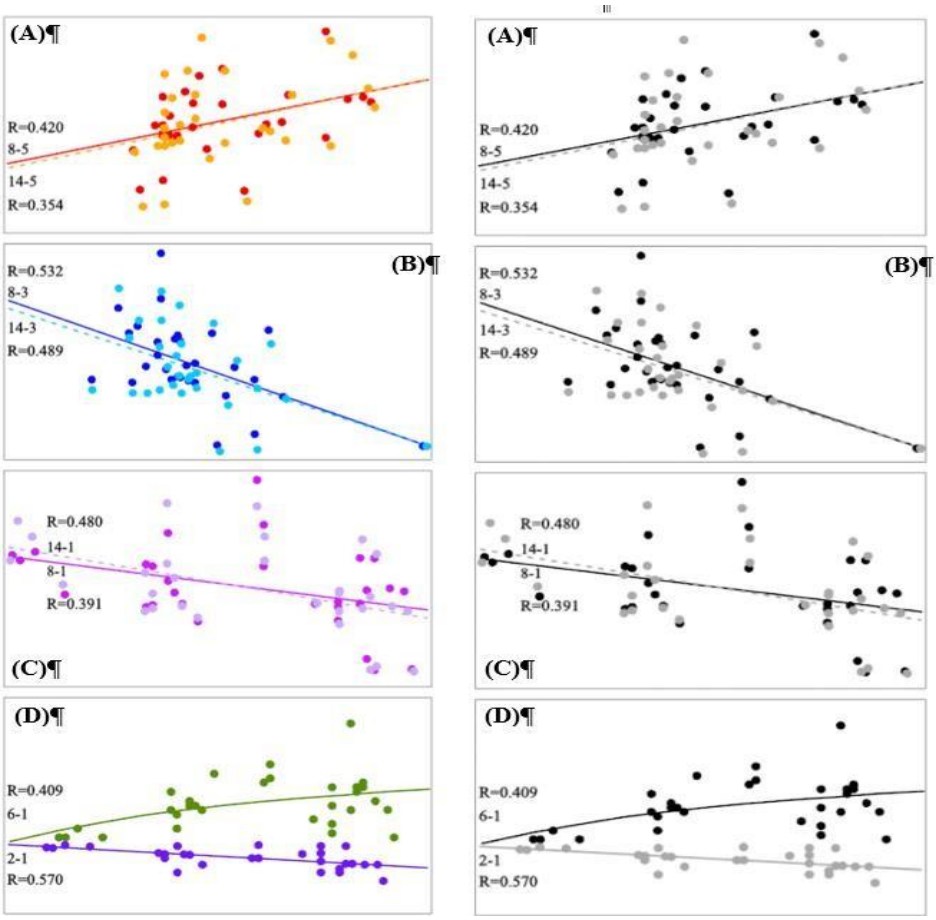

Fig. 3. Significant regression functions (R> 0.350) by unique correlation analysis. Correlations between (A) Uptake Period (5) and SUVmaxCC (8) or SUVmeanCC (14); (B) Glycemia (3) and SUVmaxCC (8) or SUVmeanCC (14); (C) Age (1) and SUVmaxCC (8) or SUVmeanCC (14); (D) Age (1) and MMSE (2) or SUVmaxAo (6)

No correlation was observed between the administered dose and any other variable, the doses being those recommended by international guidelines.

Slightly above average reverse correlations (R>0.5) were found between glycemia and cerebral SUVs, and a slightly higher between glycemia above 120 mg/dL and corresponding SUVs. The same kind of correlation was found between blood glucose and SUVs in specific ROIs SUVmax/meanGCP and SUVmax/meanP, denoting the dependence between brain glucose metabolism and plasma blood glucose.

Using only the function with the highest regression coefficient R, we obtained 34 "strong" correlations (R>0.75), of which 16 "very strong" (R≥0.9), and 3 correlations presenting "assimilable" value slopes (R>0.945).

From these 34 strong and very strong linear and exponential correlations identified between SUVs of the studied ROIs, 7 correlations have "extremely strong" R values (R>0.925) including SUVs of AD cerebral specific ROIs. Four sets of variables have an obvious correlation by the calculation formula of the maximum and average SUVs: SUVmeanP–SUVmaxP (V17-V11), SUVmeanPCG–SUVmaxPCG (V16-V10), SUVmeanCc–SUVmaxCc (V15-V9) and SUVmeanCC–SUVmaxCC (V14-V8). The correlation between SUVmeanP–SUVmaxPCG (V17-V10) is also partially related to the calculation formula. Four other sets: SUVmaxPCG– SUVmaxCC (V10-V8) (R=0.946), SUVmaxP–SUVmaxPCG (V11-V10) (R=0.939), SUVmaxP–SUVmaxCC (V11-V8) (R=0.915), SUVmaxPCG–SU-VmaxCc (V15-V10) (R=0.914) and their SUVmean correspondents are of particular significance, denoting the anatomo-pathological connection between these cerebral regions.

From the unique correlation analysis, we also withhold the average and slightly below average linear correlations (R>0.350) between SUVmaxCC (V8) or SUVmeanCC (V14) and Uptake Period (V5), Glycemia (V3) respective Age (V1). The logarithmic correlation between SUVmaxAo (V6) and Age (V1) and the linear correlation between MMSE score (V2) and Age (V1) have also statistical and medical significance (fig. 3).

Other correlations between the MMSE score and other variables didn't bring relevant information.

Further, the values of the other 16 dependent variables were obtained from the values of the independent variable V8, with regression functions (tab. IV).

Other correlations between different components of MMSE test and other variables did not brought significant data.

Several ^{18}F-FDG PET image analysis methods have been used to predict and detect aMCI conversion to irreversible AD, from qualitative, visual analysis to different quantitative methods: quantitative analysis by traditional and automated methods of deep learning-based algorithms, starting with Minoshima S *et al*. in 1995 (19) until these last years, with the work of De Carli F *et al*. in 2019 based on a Support-Vector Machine (20) or Pan X *et al*. in 2021 with theirs Multi-View Separable Pyramid Network (21). Voxel wise Cox regression based in ^{18}F-FDG PET data for prediction of aMCI conversion to AD was described by Sorensen *et al*. in 2019 (22) and Duan J *et al*. proposed BLADNet in 2023 (23). All these methods, although in different ways, have the same goal as our studies, emphasizing the importance of quantitative image evaluation. Being based also in a regression formula, Sorensen's study is the closest to our try to underline the crucial importance of quantification methods to identify the dementia irreversible starting point by regression analysis.

Our studies of quantitative evaluation of particular cerebral ROIs bring some contribution compared to qualitative evaluation. Our data demonstrated significant correlations between SUVs of specific cerebral ROIs and weak correlations between MMSE score and SUVs in the specific ROIs where modifications appear in different dementias, anatomo-pathological connections between these specific ROIs being undeniable.

The dependence between brain metabolism and glycemic levels shown in our data analysis with slightly above average reverse correlations (R>0.5) is supported by

literature (24). The total cerebral SUV and specific cerebral SUVs are values affected by glycemia, subjects with higher glucose levels presenting lower SUVmax/mean.

TABLE IV.

Single correlation regression functions for the independent variable SUVmaxCC (V8) and its degree of correlation

Weak (& vw.)	$V2(V8) = 0.486 \cdot V8 + 20.651$	$V4(V8) = -200 \cdot V8 + 191.6$	
	$V6(V8) = 0.687 \cdot ln(-9.524 \cdot V8 + 199.657) - 0.060$		$V7(V8) = -4.566 \cdot V8 + 101.826$
Medium	$V1(V8) = -9.524 \cdot V8 + 199.657$	$V3(V8) = -9.174 \cdot V8 + 239.165$	$V5(V8) = 5{,}051 \cdot V8 - 21.576$
Strong correlations	$V9(V8) = 0.505 \cdot V8 + 3.664$	$V10(V8) = 0.796 \cdot V8 + 1.176$	$V11(V8) = 0.755 \cdot V8 + 1.516$
	$V12(V8) = 0.505 \cdot \frac{0.796 \cdot V_8 + 1.176}{V} + 3.6648$	$V13(V8) = 0.505 \cdot \frac{0.755 \cdot V_8 + 1.516}{V} + 3.664$ 8	$V14(V8) = 0.298 \cdot V8 + 0.958$
	$V15(V) = e0.0535 \cdot V8 + 1.1578$	$V16(V8) = 0.479 \cdot V8 + 1.342$	$V17(V8) = 0.522 \cdot V8 + 1.940$

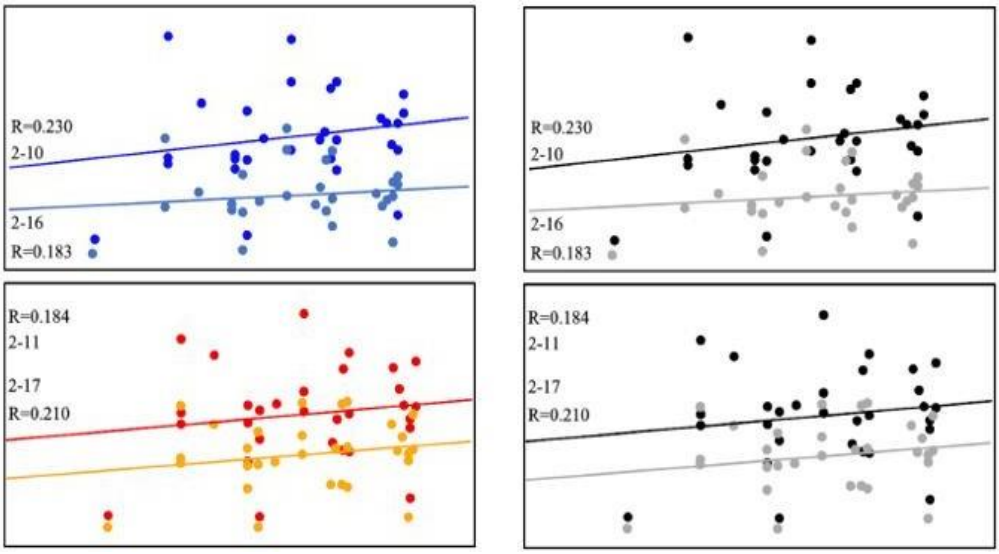

Fig. 4. Correlations between MMSE and AD specific cerebral SUVs

DISCUSSION

We observed that strictly following the protocols recommended by international guidelines, in terms of administered radiopharmaceutical dose, image acquisition protocol, and radiotracer uptake period, patient's blood glucose level before examination, is an essential step (16, 24).

It is also very important to make a judicious choice of the ROIs used for these correlations, allowing future development of some automatic correlations. It is important, also, that the patients do not have brain neoplasia diagnosis. Neurological functions

are not influenced by malignant pathology as long as patients do not have brain neoplasia, including cerebral metastases.

Different correlations between brain areas with important etiopathogenic involvement for AD suggest the usefulness of introducing an index of MCI metabolic visualization in order to identify early dementia. To obtain such an index between specific ROIs requires prospective studies on groups of patients with varying degrees of MCI and AD. Because subjects may perform ^{18}F-FDG PET repeatedly, meantime a MCI process may evolve concomitantly with the malignant pathology, this possibility supporting the usefulness of brain additional quantitative analyzes by introducing such index of MCI status.

Our studies are limited partly by the number of subjects and by the nonspecific pathologies of our patients.

We intend to extend this pilot study to a larger number of patients. Given the neoplasia patients indication for PET-CT images, a limitation of our study could be the uptake period that was established in relation to malignant pathology, also.

Other studies could be developed by improving AD neuroimaging database and by a more complex prospective predictive methodology. A direction would be the elaboration of forecasting software for aMCI to irreversible AD conversion, including multiple dependence correlation formulas with the most powerful connections between variables.

Considering the fact that many patients may be in the borderline period, still socially active, early diagnosis of predementia MCI is useful for limiting economic losses by delaying the onset of AD, thus achieving an MCI and AD precision medicine.

CONCLUSIONS

Even for the identification of a dementia irreversible starting point studies on a greater number of patients are still necessary, our results support the conclusion that the quantitative evaluation of 18F-FDG PET-CT images for cancer patients found in a stage when an MCI can evolve simultaneously would be advisable and could bring useful information for the early onset diagnostic in the precision medicine of the brain process of cognitive loss.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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RARE COMPLICATION OF RUPTURED ABDOMINAL AORTIC ANEURYSM PRESENTED IN EMERGENCY

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RARE COMPLICATION OF RUPTURED ABDOMINAL AORTIC ANEURYSM PRESENTED IN EMERGENCY(Abstract): Aorto-enteric fistula (AEF) is defined as an abnormal communication between the aorta or aortoiliac tree with the gastrointestinal tract and presents as catastrophic gastrointestinal bleeding. The reported annual incidence of primary aorto-enteric fistula is 0.007 per million (only 250 cases reported in literature). Due to the risk of progression to hemorrhagic shock and the high likelihood of surgical intervention, these patients should be closely monitored. **Materials and methods**: This paper includes 35 consecutive patients with ruptured abdominal aortic aneurysm admitted to the Department of Vascular Surgery of the "Sf. Spiridon" County Clinical Emergency Hospital, between 2020 and 2024. **Results:** From the total of 35 patients 3 cases were complicated with primary aorto-digestive fistula (8,57%). 2 of the cases had aorto-duodenal fistula and 1 case had aorto-enteric fistula. **Conclusions:** Complicated abdominal aortic aneurysm with primary aorto-enteric fistula is a rare pathology that requires prompt diagnosis and urgent surgical treatment. **Keywords:** RUPTURED ABDOMINAL AORTIC ANEURYSM, PRIMARY AORTOENTETIC FISTULA.

Aorto-enteric fistula (AEF) is defined as an abnormal communication between the aorta or aortoiliac tree with the gastrointestinal tract and presents as catastrophic gastrointestinal bleeding. The annual incidence is reported to be 0.007 per million and until present only 250 cases were found in literature (1, 2, 3). Primary aorto-duodenal fistula (PADF) is a direct communication between the abdominal aortic aneurysm (AAA) and duodenum and it is an extremely rare cause of upper gastrointestinal bleeding. This condition is often overlooked due to extremely low incidence (4). The most common predisposing conditions for primary aortoenteric fistulas are atherosclerotic abdominal aortic aneurysms or penetrating atherosclerotic ulcers. Less commonly encountered causes include inflammatory or infectious aortitis, diverticulitis, peptic ulcers, appendicitis, diverticulitis, foreign bodies and gastrointestinal malignancies (3, 5, 6, 7). Unlike secondary aortoduodenal fistula (SADF) which occurs

in patients who had previous aortic prosthetic reconstruction, primary aortoduodenal fistula is uncommon (5, 8).

Aortoenteric fistulas can occur with any part of the gastrointestinal tract, the most common location is the transverse portion of the duodenum(D3), which is involved in 60% of cases (3, 9). This is due to its relatively fixed position as compared with the rest of the bowel, and the close relationship of this part of the duodenum to the aorta (3).

The diagnostic is difficult to be recognized only through clinical findings. A classical triad of symptoms was described, including a pulsating, palpable abdominal mass, abdominal pain and gastrointestinal bleeding, but often this pathology is unsuspected until surgery or postmortem (5, 8, 10). In 30% of PADF patients, a brief and self-limiting gastrointestinal bleeding known as a "herald bleed" precedes a subsequent massive hemorrhage (5).

CT scans with contrast are the most reliable modality to evaluate an AAA, with a widely variable sensitivity of 50%-94%, a specificity of 85%-100% and a detection rate of 61% (3, 5, 6). In the appropriate clinical setting, contrast-enhanced CT is reported to have an overall specificity of 100% and a sensitivity of 50% in diagnosing an aortoenteric fistula (3, 11). Though MRI may have similar specificity and sensitivity to CT, the lack of availability in the emergency setting in addition to a longer acquisition time and need for greater technical expertise often limits its use (3).

The unreliability of clinical findings accentuates the need for a high index of suspicion for a PAEF in GI bleeding, especially upper GI bleeds (5). All reports emphasize the need for prompt imagistic investigation in patients presenting with AAA. AEF has high mortality and only prompt intervention can lead to a lifesaving outcome. (12).

Due to the evolution towards hemorrhagic shock and necessity of urgent surgical intervention, these patients should be rapidly diagnosed and admitted in the Vascular Surgery Department. Following surgical treatment patients require advanced intensive care. Prophylactic antibiotics administration is justified in special conditions, as interventions involving the intestinal tract, or the repair of traumatic injuries in contaminated wound (13). Due to the significant morbidity associated with open surgical repair, the use of endovascular repair (EVAR) for the treatment of AAA with AEF can be used as an alternative procedure (14).

MATERIALS AND METHODS

A retrospective study on patients with ruptured AAA admitted in the Vascular Surgery Department St. Spiridon Iasi Hospital between 2020-2024 was conducted. The purpose was to determine the incidence, patient characteristics and evolution in case of AAA associated with AEF.

Over the course of 5 years, a total of 35 patients with a diagnosis of ruptured AAA were admitted and underwent emergency treatment. Presentation symptoms where from discrete to severe: pale skin, diffuse abdominal pain with flank irradiation, tachycardia, tachypnoea, signs of hemorrhagic shock with hypotension, altered consciousness to confusion.

The following data where recorded: demographic characteristics (gender, age, background), perioperative clinical and biological status, presence of aortoduodenal fistula and mortality. All data was statistically analyzed using Microsoft Excel program.

RESULTS

In our study the majority where male patients (80%), the average age was 70 years and 71.42% where from the urban areas. The mortality rate was 74% (fig. 1). Of the total number of patients diagnosed with ruptured AAA through Angio-CT, only 3 cases were complicated with primary aorto-digestive fistula (8.57%) (fig. 2). Two of the cases had aorto-duodenal fistula and 1 case had aorto-enteric fistula. Similar to literature findings, the small number of digestive fistulas identified in our study supports the rarity of this complication.

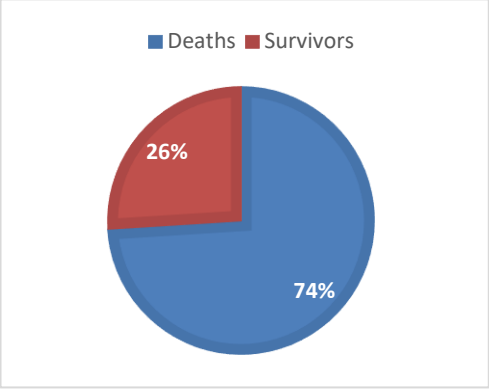

Fig. 1. The mortality rate from the total number of ruptured AAA cases

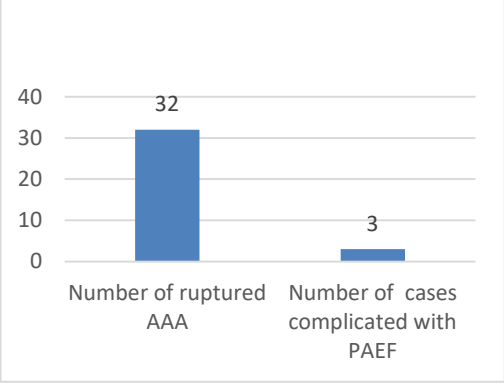

Fig. 2. The incidence of ruptured AAA complicated with PAEF

Regarding the personal pathological history of the patients hospitalized in our clinic with ruptured AAA, there is an increased prevalence of associated cardiovascular pathologies, prevalent essential arterial hypertension (62.85%), chronic heart failure (42.85%), aortic atherosclerosis (17.14%) and embolism and thrombosis of other arteries (31,42%). There is also an increased percentage of metabolic pathologies such as type 2 diabetes complicated with peripheral angiopathy (28.57%), obesity (14.28%), dyslipidemia (11.42%) but also chronic kidney disease and neoplastic pathology, both in the same percentage (14.28%). Considering the study period one risk factor taken in consideration was SARS COV 2 infection (28.57% patients) (tab. I).

TABLE I.

Pathologies associated with AAA in patients from the study group

1. Essential arterial hypertension	62.85%
2. Chronic heart failure	42.85%
3. Embolism and thrombosis of other arteries	31.42%
4. Infection with the SARS COV 2	28.57%
5. Type 2 diabetes complicated with peripheral angiopathy	28.57%
6. Aortic atherosclerosis	17.14%
7. Obesity	14.28%
8. Chronic kidney disease	14.28%
9. Neoplastic pathology	14.28%
10. Dyslipidemia	11.42%

Only 1 in 3 patients presented melenic stools as initial symptom, raising the suspicion of primary AEF.

At the time of admission in the Vascular Surgery Department, 2 of 3 patients had general influenced condition, hemodynamic

instability with need for inotropic support (Noradrenaline) and hemorrhagic shock.

We emphasize on one particular case of a 62-year-old male patient associated generalized abdominal pain with lumbar irradiation, bilateral lower limb paresthesia and melenic stools. The patient was hemodynamically stable which is why he underwent gastric endoscopy without revealing the source of bleeding. The Angio-CT exam showed a chronic partial thrombosed sacciform infrarenal AAA, with extended parietal hematoma on the right primitive iliac artery and raises the suspicion of a duodenal lesion by the contiguity of the aneurysm (fig. 3).

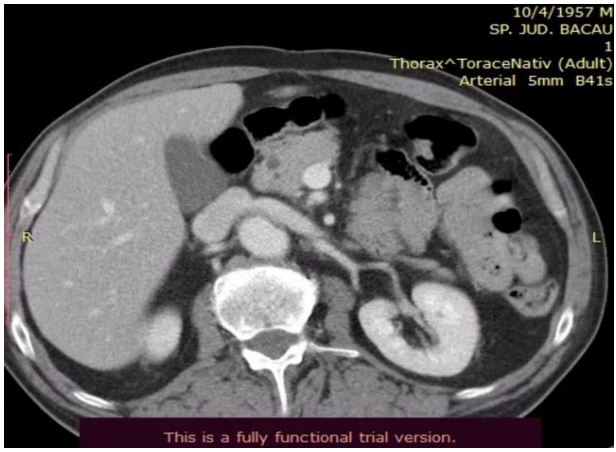

Fig. 3. Angio-CT image of ruptured AAA complicated with ADF

The patient underwent emergency surgery. The procedure included the following stages: infrarenal AAA aneurysmectomy, aorto-duodenal fistula closure, duodenorrhaphy, epiploonoplasty, left aorto-iliac bypass (left common iliac artery) and right aorto-femoral bypass (right common femoral artery) with synthetic Dacron graft (fig. 4).

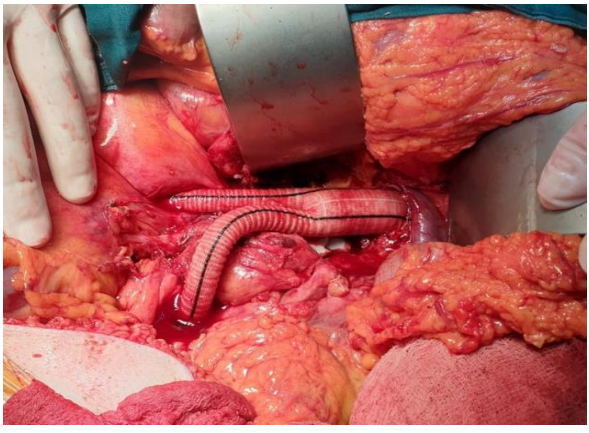

Fig. 4. Intraoperative aspect: left aorto-iliac bypass (left common iliac artery) and right aorto-femoral bypass (right common femoral artery) with synthetic Dacron graft.

Postoperative the patient was admitted in the intensive care. The evolution was favorable. Patients graft patency was initial certified by the presence of pulses in the bilateral distal arteries. The patient was discharged surgically cured. A follow-up included clinical and angio-CT evaluation.

After two years, the patient returns to the emergency department for diffuse abdominal pain and melenical stools, with SAEF observation. Immediate surgical intervention was required and it consisted in exploratory laparotomy, longitudinal duodenotomy, posterior endoluminal suture of D3, duodenorrhaphy, epiploonoplasty. The unfavorable evolution despite multiple surgical reinterventions led to the death of the patient at about1 month from admission.

DISCUSSION

Similar to literature findings, our study revealed a low prevalence of primary AEF as a complication of ruptured AAA. Given this aspect, nonspecific symptoms and rare gastrointestinal bleeding, it is hard to suspect this pathology.

The low prevalence of primary AEF can be attributed to several facts. Ruptured AAA incidence decreased due to screening and surgical or endovascular treatment prior to rupture. Also, AAA have higher success rate when treated electively (15). As we find in the literature, there is a high difference in mortality between patients who are surgically treated electively and those who benefit from emergency surgical treatment. Prevalence of abdominal aortic aneurysm in men older than age 65 has been decreasing over time, falling from about 4-8% to 2% in recent studies (15). The incidence of rupture was demonstrated to decrease as a result of active screening, increased surveillance and intervention in noncomplicated AAA.

The average age of presentation cited in the literature for patients with AAA is 63-64 years. The fact that the patients from our group are older can be attributed to the lack of screening programs leading to a later diagnostic. Similar to data in the literature AAA is being more frequent identified in men (same percentage - 80%) (6, 16, 17).

As mentioned, the clinical presentation of primary AEF is non-specific, consisting in digestive bleeding and abdominal or lumbar pain. The complete classic triad associating a palpable abdominal mass is found in only 11% of patients with AEF (3, 5, 6, 16, 18).

All patients underwent contrast CT scans for diagnosis and management planning. This imagistic investigation remains the study of choice with the highest detection rate. Imaging features suggestive of PAEF include air within the aortic wall, focal bowel wall thickening, disruption of pre-aortic fat, or the pathognomonic sign of contrast in proximity or in the small bowel (5, 6).

A review of the literature (366 cases) showed that 72.9% of PAEFs were in the duodenum, with 66% in the third portion of the duodenum and 33% in the fourth portion (D4) (19). Georgeades *et al.* shows in their study that 16 of the cases were in D3, 6 were in D4, 1 was in D2, and 13 cases had PADFs in an unspecified duodenal location (6).

When considering management of PADF, there are three main objectives - hemorrhage control, contamination / infection control, and maintenance of distal perfusion. Surgery remains the primary treatment modality of ADFs, with a periop-

erative mortality rate of 50% or higher with survival rates between 18% and 93% (6). Direct closure of the duodenal fistula appears to be the most common, but other procedures include resection with anastomosis, pyloric or duodenal exclusion, pancreatico-duodenectomy, Bilroth type I or II reconstruction, Roux-en-Y reconstruction, and others (6, 17, 18, 20-25).

Surgical technique has also been improving over time. In 2016 Ho S *et al.* reported mortality associated with PAEF repair of 34% compared with 44% prior to 1994 (5).

One important aspect of surgical repair is the use of an omental interposition flap between the aorta and duodenum. One study evaluated outcomes of ADF repair in 791 cases and noted that an omental interposition was the strongest predictor of survival. The most common cause of death is fistula recurrence, which occurs in 41.8% of patients, and has a higher frequency in those that did not have an omental interposition (6, 17).

Another important aspect in the subsequent evolution of the patients is the general condition at the time of presentation, being directly correlated with evolution. All 3 cases of ruptured AAA with primary FAE benefited from emergency surgery, regardless of clinical-biological status. Good general status and hemodynamically stable are correlated with postoperative favorably evolution.

CONCLUSIONS

Complicated AAA with primary FAE is a rare pathology that requires an increased degree of susceptibility to facilitate prompt diagnosis and initiation of specialized surgical treatment. This pathology is also associated with an increased mortality rate despite multidisciplinary treatment. Beside prompt surgical treatment and postoperative advanced intensive care, patient evolution is highly influenced by the general condition and preoperative clinical and biological parameters.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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KRUKENBERG TUMOR MIMICKING OVARIAN MASS

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KRUKENBERG TUMOR MIMICKING OVARIAN MASS (Abstract): Krukenberg disease is part of metastatic malignancies with the primary tumor usually located in the stomach, but any part of the gastrointestinal tract may be involved, including the small or large intestine. **Materials and methods:** We used statistical analysis to evaluate the link between risk factors and prognosis in 36 patients, over an 8-year period, i.e. between 2016 and 2024. **Results:** We examined the cases with ovarian metastasis due to gastrointestinal tumor over a ten-year period and found that this tumor made up 1% of the total ovarian cancers that required surgery in our department, these statistical findings being in line with literature data. **Discussion:** Managing this disease can be very difficult because of its poor prognosis. The patients have obviously a hard time understanding the pathway and the need for further investigations that include endoscopy, PET-MRI and second surgery programming, in order to improve their survival rate. **Conclusions:** An aggressive treatment is considered the key to prolong the patient's life, and the best management of this disease is to make as many studies as possible in this direction, in order to better understand and apply the interdisciplinary means of investigations and treatments. **Keywords:** KRUKENBERG TUMOR, OVARIES, ABDOMINAL PAIN.

INTRODUCTION

Krukenberg disease is part of metastatic malignancies with usually late-stage localizations in the ovary, associating a type of adenocarcinoma from the gastrointestinal tract, with cells rich in mucus and a typical ring sign presentation. In the late stages, the localization generally involves

both ovaries (in more than 80% of the cases). It was first mentioned more than 120 years ago. The starting point is usually the stomach, but any part of the gastrointestinal tract can be involved, such as the colorectal area, the appendix or the pancreas and literature shows that even the breast and the cervix can be affected. The main symptoms experienced by patients include an enlarged abdomen over a short time, flatulence, loss of appetite, weight loss, and there were reported cases where it occurred simultaneously with a pregnancy. The disease dissemination includes lymphatic and hematogenous routes, with the first one being more likely. Microscopically, it shows a first infiltration with tumoral cells on the cortex surface of the ovary, with a high number of positive lymph nodes (1, 2). The differential diagnosis is difficult to set in cases with unknown primary site, because ovarian mucinous tumors can mimic well signet-ring type cells. CA 125 is unspecific in Krukenberg cases, but may have a modest increase, up to 40 u/mL (3).

The primary development localization of the tumor is often the stomach or a colorectal cancer, in more than 70-90% of the cases. There are also other rare sites, such as the gallbladder. The appendix, breast, small intestine, pancreas or urinary bladder could also be affected. The median age at diagnosis is 40 years. The incidence rate in high risk areas with gastric cancer, such as Asia, can be up to 20% of the ovarian cancers. Pathophysiological, we describe three pathways of dissemination, such as: the lymphatic system, the hematogenous route and the trans coelomic dissemination, less recognized due to the poor infiltrates from the peritoneum, when laparotomy is performed (4-6).

The anatomopathological examination usually describes bilateral ovarian enlargement, with irregular and lumpy-looking surface. The tumors have a solid consistency, may have adhesions or near implants and could initially be considered primary cancers. Microscopy reveals a typical mucin laden sign-ring cell aspect of the tumoral cells. If this is absent, two other descriptions should be included, such as the stromal implication of the tumoral characteristics, or the sarcomatous proliferation of the ovarian stroma. Immunohistochemistry shows positive staining for cytokeratin, which is also called pan cytokeratin AE1-AE3, and positive staining for the membrane epithelial antigens (EMA). Vimentin and inhibin immunohistochemistry are negative. The most frequent cytokeratin's are 7 and 20 (7). A presurgical staging is important in describing a possible Krukenberg disease. The markers, such as CA-125, may be high and are important after the surgery, in appreciating a possible relapse.

The differential diagnosis is set after immunohistochemistry and involves other malignant ovarian tumors, but these typical markers offer a more accurate diagnosis. The prognosis is poor, less than 2 years of survival, but many efforts are made to have minimal microscopic disease dissemination during surgery. The Krukenberg disease is part of a severe oncological disease with multiorgan involvement that occurs as the final stage of a gastrointestinal primary tumor. When diagnosed, the disease has usually spread to both ovaries in more than 80% of the cases.

Our aim was to draw attention to this gastrointestinal disease that is often diagnosed during a laparotomy with an extemporaneous examination by gynecological surgeons. Before the surgery, it is important to provide an accurate anamnesis and, in cases of suspicions, to further recommend digestive endoscopic evaluation.

MATERIALS AND METHODS

We used statistical analysis to evaluate the link between risk factors and prognosis in 36 female patients, with ovarian metastasis due to gastrointestinal tumor, over an 8-year period, i.e. between 2016 and 2024, in a tertiary maternity center from Iași, Romania.

Patients total hospitalization time in our hospital ranged from 9 days to 21 days, including post-surgery hospitalization. In most cases, the diagnosis was suspected immediately intraoperatively and required extemporaneous examination. Surgery consisted of total hysterectomy with bilateral adnexectomy in 100% of the cases. Adhesiolysis was performed in 25% of the cases, and multiple biopsies of the peritoneum and great omentum were performed. In 25% of the cases, ascites was present and a cytological diagnosis was required. The extragenital macroscopic masses were localized on either the colon or the rectum and were found in 25% of the cases. During hospitalization, a gastroscopy and colonoscopy were performed postoperatively to identify the primary tumor, that was found in 25% of the cases during our hospitalization.

RESULTS

We found that this ovarian tumor made up 1% of the total ovarian cancers that required surgery in our department, these statistical findings being in line with literature data. All 36 female patients were aged between 38 and 64 and they had total hysterectomy with bilateral adnexectomy, in all cases.

The anatomopathological specimens revealed bilateral ovarian tumors in 100% of the cases. The size of the ovaries ranged from 4.5 cm to 10 cm. In all our cases, microscopy revealed cohesive tumor cells, arranged in islets, delimited by fine bands of stroma, solid areas and areas with tubular cribriform architecture. Other aspects included tumor cells with marked nuclear pleomorphism, atypical mitoses, tumor cells in signet ring and large areas of tumor necrosis. In 25% of the cases, the uterine serosa showed tumor implants, while in 25% of the cases the uterus had the same ovarian histological appearance of diffuse gastric adenocarcinoma with “signet ring” in the form of tubulo-glandular areas in mucoid or edematous stroma, with vascular lymphatic invasion. Uterine metastases involve the cervical and endometrial stroma, myometrium, and uterine wall, with frequent images of lymphatic invasion in 25% of the cases. Invasion of the parietal peritoneum or omentum with tumor implants was identified at biopsy in 25% of the cases. All the anatomopathological specimens examined showed loosely cohesive tumor cells, arranged in islets, delimited by fine bands of stroma, solid areas and areas with tubular, cribriform architecture (tab. I).

Tumor cells with marked nuclear pleomorphism, atypical mitoses, tumor cells in “signet ring” and extensive areas of tumor necrosis. In 25% of the cases, the uterine serosa showed tumor implants, and in 25% of the cases the uterus had the same ovarian histological appearance of diffuse gastric adenocarcinoma with “signet ring” in the form of tubulo-glandular areas in mucoid or edematous stroma, with vascular lymphatic invasion. Uterine metastases in the uterus involved the cervical and endometrial stroma, myometrium, and uterine walls, with frequent images of lymphatic invasion in 25% of the cases. Invasion of the parietal peritoneum or omentum with tumor implants was identified at biopsy in 25% of the cases (tab. II).

TABLE I.

Anatomopathological findings correlation with disease severity

Ovary and fallopian tube, uterus appearance	Ovary and fallopian tube, uterus appearance	Peritoneum
Macroscopic appearance	**Microscopic appearance**	**Associated histopathological appearance**
Ovarian mean dimensions 9± 2 cm (97.2%)	Ovarian metastases fallopian tube metastases uterine metastases in the uterine serosa, cervical and endometrial stroma	Peritoneal carcinomatosis
Lumpy outer surface, solid appearance in section, yellowish color, firm consistency (97.2%)	Diffuse gastric adenocarcinoma cells predominantly from "signet ring cells" arranged as pools, nests and isolated	Rectal invasion
Uterine wall with thin serosa, uterine mucosa thickened up to 3 cm, in 9 cases uterine invasion is found - in the uterine serosa, tumor implants, or in the endometrium (14.4%)	Tubulo-glandular areas included in mucoid or edematous stroma	Uterine sacral ligaments infiltration
Without intraoperative primary tumor identification (86 %)	Images of vasculo-lymphatic invasion	Bladder implants

TABLE II.

Clinical characteristics of ovarian disease progression and risk factors

Clinical characteristics	Krukenberg tumor group n=36
Age, years (mean ± SD)	50 ±4.31
Parity	Multiparous 24 (86 %)
Menopause	Premenopausal 9 (3.24%) Menopause 27 (97.2%)
Previous gynecological surgery	Uterine fibroma 4 (14.4%)
History of ovarian tumor	Previous benign ovarian cyst 5 (18%)
Important comorbidities - cardiac insufficiency, pelvic adhesions, ascites	Ascites 27 (97.2%) Cardiac insufficiency 4 (14.4%) Adhesions 27 (97.2%)

DISCUSSION

Worldwide, ovarian cancer is in the top seven oncological pathologies in women and represents the eighth cause of mortality in female patients (8, 9).

Most malignant forms that affect the ovary are the epithelial carcinoma, the germ cell tumor carcinoma, the sex cord-stromal tumor, and metastasis, like Krukenberg's tumor. The detection of the last one, this particular type of late ovarian cancer, was difficult, as there is no screening program for its early detection. The best-known cancer types that give metastasis in the ovaries are the breast, colorectal, gastric, and appendix tumors. Pregnancy and metastasis may occur simultaneously. The bilateral involvement of the ovaries is frequent at this stage (10-12).

The Krukenberg tumor from pulmonary malignancy is rare. The immunohistochemical examination of the pleural or ascites

fluid cytology can confirm the diagnosis of the main organ origin (13-17). When considering breast cancer primary tumor and metastasis to the ovary, most of the metastatic breast carcinomas are found to be positive for estrogen receptor on immunohistochemistry (17-19). Studies show that most of the gastric tumors are positive for CDX2 and only a few are positive for SATB2 markers. The colorectal and appendiceal cancers are positive for CDX2 and SATB2. GATA3 stains are positive in almost all breast primaries and in more than half of the bladder cancers. Almost all the pulmonary cancers are positive for TTF1. PAX8 is negative in gastric, colorectal and appendiceal cancers.

The management of this disease can be very difficult because of its poor prognosis. The patients obviously have a hard time understanding the pathway and the need for further investigations that include endoscopy, PET-MRI scan and a second surgery, in order to improve their survival rate. Aggressive surgery, designed to reduce the macroscopic disease and limit the number of cancerous cells, is recommended. When only one ovary is affected, bilateral oophorectomy is performed in order to decrease the risk of further dissemination. After the surgery, none of the results are considered microscopically negative, unless we perform the resection with negative margins and if a little tone or a big mass is still found in the pelvis after the operation. The differential diagnosis is difficult to set in cases with unknown primary site, because the ovarian mucinous tumors can mimic well “signet-ring” type cells. CA 125 is unspecific in Krukenberg cases, but may have a modest increase, up to 40 u/ml (19). A metastatic tumor can often be the first manifestation of a neoplastic disease. A significant number of CK7(+)/CK20(-) patients were found to have gastric cancer. The most cited and used tumor markers in the immunohistochemical diagnosis of metastatic ovarian tumors are cytokeratins: CK20 and CK7(20-24). The incidence rate of multiple primary cancers among malignancies can be up to 8%. Different immunological markers have been cited for ovarian cancers, such as PAX8 and WT1, but etiopathological pathways have yet to be surely discovered (25).

When discussing the various pathways of the spread of Krukenberg tumor, there are various theories such as: retrograde lymphatic dissemination, the main pathway of dissemination in gastric cancer metastases, hematogenous pathway spread most frequently encountered in colorectal cancer, and trans peritoneal direct spread in peritoneal carcinomatosis. Prognostic factors influence the spreading of any disease, according to parameters and factors in Krukenberg tumors disease that include peritoneal involvement, synchronous presentation, ascites, and increased serum carcinoembryonic antigen levels.

There are no apparent differences between the symptoms arising from primary and secondary ovarian malignancies. Krukenberg tumors remain asymptomatic until very advanced stages, therefore any sign of abdomen enlargement or ascites should be considered for further follow up. Although Krukenberg tumors may be induced by complex mechanisms, lymph node metastasis is considered to be the most significant risk factor for recurrence. Various articles compare patients with advanced Krukenberg tumor at a young age and establish that a functioning young ovary is prone to metastatic disease due to the rich ovarian blood supply predisposing to hematogenous metastasis.

We mostly promote early symptoms presentation, annual check-up and to encourage multidisciplinary approach when

patient symptoms are not correctly addressed and resolved.

We performed a statistical literature data review since 2021, where more than 3000 patients had the pylorus sphincter as main localization, 42.5% of the cases, and the second localization involved the colon in 26.1% of the cases. Ovarian cancers with idiopathic localization were encountered in 12.2% of the cases, while the urinary tract and bladder were found in less than 1% of the cases (25, 26).

The data reviewed involved the examination of the efficacy of surgery in patients with gastric cancer and synchronous ovarian metastases. The patients underwent resection of ovarian metastases, either alone or in conjunction with gastric resection and lymph node dissection. The newest data show the efficiency of systemic and/or hyperthermic intraperitoneal chemotherapy (HIPEC) after surgery. Median overall survival was significantly greater in those patients who underwent excision before systemic chemotherapy compared to those who underwent simultaneous gastrectomy and metastasectomy following chemotherapy. The use of HIPEC improved overall survival in patients with peritoneal metastases.

In a smaller statistical group, involving 128 patients, in the early 2000, over a 20-year period, the median age at diagnosis of the disease was 48 years, with a mean average survival rate of 16 months after the diagnosis. Data show that synchronous metastasis ($p=0.0080$), pelvic invasion ($p=0.0138$) and ascites are the main unfavorable factors (27).

CONCLUSIONS

The screening of patients with gastrointestinal symptoms is important and full abdominal ultrasound scan is recommended in order to evaluate the pelvis for metastatic diseases.

The survival rate increases if the diagnosis is set with unilateral disease. Chemotherapy can improve the quality of life of these patients and aggressive surgery is recommended in order to obtain macroscopically free lesion disease. This article is important for the need to address further investigations in cases of undetected tumor sites, in order to better perform multidisciplinary second surgeries, together with the oncological surgeon and the oncological multidisciplinary team's implication.

Ovarian metastases are present in up to 10% of the women with inoperable gastrointestinal cancer, and studies have shown that the patients' age is constantly decreasing. Therefore, early diagnosis is mandatory for the improvement of the survival rates and the patient's life quality.

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ASSOCIATION BETWEEN ALCOHOL-RELATED LIVER CIRRHOSIS AND LATE STAGES AT DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

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ASSOCIATION BETWEEN ALCOHOL-RELATED LIVER CIRRHOSIS AND LATE STAGES AT DIAGNOSIS OF HEPATOCELLULAR CARCINOMA (Abstract): Hepatocellular carcinoma (HCC) is a major complication of liver cirrhosis (LC), and its prognosis is highly dependent on the stage at diagnosis. Alcohol-related liver disease (ALD) is an increasing cause of cirrhosis worldwide, but its impact on the stage of HCC diagnosis remains unclear. **Aim** of the study: to evaluate the stage at which HCC is diagnosed and assess whether alcohol-related cirrhosis predisposes patients to a more advanced-stage presentation. **Materials and methods:** We conducted a retrospective observational study of 152 patients diagnosed with HCC at a tertiary center between January 2022 and December 2023. Patients were classified by cirrhosis etiology (alcohol-related vs. viral hepatitis-related) and categorized by HCC stage at diagnosis according to the 2022 Barcelona Clinic Liver Cancer (BCLC) classification. For analysis, patients were grouped based on curative treatment eligibility: curative stages (BCLC 0-A and selected B1 cases) and non-curative stages (BCLC B2-B3, C, and D). **Results:** Among the 152 patients analyzed, 54 (35.5%) had alcohol-related cirrhosis, and 96 (63.2%) had viral hepatitis-related cirrhosis. At diagnosis, no patients were identified at the very early stage (BCLC 0), while 18.4% were diagnosed at a curative stage and 81.6% at a non-curative stage. Notably, nearly half of all patients (46%) presented at the terminal stage (BCLC D). The proportion of patients diagnosed at a curative stage was significantly lower in those with alcohol-related cirrhosis (7.4%) compared to those with viral hepatitis-related cirrhosis (24%) ($p = 0.01$). Multivariate analysis confirmed that alcohol-related cirrhosis was independently associated with a higher likelihood of non-curative stage diagnosis. **Conclusions**: Patients with alcohol-related cirrhosis are significantly more likely to be diagnosed with HCC at a non-curative stage compared to those with viral hepatitis-related cirrhosis. This disparity underscores the need for improved HCC surveillance strategies in this high-risk population, including better patient engagement, integration of hepatology with addiction services, and enhanced screening adherence programs. Addressing these challenges could facilitate earlier diagnosis and improve treatment opportunities for patients with alcohol-related cirrhosis. **Keywords:** ALCOHOL-RELATED LIVER DISEASE, CIRRHOSIS, HEPATOCELLULAR CARCINOMA, STAGING, CURATIVE TREATMENT.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and represents a major global health burden. It is one of the most serious long-term complications of liver cirrhosis (LC), with most cases developing in patients with underlying cirrhotic disease (1). The prognosis of HCC is highly dependent on the stage at the time of diagnosis, as early-stage detection allows for potentially curative treatments such as liver transplantation, surgical resection, or locoregional therapies (2). In contrast, advanced-stage HCC is associated with limited therapeutic options and poor survival outcomes.

The development of HCC is strongly linked to various etiological factors that contribute to chronic liver disease and cirrhosis, including viral hepatitis - hepatitis B virus (HBV) with or without hepatitis D virus (HDV), and hepatitis C virus (HCV), metabolic dysfunction-associated steatotic liver disease (MASLD, formerly non-alcoholic fatty liver disease - NAFLD), and alcohol-related liver disease (ALD) (3,4). Among these, ALD remains a significant and growing cause of cirrhosis and HCC worldwide (5). However, the impact of cirrhosis etiology on the stage at which HCC is diagnosed is not fully understood.

Alcohol-related cirrhosis is associated with multiple clinical and socioeconomic factors that may contribute to delayed diagnosis, including the absence of routine surveillance, more frequent decompensation events that mask tumor-related symptoms, and differences in healthcare-seeking behavior (6). Understanding whether ALD-related cirrhosis predisposes patients to a later-stage diagnosis of HCC is critical for improving surveillance strategies and optimizing patient outcomes.

The **aim** of this study is to assess the stage at which HCC is diagnosed and to determine whether cirrhosis etiology, particularly alcohol-related cirrhosis, influences the likelihood of an advanced-stage presentation at the time of diagnosis.

MATERIAL AND METHODS

Patients. We conducted a retrospective observational study of patients diagnosed with hepatocellular carcinoma (HCC) at a tertiary reference center over a two-year period (January 2022 – December 2023). Eligible patients were identified from the hospital's electronic medical records and included if they had a confirmed diagnosis of HCC based on imaging criteria (contrast-enhanced computed tomography or magnetic resonance imaging) and/or histopathological confirmation.

Methods. We collected demographic data, including age, sex, and relevant clinical history. The etiology of liver cirrhosis was recorded for each patient and classified as alcohol-related cirrhosis, viral hepatitis (HBV with or without HDV, and HCV), MASLD, or other less common causes (including autoimmune etiology, hereditary hemochromatosis, Wilson's disease, and other rare etiologies).

The stage of HCC at the time of diagnosis was determined using the 2022 Barcelona Clinic Liver Cancer (BCLC) classification system (7). This system classifies patients into five stages (one of them comprising three substages) based on tumor burden, liver function, and performance status (Table I).

For this study, patients were categorized into two groups based on the feasibility of curative treatment: those with ***curative stages at diagnosis***, defined as BCLC 0-A and selected BCLC B1 cases eligible for liver transplantation, and those with ***non-***

curative stages at diagnosis, comprising BCLC B2-B3, C, and D.

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional ethics committee. Informed consent was waived due to the retrospective nature of the study.

TABLE I.
BCLC staging (7)

BCLC Stage	Characterization
0 (Very Early Stage)	Single, small tumors without vascular invasion and good liver function, suitable for curative treatment (e.g., surgical resection or ablation)
A (Early Stage):	Single or multiple tumors with good liver function, candidates for curative treatment (e.g., resection, liver transplantation, or ablation)
B1 (Intermediate Stage, Subgroup 1)	Multiple tumors with a low tumor burden and good liver function, potentially eligible for liver transplantation
B2 (Intermediate Stage, Subgroup 2)	Multiple tumors with a higher tumor burden, but with preserved liver function and no signs of advanced liver disease
B3 (Intermediate Stage, Subgroup 3)	Multiple tumors associated with moderate hepatic dysfunction (e.g., ascites or varices), without signs of advanced liver failure
C (Advanced Stage)	Large or multinodular tumors with vascular invasion, or extrahepatic spread, with poor liver function, typically treated with systemic therapies
D (End-Stage)	Extensive tumor burden with decompensated liver disease, managed with palliative care

Statistical analysis was performed using the *SPSS version 17.0 software* (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to summarize patient characteristics. Comparisons between groups were made using the chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

Patients' characteristics

A total of 152 patients with hepatocellular carcinoma (HCC) were analyzed (Table II). Among them, 54 patients (35.53%) had alcohol-related liver cirrhosis (LC), while 96 patients (63.16%) had a viral etiology: 30 with hepatitis B virus (HBV), 6 with both HBV and hepatitis C virus (HCV), 8 with both HBV and hepatitis D virus (HDV), and 52 with hepatitis C virus (HCV) alone. Additionally, one patient (0.66%) had metabolic dysfunction-associated steatotic liver disease (MASLD), and one (0.66%) had autoimmune-related cirrhosis.

HCC staging

According to the BCLC classification, 10 patients were diagnosed at the early stage (A), 33 at the intermediate stage (B) (5 in B1, 11 in B2, and 17 in B3), 39 at the advanced stage (C), and 70 at the terminal stage (D). Notably, no cases were detected at a very early stage (BCLC 0).

Curative treatment eligibility

When grouped by treatment eligibility, 28 patients (18.4%) were diagnosed at a curative stage (BCLC A or selected B1 cases eligible for liver transplantation), while 124 (81.6%) were diagnosed at a non-curative stage (BCLC B2-B3, C, or D). This distribution highlights a significant predominance of late (especially advanced-stage and end-stage diagnosis), limiting therapeutic options for most patients.

TABLE II.

Patients' clinical characteristics, cirrhosis etiology and stage at diagnosis

Characteristics	Total number of patients = 152
Age (years), mean +/- SD	65 +/- 10
Range (years)	44-86
Sex, male, n (%)	96 (63.2%)
Etiology of cirrhosis, n (%)	
- Alcohol-related	54 (35.53%)
- HBV	30 (19.74%)
- HBV+HCV	6 (3.95%)
- HBV+HDV	8 (5.26%)
- HCV	52 (34.21%)
- MASLD	1 (0.66%)
- Autoimmune	1 (0.66%)
Diagnostic stage, n (%)	
- Curative	28 (18.4%)
- Non-curative	124 (81.6%)

Staging by etiology

We subsequently analyzed patients based on etiology, comparing those with alcohol-related cirrhosis to those with viral hepatitis-related cirrhosis (tab. III). Due to the small numbers, we excluded the two patients with other etiologies (one with autoimmune cirrhosis and one with MASLD). Among patients with alcohol-related cirrhosis, only 4 (7.4%) were diagnosed at a curative stage, while 50 (92.6%) were diagnosed at a non-curative stage. In contrast, among patients with viral hepatitis-related cirrhosis, 23 were diagnosed at a curative stage (24%), while 73 (76%) were diagnosed at a non-curative stage. Comparative analysis using the chi-square method revealed that the proportion of patients diagnosed at a curative stage was significantly lower among those with alcohol-related cirrhosis compared to those with viral hepatitis-related cirrhosis ($p = 0.01$). This finding underscores the challenges faced by patients with alcohol-related cirrhosis in accessing timely and effective treatment options.

TABLE III.

Stage at diagnosis according to cirrhosis etiology

	Alcohol-related cirrhosis (n=54)	Viral hepatitis-related cirrhosis (n=96)	p-value
BCLC stage, n (%)			
Potentially curable stages	**4 (7.4%)**	**23 (24%)**	
0 (very early)	0	0	
A (early)	2 (3.7%)	8 (8.3%)	
B1 (intermediate 1)	2 (3.7%)	15 (15.6%)	
Stages not eligible for curative treatment	**50 (92.6%)**	**73 (76%)**	p = 0.01
	4 (7.4%)	7 (7.3%)	
B2 (intermediate 2)	2 (3.7%)	3 (3.1%)	
B3 (intermediate 3)	18 (33.3%)	19 (19.8%)	
C (advanced)	26 (48.2%)	44 (45.8%)	
D (terminal)			

DISCUSSION

A striking finding of our study is the exceptionally high proportion of patients diagnosed at non-curative stages (81.6%), with no cases identified in the very early stage and only 18.4% diagnosed at curative stages, highlighting a critical gap in early detection and intervention.

Moreover, nearly half of the patients in both alcohol-related and viral cirrhosis groups were diagnosed at the terminal stage (BCLC D). According to the current staging system, patients with severely impaired liver function are automatically classified as Stage D, regardless of tumor burden (7). This could be seen as a limitation of the actual staging system, because this means that even patients with small, potentially treatable tumors may be excluded from curative or life-prolonging interventions, because of end-stage liver disease. Nevertheless, while this classification reflects the poor overall prognosis in such cases, it also raises concerns about missed therapeutic opportunities, particularly in centers where liver transplantation is accessible. Given the evolving landscape of HCC management, with improvements in locoregional therapies and transplant eligibility criteria, a more nuanced approach that considers individual patient profiles-beyond hepatic function alone-could be beneficial in refining treatment decisions.

At the same time, most hospitalized patients fall into this D category, while very few have preserved liver function-these patients may be evaluated in outpatient settings and possibly managed directly by surgery, oncology, or interventional radiology without requiring admission to the gastroenterology department. This could partly explain the exceptionally high proportion of patients diagnosed at Stage D.

Our study demonstrates that patients with hepatocellular carcinoma (HCC) who have alcohol-related cirrhosis are significantly more frequently diagnosed at non-curative stages compared to those with viral hepatitis-related cirrhosis. This finding highlights critical disparities in disease surveillance, healthcare engagement, and modifiable risk factors between these two groups.

One of the most plausible explanations for this discrepancy is the reduced adherence of patients with alcohol-related liver disease to regular medical follow-up and surveillance programs. Chronic alcohol consumption is often associated with poor healthcare-seeking behavior, lower compliance with medical recommendations, and social or psychological factors that further hinder early diagnosis. Unlike patients with viral hepatitis, who are often identified through screening programs and may receive structured follow-up care, patients with alcohol-related cirrhosis may not seek medical attention until they develop symptoms, by which time the disease has often progressed to an advanced stage. Furthermore, alcohol use disorder is frequently accompanied by socioeconomic challenges, mental health comorbidities, and stigma (8), all of which contribute to delayed presentation and reduced access to curative interventions.

Another key factor in this disparity is the availability of antiviral therapies that can effectively suppress or even eliminate viral hepatitis, reducing the risk of HCC development and improving liver function. The advent of direct-acting antivirals (DAAs) for hepatitis C virus (HCV) and nucleos(t)ide analogs for hepatitis B virus (HBV) has significantly changed the natural history of viral cirrhosis (9,10). By decreasing viral replication, these treatments slow fibrosis progression, decrease inflammation, and, in some cases, lead to

fibrosis regression, thereby mitigating the oncogenic potential of chronic hepatitis (11). In contrast, alcohol-related liver disease remains a challenging entity with no pharmacologic intervention capable of reversing liver injury or eliminating the carcinogenic risk. Abstinence is the primary intervention, but its success depends heavily on patient motivation, psychological support, and access to addiction treatment services-factors that are highly variable and difficult to sustain in the long term.

Our findings emphasize the urgent need to enhance HCC surveillance in patients with alcohol-related cirrhosis. Given that current guidelines recommend biannual ultrasound screening for high-risk individuals (12,13), strategies to improve adherence among this population are critical. Potential solutions include integrating hepatology care with addiction services, implementing patient navigation programs, and leveraging digital health tools for appointment reminders and follow-up (14). Additionally, targeted public health interventions that address alcohol dependence and promote awareness of HCC risk may help shift the current paradigm of late-stage diagnosis in this vulnerable group.

Another aspect that deserves attention is the underutilization of liver transplantation in patients with alcohol-related cirrhosis. Despite being a well-established curative option for selected patients with HCC (15), access to transplantation remains limited for those with alcohol-related liver disease due to concerns regarding relapse and post-transplant adherence to medical care. While current selection criteria emphasize sustained abstinence (16), emerging evidence suggests that some patients with alcohol-related HCC may achieve excellent outcomes even with shorter pre-transplant sobriety periods, provided they receive appropriate psychosocial support (17). Expanding transplantation eligibility in carefully selected patients could improve survival rates and offer curative potential in a subset of patients who are otherwise classified as non-curative due to liver dysfunction.

Finally, the disparities observed in our study highlight the need for targeted public health interventions to improve HCC screening and early detection, particularly in high-risk populations. Patients with alcohol-related cirrhosis may benefit from enhanced surveillance strategies that integrate hepatology with addiction medicine, patient navigation programs to facilitate adherence, and digital health tools such as automated reminders for screening appointments. Additionally, raising awareness about HCC risk among primary care providers and implementing outreach programs for underserved populations could help bridge the gap in early diagnosis and improve outcomes in this vulnerable group.

CONCLUSIONS

The significantly higher proportion of non-curative stage diagnoses among patients with alcohol-related cirrhosis underscores the need for improved screening strategies, better patient engagement, and integrated approaches to alcohol use disorder management. Unlike viral hepatitis, where antiviral therapies can modify disease progression and reduce HCC risk, alcohol-related liver disease continues to pose unique challenges that demand a more proactive and multidisciplinary approach.

Our findings reinforce the urgent need for a more proactive, multidisciplinary approach to the detection and management of HCC in patients with alcohol-related cirrhosis. Addressing disparities in surveillance and access to care, refining staging criteria, and optimizing treatment pathways could

significantly improve outcomes for these high-risk patients. Future research should focus on identifying effective interventions to bridge these gaps and reduce the burden of advanced-stage HCC diagnoses.

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THE IMPACT OF BARIATRIC SURGERY ON OXIDATIVE STRESS IN OBESE PATIENTS

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THE IMPACT OF BARIATRIC SURGERY ON OXIDATIVE STRESS IN OBESE PATIENTS (Abstract): Given the increasing prevalence of obesity and its association with multiple systemic complications, exploring the biological mechanisms underlying this condition has become imperative. Oxidative stress is an important contributor to metabolic dysfunction and chronic inflammation. Bariatric surgery not only induces significant weight loss but may also modulate oxidative balance, offering potential benefits beyond weight reduction. **Aim:** To evaluate and compare the degree of oxidative stress (OS) in the serum of patients with morbid obesity before and after bariatric surgery (BS), in relation to non-obese control subjects. **Materials and methods:** In this longitudinal comparative study, we collected plasma samples from three groups. First, plasma was obtained from 21 patients with a BMI $\geq$ 40 kg/m² (age range: 21-65 years) to assess metabolites involved in oxidative stress (OS). We measured key OS biomarkers, including superoxide dismutase (SOD) activity, malondialdehyde (MDA), as well as total serum protein concentration, which provides additional insight into the systemic metabolic status. Second, plasma samples were collected from healthy individuals with a mean BMI of 22.1 ± 0.8 kg/m², serving as the control group. Three months after bariatric surgery, plasma samples were again collected from the obese patients who attended the follow-up visit, constituting the third group. **Results:** Three months after bariatric surgery, SOD levels showed a partial recovery (0.30 ± 0.04 U/mL), although they remained significantly lower compared to the control group. Regarding lipid peroxidation, a significant reduction in MDA levels was observed in the surgery group compared to the overweight group ($p=0.0004$), suggesting a beneficial effect of the surgical intervention on oxidative stress. Serum protein levels were significantly elevated in obese patients compared to controls and showed a marked decrease after bariatric surgery, reaching values similar to those of the control group ($p<0.05$). **Conclusions:** We have demonstrated the relationship between OS parameters and mediators in people with morbid obesity. Bariatric surgery improves the antioxidant profile and reduces oxidative stress and comorbidities. **Keywords:** BARIATRIC SURGERY, OXIDATIVE STRESS, FREE RADICALS, MORBID OBESITY.

INTRODUCTION

Obesity is a complex disease having an important public health impact worldwide, and its prevalence is increasing. It is the result of an individual complex interaction of factors, including genetic predisposition, diet, metabolism, and physical activity (1). The prevalence of obesity has risen rapidly in Romania. In a study of 2,103 participants, 31.1% were overweight and 21.3% were obese (2).Obesity rates increased with age, peaking at 41.6% among individuals aged 60-79 years (2).

It has been demonstrated that oxidative stress is involved in the pathogenesis of various metabolic, inflammatory or tumoral diseases (3). Oxidative stress plays a crucial role in the pathophysiology of obesity by altering mitochondrial regulatory factors, promoting lipogenesis, and influencing hypothalamic neurons involved in appetite control. Morbid obesity is associated with accelerated atherosclerosis, a chronic vascular disease related to OS and endothelial dysfunction. (4). Obesity disrupts mitochondrial metabolism, leading to increased production of reactive oxygen species (ROS) and oxidative stress. The accumulation of intracellular triglycerides inhibits adenosine nucleotide movement, reducing oxidative phosphorylation and facilitating electron leakage, which generates ROS. These reactive species damage tissues and initiate an inflammatory response that enhances adipokine expression, promotes macrophage infiltration into adipose tissue, and exacerbates lipid disorders through excessive ROS and cytokine production (5) (6). Although oxidative stress contributes to the development and progression of obesity, it is only one of many factors involved in this complex pathology (7). Still, the number of studies examining the oxidative stress in different stages of obesity remains small.

Oxidative stress plays a central role in the metabolic dysregulation observed in obesity, contributing to chronic inflammation and increased risk of metabolic diseases such as metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD) (8). MASLD patients often exhibit increased levels of ROS, which impair lipid metabolism and insulin sensitivity. Elevated levels of ROS lead to lipid peroxidation, mitochondrial dysfunction, and insulin resistance, which further exacerbate metabolic complications. Among the key biomarkers of oxidative stress, superoxide dismutase (SOD) serves as a critical antioxidant enzyme that neutralizes superoxide radicals, while malondialdehyde (MDA) acts as a marker of lipid peroxidation. Studies have shown that obese individuals exhibit increased oxidative stress, characterized by decreased SOD activity and elevated MDA levels, which correlate with systemic inflammation and metabolic disturbances (9). Oxidative stress is also recognized as a major factor in the pathogenesis of irritable bowel syndrome (IBS). Studies have shown that both obesity and IBS are associated with gut dysbiosis and immune activation, which exacerbate oxidative stress and increase the risk of metabolic comorbidities (10).

Furthermore, interventions such as bariatric surgery have demonstrated potential in restoring oxidative balance by enhancing SOD activity and reducing MDA levels, ultimately improving metabolic profiles and reducing comorbidities (11).

The aim of our study was to evaluate the specific activity of the peripheral antioxidant SOD and the lipid peroxidation marker MDA in the serum of obesity patients, both before and three months after bariatric surgery, compared to matched control subjects.

MATERIAL AND METHODS

Patients. We conducted a prospective study involving 21 consecutive patients with a confirmed diagnosis of morbid obesity (BMI > 40 kg/m²) (12), all of whom underwent bariatric surgery at the Surgery Department III of St. Spiridon Hospital in Iași, Romania. Among the bariatric procedures performed, seven patients underwent laparoscopic sleeve gastrectomy, whereas fourteen underwent Roux-en-Y gastric bypass. The control group comprised 10 non-obese persons with mean BMI 22.1 ± 0.8 kg/m², in the age group 30 to 35 years.

Inclusion criteria for the obese group (n = 21) included a confirmed diagnosis of morbid obesity (BMI ≥ 40 kg/m²), eligibility for bariatric surgery, an age range between 21 and 65 years, and the provision of informed written consent to participate in the study. Inclusion criteria for the control group were: absence of alcohol abuse, no history of infectious diseases, and no use of vitamin supplementation. In addition, participants continued their usual medication regimen prior to testing.

Exclusion criteria for both the obese and control groups included: current use of antioxidants or anti-inflammatory medications, recent surgery or trauma (within the past 6 months), pregnancy or lactation, and adherence to special diets (e.g., ketogenic, vegan, or detox regimens) that could influence oxidative stress parameters.

All participants provided written, informed consent. The study was approved by the local ethics committee.

Methods. Blood samples were collected in the morning, prior to breakfast, from both the baseline group and the patients who returned for the 3-month follow-up. Samples were allowed to clot and were centrifuged immediately thereafter. Serum was aliquoted into Eppendorf tubes and stored at -40ºC prior to measurement. All samples were measured in duplicate and averaged.

Determination of superoxide dismutase (SOD)

SOD activity was determined using the SOD Determination Kit (Fluka, 19160) based on the reduction of the water-soluble tetrazolium salt WST-1, which yields a formazan dye upon reaction with superoxide anions. The reaction is mediated by xanthine oxidase, and its inhibition by SOD reflects the enzymatic activity. Each reaction was carried out in a 96-well plate by mixing the sample with WST Working Solution and Enzyme Working Solution, followed by incubation at 37°C for 20 minutes. Absorbance was measured at 450 nm using a microplate reader. SOD activity was calculated as the percentage inhibition of the colorimetric reaction, according to the formula provided by the manufacturer, and results were normalized to the protein concentration, expressed as SOD activity units.

Determination of malondialdehyde (MDA)

MDA levels were determined using the Lipid Peroxidation (MDA) Assay Kit (MAK568). Briefly, 20 µL of serum or plasma was mixed with 500 µL of 42 mM sulfuric acid, followed by the addition of 125 µL phosphotungstic acid solution and vortex mixing. After incubation at room temperature for 5 minutes, the samples were centrifuged at 13,000 x g for 3 minutes, and the supernatant was discarded, retaining the pellet. The pellet was resuspended in a mixture of 2 µL BHT solution and 100 µL cold ultrapure water, adjusting the final volume to 200 µL. The assay reaction was performed by adding 600 µL of TBA solution to 200 µL of sample or standard, incubating at 95°C for 1 hour, and cooling to room temperature. The absorbance was measured at 532 nm, and

MDA concentrations were determined using a standard curve, with results expressed as nmol/mL.

Determination of proteins level

Serum protein levels were measured using the Bradford method, which relies on the specific binding of Coomassie Brilliant Blue G-250 dye to proteins, resulting in a quantifiable color change. This assay measures the absorbance at 595 nm, allowing protein concentration to be determined by comparing sample absorbance to a standard calibration curve, typically generated with bovine serum albumin (BSA). The Bradford method is valued for its rapidity, high sensitivity, and low reagent consumption; however, its accuracy may be affected by the presence of detergents or other substances that interfere with dye binding (13).

TABLE I.

Demographic and clinical data in control, baseline, and post-surgery groups (3 months after surgery)

	Control (n=10)	Baseline (n=21)	After 3 Months Group (n=10)	p Value[b]
Age [a]	32.2 ± 5.95	37 ± 1.30	41.2 ± 15.1	
Gender (Male/Female)	3/7	1/20	0/10	0.04
Active Smokers	20%	33.3%		0.60
BMI [a]	29 ± 1.8	50.5 ± 6.3	39.0 ± 7.6	0.00085

a. Each value represents mean ± standard deviation b. Analysis of covariance

RESULTS

Demographic and clinical characteristics of participants

A total of 31 participants were included in the study, divided into three groups: the control group (n = 10), consisting of non-obese individuals with a mean BMI of 22.1 ± 0.8 kg/m²; the baseline group (n = 21), composed of patients with morbid obesity (BMI > 40); and the post-surgery group, consisting of only a subset of the initial obese cohort (n = 10), evaluated three months after undergoing bariatric surgery. The mean age of the participants ranged between 30 and 37 years, with a predominance of females, especially in the obese group (1 male and 20 females). Chronic treatments were reported in 42.9% of obese patients, most commonly addressing hypertension, type 2 diabetes mellitus (T2DM), and gastroesophageal reflux disease (GERD). Regarding medication, metformin and pantoprazole were each used by 14.28% of patients, while selective β1-blockers and levothyroxine sodium were also noted. After surgery, patients experienced a marked decrease in BMI (from 50.5 ± 6.3 to 39.0 ± 7.6), suggesting a significant early weight loss following the intervention. The analysis of covariance showed that the patients from overweight group did not differ significantly from the healthy comparison subjects with respect to age, smoking habits (tab. I).

Changes in SOD activity post-surgery

Regarding the specific activity of SOD, which is the first line of defense against oxidative stress development, we noticed a significant decrease in the overweight group compared to the control group (Fig. 1). The control group exhibited the highest SOD activity levels (0.58 ± 0.13 U/mL), whereas the overweight group demonstrated a marked reduction (0.20 ± 0.07 U/mL).

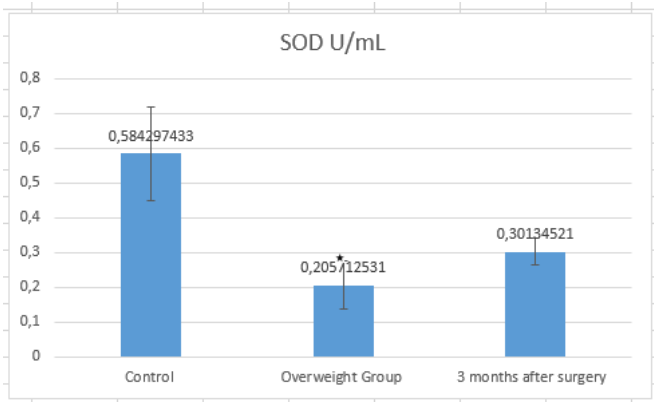

Fig. 1. Superoxide dismutase specific activity in the serum of Control (n=10) Overweight (n=21) and 3 months after surgery (n=10) patients.
The values are expressed as mean ± SEM (Standard Error on the Mean) $^{*}p = 0.03$

Three months after bariatric surgery, SOD levels showed a partial recovery (0.30 ± 0.04 U/mL), but remained significantly lower than in the control group. Post hoc analysis confirmed significant differences between the control and overweight groups ($p<0.05$), while the difference between the post-surgery and overweight groups was also statistically relevant ($p <0.05$), suggesting an improvement in antioxidant defense following surgical intervention.

Lipid peroxidation changes

Concerning the lipid peroxidation, a significant increase of the MDA concentration was observed in the overweight group compared with the control group ($p < 0.05$). The comparison between the control and surgery groups revealed a non-significant difference ($p = 0.0539$), indicating that after surgery, MDA levels approximate those of the control group, suggesting a potential restoration to baseline values (fig. 2).

Also, the comparison between the overweight and surgery groups showed a significant difference ($p = 0.0004$), suggesting that the surgical intervention led to a notable reduction in MDA levels compared to the overweight group. The post-hoc analysis for MDA revealed significant differences, indicating that MDA levels were higher in the overweight group and significantly decreased after surgery, approaching control levels.

Protein level changes post-surgery

The assessment of protein levels in this study offers crucial insights into the metabolic profile and physiological adaptations of both obese individuals and those who have undergone bariatric surgery. Regarding protein levels, a significant increase was observed in the overweight group compared to the control group ($p < 0.05$). The comparison between the control and surgery groups revealed a non-significant difference ($p = 0.8316$), indicating that after surgery, protein levels approximated those of the control group, suggesting a potential return to baseline values (fig. 3). Additionally, the comparison between the overweight and surgery groups showed a significant difference ($p < 0.05$), indicating that surgical intervention led to a notable reduction in protein levels compared to the overweight group. The post-hoc analysis for protein levels confirmed significant differences, demonstrating that protein

concentrations were higher in the overweight group and significantly decreased after surgery, reaching levels comparable to the control group.

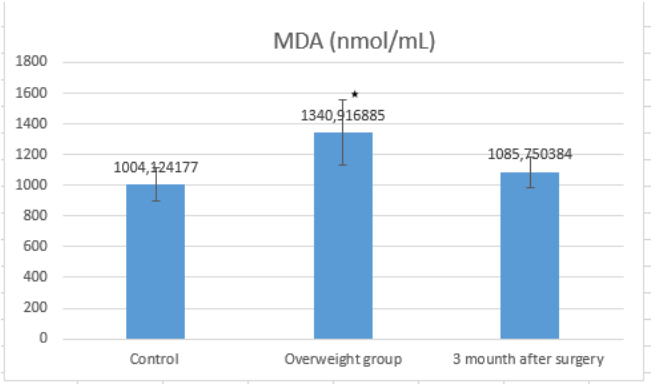

Fig. 2 Malondialdehyde specific activity in the serum of Control (n=10) Overweight (n=21) and 3 months after surgery (n=10) patients. The values are expressed as mean ± SEM (Standard Error of the Mean) $^{*}p < 0.05$

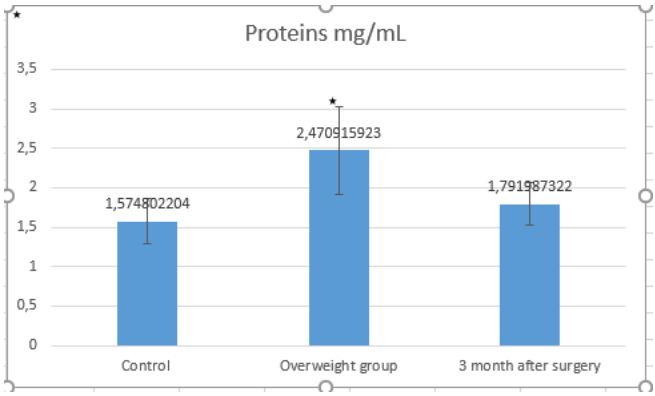

Fig. 3 Protein levels in the serum of Control (n=10) Overweight (n=21) and 3 months after surgery (n=10) patients. The values are expressed as mean ± SEM (Standard Error of the Mean) $^{*}p < 0.05$.

Correlations between oxidative enzymes and protein levels

Analyzing the correlation between the two measured enzymes with the value of protein in serum, we observed in the case of SOD a very weak, non-significant correlation **(**n = 41, r = 0.029, p = 0.859), suggesting that SOD levels do not show a meaningful relationship with protein concentration. This contrasts with studies where SOD was moderately correlated with inflammatory markers (e.g., CRP) (14), indicating that additional factors may influence these oxidative stress parameters in our study population. A small, non-significant positive correlation in the case of MDA, (n = 41, r = 0.205, p = 0.199), indicating that MDA levels are slightly associated with protein concentrations, but not at a statistically significant level.

DISCUSSION

Oxidative stress is a key factor in obesity-related metabolic dysfunctions (1). In this study, we assessed oxidative stress biomarkers in three groups: a control group, an obese group before bariatric surgery, and the same obese individuals three months post-surgery. SOD levels were significantly elevated in obese patients compared to controls, suggesting a compensatory enzymatic response to increased oxidative stress. However, three months post-surgery, SOD levels decreased, indicating a partial recovery of redox homeostasis. Similarly, MDA, a marker of lipid peroxidation, was highest in obese patients and decreased following bariatric surgery, though it remained elevated compared to controls. Additionally, protein levels were significantly lower in obese individuals but showed a modest increase post-surgery.

In the group of overweight patients, significantly elevated protein levels suggest the presence of a chronic systemic inflammatory status, characteristic of obesity. This may be correlated with oxidative stress and altered liver function (15). This aspect was highlighted a study (16), which shows that oxidative stress associated with mitochondrial dysfunction enhances the glycation of hepatic proteins, thereby exacerbating inflammation-induced liver damage.

At three months postoperatively, protein levels were significantly reduced compared to the overweight group, suggesting either a normalization of protein metabolism through a balance between protein synthesis and degradation, or a physiological adaptation to the new metabolic conditions imposed by dietary restrictions and post-bariatric hormonal changes. This was also highlighted in a comparative study (17) that analyzed changes in the protein profile of two groups: one following a hypocaloric diet and the other undergoing bariatric surgery. A significant decrease in leptin levels was observed in the surgical group, suggesting improved appetite regulation postoperatively ($p = 0.005$).

As shown in Table 1, 19% of the patients in our study have type II diabetes, a condition known to be associated with increased oxidative stress (18). This relationship is further supported by a study investigating lipid peroxidation and protein oxidation levels (19), which demonstrated that diabetes, particularly when combined with glucose-6-phosphate dehydrogenase (G6PD) deficiency, leads to a significant increase in MDA levels, a key marker of oxidative stress. In patients with G6PD deficiency, MDA levels were elevated by approximately 28-41%, indicating an enhancement of lipid peroxidation under the combined influence of these pathological conditions. The postoperative increase in SOD levels observed in our patients, including those with type II diabetes, may contribute to a hypoglycemic effect. This is supported by experimental evidence from a diabetic rat model (20), where elevated levels of MDA and GSSG/GSH ratio indicated pronounced oxidative stress. Oral administration of liposome-embedded SOD (L-SOD) in these rats led to a reduction of MDA by 34.74% and GSSG/GSH ratio by 45.4%. After four weeks of intervention, blood glucose levels significantly decreased in the treated groups, suggesting a potential role of SOD in improving glycemic control through attenuation of oxidative stress.

The analysis of LPO levels, assessed via MDA content, has yielded contradictory findings. In our study, MDA levels were reduced three months postoperatively, indi-

cating an improvement in oxidative status. Similar results were reported by Bell *et al.*, where hepatic MDA content-a marker of lipid peroxidation-decreased significantly after bariatric surgery, from 34.9% ± 18.3% to 22.9% ± 13.8% (21). However, the evaluation of long-term outcomes appears less favorable. A separate study (22) assessing global oxidative stress markers found that TBARS (thiobarbituric acid reactive substances), an indirect indicator of MDA levels, did not remain decreased four years after surgery. In some patients, TBARS levels even increased, suggesting a potential re-emergence of oxidative stress. Contributing factors identified included dietary habits, physical activity levels, and the use of antioxidant supplements.

These findings suggest that BS contributes to a reduction in oxidative stress but does not fully restore normal metabolic balance within three months. Further long-term studies are needed to evaluate the full extent of oxidative stress recovery following bariatric intervention.

The results of our study are consistent with previous findings by Min *et al.* (22) Horn *et al.*, (11) and Cabrera *et al.*, (23) which demonstrated that bariatric surgery contributes to a reduction in oxidative stress in obese patients, primarily through decreased LPO and enhanced antioxidant defense mechanisms.

There are several limitations inherent in this study. First, the relatively small sample size may limit the generalizability of our findings. Second, not all 21 patients returned for follow-up at three months postoperatively, which could have influenced the consistency of the longitudinal comparisons. Additionally, individual variability in oxidative stress markers may have been influenced by unmonitored factors such as dietary changes, physical activity levels, or unreported supplement intake during the postoperative period.

CONCLUSIONS

Given the changes observed in oxidative stress markers following bariatric surgery, our study confirms that excessive body mass significantly contributes to elevated production of ROS. The notable decrease in MDA levels and partial recovery of SOD activity three months postoperatively suggest that surgical intervention, combined with weight loss, contributes to the restoration of redox balance. Furthermore, the normalization of protein levels may indicate an attenuation of systemic inflammation and improved metabolic function.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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THE RELATIONSHIP BETWEEN DENTAL ANXIETY AND THE SOCIAL IMPACT OF ORAL DISORDERS ON WELL-BEING IN PATIENTS ATTENDING A PUBLIC DENTAL EMERGENCY UNIT. A SINGLE-CENTER STUDY

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THE RELATIONSHIP BETWEEN DENTAL ANXIETY AND THE SOCIAL IMPACT OF ORAL DISORDERS ON WELL-BEING IN PATIENTS ATTENDING A PUBLIC DENTAL EMERGENCY UNIT. A SINGLE-CENTER STUDY (Abstract): **The aim** of this study was to identify the characteristics of patients who access public dental emergency units in the Oradea area, in relation to the presence of dental anxiety and the impact of oral disorders on general well-being. **Materials and methods**: A total of 105 patients admitted to the emergency dental unit were included in this study. Demographic data, socioeconomic information, medical problems, and self-evaluations of oral health were recorded. To evaluate the social impact of oral disorders on well-being, *The Oral Health Impact Profile* was applied, and the *Norman Corah's Dental Anxiety Scale* was used to score the dental anxiety level. Statistical analysis was performed using the IBM Statistical Package for Social Sciences, version 26. **Results**: More than 10% of the patients showed high and severe dental anxiety. No significant differences in participants' sex, marital status, or living environment were found to be related to *Norman Corah's Dental Anxiety Scale.* Women registered higher scores than men on the physical, psychological and social disability subscales of *The Oral Health Impact Profile*. The OHIP score was strongly and positively correlated with the DAS-R score. The results related to the level of education, financial income, dental hygiene, alcohol consumption, and analgesic use are discussed. **Conclusions**: The impact of oral health on the physical, psychological, and social quality of life is important. In prevention campaigns, partners and institutions should consider different factors to manage the level of dental anxiety and increase the quality of dental health. **Keywords:** DENTISTRY, DENTAL EMERGENCY UNIT, PUBLIC HOSPITAL, DENTAL ANXIETY, ORAL DISORDERS, QUALITY OF THE ORAL HEALTH.

INTRODUCTION

The World Health Organization advocates coverage of healthcare costs for all categories of people. (1) Studies in medical sociology and health management have shown that several factors affect the use of health services, including costs, level of health education, and distance from the health center. Sociological models related to health have been considered to identify

vulnerable populations and provide them with easier access to preventive, diagnostic, and treatment services.

Andersen's model provides a theoretical framework for understanding and predicting the use of health services. Over the years, three main determinants have been considered: a) *predisposing factors* that determine the inclination or likelihood of an individual to use health services when they need them, such as demographic characteristics (age, sex, and family size), social characteristics (education, income and marital status) and beliefs about health (attitudes, values, and perceptions about health and illness); b) *enabling factors* are resources or barriers that facilitate or hinder the use of health services. These can be individual (income and health insurance) or community-level or community resources (availability and accessibility of health services in a geographic area), and c) *needs* referring to the perceived or assessed need for care, determined conditions, symptoms, or physical limitations (2-4).

In addition to interventions to relieve pain, some studies have shown that patients access emergency dental services for psychological reassurance, that the problem is not serious, and seek counseling. Therefore it is important for dentists to offer psychoemotional support in addition to medical intervention and in terms of the duration of the intervention. Communication in emergency services is extremely important, especially if the patient is in a serious condition due to avoidant behavior or the presence of phobia. In addition, as we have shown above, it is possible that the patient presented to the emergency system because the postponement of care was determined by poor socioeconomic conditions or the distance from a dental care center (5-8).

Poor untreated dental health leads to a low quality of life, and most oral diseases tend to be progressive and cumulative without proper intervention. Oral health determines general quality of life, and ensures good physical, psychological and social balance (9).

The goals of this study were to shape the profile of the patient that accesses the public dental emergency units in the Oradea area, in relation to the presence of dental anxiety and to identify the impact of oral disorders on general well-being. A previous study by the research team identified oral behaviors among patients consulting emergency units (10). The present analysis of data will focus on: a) the relationship between stress and various aspects of dental health and dental health-related behaviors; b) differences in dental anxiety level based on demographic and socioeconomic characteristics; c) the relationship between dental health behaviors and dental anxiety; d) the relationship between satisfaction with oral health and various aspects of functional limitations, physical pain, and psychological discomfort; and e) to identify whether the subscales of the oral health profile (functional limitations, physical pain, psychological discomfort, physical disability, psychological disability, social disability, and obstacles) are significant predictors of dental anxiety.

MATERIALS AND METHODS

Participants and Data Collection. In 2021, 2026 patients were accepted at the dental emergency unit of the Oradea Emergency, a public hospital in North-Western Romania. Between June 20th and July 20th, 197 patients were registered, and printed questionnaires were distributed to them before consultation. All participants were

informed about the purpose of the research and that the data collected would be confidential. No incentives were provided to participants in the study. To ensure the confidentiality of personal data, the surveys were collected in a sealed box located in the main hall.

The inclusion criterion was patients older than 18 y.o. who fully filled in questionnaires. Only questionnaires filled in in person were accepted in order to select patients (literate, no cognitive or mental disability, self-signing informed consent). A total of 105 questionnaires were included in this study.

Instruments. The questionnaire collected data on demographic characteristics, financial status, health-related behaviors, level of stress, quality of dental life, consumption of alcohol or tobacco, dental hygiene routine, and use of pain drugs. Supplementary data regarding diagnostic and self-rated items, focusing on identifying the level of satisfaction with health, were also collected.

Two psychological tools were added to investigate the presence of dental anxiety and impact of oral health-related problems on general well-being.

Norman Corah's Dental Anxiety Scale (DAS-R) is a validated instrument used by dentists to assess the level of dental anxiety in patients. The scale consists of four questions, and each item is constructed as a scenario with different possible reactions (for example: If you had to go to the dentist tomorrow for a check-up, how would you feel about it? a) I would look forward to it as a reasonably enjoyable experience. b) I would not care about one way or the other. c) I would be a little uneasy about this. d) I would be afraid that it will be unpleasant and painful. e) I would be very frightened about what the dentist would do). Responses were scored on a scale of 1 to 5, with a maximum total score of 20. The score obtained by the patient generates the following situations: 912 points = *moderate anxiety level* with some specific stressors that should be discussed and managed together with the patient, 1314 points = *high anxiety* and a score over 1520 = *severe anxiety (or phobia*) (11). In the present study, the Cronbach's alpha was 0.898, indicating good internal consistency.

The Oral Health Impact Profile (OHIP-14), developed by Slade in 1997 (12) is a self-administered questionnaire consisting of 14 items designed to measure the social impact of oral disorders on well-being. The questionnaire was organized into seven dimensions, each represented by two questions. These dimensions are: a) functional limitation, b) physical pain, c) psychological discomfort, d) physical disability, e) psychological disability, f) social disability, and g) handicap. Participants were asked to rate the frequency of specific problems they experienced, relating to these dimensions in their daily lives. Responses were recorded on a 4-point Likert-type scale, where 0 = *never*, 1 = *almost never*, 2 = *occasionally*, 3 = *often*, and 4 = *very often*. Each dimension's score ranged from 0 to 8 and was calculated by summing the scores of the two corresponding items. The total OHIP-14 score was the sum of all individual item scores, ranging from 0 to 56. Lower scores indicate a higher quality of life, with scores closer to 0, suggesting a minimal impact of oral health issues on daily life. Conversely, higher scores (e.g., eight for the total score for each dimension and 56 for the total score for the OHIP-14) indicated a more significant negative impact on quality of life.

In the present study, Cronbach's alpha was 0.710 for the functional limitation dimension, 0.670 for the physical pain dimension, 0.817 for the psychological discomfort dimension, 0.845 for the physical disability dimension, 0.811 for the psychological disability dimension, 0.824 for the social disability dimension, and 0.648 for the obstacles dimension. A Cronbach's alpha of 0.932 for all items suggested greater internal consistency.

Statistical Analyses. The statistical analysis was conducted using IBM Statistical Package for Social Sciences (SPSS) version 26 (SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD), as well as frequencies and percentages, were used for descriptive analysis of the data. An independent T test was performed for comparative analysis. Correlation analysis was done using Pearson's correlations, and the level of statistical significance was set at $p < 0.05$.

Ethical Approval. The present study was conducted in accordance with the Declaration of Helsinki, and the research protocol was approved by the Ethical Committee of the Emergency Clinical Hospital of Oradea, Bihor County, Romania (No. 14518/16.06.2021).

RESULTS

Sociodemographic and health-related data. The participants included in the research are 55 female (52.4%) and 50 males (47.6%), aged 37.03 ± 14.45, with a minim of 18 and a maximum of 84 years old. Most participants were from urban areas (N = 64, 61%). Almost three-quarters declared that they were employed (N = 73, 69.5%). Regarding their level of education, we identified that N = 29 (27.6%) had a middle school education level, N = 60 (57.1%) had graduated from high school and N = 16 (15.2%) had a university level of education. By investigating their financial status, we found that 86 (82.8%) earned less than 3,000 RON (600 euros) per month.

A series of items investigated the behaviors associated with oral health and daily dental routines. More than a quarter of them (N = 30, 28,6%) declared that they consumed alcohol, and more than half of the participants sustained that they did not smoke (N = 66 (62.9%). Ten patients (9,5%) presented with abscesses and 5 (4,8%) were diagnosed with gingivitis.

Regarding their teeth brushing routine, we identified that around one-third of them brushed their teeth daily, in the morning or at night, with morning brushing being more frequent than evening brushing. By asking participants to rate their level of satisfaction with dental health on a scale from 1 (very dissatisfied) to 5 (extremely satisfied) we obtained an M = 2.97 ± 1.11. The detailed results of the dental hygiene routine are presented in first table.

TABLE I.
Dental Health Routine

	Do you brush your teeth in the morning?	Do you brush your teeth in the evening?
Never	0	0
Very rare	6 (5.7)	11 (10.5)
Sometimes	21 (20.0)	28 (26.7)
Almost every morning	30 (28.6)	32 (30.5)
Every morning	48 (45.7)	34 (32.4)

[1] Number of answers (N) and percentage (%)

Dental Anxiety and Oral Health-Related Quality of Life

Corah's Dental Anxiety Scale - the total score for DAS-R scale was M = 8.51 ± 3.34, ranging from 4 (16.2%) to 20 (1.0%). More than one-third of participants had

moderate levels of anxiety (tab. II).

TABLE II.
Results for the DAS-R scale[1]

DAS-R Scale	N (%)
Mild level of anxiety	50 (47.6%)
Moderate anxiety	44 (41.9%)
High anxiety	7 (6.7%)
Severe anxiety/Phobia	4 (3.8%)

[1] Number of answers (N) and percentage (%)

No significant differences were found in terms of participants' sex, marital status, or living environment.

There was a statistically significant difference in the mean DAS-R score between those who very rarely brush their teeth in the morning and those who brush their teeth every morning - 0.35 (95% CI, 0.22 to 6.36), $t(51) = 2.155$, $p = .036$; those who rarely brush their teeth in the morning have a higher level of anxiety (11.17 ± 4.75) compared to those who have oral hygiene every morning (7.87 ± 3.36).

Oral Health-Related Quality of Life Scale: The analysis of answers showed that respondents had low levels of oral health impact. The total OHIP-14 score was, on average, M = 17.12 ± 11.46, with scores ranging from 0 (3.8%, N = 4) to 53 (1%, N = 1) (tab. III).

An independent-samples t-test was run to determine if there were differences in the OHIP scores between males and females.

-women reported a higher mean for total score of social impact of oral disorders on well-being (19.96 ± 10.73) compared with men (14.00 ± 11.52), a statistically significant difference of 5.96 (95% CI, 10.27 to 1.65), $t(103) = -2.745$, $p = .007$,

-women reported a higher mean number of psychological discomfort (4.09 ± 1.99) compared with men (2.92 ± 2.18), a statistically significant difference of 0.30 (95% CI, -1.97 to -0.36), $t(103) = -2.873$, $p = .005$,

-women reported higher scores for social disability (2.63 ± 1.92) compared with men (1.54 ± 1.83), a statistically significant difference of 0.86 (95% CI, -1.82 to -0.36), $t(103) = -2.980$, $p = .004$,

-women reported a higher mean number of psychological disabilities (3.01 ± 2.13) compared with men (2.16 ± 1.83), a statistically significant difference of 0.43 (95% CI, -1.63 to -0.83), $t(103) = -2.197$, $p = .030$.

TABLE III.
Scores for OHIP subscales

OHIP subscales	Items	Means ± Standard deviation
Functional Limitation	*Have you had trouble pronouncing any words because of problems with your teeth, mouth, or dentures?*	M = 1.39 ± 1.76
	Have you felt that your sense of taste has worsened because of problems with your teeth, mouth, or dentures?	
Physical Pain	*Have you experienced pain in your mouth?*	M = 3.12 ± 2.12
	Have you found it uncomfortable to eat any food because of problems with your teeth, mouth, or dentures?	
Psychological Discomfort	*Have you been self-conscious of your teeth, mouth, or dentures?*	M = 3.53 ± 2.15
	Have you felt tense because of problems with your teeth, mouth, or dentures?	

OHIP subscales	Items	Means ± Standard deviation
Physical Disability	*Has your diet been unsatisfactory because of problems with your teeth, mouth, or dentures?*	M = 2.61 ± 2.30
	Have you had to interrupt meals because of problems with your teeth, mouth, or dentures?	
Psychological Disability	*Have you found it difficult to relax because of problems with your teeth, mouth, or dentures?*	M = 2.60 ± 2.03
	Have you been slightly embarrassed because of problems with your teeth, mouth, or dentures?	
Social Disability	*Have you been slightly irritable with other people because of problems with your teeth, mouth, or dentures?*	M = 2.11 ± 1.95
	Have you had difficulty doing your usual job because of problems with your teeth, mouth or dentures?	
Handicap	*Have you felt that life in general was less satisfying because of problems with your teeth, mouth, or dentures?*	M = 1.73 ± 1.75
	Have you been completely unable to function because of problems with your teeth, mouth, or dentures?	

There were differences in the OHIP scale depending on the use of analgesics. People taking painkillers reported a higher OHIP score (20.54 ± 11.46) compared with people who did not take painkillers (12.38 ± 9.73), a statistically significant difference of 0.75 (95% CI, 3.92 to 12.38), $t(103) = 3.826$, $p < .001$.

There was a statistically significant difference in the mean OHIP scale scores between those who very rarely brushed their teeth in the morning and those who brushed their teeth every morning - 0.85 (95% CI, 1.79 to 19.58), $t(51) = 2.413$, $p = .019$, those who rarely brushed their teeth in the morning had a higher OHIP score (26.50 ± 9.81) than those who brushed their teeth every morning (15.80 ± 10.26).

The results showed differences in OHIP scores between those who did not drink alcohol and those who drank alcohol very rarely. Respondents who did not drink alcohol reported a higher OHIP score (19.00 ± 11.52) than respondents who drank alcohol very rarely (12.66 ± 10.25), a statistically significant difference of 0.33 (95% CI, 0.81 to 11.85), $t(94) = 2.277$, $p = .025$). Physical pain was negatively correlated with alcohol consumption ($r = -0.27$, $p = 0.005$) and analgesic pill use ($r = -0.35$, $p < 0.001$), indicating that as physical pain increased, alcohol and toothache medication consumption decreased.

The DAS-R score was negatively correlated with the degree of satisfaction regarding dental health ($r = -0.27$, $p = 0.005$), indicating that the higher the level of dental anxiety, the less satisfied the participants were with the health of their teeth.

The OHIP score was strongly positively correlated with the DAS-R score ($r = 0.46$, $p < 0.001$) in the sense that the higher the level of dental anxiety, the greater the social impact of oral disorders on well-being.

Statistical analysis of the data revealed that the OHIP score was negatively corre-

lated with the degree of satisfaction regarding dental health ($r = -0.40$, $p < 0.001$) and income ($r = -0.27$, $p = 0.005$) indicating that the greater the impact of dental health on patients' well-being, the less satisfied they are with their dental health and the lower their income.

Positive correlations were observed between the OHIP score and the intensity of pain felt by patients ($r = 0.23$, $p = 0.015$, indicating that the greater the social impact of dental health on patients' well-being, the higher the pain felt by patients.

A negative correlation between functional limitation and the level of education of the patients ($r = -0.28$, $p = 0.006$) was identified, in the sense that the higher the functional limitation, the lower the level of education.

There was a negative correlation between physical disability and abscess diagnosis ($r = -0.20$, $p = 0.032$), indicating that as physical disability increased, abscess diagnosis decreased.

DISCUSSION

Our results are similar to those of Frichembruder *et al*. (13), who identified that greater use of the emergency service was registered by people in great pain, usually women and adults, coming from urban areas, having a lower income, and being less educated.

In our study, women seemed to be more affected than men in terms of social and psychological disabilities and presented with higher psychological discomfort. Therefore, in general, they are more psychologically and socially affected by poor oral health. These results are similar those of Shamim *et al*. (14), Skośkiewicz-Malinowska *et al*. (15) and Närhi *et al*. (16) who identified sex differences in the impact of oral health among men and women. Aesthetic reasons are related to the level of satisfaction with body image, meaning that women will be more preoccupied with their dental health to maintain satisfaction with their body image.

Oral health has been defined as "a standard of health of the oral and related tissues that enables an individual to eat, speak or socialize without active disease, discomfort or embarrassment and which contributes to general well-being". (17) Women were found to be more preoccupied than men when it came to using toothpaste and mouthwash. Brushing twice a day and using a mouthwash were statistically higher in female than male. (18) A low level of satisfaction with body image is closely linked to satisfaction with facial aesthetics, including teeth. Studies have shown that facial images are among the most important aspects that make patients feel satisfied with their physical appearance. When it comes to the face (nose, eyebrows, lips, cheekbones and teeth), the teeth occupy the main place. Therefore, it is understandable why women who are dissatisfied with the appearance and health of their teeth have lower overall satisfaction with their body image. Thus, a low level of satisfaction with body image generates a high level of psychological discomfort and psychological and social disabilities.

We found that the DAS-R score was negatively correlated with the degree of satisfaction with dental health, and that patients with a higher level of anxiety rarely brushed their teeth in the morning. These results can be explained by the vicious circle in which a patient with dental anxiety finds themselves. Although some studies

have shown that patients with dental anxiety avoid regular check-ups at the dentist, invest heavily in expensive products to care for their teeth, and take over-the-counter medications to reduce dental pain, they still do not have proper dental hygiene. Teeth brushing is an important step in oral hygiene. Brushing less often results in poorer oral health, which subsequently results in poorer overall health (due to consequences on overall health, such as oral, cardiovascular, and digestive diseases). Therefore, people are less satisfied with their health and body image. Folayan *et al*. (19) showed that the frequency of toothbrushing during the pandemic decreased as the psychological wellbeing increased.

Cohen *et al*. (20) identified that people with high levels of dental anxiety, despite ongoing concerns about oral health, have poor oral quality of life. Winkler *et al*. (21) found a significant correlation between high dental anxiety levels and reduced toothbrushing behavior. The team also found that the use of dental solutions was not affected by the intensity of anxiety. However, in general, a substantial decline in quality of life was identified.

Other studies have shown that, although younger female patients reported higher levels of dental anxiety, the desire for a perfect facial selfie may reduce dental neglect behavior. (22)

The results of this study showed that more than 10% of the participants had high and severe levels of dental anxiety. The prevalence of dental anxiety in the general population varies between 4% and 20%, regardless of the population, culture, or country. (23) Our study revealed no differences in variables such as sex, marital status or living environment. Other studies have shown that women present higher levels of dental anxiety than men do. (24)

Strengths and limitations: The results of this study are important because they outline a profile of patients accessing emergency services, thus enabling dentists to be prepared to care for this category of patients. Sociodemographic and personality factors must be considered when providing medical services, to ensure the best possible care and a higher level of patient satisfaction. As a limitation of this study, we note that the research was conducted during the COVID-19 pandemic, when access to dental services was restricted or diminished. Therefore, it is possible that patients who accessed emergency dental services were forced to do so (due to lack of access, worsening oral health, etc.) which is one of the explanations why, in general, the patients in the study did not present high scores on the scales used. Finally, as this was a single-center study, the results cannot necessarily be generalized to the entire population as it was an urban university center. It is likely that in areas with no access to dental services, differences in the characteristics of the investigated group would appear.

Reflections and Planning. Identifying the sociodemographic and psychological characteristics of patients accessing dental services, along with information regarding the presence of dental anxiety and the psychological and social impact that poor oral health has on the patient's overall health and satisfaction with general health and body image, is important. The results make us more aware of the usefulness of training dentists to work with different social categories and treat patients who present with dental anxiety.

CONCLUSIONS

The reasons why patients end up in the dental emergency system are diverse, including painful incidents, lack of hygiene behaviors, inability to access medical services (due to low health education or poor financial situation), neglect of dental health, and high dental anxiety scores that encourage patients to avoid regular check-ups. The impact of oral health on the physical, psychological and social quality of life is important. In prevention campaigns, partners and institutions should consider all factors that can diminish the level of oral health and, implicitly, the patient's health in general.

CONFLICT OF INTERESTS AND FUNDING

The authors declare no conflict of interests. The research did not receive external funding.

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ADVANCING TRAINING FOR GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI ENTRANCE EXAMINATION, BASED ON EDUCATIONAL, EMOTIONAL, AND SOCIAL DATA OF FUTURE CANDIDATES

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ADVANCING TRAINING FOR GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI ENTRANCE EXAMINATION, BASED ON EDUCATIONAL, EMOTIONAL, AND SOCIAL DATA OF FUTURE CANDIDATES (Abstract): The aim of this article is to evaluate the perception of future candidates at the Grigore T. Popa University of Medicine and Pharmacy Iasi regarding the initiative of our institution to organize free preparatory courses in human anatomy and physiology for the admission exam. Secondary objectives included characterizing participants' demographics, educational backgrounds, options for the entrance exam, learning effort and engagement patterns. **Materials and methods:** The study included 119 voluntary participants over two consecutive academic years (60 in 2024, 59 in 2025) who provided explicit consent for anonymous data processing. In 2023-2024, preparatory courses were conducted in an intensive format (March-May 2024), while in 2024-2025, the program was extended to a more in-depth format (October 2024-May 2025). Both courses comprehensively covered all 23 chapters of Barron's Human Anatomy and Physiology textbook. A Google Forms questionnaire assessed multiple domains: demographics, geographical domicile, educational background, time allocation to study examination subjects, and perceived difficulty of specific anatomical topics (embryology, spatial relationships between structures). Participants rated course usefulness on a 5-point Likert scale (1 = "Not useful" to 5 = "Very useful"). Data analysis employed descriptive and comparative statistics. Complete anonymized raw data, statistical analyses with correlations, and supplementary materials (> 50 pages) are publicly available via Mendeley Data, a subsidiary of Elsevier (DOI: 10.17632/2y8nyy4yyy.1). **Results:** Participants had a mean age of 18.96 years (median 18), with 91.6% selecting General Medicine as their first choice. The cohort comprised 54.6% current high school students and 14.3% university graduates seeking career transition. Over 60% of participants allocated 50-90% of their total study time to admission exam preparation. Geographic diversity was observed, with 26.1% of participants traveling from neighboring counties. Regarding course usefulness, a very high proportion of participants rated the courses as "Useful" or "Very useful," with a slight increase from 80.0% in 2024 to 83.1% in 2025. Notably, the proportion rating the courses as "Very useful" increased from 45.0% to 61.0% between years, reflecting the program's ongoing refinement. **Conclusions:** The highly positive feedback from participants validated the preparatory program's effectiveness and triggered informed evidence-based expansion from an intensive to an adequately-paced format. Anonymous questionnaires proved effective for capturing multiple

dimensions of participants' perception regarding study effort and commitment. Our university pre-admission training initiative demonstrates institutional responsiveness to learner needs and provides a replicable model for other medical universities. **Keywords:** ANATOMY AND PHYSIOLOGY, FREE PREPARATORY COURSES, MOTIVATION, LEARNING PROGRAM, SATISFFACTION, ADMISSION EXAM, MEDICINE.

INTRODUCTION

The admission examination for medical and dental medicine faculties represents a critical milestone for high school graduates and constitutes a rigorous filter in selecting the most qualified candidates. Up to and including the summer of 2022, this exam at Grigore T. Popa University of Medicine and Pharmacy Iasi was based on the 11th-grade high school textbook for Anatomy and Physiology. Beginning with the July 2023 admission cycle, the curriculum transitioned to Barron's Human Anatomy and Physiology textbook by Barbara Krumhardt and I. Edward Alcamo, translated and republished in 2022 by University Press of George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș.

This transition represented a coordinated effort among Romanian medical universities to align admission requirements with more rigorous international standards, ensuring that the selection process better reflects prerequisite comprehensive anatomical and physiological knowledge of all body systems, essential for first-year medical students. The examination employs multiple-choice question (MCQ) format, requiring not only content mastery, but also analytical reasoning skills to navigate complex question types.

The expanded scope and complexity of Barron's textbook may present significant challenges for prospective students, and this is the reason why beginning with the 2023-2024 academic year, our university initiated free-access preparatory courses for future candidates. These courses were intended to provide equitable access to structured learning opportunities, particularly for candidates without access to private tutoring or other preparatory programs.

The program evolved from the initial intensive format (March-May 2024), towards an in-depth approach, spanning two academic semesters (October 2024-May 2025). This extension allowed more comprehensive content delivery and additional practice. However, the effectiveness of such institutional initiatives requires a systematic evaluation beyond anecdotal impressions. Therefore, this study aimed to systematically evaluate future candidates' perception of the free preparatory course initiative at our university. Secondary objectives included characterizing participants' demographics, educational backgrounds, and entrance examination preferences, in parallel with perceived learning benefits and satisfaction levels. Understanding all these aspects is essential for evidence-based program refinement and potential replication at other higher schools of medicine.

MATERIALS AND METHODS

Preparatory Course Structure. The preparatory program offered comprehensive coverage of all 23 chapters from Barron's textbook through systematic instruction by senior experienced faculty members from the Department of Anatomy and Embryology. Recognizing visualization chal-

lenges inherent in the Barron's monochrome illustrations, instructors supplemented lectures with highly didactic color images from relevant anatomical atlases, facilitating comprehension of three-dimensional spatial relationships and complex structural features, such as saddle-type joints, which display both concavity and convexity on each contact surface.

The course also integrated structured multiple-choice question (MCQ) exercises with varying difficulty levels, training the candidates for analytical approaches to anatomical associations, multiple selections, structural exclusions, spatial relationships between anatomical structures, and image-based identification skills that are essential for a successful performance in the exam.

The preparatory course program utilized a weekly registration system managed through university enrollment forms on a first-come, first-served basis, with each enrollment form published online at the beginning of the week, for lectures held on weekends (Saturday or Sunday). Enrollment was limited to amphitheater capacity (maximum 110 participants per session) to ensure adequate instructional quality.

The program format evolved between study years. The 2023-2024 iteration employed an intensive delivery model spanning three months (March-May 2024), providing focused pre-examination preparation. Based on preliminary participant feedback, the 2024-2025 program was redesigned as an extended in-depth format spanning two academic semesters (October 2024-May 2025), allowing more gradual content delivery, enhanced opportunities for concept reinforcement, and additional practice with demonstrative MCQ assessments administered through direct instruction and Google Forms platform.

Study Design, Participants and Data Collection Instrument. This prospective observational study employed a two-year comparative design conducted during the 2023-2024 and 2024-2025 academic years at the Grigore T. Popa University of Medicine and Pharmacy Iași.

The study population comprised voluntary participants who attended the preparatory courses and completed an anonymous evaluation questionnaire, administered via Google Forms. Questionnaire submission remained open during the program duration and shortly thereafter, allowing participants to provide feedback based on their course experience. In 2024, submissions were collected from March to June, while in 2025, submissions spanned from January to May, allowing participants sufficient exposure to form informed impressions while maximizing response rate.

The instrument featured adaptive branching logic to suit different participant categories (current high school students, university graduates seeking career transition, employed professionals) and assessed multiple domains: demographic characteristics (age, citizenship), geographical domicile, entrance examination preferences (first- and second-choice program selection), time allocation patterns for examination preparation, perceived difficulty (Likert scale, 1 = "Very easy" to 5 = "Very difficult") of specific anatomical content areas (embryology, spatial relationships between structures), and overall course usefulness rated also on a 5-point Likert scale (1 = "Not useful" to 5 = "Very useful").

A total of 119 individuals completed the questionnaire (60 participants in 2024, 59 in 2025). All participants provided explicit

informed consent for anonymous data processing prior to questionnaire submission. Inclusion criteria comprised active enrollment in the preparatory course program and willingness to complete our questionnaire.

Importantly, this questionnaire design was separated from the university enrollment forms system, ensuring no linkage between enrollment forms and this study's responses. We did not collect any names, personal identification numbers, email addresses or phone numbers, encouraging candid responses and protecting participant confidentiality in compliance with EU General Data Protection Regulation (GDPR) principles.

Statistical Analysis. Data analysis employed descriptive and inferential statistics to characterize participant demographics, study behaviors, and satisfaction levels. Continuous variables were summarized using means ± standard deviation and medians, while categorical variables were expressed as frequencies and percentages. Comparative analysis between the 2024 and 2025 cohorts was performed using Student's t-test for continuous variables with normal distribution, Pearson Chi-squared test for categorical variables, and Kruskal-Wallis test for ordinal data and non-normally distributed continuous variables. Statistical significance was established at a 95% confidence interval ($p < 0.05$). Statistical computations were performed using *SPSS* software.

Ethical Considerations and Data Availability. The study adhered to ethical principles of voluntary participation, informed consent, and data anonymization. All participants provided explicit consent for anonymous data processing, with no identifiable information collected.

Complete anonymized raw data, detailed statistical analyses with correlations between certain findings, and supplementary materials with graphics (> 50 pages) are publicly accessible via Mendeley Data, a subsidiary of Elsevier (DOI: 10.17632/2y8 nyy4yyy.1), promoting transparency and enabling independent analysis and verification of findings. Reference to specific information in this repository is indicated in text as an abbreviation (MendRep, DocXX, Table YY / Figure YY) throughout the manuscript, together with corresponding reference (1).

RESULTS

Participant Demographics

The study enrolled 119 participants, distributed equally between the two cohorts: 60 participants in 2024 and 59 in 2025. The mean age was 18.96 years (SD = 3.123, range: 16-43 years), with no significant age difference between cohorts (2024: 18.82 ± 2.541 years *vs.* 2025: 19.10 ± 3.637 years; $t = -0.496$, $p = 0.621$). Nearly all participants were Romanian citizens (99.2%, n = 118), with one participant holding dual Romanian and Moldovan citizenship (MendRep, Doc05, Table 1) (1).

Medicine was the overwhelming first choice for admission (91.6%, n = 109), followed by Dental Medicine (4.2%, n = 5), Nursing (2.5%, n = 3), and Bioengineering (0.8%, n = 1). The distribution of first-choice specialization remained stable between cohorts ($\chi^2 = 2.754$, $p = 0.600$). The majority of participants (63.0%, n = 75) were currently enrolled in high school, with 42.0% in 12th grade and 20.2% in 11th grade. High school enrollment status did not differ significantly between 2024 and 2025 cohorts ($\chi^2=0.203$, $p=0.653$) (Mend Rep, Doc05, Table 2) (1).

Geographic distribution revealed that 63.0% (n = 75) of participants attended schools in Iași County, while 26.1% (n = 31) commuted from other counties to attend the preparatory courses. Participants came from diverse educational backgrounds, with 66.4% enrolled in National Colleges without specialized domains and 20.2% in Theoretical High Schools (MendRep, Doc05, Table 2) (1).

Study Habits and Exam Preparation. Participants allocated a mean of 19.99 hours weekly (SD = 11.015, range: 2-50 hours) to studying anatomy and physiology from Barron's textbook, and 9.58 hours weekly (SD = 6.774, range: 0-40 hours) to the second exam subject (MendRep, Doc05, Table 3) (1). Organic Chemistry was the most popular second exam choice (67.2%, n = 80), followed by Physics (27.7%, n = 33) (MendRep, Doc05, Table 7) (1).

A substantial proportion (33.6%, n = 40) dedicated 75-90% of their total weekly study time to UMF admission preparation, while 26.9% (n = 32) focused exclusively (100%) on entrance exam preparation (MendRep, Doc05, Table 5) (1).

Among 69 participants who took the admission simulation exam, the mean score was 6.88 ± 1.66 (range: 2.03-9.60) (MendRep, Doc05, Table 9) (1). Most students perceived this grade as reflective of their knowledge (28.6%, n = 34), while 24.4% (n = 29) felt they knew the material better than their score indicated (MendRep, Doc05, Table 10) (1).

Perceived Difficulty and Course Satisfaction. Participants were asked to rate the difficulty of selected chapters (Likert scale, 1 = very easy, 5 = very difficult), traditionally considered challenging by first-year university students. For the musculoskeletal system, ratings were predominantly moderate to high, with 42.9% (n = 51) rating it as "4" and 31.9% (n = 38) as "5" (MendRep, Doc05, Table 11) (1). The perception of difficulty did not differ significantly between the 2024 and 2025 cohorts (χ^2 = 6.019, p = 0.111) (tab. I). Cell division, gamete formation, and intrauterine development received more varied difficulty ratings, with 34.5% (n = 41) rating these chapters as "3" and 26.1% (n = 31) as "4" (MendRep, Doc05, Table 12) (1), again with no significant difference between cohorts (χ^2 = 0.191, p = 0.996) (tab. II).

TABLE I.

Perceived difficulty of the chapters on the skeletal and muscular system, by cohort year (2024 *vs.* 2025)

How difficult do you think the chapters on the skeletal and muscular system ("bones and muscles") are?		Year			
		2024		2025	
		n	%	n	%
Likert scale: 1 = very easy 3 = neutral 5 = very difficult	1	0	0%	0	0%
	2	1	1.7%	5	8.5%
	3	9	15.0%	15	25.4%
	4	27	45.0%	24	40.7%
	5	23	38.3%	15	25.4%
Total		60	100.0%	59	100.0%
Pearson Chi-square = 6.019 / p = 0.111					

TABLE II.

Perceived difficulty of the chapters on cell division, gamete formation, and intrauterine development, by cohort year (2024 *vs.* 2025)

How difficult do you think the chapters on cell division, gamete formation and intrauterine development are?		Year			
		2024		2025	
		n	%	n	%
Likert scale: 1 = very easy 3 = neutral 5 = very difficult	1	4	6.7%	3	5.1%
	2	11	18.3%	11	18.6%
	3	21	35.0%	20	33.9%
	4	15	25.0%	16	27.1%
	5	9	15.0%	9	15.3%
Total		60	100.0%	59	100.0%
Pearson Chi-square = 0.191 / p = 0.996					

Course usefulness showed a notable improvement in maximum satisfaction on the Likert scale. The combined proportion of participants rating the courses as highly useful (ratings 4 and 5) increased slightly from 80.0% in 2024 to 83.1% in 2025, though this difference did not reach statistical significance (χ^2 = 3.360, p = 0.339). Across both cohorts, 81.5% of all participants (n = 97) rated the courses as either "4" or "5" on the 5-point usefulness scale (tab. III).

TABLE III.

Perceived course satisfaction in terms of usefulness, by cohort year (2024 *vs.* 2025)

How useful do you think these free courses in human anatomy and physiology, organized by Grigore T. Popa University of Medicine and Pharmacy, are for you?		Year			
		2024		2025	
		n	%	n	%
Likert scale: 1 = not useful 3 = neutral 5 = very useful	1	0	0%	0	0%
	2	1	1.7%	1	1.7%
	3	11	18.3%	9	15.3%
	4	21	35.0%	13	22.0%
	5	27	45.0%	36	61.0%
Total		60	100.0%	59	100.0%
Pearson Chi-square = 0.191 / p = 0.996					

Importantly, the proportion of participants rating the courses as "5" (very useful) varied from 45.0% (n = 27) in 2024 to 61.0% (n = 36) in 2025, representing a 36% relative increase. Conversely, ratings of "4" decreased from 35.0% (n = 21) to 22.0% (n = 13), and ratings of "3" decreased from 18.3% (n = 11) to 15.3% (n = 9), illustrating a shift toward higher satisfaction in the extended program format (tab. III).

Correlation Analyses. We performed extensive correlation and association analyses to explore potential relationships between participant characteristics and their perceptions of course content difficulty and overall usefulness. The complete set of correlation matrices and statistical tests (Tables 19-58) is available in the supplementary data repository (MendRep, Doc05) (1).

While most demographic and behavioral variables showed no significant associations with outcome measures, four relationships emerged with statistical significance ($p < 0.05$), which we will examine in detail.

No significant relationship was found between age and the following: (a) difficulty of the chapters on the skeletal and muscular system (Kruskal-Wallis H = 2.204, p = 0.531), (b) difficulty of the chapters on cell division, gamete formation, and intrauterine development (Kruskal-Wallis H = 6.281, p = 0.179), and (c) course usefulness ratings (Kruskal-Wallis H = 0.694, p = 0.875), suggesting that our preparatory lectures were equally beneficial across different age groups (MendRep, Doc05, Table 19) (1).

Similarly, current high school enrollment status did not significantly affect perceptions of course usefulness (χ^2 = 2.860, p = 0.414) (MendRep, Doc05, Table 25) (1). The number of weekly study hours allocated to anatomy did not significantly correlate with perceived difficulty of the musculoskeletal system (Kruskal-Wallis H = 4.979, p = 0.173) (MendRep, Doc05, Table 32) (1) or of the chapters on cell division, gamete formation, and intrauterine development (Kruskal-Wallis H = 1.537, p = 0.820) (MendRep, Doc05, Table 33) (1).

In contrast, four variables demonstrated statistically significant associations with either perceived content difficulty or course usefulness ratings.

First finding refers to how current high school students rated cell division and embryology topics as more difficult compared to graduates (χ^2 = 10.738, p = 0.030), with a higher proportion rating them as 4 or 5 (41.4% *vs.* 40.9% combined for 4+5), but notably fewer rating them as "very easy" (1.3% *vs.* 13.6%) (tab. IV).

TABLE IV.

Perceived difficulty of cell division, gamete formation, and intrauterine development chapters by current high school enrollment status

How difficult do you think the chapters on cell division, gamete formation and intrauterine development are?		Do you currently attend a high school?			
		Yes		Not	
		n	%	n	%
Likert scale: 1 = very easy 3 = neutral 5 = very difficult	1	1	1.3%	6	13.6%
	2	16	21.3%	6	13.6%
	3	27	36.0%	14	31.8%
	4	17	22.7%	14	31.8%
	5	14	18.7%	4	9.1%
Total		75	100.0%	44	100.0%
Pearson Chi-square = 10.738 / p = 0.030*					

Secondly, high school specialization significantly influenced perceived difficulty of the chapters on cell division, gamete formation, and intrauterine development (χ^2 = 28.915, p = 0.025) (tab. V), whereas no significant association was found with perceived difficulty of the musculoskeletal system chapters (χ^2 = 14.374, p = 0.277) (MendRep, Doc05, Table 29) (1). Thus, high school students from Natural Sciences profiles rated embryology content as relatively easier, with 28.6% finding it "Easy" (rating 2) compared to other specializations (tab. V).

TABLE V.

Perceived difficulty of cell division, gamete formation, and intrauterine development chapters by high school specialization

How difficult do you think the chapters on cell division, gamete formation and intrauterine development are?		What profile/specialization do you follow in high school?									
		Another specialization that is not found above		Mathematics - Computer Science		Human Profile - Philology		Vocational profile		Natural Sciences	
		n	%	n	%	n	%	n	%	n	%
Likert scale: 1 = very easy 3 = neutral 5 = very difficult	1							1	25.0%		
	2			4	16.0%	1	12.5%	1	25.0%	10	28.6%
	3			9	36.0%	4	50.0%	2	50.0%	12	34.3%
	4	1	33.3%	8	32.0%	1	12.5%			7	20.0%
	5	2	66.7%	4	16.0%	2	25.0%			6	17.1%
Total		3	100.0%	25	100.0%	8	100.0%	4	100.0%	35	100.0%
Pearson Chi-square = 28.915 / p = 0.025*											

Thirdly, prior exposure to school-based or biology-teacher-led preparatory sessions significantly influenced our university course usefulness ratings (χ^2 = 18.793, p < 0.001) (tab. VI), though notably, only a small percentage of participants (11.0%, n = 8 of 73 current 11th-12th graders) had access to such sessions at their schools. Among this limited group with prior preparatory experience, 25.0% rated the courses as "Not very useful" (rating 2), while 62.5% rated them as "Very useful" (rating 5). In contrast, among high school pupils without prior preparatory session experience (n = 65), none gave the first-lowest or the second-lowest rating, 21.5% rated the courses neutrally (rating 3), and a great majority rated them "Useful" (27.7%) and "Very useful" (50.8%) (tab. VI).

TABLE VI.

Perceived course usefulness by prior exposure to school-based or biology-teacher-led preparatory sessions

How useful do you think these free courses in human anatomy and physiology, organized by Grigore T. Popa University of Medicine and Pharmacy Iasi, are for you?		If you are in the 11th or 12th Grade, is your school (or your biology teacher) organizing a weekly preparation / class with the subjects from Barons?			
		Yes		Not	
		n	%	n	%
Likert scale: 1 = not useful 3 = neutral 5 = very useful	1	0	0%	0	0%
	2	2	25.0%	0	0%
	3	0	0%	14	21.5%
	4	1	12.5%	18	27.7%
	5	5	62.5%	33	50.8%
Total		8	100.0%	65	100.0%
Pearson Chi-square = 18.793 / p < 0.001**					

Lastly, commute distance significantly influenced course usefulness ratings (χ^2 = 14.203, p = 0.027). Participants commuting from other counties rated the courses as "Very useful" (rating 5) at notably higher rates (71.0%) compared to those living in Iasi Municipality (48.8%) (tab. VII).

TABLE VII.

Perceived course usefulness by commute distance to our university

How useful do you think these free courses in human anatomy and physiology, organized by Grigore T. Popa University of Medicine and Pharmacy Iasi, are for you?		Do you have to commute to participate in the admission preparation courses organized by Grigore T. Popa University of Medicine and Pharmacy Iasi?					
		Yes, I live in another county		Yes, I live in Iasi County, but at a distance of more than 15 km from Iasi		No, I live in the Municipality of Iasi (including the surrounding areas)	
		n	%	n	%	n	%
Likert scale: 1 = not useful 3 = neutral 5 = very useful	1	0	0%	0	0%	0	0%
	2	0	0%	0	0%	2	2.4%
	3	4	12.9%	0	0%	16	19.5%
	4	5	16.1%	5	83.3%	24	29.3%
	5	22	71.0%	1	16.7%	40	48.8%
Total		31	100.0%	6	100.0%	82	100.0%
Pearson Chi-square = 14.203 / p = 0.027*							

DISCUSSION

In the Romanian exam-based medical school admission systems, high-stakes standardized testing can create unique challenges for prospective students, particularly those from underserved communities or regions with limited access to high-quality preparatory resources (2, 3). Free, university-led preparatory initiatives address these aspects by providing equitable access to structured exam preparation and ensuring that admission success reflects academic potential rather than socioeconomic advantage (4).

This study examined the perceptions and learning experiences (tabs. I, II) of participants in free preparatory courses for the Grigore T. Popa University of Medicine and Pharmacy Iași entrance examination across two consecutive academic years (2024 and 2025). Participant demographics reflected the typical profile of prospective medical students, with a mean age of 18.96 years (range 16-43), predominantly comprised of current high school students (63.0%) and Romanian citizens (99.2%). The majority selected General Medicine as their first choice (91.6%), and demonstrated substantial commitment to entrance exam preparation, with 89.1% dedicating over half of their total study time to anatomy and physiology (MendRep, Doc05, Tables 1, 2, 5) (1).

Understanding student perspectives on educational interventions is fundamental to evidence-based program development and refinement, as participant experiences directly inform pedagogical effectiveness and institutional decision-making (5-7).

Our findings reveal consistently high

satisfaction with the preparatory courses, with notable improvements in perceived usefulness from 2024 to 2025 (tab. III), alongside significant correlations between demographic and educational variables and both perceived difficulty of exam content and course usefulness ratings.

Current high school students rated the topics on cell division, gamete formation, and intrauterine development as significantly more difficult compared to responders who finished high school ($\chi^2 = 10.738$, $p = 0.030$) (tab. IV). While both groups showed similar proportions rating this content as difficult or very difficult (ratings 4+5: 41.4% *vs.* 40.9%), responders who had already finished high school were significantly more likely to rate it as "very easy" (13.6% *vs.* 1.3%), suggesting that their additional educational experience or cognitive maturation facilitated comprehension of these embryology topics. Moreover, high school specialization significantly influenced these difficulty perceptions ($\chi^2 = 28.915$, $p = 0.025$) (tab. V), with students from Natural Sciences profiles rating this content as relatively easier - 28.6% finding it "easy" (rating 2) compared to other specializations. In contrast, Mathematics-Computer Science and Humanities students showed more varied difficulty perceptions, suggesting that prior exposure to biological concepts in Natural Sciences curricula provided foundational knowledge that facilitated comprehension of embryology topics.

The significant association between prior preparatory experience and our program usefulness ratings ($\chi^2 = 18.793$, $p < 0.001$) revealed a notable pattern (tab. VI). Participants with a comparative framework for evaluating preparatory instruction, having experienced similar classes at their high schools, were better positioned to assess the quality and pedagogical value of the university-level courses, despite representing only 11.0% of current high school participants. While a quarter of this small group evaluated the courses more critically, their great majority (75%) provided validation of our program quality. Moreover, we observed that only a small fraction of high school pupils has access to school-based preparatory sessions, emphasizing the critical importance of university-led initiatives in providing equitable access to high-quality exam preparation (tab. VI).

Commute distance correlated significantly with course usefulness ratings ($\chi^2 = 14.203$, $p = 0.027$) (tab. VII). Participants who invested greater time and effort to commute from other counties valued the program more highly, potentially reflecting both self-selection of highly motivated individuals and tangible appreciation for educational access that would otherwise be unavailable in their home regions.

The observed relative increase of 36%, in participant satisfaction rating the courses as "5" (very useful), from 45.0 % to 61.0% between its first two years of implementation, likely reflects the program's evidence-based evolution. Our preparatory program evolved significantly from the initial 2024 intensive format (March-May 2024) to the 2024-2025 approach over two academic semesters (October 2024-May 2025), thus achieving a thorough curriculum coverage. The extension decision was taken by our university, based on the preliminary feedback and pedagogical considerations (MendRep, Doc03) (1).

The transition from intensive to extend-

ed format required both organizational commitment and faculty members mobilization, but the demonstrated improvements in participant satisfaction validated its success. The program's sustainability is evidenced by the continuation of this extended format for the new academic year of 2025-2026, with the first lecture for the 2026 admission exam being delivered in October 2025.

Future program development could address the current curricular gap by incorporating physics courses alongside the existing human anatomy and physiology, and organic chemistry preparation modules, thereby supporting the minority of candidates who select physics instead of organic chemistry as their second examination subject at the Faculties of Medicine and Dental Medicine. Another pathway for program expansion would be increasing participant capacity through technology-enhanced delivery methods, such as simultaneous audio-visual transmission to nearby dual amphitheaters. This approach would preserve in-person interactive instruction with the teacher during breaks and at the end of the lecture, a pedagogical approach long recognized as beneficial for student outcomes (8).

Several limitations of our research warrant consideration. First, the fully anonymous questionnaire design, while essential for protecting participant confidentiality and encouraging candid responses, precluded technical prevention of duplicate submissions. Although appeals to honesty were made to minimize duplicate responses, the anonymous nature of data collection made verification of unique respondents impossible. However, given the educational context, voluntary participation, and absence of incentives for multiple submissions, we assume the potential for duplicate responses to be minimal and unlikely to substantially alter the study's primary findings. This trade-off between anonymity and duplicate prevention represents an inherent methodological constraint in anonymous survey research that prioritizes participant privacy (9).

CONCLUSIONS

The highly positive feedback from participants validated the preparatory program's effectiveness and triggered informed evidence-based expansion from an intensive format to an in-depth material presentation; further expansion could include physics preparation to support the minority of candidates who select this as their second examination subject.

Anonymous questionnaires proved valuable tools for capturing multiple dimensions of participants' perception regarding study effort and commitment.

Our university pre-admission training initiative reflects institutional responsiveness to learner needs and provides a replicable model for other medical universities in Romania where competitive entrance examinations are in place.

The participation of future candidates from other counties demonstrates the program's regional reach and its value for supporting exam preparation across an extended geographical area.

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CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding for this study.

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TROSPIUM CHLORIDE *VERSUS* TAMSULOSIN IN THE TREATMENT OF DOUBLE-J STENT RELATED LOWER URINARY TRACT SYMPTOMS (LUTS)

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TROSPIUM CHLORIDE *VERSUS* TAMSULOSIN IN THE TREATMENT OF DOUBLE-J STENT RELATED LOWER URINARY TRACT SYMPTOMS (LUTS): A CASE-CONTROL STUDY (Abstract): Double-J ureteral stents frequently cause lower urinary tract symptoms (LUTS), including urinary urgency and pollakiuria, which significantly affect patient comfort. This study compared the efficacy and safety of trospium chloride versus tamsulosin in patients with stent-related LUTS. **Materials and methods:** We conducted a retrospective 6-year study comparing patients with LUTS in the presence of double-J stents who had received trospium for 4 weeks (39 patients) with patients who had received tamsulosin (52 patients). **Results:** Both trospium and tamsulosin relieved LUTS symptoms ($p<0.05$). Trospium relieved pollakiuria (82.05% *vs.* 61.53%, $p=0.034$) and urinary urgency (87.17% *vs.* 59.61%, $p=0.003$) in a higher percentage than tamsulosin. However, there was no statistically significant difference in the improvement of urinary urgency incontinence (80% *vs.* 47.05%, not significant at $p<0.05$), hypogastric pain (64% *vs.* 40%, $p=0.103$), foreign body sensation (64.28% *vs.* 38.09%, $p=0.128$) and macroscopic hematuria (37.5% *vs.* 18.18%, not significant at $p < 0.05$); side effects occurred rarely, with no statistically significant difference between the two groups (5.12% *vs.* 5.76%, not significant at $p<0.05$). **Conclusions:** Trospium chloride is a safe and effective option for managing LUTS associated with double-J stents and provides superior improvement in pollakiuria and urinary urgency compared with tamsulosin. **Keywords:** TROSPIUM CHLORIDE, TAMSULOSIN, DOUBLE-J STENT, LOWER URINARY TRACT SYMPTOMS, LUTS, STENT-RELATED SYMPTOMS.

INTRODUCTION

Trospium chloride is a quaternary ammonium derivative of nortropanol (1), a competitive cholinergic receptor antagonist (2). Its activity is predominantly peripheral, non-selective antimuscarinic, and it has no effects on the central nervous system (3). Trospium is commonly used for overactive bladder to relieve urinary incontinence associated with urinary urgency and pollakiuria (4). It is also used to relieve these symptoms in neurogenic detrusor overactivity (5) and to reduce postoperative bladder irritation and radiation-induced cystitis (6). Trospium is an anticholinergic agent that has been used in Europe since the 1990s and in the USA since 2004 (7). Over the past 30 years of experience, only a few

side effects have been reported, the most common being dry mouth, constipation, and headache (8, 9). This safety profile has been confirmed in studies involving more than 10,000 patients (1). Trospium can be administered orally in one or more doses not exceeding 45-60 mg per day (9-11).

Urinary catheters or stents are commonly used in clinical practice, and many patients wear them for weeks, months, or even years (12). The presence of these catheters predisposes patients to urinary tract infections (13, 14), hematuria, pollakiuria, urinary urgency, and urge incontinence (15, 16). They relax the smooth muscle fibers of the bladder neck and the prostate, which leads to lower micturition pressure and urinary reflux (17). The effect can be measured by urodynamic studies, which have shown normalization of intravesical pressure and increased cystometric bladder capacity (18). Several drugs have been proposed to treat these symptoms, including tamsulosin, silodosin, or their combination (19, 20). Tamsulosin is an alpha-blocker used not only to treat LUTS associated with benign prostatic hyperplasia (BPH), but also to promote the passage of ureteral stones or fragments formed during extracorporeal lithotripsy and to treat symptoms associated with double-J catheters (21). However, both tamsulosin and solifenacin have certain side effects, such as arterial hypotension and retrograde ejaculation in the case of tamsulosin, and central nervous system effects-particularly in the elderly-in the case of solifenacin (22, 23).

As trospium chloride is the only antimuscarinic drug that has no effects on the central nervous system (22) and lacks the adverse effects associated with tamsulosin (orthostatic hypotension, retrograde ejaculation), we aimed to determine whether it could be a viable alternative for the treatment of secondary LUTS in double-J stent carriers. To our knowledge, this is the first study in the literature to evaluate the efficacy and safety of trospium in this specific patient group.

MATERIALS AND METHODS

Study Population and Design. We conducted a retrospective case-control study of patients with double-J ureteral stents who underwent Extracorporeal Shock Wave Lithotripsy (ESWL) at Elytis Hospital between 1 January 2018 and 31 December 2024. Of the total number of urological patients admitted, 289 had double-J stents. Among these, 42.56% (123 patients) presented with bladder symptoms related to the presence of the double-J stents. All patients had only one double-J stent inserted. These patients were eligible for inclusion in the study. The study was approved by the Ethics Committee of Elytis Hospital (Protocol No. 3927/07.05.2025).

Patients with LUTS who also had benign prostatic hyperplasia (3 patients), those who received tamsulosin in combination with solifenacin or other drugs for LUTS due to the presence of double-J stents (6 patients), those who developed urinary tract infections during treatment (2 patients), and patients with incomplete data in hospital records (21 patients) were excluded. In all patients, urine culture was performed, to exclude urinary tract infection, a well-known complication of double-J stents, even with multidrug-resistant bacteria (24).

Study Procedure. The study group consisted of patients with LUTS and double-J stents who were administered trospium chloride (Inkontan™, Montavit, Austria), and the control group consisted of patients with LUTS and double-J stents

who were administered tamsulosin (Fokusin™, Zentiva, Czech Republic). The indication for treatment with tamsulosin was the presence of symptoms secondary to the double-J stents, as well as to promote the elimination of lithiasis fragments smaller than 4 mm occurring after ESWL.

Trospium chloride was administered at a dose of 15 mg twice daily (morning and evening), and tamsulosin was administered 0.4 mg once daily in the evening. No patient received 15 mg of trospium three times daily, so the maximum recommended dose of 45 mg per day was not reached, in order to avoid known anticholinergic side effects.

Patients who underwent ESWL and had LUTS started treatment three days after the procedure. During this period, they completed a voiding calendar to assess the severity of pollakiuria. Treatment with trospium or tamsulosin lasted four weeks, after which patients returned for follow-up. Before each new ESWL session and follow-up, an ultrasound scan and X-ray of the kidneys were performed to detect calcifications or misplacement of the double-J catheters, especially at the distal intravesical end, as these could influence treatment response and represent potential bias.

The ureteral catheters were made of polyurethane, 6-7 Ch thick and 24-28 cm long, adjusted to the individual patient height and according to the manufacturer specifications. The length of the distal end in the bladder was not measured, but all patients had a complete distal loop of the double-J stent in the bladder.

Patient Assessment and Outcome Measurement. The following variables were collected from hospital records: age, gender, history of urinary tract infections, underlying condition requiring stent insertion, right-left localization of the double-J catheter, and comorbidities potentially influencing LUTS, such as diabetes and neurological disorders. In both groups, the drug dose, administration frequency, and treatment duration were analyzed. The presence of hematuria, pollakiuria, urinary urgency and urge incontinence, intravesical foreign body sensation, and hypogastric pain (bladder tension or persistent pain) were recorded.

Visual Analog Scale (VAS) questionnaires for pain were not used, as facial expression was considered subjective and patients generally requested removal of the stent if discomfort was significant. Therefore, all included patients were presumed to have moderate pain. To assess the reduction in daily micturitions, a voiding calendar was used for three consecutive days before and at the end of treatment. Treatment discontinuation due to side effects or lack of efficacy was also recorded.

All patients were evaluated and managed by the same two attending urologists, who followed identical departmental protocols for the treatment of double-J stent-related symptoms. Tamsulosin was prescribed primarily to relieve stent-related LUTS and to facilitate the passage of residual stone fragments <4 mm after ESWL, whereas trospium chloride was preferentially used in patients whose predominant symptoms were urinary urgency and frequency without the need for expulsive therapy. Baseline symptom presence was similar across groups.

Patients with LUTS following ESWL were treated for one month with either trospium or tamsulosin and then re-evaluated. The presence of symptoms and subjective improvement were recorded at baseline and after one month of therapy.

The micturition calendar was reviewed during the control visit.

Statistical Analysis. Quantitative variables were described by mean and standard deviation, and qualitative variables by percentages. Quantitative variables were compared using the t-test for normally distributed data and the Mann-Whitney U-test for non-normally distributed data. Normality of data distribution was verified using the Kolmogorov-Smirnov test. Qualitative variables were compared using the chi-square test or Fisher's exact test when fewer than five observations were present. Statistical significance was considered at $p < 0.05$. Data were processed using *Microsoft Excel version 2019* (Microsoft, Redmond, WA, USA) and *SPSS version 24.0* (IBM, Chicago, IL, USA).

RESULTS

During the study period, a total of 91 patients underwent ESWL and experienced LUTS due to the presence of double-J stents. Of these, 39 patients received trospium chloride (study group) and 52 patients received tamsulosin (control group).

Demographic and Clinical Characteristics

First table presents the demographic and clinical characteristics of patients in both groups.

There were no statistically significant differences in age, sex, laterality of the stent, stone location, or comorbidities such as diabetes or obesity between the two groups. No patient had neurological disorders that could have influenced LUTS or treatment response.

TABLE I.
Demographic and Clinical Characteristics of Patients in the Trospium and Tamsulosin Groups

Characteristic	Trospium Group (n = 39)	Tamsulosin Group (n = 52)	p-value	Test
Age (years, mean ± SD)	48.85 ± 14.12	47.69 ± 11.94	0.677	Student's *t*-test
Sex (Male/Female), n (%)	15 / 24 (38.5% / 61.5%)	22 / 30 (42.3% / 57.7%)	0.877	Chi-square
Indication for stent insertion				
Infected hydronephrosis, n (%)	34 (87.2%)	44 (84.6%)	0.729	Chi-square
Recurrent renal colic, n (%)	5 (12.8%)	8 (15.4%)	0.729	Chi-square
Stent laterality				
Right ureter, n (%)	17 (43.6%)	26 (50.0%)	0.544	Chi-square
Left ureter, n (%)	22 (56.4%)	26 (50.0%)	0.544	Chi-square
Stone location (pre-ESWL)				
Ureteral calculus, n (%)	23 (59.0%)	34 (65.4%)	0.531	Chi-square
Pyelocaliceal calculus, n (%)	16 (41.0%)	18 (34.6%)	0.531	Chi-square
Comorbidities				
Diabetes mellitus, n (%)	2 (5.1%)	3 (5.8%)	N.S.	Fisher's exact
Obesity (BMI > 30), n (%)	4 (10.3%)	5 (9.6%)	N.S.	Fisher's exact

N.S.-non-significant

There were no statistically significant differences in age, sex, laterality of the stent, stone location, or comorbidities such as diabetes or obesity between the two groups. No patient had neurological disorders that could have influenced LUTS or treatment response.

Baseline Symptoms

In the second table are presented the baseline prevalence of stent-related LUTS prior to treatment.

TABLE II.

Baseline Prevalence of Stent-Related Lower Urinary Tract Symptoms in Each Group (Pre-Treatment)

Symptom	Trospium Group (n = 39)	Tamsulosin Group (n = 52)	p-value	Test
Urinary urgency, n (%)	39 (100%)	52 (100%)	N/A	—
Urge urinary incontinence, n (%)	10 (25.6%)	17 (32.7%)	0.619	Chi-square
Urinary frequency (pollakiuria), n (%)	39 (100%)	52 (100%)	N/A	—
Voids per day (mean ± SD)	13.55 ± 4.31	13.45 ± 3.88	0.771	Mann-Whitney U
Suprapubic (hypogastric) pain, n (%)	25 (64.1%)	35 (67.3%)	0.749	Chi-square
Intravesical foreign body sensation, n (%)	14 (35.9%)	21 (40.4%)	0.663	Chi-square
Gross hematuria, n (%)	8 (20.5%)	11 (21.2%)	0.940	Chi-square

The frequency of the six symptoms recorded in the study was similar in both groups, with no statistically significant differences between the groups. We found that the only symptoms that occurred in all patients in both groups were pollakiuria and urinary urgency. There were no differences between the two groups in the total number of micturitions per day. The other symptoms occurred in much smaller percentages, namely urge urinary incontinence and hematuria, which occurred in about a quarter of patients in both groups.

Symptom Improvement After Four Weeks

Table III summarizes symptom improvement after four weeks of treatment.

TABLE III.

Patients Reporting Improvement of Symptoms After 4 Weeks of Treatment

Symptom	Trospium Improved n/N (%)	Tamsulosin Improved n/N (%)	p-value	Test
Urinary urgency	34/39 (87.2%)	31/52 (59.6%)	0.003	Chi-square
Urge urinary incontinence	8/10 (80.0%)	8/17 (47.1%)	N.S.	Fisher's exact
Urinary frequency (pollakiuria)	32/39 (82.1%)	32/52 (61.5%)	0.034	Chi-square
Suprapubic pain	16/25 (64.0%)	14/35 (40.0%)	0.103	Fisher's exact
Intravesical foreign body sensation	9/14 (64.3%)	8/21 (38.1%)	0.128	Fisher's exact
Gross hematuria	3/8 (37.5%)	2/11 (18.2%)	N.S.	Fisher's exact

N.S.-non-significant

In the study group, only pollakiuria, urinary urgency and urge incontinence improved by more than 80%, while macroscopic hematuria, hypogastric pain and foreign body sensation improved in more than 60% of patients. In the control group, on the other hand, the percentage of patients reporting an improvement in symptoms was significantly lower than in the study group, with only pollakiuria and the percentage of improvement of more than 50% was achieved. For urge incontinence, hypogastric pain and intravesical foreign body sensation, the percentage was less than 50%, and for macroscopic hematuria even less than 20%. However, statistically significant differences were found only for pollakiuria (p = 0.03) and urinary urgency (p = 0.003).

For urge incontinence, hypogastric pain, foreign body sensation and hematuria, there was no statistically significant difference in the number of patients who reported an improvement in these symptoms, while the total percentage of patients who subjectively reported an improvement showed a statistically significant difference in favor of the group receiving Trospium 30mg/day.

The mean number of micturitions/day calculated from the micturition calendar before treatment and at the end of treatment was significantly reduced in both the study group (13.68 ± 4.08 *vs.* 10.43 ± 5.28, p<0.001) (Wilcoxon test) and the control group (13.67 ± 3.87 *vs.* 11.08 ± 5.08, p<0.001) (Wilcoxon test).

Adverse Events

Treatment-related side effects are presented in the fourth table.

TABLE IV.

Treatment-Related Adverse Events Reported in Each Group

Adverse Event	Trospium Group (n = 39)	Tamsulosin Group (n = 52)	p-value	Test
Dry mouth	1 (2.6%)	0 (0%)	N.S.	Fisher's exact
Constipation	0 (0%)	0 (0%)	N/A	—
Dizziness (lightheadedness)	0 (0%)	3 (5.8%)	N.S.	Fisher's exact
Diarrhea	1 (2.6%)	0 (0%)	N.S.	Fisher's exact
Total side effects	**2 (5.1%)**	**3 (5.8%)**	N.S.	Fisher's exact

N.S.-non-significant

The incidence of side effects was low in both groups, with no statistically significant differences. Despite the occurrence of these side effects, this did not lead to discontinuation of treatment by the patients, with the exception of one patient with diarrhea in the study group who discontinued treatment after 10 days. We note that in the control group, no patient reported constipation and only one patient reported dry mouth, which are recognized side effects of parasympathomimetic. In the control group, a small number of patients reported dizziness. Although retrograde ejaculation is a common complication in male patients, we did not investigate its occurrence due to the mixed-sex group. However, no patient discontinued tamsulosin treatment due to the presence of this side effect.

DISCUSSION

To our knowledge, this is the first

study to evaluate the efficacy and safety of trospium chloride in patients with LUTS due to double-J stents. We found that trospium demonstrated superior efficacy compared to tamsulosin in the treatment of pollakiuria and urinary urgency, with both drugs being well tolerated and associated with a low rate of side effects.

The demographic characteristics of patients were similar between groups in terms of age, sex, stent laterality, and stone location. The mean age of participants was consistent with other studies investigating trospium (25, 26), typically ranging between 45 and 60 years. Equal distribution of diabetic patients and the absence of neurogenic bladder disorders minimized confounding factors affecting LUTS or drug response. We included only patients with lithiasis and double-J stents, excluding those with other causes of LUTS such as radiotherapy or urinary tract infections, to further reduce bias.

Although most studies investigating trospium for overactive bladder used doses of 40-60 mg/day (27, 28), in our study we administered 30 mg/day in two doses to minimize side effects. Increasing the dose to 45 mg/day might have enhanced efficacy, as previous reports suggest 40 mg/day as optimal for balancing efficacy and tolerability (30), with potential additional benefits at 60 mg/day (30). Trospium is known for good safety even in elderly patients with dementia or Parkinson's disease (22, 25), although such patients were not included in our analysis.

In the literature, only one study has assessed trospium for catheter-related LUTS, specifically in patients with Foley urethral catheters (12), but none have focused on double-J stents. Similar to other investigations (12, 19, 27, 28), we evaluated improvement in individual symptoms rather than overall pain or quality-of-life scores (20, 29), allowing a clearer understanding of specific drug effects.

All patients continued therapy, except one in the trospium group who discontinued due to diarrhea. The discontinuation rate for trospium is reported to be lower than for other anti-muscarinics (31).

Our findings showed significantly greater improvement in pollakiuria and urinary urgency with trospium compared to tamsulosin. Pollakiuria improved by an average of 3.19 micturitions per day, consistent with previous studies (9), and higher than those reporting a reduction of fewer than two micturition per day (19). The proportion of patients reporting improvement (> 80%) was greater than in other studies (approximately 62%) (27). Because we assessed total daily micturition, we could not differentiate between day and night improvements; other research indicates greater benefit during daytime (28). We defined pollakiuria as ≥ 10 micturitions per 24 hours, compared with thresholds of > 8 (19, 27) or even > 30 (32) used elsewhere. Clinically, patients with <10 micturitions per day often did not require therapy, while those exceeding 30 generally needed stent removal rather than medication.

Urinary urgency improved in both groups but more markedly with trospium (87.17% *vs.* 59.61%). This difference of nearly 30% highlights trospium's superior efficacy. Comparable benefits have been reported in other studies examining trospium for overactive bladder (5, 9, 25).

For urge urinary incontinence, the improvement was greater with trospium, though not statistically significant, likely

due to the small number of affected patients. We did not count daily incontinence episodes as in some trials (9) but relied on subjective reporting, since patient self-monitoring of these episodes is often unreliable.

Hypogastric pain and intravesical foreign body sensation improved less than pollakiuria and urgency in both groups, possibly reflecting subjective variability in symptom perception. Some patients may have expected complete symptom resolution, while others reported satisfaction with partial relief. Similar findings have been observed in studies of Foley catheter users (12).

Hematuria showed the least improvement, with no difference between groups. This likely reflects mechanical irritation from the stent, which persists despite pharmacologic relaxation of bladder smooth muscle. No cases of clot-forming hematuria occurred, and this symptom did not lead to treatment discontinuation.

Adverse effects were mild in both groups. In the trospium group, one case each of dry mouth and diarrhea was reported, with no constipation, neurological impairment, or other systemic symptoms, consistent with the literature (5, 26). No sedation or sleep disturbances occurred (33). In the tamsulosin group, dizziness was reported in a few cases, likely secondary to orthostatic hypotension. Retrograde ejaculation, although a known adverse event, was not investigated due to the mixed-sex population, but no patient discontinued tamsulosin therapy due to such complication.

Overall, trospium chloride demonstrated superior efficacy to tamsulosin in reducing urinary urgency and frequency among double-J stent carriers, with similar tolerability and safety.

This study has some limitations. First, its retrospective design inherently restricts the ability to control for potential confounding variables, including treatment selection bias arising from routine clinical prescribing patterns rather than random allocation. Second, symptom assessment relied on patient-reported improvement without the use of standardized instruments such as the USSQ, which were not validated in Romanian language at the time of patient's admission. Only pollakiuria could be quantified objectively through the three-day voiding calendar.

CONCLUSIONS

Trospium chloride showed superior efficacy compared to tamsulosin in the treatment of pollakiuria and urinary urgency among patients with double-J stents. It demonstrated similar efficacy to tamsulosin in relieving urinary urge incontinence, intravesical foreign body sensation, hypogastric pain, and hematuria. Adverse effects were rare and did not significantly increase the rate of treatment discontinuation.

Overall, trospium chloride represents a safe and effective therapeutic option for managing LUTS associated with double-J stents. Prospective studies with larger patient populations are needed to validate these findings and to better define the role of trospium chloride in the pharmacologic management of stent-related urinary symptoms.

CONFLICT OF INTEREST AND FUNDING

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SELLAR AND PARASELLAR MENINGIOMAS - CLINICOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMICAL PREDICTORS OF TUMOR GRADE

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SELLAR AND PARASELLAR MENINGIOMAS - CLINICOPATHOLOGICAL FEATURES AND IMMUNOHISTOCHEMICAL PREDICTORS OF TUMOR GRADE. INSIGHTS FROM A RETROSPECTIVE CASE SERIES (Abstract): Sellar and parasellar meningiomas (spMs) represent a rare but clinically challenging subgroup of intracranial tumors due to their proximity to critical neurovascular and endocrine structures. This study **aimed** to provide a comprehensive clinicopathological and statistical evaluation of spMs, with particular emphasis on biological markers associated with tumor aggressiveness. **Materials and methods**: We retrospectively analyzed 15 patients with histologically confirmed spMs treated surgically at Prof. Dr. N. Oblu Emergency Clinical Hospital, Iași, between 2021 and 2025. Demographical, clinical, radiological, surgical, and immunohistochemical data [Ki-67 labeling index (Ki67 LI), progesterone receptor (PR), and p53 expression] were evaluated. Statistical analysis included comparative tests and logistic regression modeling for predictors of WHO grade. **Results:** The cohort showed a marked female predominance (80%), with a mean age of 60.3 ± 13.2 years. Most tumors originated in the sellar region (66.7%). Gross total resection was achieved in 66.7% of cases. Histopathologically, WHO grade 1 spMs predominated (73.3%), while grade 2 spMs accounted for 26.7%. Ki-67 LI values were significantly higher in grade 2 tumors (12.0 ± 2.2 vs. 3.4 ± 1.3; p=0.003), whereas PR expression was significantly reduced (42.5 ± 18.9 vs. 81.4 ± 14.8; p=0.010). Logistic regression identified Ki-67 LI (OR=1.85) as an independent risk factor and PR (OR=0.92) as a protective factor for higher tumor grade. **Conclusions**: These findings highlight the central role of tumor biology in sellar and parasellar meningiomas. Ki-67 LI and PR expression represent complementary and clinically relevant biomarkers that improve risk stratification and may guide surgical and postoperative management. **Keywords**: SELLAR MENINGIOMA, KI-67, PROGESTERONE RECEPTOR, TUMOR GRADE, PREDICTIVE BIOMARKERS.

INTRODUCTION

Meningiomas are the most frequent primary intracranial tumors in adults, representing 36-41% of these (1, 2). Intracranial meningiomas are currently classified according to the 2021 World Health Organization (WHO) Classification of Central Nervous System Tumors into grades 1–3,

based on histopathological criteria and selected molecular alterations (3). Although most meningiomas are WHO grade 1 tumor, a clinically relevant subset shows atypical or aggressive behavior, with increased recurrence risk and need for closer postoperative surveillance (3-5). Morphological grading of meningiomas does not always reflect the actual biological behavior, which is why immunohistochemical biomarkers become essential in prognostic stratification (6).

Ki-67/MIB-1 is widely used as a proliferation marker and has repeatedly been associated with higher grade, increased recurrence risk and more aggressive biological behavior (7-9).

Progesterone receptor (PR) expression, in contrast, is generally associated with more differentiated tumor biology and more favorable behavior, whereas reduced or absent PR expression is more frequently observed in higher-grade meningiomas (10).

One small group of intracranial meningiomas are the sellar and parasellar meningiomas (spMs) that may develop from arachnoid cap cell located on the level of the tuberculum sellae, planum sphenoidale, anterior or clinoid processes, sphenoid wing, cavernous sinuses, petroclival region, diaphragm sellae, or clivus (11).

SpMs represent a particularly challenging subgroup of skull base tumors because of their close anatomical relationship with the optic apparatus, pituitary region, cavernous sinus, internal carotid artery and anterior skull base structures (12). On the other hand, these tumors are quite rare, representing approximately 1%-2% of sellar lesions, but are the second most common sellar tumor after pituitary adenomas (13, 14).

In these locations, surgical management must balance maximal tumor removal with preservation of visual, endocrine and neurovascular function (15-17).

However, spMs are increasingly understood as heterogeneous tumors due to their anatomical origin heterogeneity, clinical variability and sometimes coexistence with other tumors, such as pituitary adenoma (18) or sellar Rathke cleft cyst (19).

The aim of this study was to perform a comprehensive clinicopathological and statistical analysis of a consecutive institutional series of sellar and parasellar meningiomas, with particular focus on tumor biology, as there are only few data on this issue in literature as far as we know.

MATERIALS AND METHODS

We retrospectively identified 15 patients with histologically confirmed sellar and parasellar meningiomas that were surgically treated in Neurosurgical Clinics, Prof. Dr. N. Oblu Emergency Clinical Hospital, Iasi, Romania, in a period of 5 years (January 1, 2021 – December, 31, 2025).

The following demographic, clinical and radiological data were retrospectively extracted from the electronic observation sheets located in the hospital electronic archive: patients' age and gender, tumor localization, meningioma histological subtype, immunohistochemical assessment of Ki67 Labeling Index (Ki67 LI), p53 and Progesterone Receptor (PR) expression, grade of malignancy according to WHO Classification 2021, 5th Edition, type of surgical approach, and extent of tumor resection.

The data was analyzed using *SPSS Statistics version 27.0* (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Descriptive statistics were calculated for all variables.

The values were measured as median, mean, and SD according to the considered variables. Comparative analyses between WHO grade 1 and grade 2 tumors were performed using the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables. Statistical significance set at $p<0.05$. Logistic regression analysis was used to identify independent predictors of tumor grade.

RESULTS

The study cohort comprised 15 patients diagnosed with sellar and parasellar meningiomas (tab. I), with a marked predominance of female patients (80%), compared to males (20%), resulting in a female-to-male ratio of 4: 1. The mean age at diagnosis was 60.3 ± 13.2 years, with a median of 63 years (IQR: 52–71), and an age range spanning from 32 to 76 years. There was a clear predominance of lesions arising from the sellar region [tuberculum sellae (Fig. 1) and sellar diaphragm] (66.7% of all cases), while the remaining tumors (33.3%) were located in parasellar region (Fig. 2), including the anterior clinoid process of the sphenoid bone and cavernous sinus.

The most frequently used approaches were pteryonal and fronto-temporal (46.7%), followed by frontal and bifrontal (33.3%), and endoscopic endonasal approaches (20%). Complete resection was achieved in 66.7% of patients, subtotal resection in 20% of cases, and partial resection in only 13.3% of cases.

TABLE I.

Demographic, clinical, and surgical characteristics of patients with sellar and parasellar meningiomas (n=15).

Variable	No. of cases	Percentage
Age (years)		
mean ± SD	60.3 ± 13.2	
median	63 (52-71)	
Gender		
Female	12	80%
Male	3	20%
Tumor location		
Sellar region (tuberculum sellae, sellar diaphragm)	10	66.7%
Parasellar (clinoidian, cavernous sinus)	5	33.3%
Surgical approach		
endonasal endoscopy	3	20%
pteryonal / frontotemporal	7	46,7%
frontal / bifrontal	5	33.3%
Tumor resection type		
total (macro-/microscopical)	10	66.7%
subtotal	3	20%
partial	2	13.3%
Total patients	15	100%

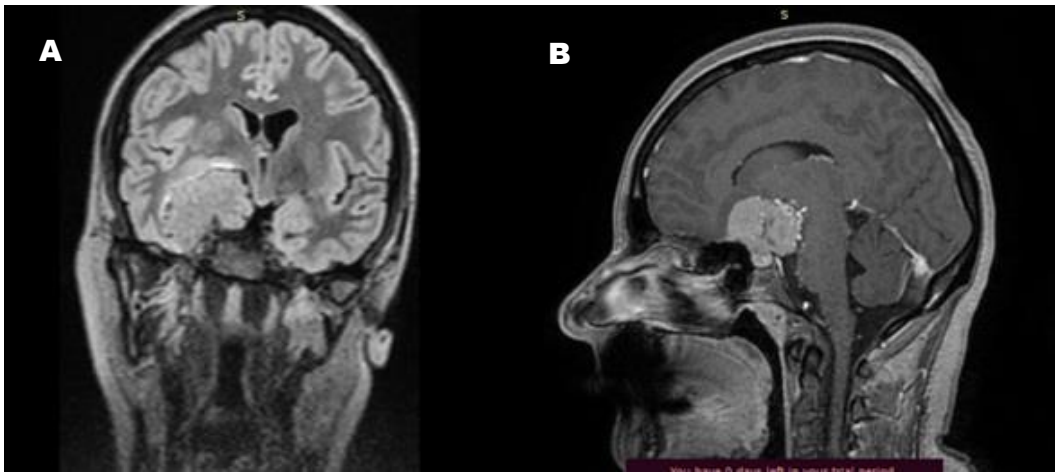

Fig. 1. Craniocerebral Magnetic Resonance Imaging (MRI) of a tuberculum sellae meningioma. (A) sagittal section.; (B) coronal section.

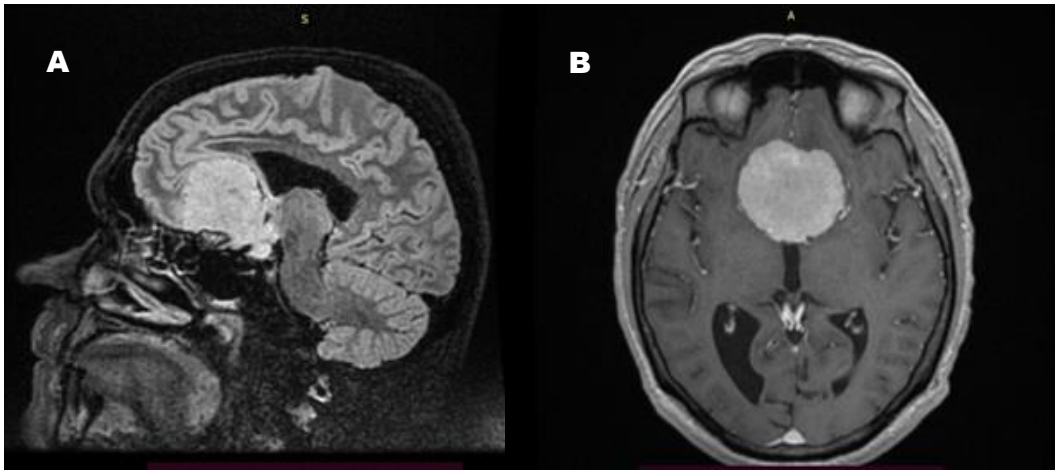

Fig. 2. Craniocerebral Magnetic Resonance Imaging (MRI) of a parasellar meningioma. (A) sagittal section.; (B) axial section.

Final histopathological analysis (tab. II) revealed a predominance of WHO grade 1 spMs (73.3%) (fig. 3), while WHO grade 2 spMs accounted for 26.7% of cases (fig. 4).

The meningothelial subtype was the most frequent (60%), followed by transitional (26.7%) (fig. 3) and secretory subtypes (6.7%). Atypical subtype represented 26.7% of all cases (fig. 4).

Dividing patients into two subgroups according to the degree of malignancy of spMs (tab. III), we found that grade 2 spM patients were younger than grade 1 spM patients (57.5 ± 18.5 years vs. 60.5 ± 12.5) but no statistical significance was found. 81.8% of cases with grade 1 spM and 75% of cases with grade 2 spM were female patients, but no statistical significance was found.

TABLE II.

Pathological characteristics of patients with sellar and parasellar meningiomas (n=15).

Variable	No. of cases	Percentage
Tumor grade (WHO, 2021)		
Grade 1	11	73.3%
Grade 2	4	26.7%
Histological subtype		
meningothelial	9	60%
transitional	4	26.7%
secretory	1	6.7%
atypical	4	26.7%

Mean percentual value of Ki-67 LI was lower (3.4 ± 1.3) in WHO grade 1 spMs (fig. 3D) and markedly increased (12.0 ± 2.2) in WHO grade 2 spMs (fig. 4D) and these data had statistical significance (p = 0.003) (tab. III, fig. 5). Mean percentual value of PR was higher (81.4 ± 14.8) in WHO grade 1 spMs (fig. 3B) and markedly decreased (42.5 ± 18.9) in WHO grade 2 spMs (fig. 4B), and these data had statistical significance (p = 0.003) (tab. III, fig. 5). Positivity of nuclear p53 was more frequently identified in WHO grade 2 sellar and parasellar meningiomas (Fig. 4C), but without statistical significance (p = 0.077) (tab. III).

TABLE III.

Comparison of demographic and surgical features, as well as immunohistochemical markers of sellar and parasellar meningiomas (n=15) according to their WHO grade.

Variable	Who Grade 1 (N = 11)	Who Grade 2 (N = 4)	p-Value
Age, years, mean ± SD	60.5 ± 12.5	57.5 ± 18.5	0.743
Age, median (IQR)	63 (56–69)	61 (52.3–66.3)	-
Female	9/11 (81.8%)	3/4 (75%)	1.000
Male	2/11 (18.2%)	1/4 (25%)	-
Ki-67, %, mean ± SD	3.4 ± 1.3	12.0 ± 2.2	0.003
Ki-67, median (IQR)	3 (3–3.5)	12.5 (11.3–13.3)	-
PR, %, mean ± SD	81.4 ± 14.8	42.5 ± 18.9	0.010
PR, median (IQR)	90 (75–90)	35 (30–47.5)	-
p53 positive	2/11 (18.2%)	3/4 (75%)	0.077
Complete resection	7/10	2/4 (50%)	0.580

We performed a logistic regression analysis for the prediction of WHO Grade 2 meningioma for the present cohort (n = 15) using tumor grade as the dependent variable (0 = Grade 1; 1 = Grade 2) and as independent variables we chose the biological variables Ki-67 LI (%), PR (%), and p53 (positive vs. negative).

Table IV shows that for every 1% increase in Ki-67 LI, the risk of diagnosing a grade 2 spM will increase by 85% and that a 1% increase in PR will decrease the risk of identifying a grade 2 spM by 8%. Regarding p53, an increased OR (≈4.5) was identified, but without statistical significance.

The model combining Ki-67, progesterone receptor expression, and p53 status demonstrated excellent discriminative ability (AUC = 1.00) for prediction of WHO grade 2 meningiomas (fig. 2), although the small sample size may lead to overestimation of performance.

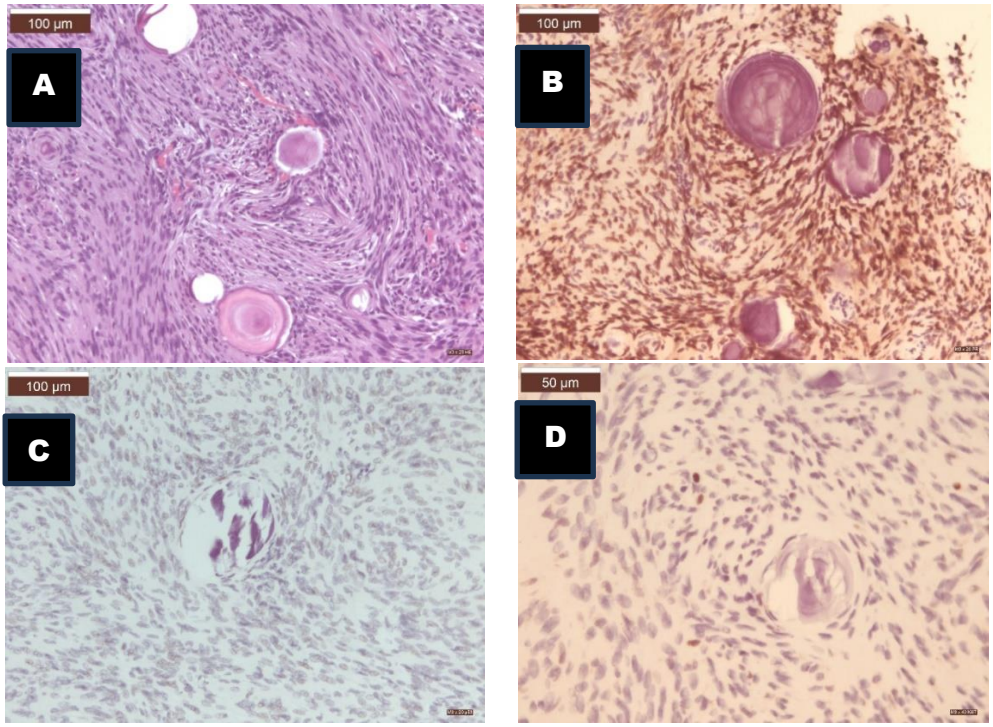

Fig. 3. Microphotographs of a grade 1 parasellar meningioma, transitional subtype. (A). Meningothelial tumor cells with fibroblastic appearance formed syncytial structures and were disposed in fascicles or whorls. The tumor cells presented indistinguishable cell membranes and bland nuclear features. Numerous psammomatous bodies were randomly scattered throughout the tumor mass. (HE, x200). (B). Almost all cells showed intense nuclear immunopositivity for Progesterone Receptor (Immunohistochemistry, x200); (C) Tumor cells did not demonstrate nuclear immunostaining for p53 (Immunohistochemistry, x200); (D). Ki67 LI exhibited low values (≤4%) (Immunohistochemistry, x400).

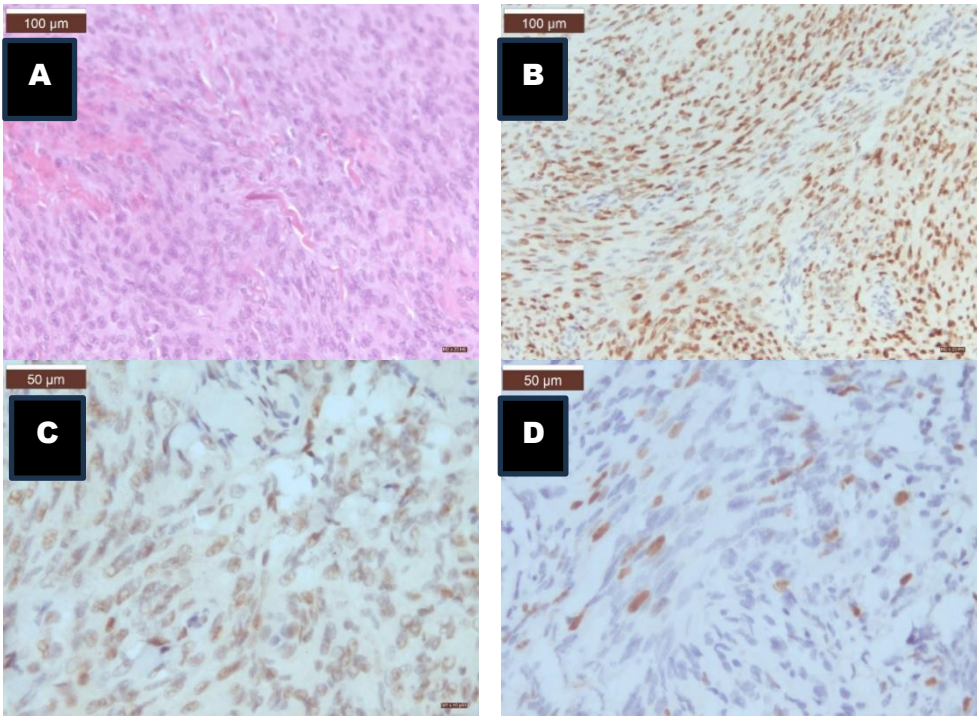

Fig. 4. WHO Grade II meningioma, atypical subtype. (A). Meningothelial tumor showing sheeting architecture, increased cellularity, and macro nucleoli (HE, x200); (B) The tumor showed nuclear immunopositivity for Progesterone Receptor varying from one area to another, with some areas having immunopositivity in 100% of the nuclei, others having it in 50% of the nuclei (Immunohistochemistry, x200); (C) Tumor cells demonstrated moderate nuclear immunostaining for p53 in almost all tumor cells (Immunohistochemistry, x400); (D). Ki67 LI exhibited higher values (Immunohistochemistry, x400).

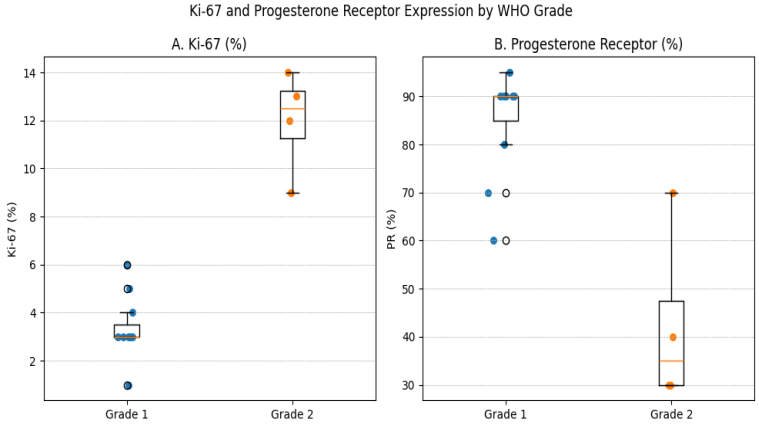

Fig. 5. Distribution of Ki-67 LI values (A) and progesterone receptor (PR) expression (B) in sellar and parasellar meningiomas according to their WHO grade.

TABLE IV.

Logistic regression analysis of immunohistochemical predictors of WHO grade 2 sellar and parasellar meningiomas in the present study

Variable	Odds Ratio (OR)	C.I. 95%	p-Value
Ki-67 (%)	1.85	1.20 – 3.90	0.01
PR (%)	0.92	0.86 – 0.98	0.02
Positive p53	4.50	0.70-35.0	0.11

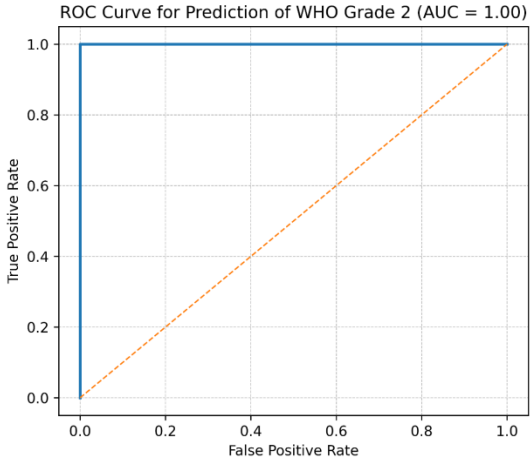

Fig. 5. Receiver operating characteristic (ROC) curve for the prediction of WHO grade 2 sella and parasellar meningiomas in the present study.

DISCUSSION

The present study provides a comprehensive clinicopathological and statistical evaluation of sellar and parasellar meningiomas. The majority of cases in our study were clustered in the sixth and seventh decades of life, which represent a decade (20) or even two decades later (21) than previous studies realized on spMs thus reflecting a particularity for the present cohort.

The majority of the patients in the present study were females, and this result was similar to the gender ratios in studies realized on meningiomas in general who found out a F: M ratio=3.8: 1 (22). But studies focusing on spMs revealed an even higher ratio. Kwancharoen *et al*. (20) highlighted the fact that spM are common in females, with F: M ratio of 6: 1.

In our study, tumor anatomical distribution highlights the predilection of spMs for development in close proximity to the optic apparatus and pituitary region, i.e. tuberculum sellae and sellar diaphragm, explaining the frequent visual and endocrinological symptoms observed in clinical practice (23, 24).

Our study also revealed a heterogeneous but anatomically tailored surgical strategy, and these data are similar to those reported by other authors (25). The fronto-temporal approach was the most used approach, and the same data was reported by some other authors in order to reduce post-surgical

morbidity (26). Our findings reflect both the technical feasibility of surgery in this region and the anatomical constraints imposed by critical neurovascular structures, particularly in parasellar tumors. SpM resection can vary, depending mostly on the tumor extension, and the pattern of growth (25, 27). In our study, a high rate of complete resection was identified and was associated with grade 1 spMs. Subtotal and partial resections were more frequently encountered in grade 2 spMs, indicating a tendency toward more complex surgical management due to infiltration of the surrounding structures.

Intracranial meningiomas, regardless of their location, are more common of the meningothelial (6) or transitional type (22, 28). Posterior fossa meningiomas are mostly of fibrous subtype (29). In the present series, which included only spMs, we identified a preponderance of the meningothelial subtype as found by other studies focused on parasellar meningiomas (17, 30).

Grade 1 spMs from the present study represented the majority of cases, followed by grade 2 spMs that represented a quarter of all analyzed cases. This proportion of higher-grade tumors is slightly elevated compared to other studies based on intracranial meningiomas in general (1, 31, 32). Thus, our results can suggest either referral bias toward complex cases or a true enrichment of biologically aggressive lesions in this anatomical region.

When comparing the subgroup of grade 1 and grade 2 spMs, we did not identify significant differences for age, sex, or extent of resection in the present cohort. Ki-67 LI showed significantly higher values in grade 2 spMs compared to grade 1 spMs, supporting the role of Ki-67 as a proliferative marker associated with histological aggressiveness. PR expression showed the opposite pattern, with higher values in grade 1 spMs and significantly reduced expression in grade 2 spMs. p53 expression was more frequent in grade 2 spMs but did not reach a statistical significance. Thus, the present study highlights the importance of integrating immunohistochemical markers into the evaluation of spMs. However, Ki-67 is a well-established marker of proliferative activity and has been consistently associated with tumor grade and recurrence (2, 33). Guresci *et al*. (34) found that the median survival of patients with meningiomas harboring Ki-67 ≤13.2% was 122 months and of those with meningioma revealing Ki67 LI > 13.2% was only 60 months ($p = 0.004$). Also, the prediction of recurrence based on Ki-67 LI had an acceptable discrimination (AUC = 0.74).

For the present cohort, Ki-67 Li was also shown to be an independent predictor for spMs. Ki-67 LI is not just a descriptive marker, but a major biological determinant of tumor aggressiveness. On the other hand, the results of the present study also showed that PR has an independent protective role for spMs, an aspect also confirmed by Aung *et al*. (35), who showed that high values of PR in meningiomas were associated with longer recurrence-free survival.

We found an inverse correlation between PR expression and malignancy grade of spMs and this fact is also clinically relevant being of interest in predicting tumor aggressiveness and guiding postoperative management. Loss of PR expression may indicate dedifferentiation and increased proliferative potential, i.e. tumor aggressiveness. Similar findings were reported in large cohorts and meta-analyses (35-38).

In the present study, although p53 was

more frequently positive in grade 2 spMs, it did not reach statistical significance in the adjusted model. Therefore, in our cohort, p53 acted as an auxiliary marker, not as a primary determinant. Also, in the literature, p53 is considered a marker of genomic instability, an aspect previously observed by some of the authors of the present study in other types of brain tumors (39), and not an independent predictor for tumor aggressiveness (2, 33). Its role is more evident in grade 3 meningiomas or recurrent tumors (40, 41).

In the present research, using logistic regression analysis, we identified Ki-67 LI and PR expression as independent predictors of WHO grade 2 spMs. The same results were obtained by Akca and Gökçe for intracranial meningiomas (37).

Our predictive model provides a practical clinical tool for estimating the probability of higher-grade spMs based on routinely available parameters.

Combining histology, immunohistochemistry, and statistical methods, these findings align with the broader trend in neuro-oncology (42) toward integrative predictive models to improve clinical decision-making.

CONCLUSIONS

The present cohort of sellar and parasellar meningiomas demonstrated a typical epidemiological and anatomical profile, combined with a relatively high proportion of grade 2 tumors, and a biologically meaningful immunohistochemical pattern. The present study demonstrates that the behavior of sellar and parasellar meningiomas is primarily driven by tumor biology rather than age, gender or anatomical location. Ki-67 Labeling Index and Progesterone Receptor expression are complementary and independent predictors of tumor grade in sellar and parasellar meningiomas. Their combined assessment provides valuable insights into tumor biology and may improve clinical decision-making. The integration of these two immunohistochemical markers into routine evaluation supports a more personalized approach to meningioma management.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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MULTIDISCIPLINARY MANAGEMENT OF COMPLEX BALLISTIC CERVICAL TRAUMA. CASE REPORT

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MULTIDISCIPLINARY MANAGEMENT OF COMPLEX BALLISTIC CERVICAL TRAUMA. CASE REPORT (Abstract): Accidental explosion of a projectile in peacetime is very rare. Ballistic trauma can be fatal or cause long-term consequences. Cervical trauma is associated with comminuted bone fracture, dilacerations of muscle fibers, vascular and nerve compression with or without complex lesions of the upper aerodigestive and respiratory tract. It is a rare situation that a projectile fragment passes through the face and neck without damaging vital structures. Considering the multiple lesional aspects, especially at the level of the cervical area where there are many vital visceral structures, a concise clinical, laboratory and multidisciplinary assessment is required to ensure the therapeutic success. We present such a case from our experience managed with a multidisciplinary team approach.
Keywords: BALLISTIC TRAUMA, FOREIGN BODY, MULTIDISCIPLINARY APPROACH.

Complex lesions of the head and neck of ballistic etiology are rarely described in the literature, generally in case reports from armed conflict zones. However, there are fewer reports of accidentally exploding shells dating from the World War II in areas outside the war zones (1). Most of the deaths occurring after ballistic cervical trauma are caused by vascular injuries in the aerodigestive and respiratory tracts, vertebral column or calvarium (2). The questions surrounding the optimal management of cervical trauma are related to exploratory surgery in emergency or to selective surgical approach after some investigations to decrease the rates of morbidity and mortality. In this paper we present the approach and the management of a patient who was admitted in the emergency department with complex craniofacial and cervical ballistic trauma.

CASE REPORT

In the present study we report the case of a 52-year-old, male patient which was brought into the Emergency Room of the

"Sf. Spiridon" County Clinical Emergency Hospital in March 2015, with complex injuries caused by an accidental explosion of a 76 millimeters projectile from World War II. At the first examination, the patient was in a serious condition, being intubated and mechanically ventilated. The clinical examination revealed right periorbital hematoma, asymmetric face, discontinuous bone contours, abnormal bone mobility of the right part of the face, right-side chin concussion wound in the 1/3 superior part, tenderness of the cutaneous and subcutaneous plans in upper posterior third of the neck, without any identified exit wound of the projectile fragment.

Initially we performed a neck X-ray (fig. 1) and a computed tomography which revealed comminuted fracture with displacement of the lateral and anterior wall of the right maxillary sinus (fig. 2), right zygomatic arch, lateral wall of right orbit, external process of the right pterygoid apophysis, and mandibular fracture of the right ascending ranch. It was also observed a metallic foreign body with a maximum diameter of 34 mm, positioned on the soft tissues from the right posterior cervical region at C1, C2, C3 level (fig. 3). The CT showed a fracture of the transverse process of the atlas and possible fracture of the right carotid channel with gas bubbles, fluid collection with maximum dimensions of 10.2/30.2 mm and a 55 mm craniocaudal extension of the head on the right side, located between posterior cervical muscles, semi spinal, and splenius. One of the right posterior veins, accompanying the splenius muscle was dilated in its lower part, and its upper part was apparently intercepted by the metallic foreign body.

Fig. 1. X-ray-Foreign bogy located in the right posterior cervical region.

Fig. 2. CT-comminuted fracture with displacement of the lateral and anterior wall of the right maxillary sinus

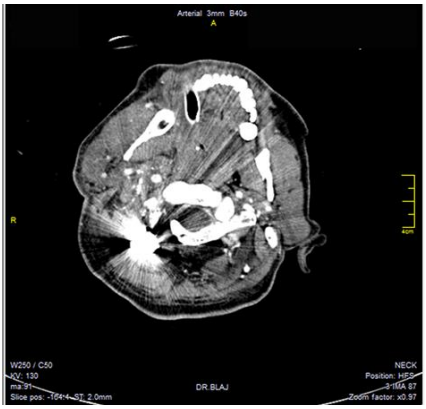

Fig. 3. CT-metallic foreign body located in the right posterior cervical region.

The patient was evaluated by a multidisciplinary surgeons' team: ENT, maxillofacial, ophthalmologist and neurosurgeon. The primary surgical approach consisted in an eschilectomy, reduction and immobilization of the orbital wall through the maxillary sinus approach and osteosynthesis of right ascending part of the mandible by the oromaxilofacial surgeon. Postoperatively, the patient showed confusional state, dysphonia and marked dysphagia. ENT evaluation and fibroscopy examination detected an adductor paralysis of the right vocal cord, salivary stasis in the right piriform sinus without swelling and hematoma of the oro- and hypopharynx. The upper gastrointestinal endoscopy performed during the gastroenterological exam excluded an intrinsic or extrinsic obstructive cause at this level. Computed tomographic reassessment highlighted-besides the presence of the foreign body and cervical collection-the presence of an 42 mm wide edema in the upper portion of right para pharyngeal space with bulging of pharyngeal wall and obliteration of the fat layer at the level of right carotid space, without perivascular extravasated during injection, right jugular vein compression in the upper portion from the exit of the skull base, deficiency filling for a distance of 45 mm, including the jugular foramen.

The second surgical phase involved an intervention performed by a mixed surgical team of ENT and neurosurgeon. The collection was located intraoperatively in the right paravertebral space C1-C4 and after aspiration of secretions was identified the foreign body with a prismatic shape of 3.0/2.0/2.5 cm, metallic nature, ash-gray color. The foreign body was extracted, and the remnant cavity was explored internally – in intimate contact with the lateral portion of C1-C4, externally-the muscle plane, anterior-prior to about 8 cm continuing with paths that dissected away the right retrostilian part of para pharyngeal space. All plans showed necrotizing fasciitis and identified a bone splinter with sharp edges about 2.0/1.5 cm, which was extracted. The surgical team performed a surgical toilet of the plans, washing with diluted Betadine solution, hemostasis, debris removal; they placed a drain in the wound cavity and realized a partial suture. Subsequently, the patient received antibiotic, analgesic, steroid anti-inflammatory, and anticoagulant therapy, anti-tetanus vaccination, nutritional supplement and topical wound bandage. The evolution was favorable permitting drain suppression, secondary suture of the wound and hospital discharge after 30 days. The 3-month follow–up of the patient showed improvement of right vocal cords mobility.

DISCUSSION

The neck is divided into three zones, by imaginary horizontal lines traced through the collarbone, cricoid cartilage, mandible angle and base of skull. These are used in the evaluation and treatment of penetrating neck injuries (3, 4). The classification system of penetrating facial trauma involves two entry areas: the face and mandible (4, 5, 6). To understand the complexity of the injuries caused by exploded projectiles, some notions of ballistic physics are necessary: external lesion, the path and terminal lesion. Once the projectile fragment penetrates the skin it will destroy and crush the tissues, the complexity of the wound being directly proportional to the input speed and with the shock wave that radiates outwards resulting in stretching, shearing and then collapse them. A small gateway can hide

massive internal injuries that are not visible at first medical evaluation (1). Cervical region involves a vital risk because of the anatomical noble elements, both visceral and neurovascular, which lack the protection of a skeleton bone (7, 8, 9).

The appearance of gunshot entrance wounds on the body depends upon many factors including, the type of firearm, the type of ammunition, the location of the wound on the body, and the circumstances of how a wound was sustained (10). The capacity of the penetrating projectile to destroy and dislocate tissue is defined by its kinetic energy, but the real tissue injury is related to the manner of energy release during the contact between the projectile and the tissue and to the physical characteristics of tissues and organs involved (resistance to tension, physical sizes of an organ, and the existence or absence of surrounding anatomical limitations (2,11). Therefore, the ratio of energy transmission to the tissues along the wound trajectory is influenced by some projectile features, as profile, structure, and stability. At the level of tissue injury can be recognized two areas: a cavity created by the passage of the projectile and the contused tissue surrounding it, created principally by transitory cavitation which is an indicator of real high-energy assignment to tissue. Consequently, the wound assessment and the type of necessary treatment must be centered on the understanding of the mechanisms contributing to tissue impairment (12, 13). The surgeon can promptly evaluate the tissue injury in a patient if he knows the type of missiles and features related to wound profile as the penetration complexity, the ammunition distortion/fragmentation, diameter of the permanent and temporary wound cavity (13).

Primary evaluation, in emergency, involves stabilization of breathing and circulatory functions, as well as injuries that may be life threatening. In most cases, medical history, physical examination, laboratory investigation, and treatment are done simultaneously. They are performed by a multidisciplinary team consisting of surgeons (ENT, maxillofacial, neurosurgery, vascular, plastic) and intensive care specialists, who will determine lesion characteristics and treatment priorities. The ideal time and treatment choice remain a continuous discussion within this subject (14, 15). In our case, the surgical extraction of the projectile fragment was not a priority because it has not started to migrate, and it was not a danger for the vital structures of the neck. Subsequently, priority was given to the maxillary and mandibular fixation. Although, the belief that projectiles are sterilized by the heat is false, and the antibiotic administration is questionable (16), in this case we administered intravenous doses of vancomycin, meropenem and ceftriaxone.

CONCLUSIONS

Our study confirms that ballistic cervical trauma is associated with high risks, requiring emergency treatment and a multidisciplinary approach for collaboratively developing a treatment plan. Particularities of this case consist in defining the mechanism of cervical trauma, accidental explosion of a projectile outside armed conflict areas and surgical therapeutic approach with a mixed team to ensure a high survival rate, optimal healing with prevention of postoperative complications and sequels.

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ROLE OF COMPUTED TOMOGRAPHY IN THE EVALUATION OF THE PATIENTS WITH HEPATITIS C-RELATED LIVER CIRRHOSIS

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ROLE OF COMPUTED TOMOGRAPHY IN THE EVALUATION OF THE PATIENT WITH HEPATITIS C-RELATED LIVER CIRRHOSIS (Abstract): Cirrhosis is an increasing cause of morbidity and mortality, one of the most common causes being hepatitis C virus (HCV) infection. Diagnosis, staging and complication assessment are equally important in patients' management, as well as establishing the right indication for antiviral treatment and further follow-up. So far, it is well recognized the role of multidetector computed tomography (MDCT) in the evaluation of focal liver lesions in the cirrhotic liver, while its capabilities of describing morphological changes of the liver and the extrahepatic response to increased portal pressure is less extensively used. The aim of this review is to underline the utility of MDCT as a useful complementary tool in the evaluation of patients with hepatitis C-related cirrhosis. **Keywords:** CIRRHOSIS, HEPATITIS C VIRUS, COMPUTED TOMOGRAPHY, HEPATOCELLULAR CARCINOMA, MORPHOLOGICAL CHANGES OF LIVER.

Cirrhosis is the final stage of chronic liver diseases. Even if there are many possible causes of chronic liver injury, the histological pathway is similar, characterized by fibrosis and change of normal liver structure into a distorted architecture consisting in regeneration nodules. Moreover, there is vascular remodeling, which results in the formation of intrahepatic shunts of the arterial and portal vessels into the central vein and impairment of the exchange between liver parenchyma and sinusoids (1).

In the more developed countries, the main causes of cirrhosis are: hepatitis C virus (HCV) infection - the leading cause of liver-related morbidity and mortality worldwide (2), alcoholic liver disease, non-alcoholic fatty liver diseases, hepatitis B virus, and others (the most common being autoimmune hepatitis, hereditary liver diseases, drug-induced liver disease) (3, 4). A recent study covering the geographic area of Europe has estimated that the prevalence of HCV ranges from 2.4% in Western and Central Europe to 2.9% for Eastern Europe (5). The risk and progression of fibrosis associated with HCV have been correlated with host factor rather than viral factor: there is no relationship between viral load and genotype and the severity of fibrosis, the human promoter polymorphism appears to correlate with fibrosis and host immune phenotype may be critical, with more rapid progression in

immunosuppressed patients (6).

In the present days, interferon (IFN)-free treatment represents the paradigm for treatment of chronic hepatitis C with high rates of sustained virologic response (SVR). Several recent and ongoing studies show that IFN-free regimens using direct-acting antiviral (DAA) combinations can cure most chronically infected HCV patients (7).

The progression of liver fibrosis is not a continuous process but rather has a non-linear course depending of various factors like sex, age, alcohol consumption and obesity. The former gold standard liver biopsy is currently considered insufficient for frequent monitoring because of its disadvantages: it is expensive, may have complications and its accuracy suffers from sampling variation. The ideal noninvasive liver fibrosis assessment should be simple, accurate, inexpensive and widely available (8). The new noninvasive methods in use today have two different but complementary approaches: the biological approach based on the quantification of biomarkers in serum samples and the physical approach based on the stiffness of liver parenchyma, a more specific marker (9). Liver stiffness is measured by using transient elastography, acoustic radiation force impulse imaging (ARFI) and magnetic resonance (MR) elastography. These noninvasive methods are widely used and have equal advantages and limitations. Serum tests are not specific to the liver, unable to discriminate between intermediate stages of cirrhosis, and the results are not immediately available. Transient elastography requires a dedicated device and region of interest cannot be chosen, has low applicability in obesity and ascites. MR elastography is time-consuming and costly, and is not applicable in iron load (9, 10).

CT has been considered insufficient as a non-invasive method of staging fibrosis in comparison with ultrasound elastography and laboratory testing, or magnetic resonance (11). Along with the advantages of being a widely used, reliable and affordable examination, nowadays there is some progress in new approach techniques that may promise the utility of CT in the detection and precise staging of fibrosis.

The main use of CT examination is the detection of hepatocellular carcinoma (HCC), the most fearsome complication, especially in patients with high serum α-fetoprotein levels, which are at the highest risk. Also, there are some concerns related to the higher incidence of HCC following IFN-free antiviral therapy, most likely a consequence of the removal of the upregulated inflammatory status as a control mechanism of the new malignant cell growth (12).

The aim of this article is to illustrate the main aspects of the pathological process as they appear on CT examination, underline the morphological transformation of the liver in volume and shape, the structural and textural changes.

Liver assessment

Morphological changes in the liver vary with the stage of fibrosis. Hepatomegaly is the early sign in more than 60% of patients (13). As the disease progresses there is an architectural change and, due to the alteration in portal blood flow, one theory suggests that the blood does not mix well and "streams" do occur (14). The right lobe receives most of its blood from the superior mesenteric vein so it is more exposed to toxins and the consequence is the atrophy

of the right lobe posterior segments (VI and VII) and medial segment of the left lobe (IV). On the other side, the left lobe receives blood from the splenic vein and is more exposed to the pancreatic hormones that promote hypertrophy, so there will be an enlargement of the lateral segments of the left lobe (II and III). Caudate lobe enlargement is due to the supply of left branches or bifurcation of portal vein, and because the flow is increased by the shorter intrahepatic course of the branches (15).

The typical appearance of the cirrhotic liver on CT is transversal enlargement of lateral segments and atrophy of the medial segment of the left lobe and atrophy of the posterior segments of right lobe and hypertrophy of the caudate lobe (fig. 1).

An important consequence of this liver remodeling is the presence of right hepatic notch sign defined as a sharp indentation developed between the caudate and right lobe of the liver (fig. 2). The presence of hepatic notch is a qualitative method for assessing the hypertrophy of the caudate lobe and atrophy of the right lobe (16).

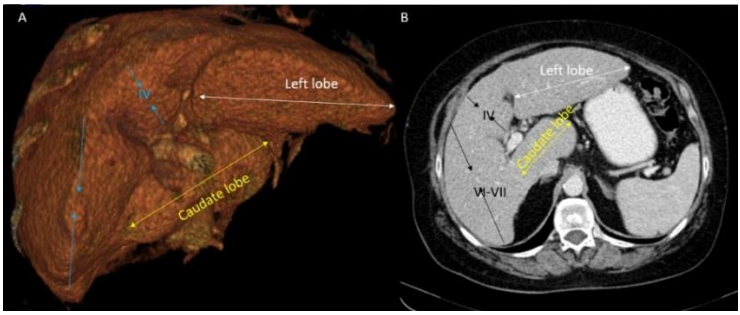

Fig. 1. Volume rendering image (A) and axial section (B). Typical appearance of the cirrhotic liver: enlargement of the lateral segment of the left lobe and atrophy of the medial segment with classical "tongue-like" appearance of the left lobe, enlargement of the caudate lobe and shrinking of the posterior segments of the right lobe.

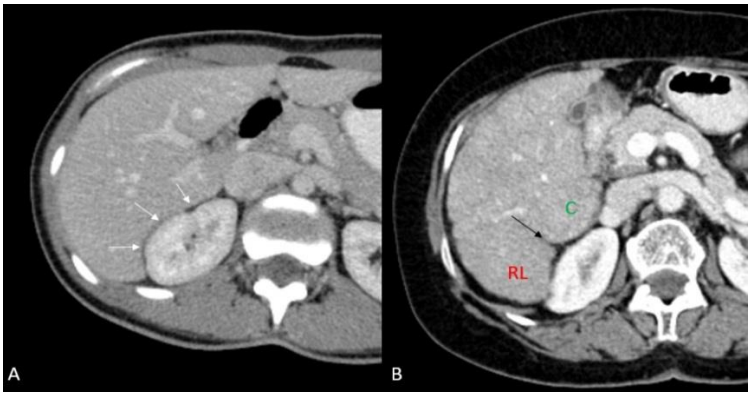

Fig. 2. (A) The posterior - inferior surface of the right hepatic lobe at the level of the right kidney is normally concave owing to the renal impression (white arrows) (B) In patients with cirrhosis a sharp indentation develops between the caudate and the right lobe of the liver.

Other morphological changes seen in cirrhosis are: blunt liver margins, widening of the periportal space, expansion of the pericolecystic space and enlargement of the interlobar fissure (17).

Widening of the periportal space is a consequence of segment IV atrophy. Although the exact mechanism remains unclear, it is believed to be related to portal vein hypoperfusion. An enlarged hilar periportal space, defined as a distance between the right portal vein and the posterior edge of segment IV greater than 10 mm, may be the only morphological sign in earlier stages of cirrhosis.

In normal liver, the gallbladder fossa is delimited laterally by the right lobe and medially by segment IV, and contains little fat. In cirrhotic liver, the gallbladder fossa can be enlarged. The expanded gallbladder fossa in cirrhosis may be the consequence of four factors: atrophy of the medial segment of the left hepatic lobe and hypertrophy of the caudate lobe, atrophy of the anterior segment of the right hepatic lobe with counterclockwise rotation of the major interlobar fissure and enlargement in the cephalocaudal direction of the left hepatic lobe (13, 18).

Hypertrophy of the caudate lobe and atrophy of the right lobe were at the basis of the attempts to stage fibrosis. There are few studies investigating the accuracy of CT findings in liver fibrosis: In 1980 Harbin *et al.* (19) developed a cirrhosis score using the ratio of the width of the transverse caudate lobe to the width of the transverse right lobe, a ratio > 0.65 is 96% and > 0.73 is 99% likely to be cirrhotic (fig. 3). Because the hypertrophied caudate lobe extended beyond the main portal vein bifurcation, in 2002 Awaya *et al.* (15) used a modified version of this ratio with much more sensitivity using the bifurcation of the right portal vein as a landmark.

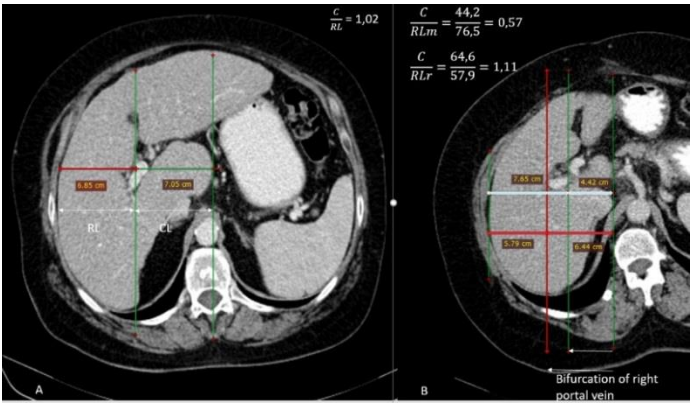

Fig. 3. (A) Harbin method image: axial slice immediately below the bifurcation of the main portal vein line 1: parasagittal line drawn through the right lateral border of the portal vein, line 2: parasagittal line drawn through the left lateral border of the caudate lobe, line 3: line orthogonal to lines 1 and 2 midways between the portal vein and the IVC extended to the right liver edge. RL measurement: along line 3, from right liver edge to line 1. C measurement: along line 3, between line 1 and line 2. Caudate-right lobe ratio: C/RL. (B) Hypertrophied caudate lobe extended beyond the main portal vein bifurcation and modified C/RL uses the bifurcation of the right portal vein as a landmark. A ratio greater than 0.55 suggested a diagnosis of cirrhosis.

The latest attempt was in 2014, when Huber *et al.* (20) used the sum diameters of the hepatic vein divided by modified caudate - right lobe ratio with a sensitivity of 83% and a specificity of 76% for precirrhotic liver fibrosis, and a sensitivity of 88% and a specificity of 82% for cirrhotic liver fibrosis (fig. 4).

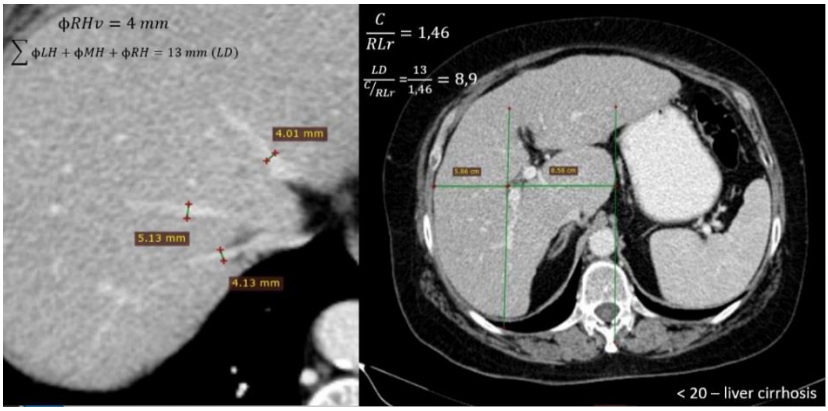

Fig. 4. Patient with right hepatic vein smaller than 9 mm, the ratio of the sum of hepatic vein divided by the modified caudate-right hepatic lobe diameter is 8.9, and is suggestive for cirrhosis.

Contour and texture changes

Diffuse fibrosis is the main cause of heterogeneity of the liver. It is better seen on unenhanced CT and may have four different patterns of distribution: poorly defined region of low attenuation, thin perilobular bands of low attenuation, thick bridging bands of low attenuation, and diffuse fibrosis causing perilobular cuffing (21) (fig. 5).

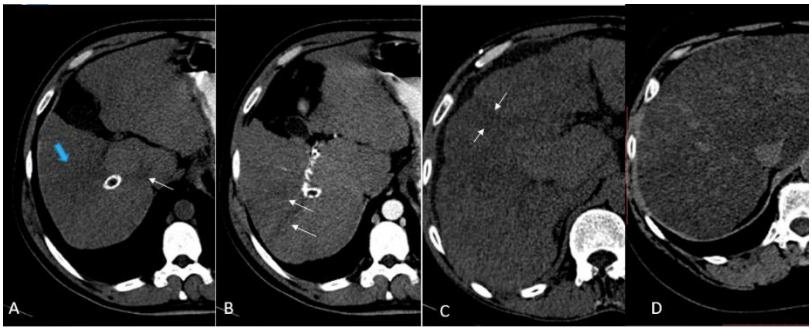

Fig. 5. Different patterns of diffuse fibrosis are seen on CT: (A) Patchy, poorly defined region of low attenuation, (B) Thin perilobular bands of low attenuation, (C) Thick bridging bands of low attenuation (D) Diffuse fibrosis with perilobular cuffing.

Surface nodularity is a direct consequence of the presence of regenerative nodule. The liver contour may be smooth, nodular, fine to coarse if they are < 3 cm,

or grossly lobular if they are more the 3-cm diameter (22) (fig. 6). This direct relationship between hepatic distortion and the surface embossing may predict the underlying hepatic fibrosis.

Recent studies tried to use liver surface nodularity as a biomarker to allow CT examination alone to accurately discriminate between stages of liver fibrosis comparable to elastography (23, 24). Moreover, a multiparametric analysis is possible in a near future, using a combination between the liver surface nodularity quantification and other CT biomarkers that are already available, like the volumetric and textural features (24).

The diagnosis of liver cirrhosis based on selected independent variables, such as irregular and nodular surface, parenchymal abnormalities, morphological changes, and manifestation of portal hypertension has in one study by Kudo et al (25) an accuracy of 71.9%, sensitivity of 77.1% and specificity of 67.5%.

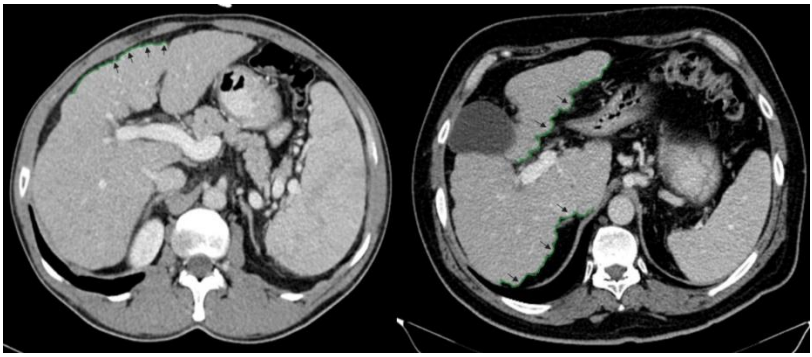

Fig. 6. Fine nodularity of liver contour, (left image) "cobblestone" margin (right image).

Regenerative nodules, dysplastic nodules, and hepatocellular carcinoma

Regenerative nodules (RNs) are defined as an area of liver parenchyma enlarged in response to necrosis and altered circulation or other stimuli, and are surrounded by fibrous septae. According to size, RNs are micronodules if they have less than 3 mm, or macro nodules if they are equal or more than 3 mm. If greater than 15 mm they may harbor dysplastic or malignant foci (26). RNs are present in all cirrhotic livers, but they are revealed in only 25% of unenhanced CT scans. Nodules are difficult to detect on CT because they are too small and like the surrounding tissue (fig. 7). They are detectable when surrounded by hypodense fibrotic bands or when they accumulate iron (siderotic nodules) (21).

A dysplastic nodule (DN) is defined as a nodular region of dysplastic hepatocytes of at least 1 mm in diameter without histologic features of malignancy. DNs are considered an intermediate stage in the progression to hepatocellular carcinoma. They are classified according to histological atypia into low-grade dysplastic nodules, which are composed of hepatocytes that are minimally abnormal, and high-grade dysplastic nodules which have any of the features of low-grade nodules but in addition have one or more of the following: high nuclear-cytoplasmic ratio, irregular nuclear contour, nuclear hyperchromatic, rare mitotic figures, pseudo-gland formation, cytoplasmic basophilia, and resistance to iron accumulation (27).

The prevalence of DNs in patients with cirrhosis ranges from 14% to 37%, but they are not easily distinguishable from the regenerative nodules in the surrounding liver (28). Most of DNs are undetectable by CT. Occasionally there may be a slightly hyperattenuating nodule on non-enhanced scan but without postcontrast enhancement. Tumor angiogenesis seems to be a required step in the progression from regenerative nodule to dysplastic nodule (low-grade to high-grade) and, finally to HCC. During this process, there is a conversion of the vascular supply with an increase in the arterial and decrease in the portal supply. The less differentiated nodules enhance more on postcontrast in early arterial phase and may mimic HCC on CT scan.

HCV promotes carcinogenesis having a direct and indirect role, especially due to fibrosis. In this situation, the chromosomal alteration that occurs in fibrotic tissue is associated with tumor genesis. Another way to promote carcinogenesis is through chronic inflammation of the liver which has been associated with a shift in signaling from tumor suppression to fibrosis and carcinogenesis (29).

HCC is a malignant neoplasm composed of cells with hepatocellular differentiation and is histologically classified as trabecular, pseudo glandular, compact and scirrhous. The most common is the trabecular type.

The classical CT features of the HCC are a solitary nodule on non-enhanced scan, often inhomogeneous due to necrosis. Almost all HCCs are highly vascular lesions and exhibit intense contrast enhancement during the arterial phase. Due to the alteration in vascular supply with an increased arterial and decreased portal blood supply, they exhibit washout in the portal phase. Another important CT finding is the presence of the fibrous capsule which is usually enhanced on delayed equilibrium phase images.

Another less common appearance is as an infiltrating mass, a multicentric lesion (mass with daughter lesions) or multifocal lesion (may mimic metastasis).

Liver Imaging Reporting and Data System (LI-RADS) was created by the American College of Radiology and is both a set of standardized terminologies and a classification system for imaging findings in liver lesions. The classification system is meant to be used in livers which have risks factor for HCC. The most suitable are the patients with liver cirrhosis, especially those with HCV infection who are at the greatest risk.

The latest version in use is ver. 2014 applicable to CT and MR and for contrast enhanced ultrasound (CEUS LI-RADS). The advantages of the method are the decrease in error of interpretation by applying the consistent terminology and enabling the communication with the clinician and increased therapeutic performance.

The "observation" is the preferred term for the CT findings which is described using major criteria and ancillary features. Major criteria are: hepatic phase enhancement, washout, capsule, or pseudo capsule and threshold growth. For each of these we have a binary categorization: present or not present. In the dubitative situation, the ancillary features mainly on MR evaluation which may favor malignancy or benignity are used. If assignment is unclear, ancillary findings may be used as "tie-breaker". The LI-RADS scores range from L-1 which favor benignity to L-5 which favor malignancy (30, 31).

The first two categories are LR-1 benign

and LR-2 probably benign. Such lesions as cysts, hemangioma, vascular anomaly, perfusion alteration, hypertrophic pseudo mass, confluent hepatic fibrosis, focal scar, and lesions which disappear at follow-up without treatment are LR-1. Lesions which have an atypical appearance of the benign entities and cirrhosis-associated nodules are categorized as LR -2.

Observations that are neither definitely or probably benign are categorized based on their diameter and the following features: washout, capsule, or threshold growth in one of the category.

The intermediate probability for HCC is assigned as LR-3 observation, which has a moderate probability of both benign entity and HCC (fig. 8).

LR-4 category is assigned when the imaging features are suggestive, but not diagnostic, of HCC (fig. 9).

LR-5 category is assigned only when there is 100% certainty that the observation is HCC or is proven to be HCC at histology. LR-5V category is assigned when there is definite enhancing soft tissue in a vein irrespective of the presence or absence of visible intraparenchymal HCC. Finally, LR-M is a special category assigned to observations that are probably malignant but not specific for HCC, like metastasis, lymphoma, cholangiocarcinoma (fig. 10).

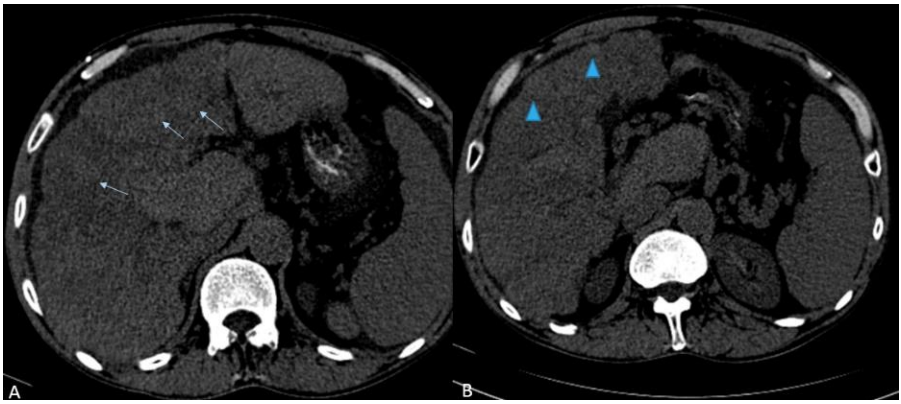

Fig. 7. (A) Regenerative nodules (arrow) surrounded by lower attenuation fibrosis, (B) High-attenuation nodule (arrowhead).

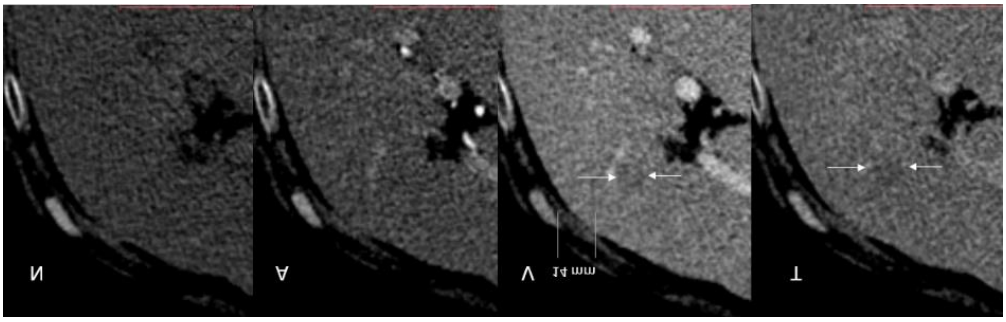

Fig. 8. LR-3 observation. Liver nodule (< 20 mm) isoattenuation to the surrounding liver in arterial phase, and hypoattenuating in the portal and equilibrium phase (washout).

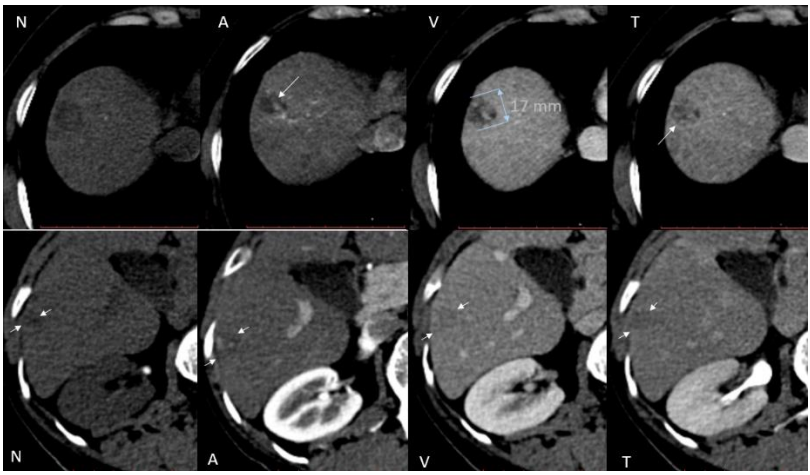

Fig. 9. LR-4 observation. First row: 17-mm nodule with partial hyperenhancement in arterial phase and capsule in delayed phase;
Second row: nodule with partial hyperenhancement in arterial phase and hypo enhancement in venous and delayed phase (washout) but without capsule in delayed phase.

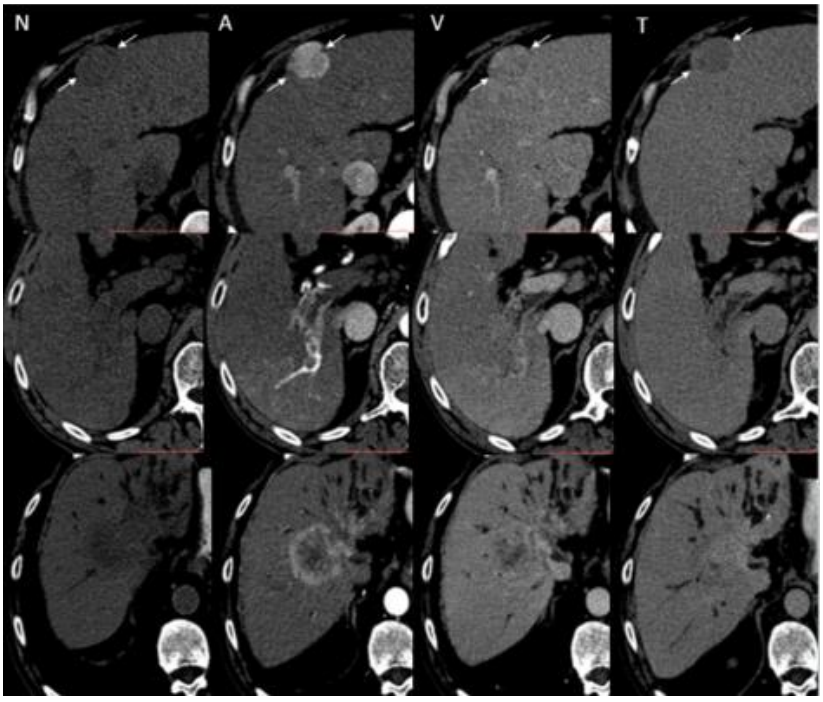

Fig. 10. LR-5 observation (first row) - a large subcapsular mass with arterial hyperenhancement, washout and capsule in delayed phase. LR-5V (second row) - enhanced tumor tissue into right portal vein. LR-M (bottom row) - mass with dynamic enhancement suggestive for cholangiocarcinoma. Note the dilated bile ducts in the left liver lobe.

Repercussion on the extrahepatic abdominal organs in cirrhosis

Extrahepatic abnormalities associated with cirrhosis are mainly a consequence of portal hypertension defined as a pressure greater than 5-10 mm Hg. As the pressure in the portal system increases due to the distortion of the hepatic vasculature and fibrosis, the blood flow is shunted away from the liver to the low pressure systemic vessels and the portosystemic collaterals develop.

The main abdominal manifestations are dilated portal vein, varices, splenomegaly, ascites and bowel wall and mesenteric edema.

Varices appear as a serpentine or tubular structure that enhance after contrast administration. They are located most frequently in the wall of the esophagus and appear as s nodular intraluminal protrusion. Gastroesophageal bleeding is the most common clinical presentation. Another frequent location is in lesser omentum from the left gastric collaterals present in 80% of the patients. Varices can occur on the collateral paraumbilical vessel which communicates with the vessel located in the subcutaneous abdominal wall and reassembling a radial distribution called *caput medusae*. Portosystemic shunts commonly involve the gastrorenal and splenorenal systems (fig. 11). Nonmalignant portal vein thrombosis may occur in cirrhotic patients and is correlated with the severity of liver disease (32).

Splenomegaly is a result of portal congestion, tissue hyperplasia, and fibrosis. There is a vicious circle: the enlarged spleen requires an increased blood flow which perpetuates the underlying portal hypertension (33).

The exact pathophysiologic mechanism leading to edema of the gallbladder wall is due to a combination of factors: elevated portal venous pressure, elevated systemic venous pressure, and decreased intravascular osmotic pressure. In cirrhotic patients, the gallbladder wall is often thickened measuring more than 5 mm and hypodense (fig. 11).

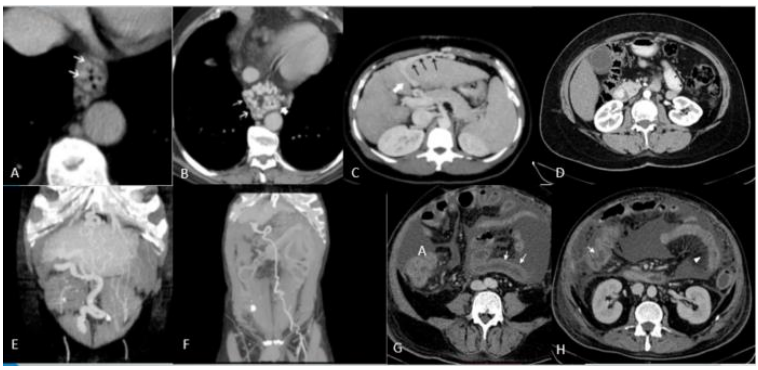

Fig. 11. CT appearances of esophageal varices. Intraluminal protrusion with scalloped margins (arrows) (A). Paraoesophageal varices (white double arrows) appear as well defined, round, tubular, or serpentine structures situated outside the wall of the esophagus in the mediastinum (B). Dilated paraumbilical veins (C) anterior to the left hepatic lobe (black arrows) arising from the left portal vein (white arrow). Abdominal wall varices radiating from the umbilicus. Connection with inferior epigastric veins (F) and multiple vessels forming *caput medusae* (E). Thickened gallbladder wall (D). Small bowel wall thickening in a patient with hepatic cirrhosis. CT images show diffuse thickening of the small bowel and colon wall and folds (arrows). Ascites (G) and mesenteric edema (arrowheads) are noted (H).

Ascites develops when the portal venous pressure exceeds 12 mmHg and is due to increased intravascular hydrostatic pressure and decrease in oncotic pressure. Ascites is visible as a large fluid accumulation into the peritoneal cavity with central displacement of the small bowel and mesentery.

Gastrointestinal wall thickening is mainly caused by edema and has a multisegmental distribution. The jejunum and ascending colon are most often involved and the haustral thickening of the colon is more common than wall thickening. The involvement of other digestive segments such as the duodenum, jejunum, or transverse colon without involvement of the jejunum or ascending colon may have a non-cirrhotic cause like inflammatory, ischemic, and neoplastic disease. Such CT findings should determine the search for another coexisting cause of thickening (34).

Diffuse intra- and retroperitoneal edema may have multiple causes but the most obvious mechanism is portal hypertension which causes increased hydrostatic pressure in the mesenteric vessel and extravasation of the fluid into the mesentery. Often, mesenteric edema is mild and focal and it becomes more diffuse as its severity increases. Presence of omental and retroperitoneal edema are signs of severity and correlates with other findings like severe ascites, subcutaneous edema or pleural effusion (35).

CONCLUSIONS

In our opinion, there is a need for an imaging tool able to monitor the cirrhotic patient, especially after antiviral treatment, and CT might be this tool. CT has been considered inferior to other methods (such as Fibroscan®, serum test and MR) for the noninvasive diagnosis of liver fibrosis, but is fast, widely accessible, and affordable and now there are perspectives in new applications available in the last years, such as contour or texture analysis, and perhaps a combined analysis will provide a more accurate staging of fibrosis.

Moreover, besides the reasonable assessment of fibrosis, CT provides a complete assessment of the liver including the architectural and structural changes such as the vascular and biliary tract changes, and focal and diffuse lesion assessment. For the patients with sustained virologic response it is necessary to redefine a new semiology related to liver remodeling due to regression of fibrosis.

In patients responsive to antiviral therapy, there is a need for more elaborated techniques for the prediction and detection of hepatocellular carcinoma, especially in the context of liver remodeling.

Taking into consideration these advantages, we strongly believe that CT examination provides valuable insights into the extent of the hepatic injuries of cirrhosis and extrahepatic abdominal complication due to portal hypertension and should be used.

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NEWS

OBSTETRIC AND PERINATAL OUTCOMES OF SINGLETONS AFTER SINGLE BLASTOCYST TRANSFER: IS THERE ANY DIFFERENCE ACCORDING TO BLASTOCYST MORPHOLOGY?

A strong correlation between blastocyst morphology and morpho kinetics parameters and implantation has been shown by recent studies. The consequences and effects of assisted reproductive techniques on children's short and long-term health have major impact on the subject and focus on reproduction. The obstetric and perinatal outcome of singletons according to blastocyst morphology has rarely been evaluated. The aim of this observational study is to determine whether a relationship exists between blastocyst morphology and obstetric and perinatal outcomes. A total of 799 singleton clinical pregnancies were analyzed after transfer of a single fresh blastocyst - (day 5) between 2006 and 2013. Blastocysts were divided into four groups based on their morphology on day 5: group 1 = good morphology blastocysts; group 2 = fair morphology blastocysts; group 3 = poor morphology blastocysts and group 4 = early (B1/B2) blastocysts. Obstetric and perinatal outcomes were compared between the four groups of this study. Some confounding variables were adjusted but main obstetric and perinatal outcomes after transfer of blastocysts with poor morphological characteristics were not associated with increased adverse obstetric and perinatal events. Sex ratio was significantly higher in group 1 compared with groups 2, 3 and 4, and in Group 2 compared with Group 3 ($P < 0.001$) even after adjustment ($P < 0.05$) (Céline Bouillon, Noémie Celton1, Sandra Kassem, Cynthia Frapsauce, Fabrice Guérif. Obstetric and perinatal outcomes of singletons after single blastocyst transfer: is there any difference according to blastocyst morphology? *Reprod BioMed Online* 2017; 35(2): 197-207.

Emil Anton

PATHOPHYSIOLOGICAL ISSUES INVOLVED IN THE EARLY ONSET OF OSTEOPOROSIS ASSOCIATED WITH RHEUMATOID ARTHRITIS

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PATHOPHYSIOLOGICAL ISSUES INVOLVED IN THE EARLY ONSET OF OSTEOPOROSIS ASSOCIATED WITH RHEUMATOID ARTHRITIS (Abstract). Osteoporosis is a major global health problem, as it is one of the leading causes of disability due to the complications associated with fractures. The mechanisms involved in the onset of osteoporosis are complex; there is significant interference with the immune system, in most cases osteoporosis accompanying the autoimmune joint diseases. Rheumatoid arthritis is the most common systemic articular inflammatory disease, characterized by articular cartilage and periarticular bone damage. Moreover, patients suffering from rheumatoid arthritis associate a decrease in bone mineral density in the axial and peripheral skeleton, posing a high risk of fractures. The discovery of the RANKL/RANK/OPG system led to the elucidation of the pathophysiological mechanisms of metabolic bone diseases by clarifying the communication mechanisms operating between osteoblast and osteoclast. This system led to a better understanding of the molecular processes involved in osteoclastogenesis and thus increased the possibility to intervene in osteoclast formation and to limit exaggerated bone resorption in such metabolic bone diseases as osteoporosis, rheumatoid arthritis, arthrosis, or Paget's disease. **Keywords**: OSTEOPOROSIS, RHEUMATOID ARTHRITIS, BONE RESORPTION, OSTEOCLAST.

Over the last decades, osteoporosis has become one of the major global health problems due to the constant increase in life expectancy, entailing vast, even alarming, health care expenses, with a negative impact on government budgets. It is currently believed that more than 50% of women over age 50 and 13% of men over age 70 will suffer from an osteoporosis-related fracture during their lifetime. It is necessary to improve osteoporosis diagnosis strategies, diagnosis and treatment guidelines, and to extend the scope of evaluation aimed at discovering the disease from its very onset in patients with risk factors, to optimize the management of patients with this condition (1).

BONE MATRIX FORMATION AND REMODELLING

The bone is subject to a continuous remodeling process. Thus, approximately 5-

25% of bone surface is permanently active (1). A bone remodeling cycle is achieved by an active basic multicellular unit (BMU) and has several phases: activation, resorption, reversal and formation. The mechanical or structural destruction stimuli can stimulate osteocytes and thus initiate their apoptosis. Osteocyte biology could also be altered by systemic changes in hormone homeostasis or by corticosteroid action on bone cells. Hauge *et al* (2) highlighted the fact that bone surface lining cells receive signals from the osteocytes and thus influence the microenvironment in order to determine the bone remodeling nucleus. Usually, osteocytes secrete a series of factors, among which TGF-β, which inhibit osteoclast formation. Osteocyte apoptosis stimulation leads to a decrease in the secretion of these factors and, implicitly, could contribute to attracting osteoclast precursor cells. Aguirre *et al* showed that, after a three-day period of immobilization, osteocyte apoptosis preceded the formation of osteoclasts and was followed by approximately two weeks of osteoclastogenesis (3). Li *et al* (4) demonstrated that the osteoblast also plays an important role in the recruitment of osteoclast precursors by secreting more products, the most important of which are MCP-1 (monocyte chemoattractant protein-1) and RANKL (receptor activator of nuclear factor kappa-B ligand). At the level of the active surface, these precursors are transformed into mature osteoclasts, having a role in bone resorption. Osteoclasts are giant polykarion cells, which develop from the line of monocyte/macrophage hematopoietic cells. To this day, they are the only cells capable of destroying the mineralized bone, the dentin and the calcified cartilage (5). After attracting and activating mononuclear precursors, another important step in the formation of mature osteoclasts is their expression of M-CSF receptor c-fms, and its activation with the ligand. Thus, proliferation is induced and the next phase commences, i.e. the expression of the receptor activator of nuclear factor kappa B (RANK). The presence of the ligand for RANK, RANKL is essential to ensure the formation and fusion of multinucleated cells. RANK signaling determines the activation of transcription factors with a major role in osteoclastogenesis. These include protein-1, NF-kB or NFAT (nuclear factor of activated T-cells). Several proteins, including cathepsin K, calcitonin receptor (CTR), TRAP (tartrate-resistant acid phosphatase) and integrin receptors are expressed on the surface of mature osteoclasts (5). To maintain the acidity of the microenvironment necessary for bone resorption, the osteoclasts are attached very tightly to the matrix by means of integrins. The degradation of the organic matrix is achieved by many enzymes, including cathepsin K, TRAP and matrix metalloproteinases MMP-9.

THE RANKL/RANK/OPG SYSTEM

The discovery of the RANKL/RANK/OPG system led to the elucidation of the pathophysiological mechanisms involved in metabolic bone diseases by clarifying the communication mechanisms between osteoblast and osteoclast. It also contributed to the development of innovative therapies for osteoporosis, such as human antibody RANKL (6). By clarifying the molecular processes of osteoclastogenesis, the discovery of RANKL and of its receptors, RANK and OPG (osteoprotegerin), increased the possibility to intervene in the formation of osteoclasts and in limiting excessive bone resorption in such metabol-

ic disorders as osteoporosis, rheumatoid arthritis, arthrosis or Paget's disease (1). The first discovered protein belonging to this system was OPG, so called due to its bone-protective effects, and its ability to inhibit osteoclast development and activation. To date, this is its most important role. Osteoprotegerin is part of the TNF receptor superfamily; it is expressed both at bone-level, as well as in many tissues such as kidney, stomach, heart, lung, brain, bowel, thyroid gland, or spinal cord. Following these discoveries, some researchers (1) attributed an important role to this protein in endothelial cell survival. Moreover, in 2006, Rasmussen discovered the involvement of OPG in vascular calcification.

The next protein belonging to this system was represented by RANKL, eventually named after the initials of the four researchers who identified it. It is a protein from the TNF superfamily and is expressed in many tissues, essentially in the bone (preosteoblast/stromal cells, but also in activated T cells), kidney, lung, heart, brain, liver, skeletal muscle, bowel, placenta, mammary tissue, thymus, spleen and testicles. Even though the main role of RANKL is linked to bone remodeling, its extended distribution throughout the body indicates its involvement in many other functions. RANKL is present in tissues in three isoforms. The first two, RANKL 1 and RANKL 2, are type II transmembrane proteins, while the third, RANKL3, is a soluble protein. It is assumed that RANKL3 is sectioned from the transmembrane form by TACE (metalloproteinase, enzyme that converts TNFα). The third member of this system is RANK, a protein which, like OPG, belongs to the TNF receptor superfamily. RANK is a type I transmembrane protein. It is most commonly encountered in osteoclasts and dendritic cells, but it is also expressed in all other tissues in which RANKL can be found as well (7). Mice that lack the RANK protein have similar phenotypes to those that cannot express RANKL, the latter presenting osteopetrosis and lacking dental eruption and lymph nodes. RANKL binds to the extracellular domain of RANK and, with the help of TRAF6 (TNF receptor associated with factor 6), can induce the RANK signaling pathway.

Furthermore, Rauner *et al* (8) showed that there are many mediators that can lower TRAF levels, the most important of which are A AP-1, NF-Kb, NFATc1 (nuclear factor of activated T-cells), but also the mitogen-activated protein kinase pathway (MAPK), including JNK (c-Jun N-terminal kinase), p38 stress kinase, and ERK (extracellular signal–regulated kinase). Leibbrandt and Penninger (9) described a series of local and systemic factors that influence the expression of OPG and RANKL. Among such factors that stimulate the expression of RANKL, they describe the parathyroid hormone, glucocorticoids, 1,25(OH) vitamin D, IL-1, 6, 8 and prostaglandin E. The category of factors involved in stimulating the expression of OPG includes estrogens, INF-γ, bone morphogenetic protein 2 and TGF-β.

Both in physiological as well as in pathological conditions, many cytokines and chemokines are involved in bone remodeling. Lorenzo *et al* (10) described, on the one hand, pro-inflammatory cytokines, that stimulate the expression of RANKL on the surface of the osteoblast, or stimulate the formation and activation of osteoclasts and, thus, increase the loss of bone mass. On the other hand, they described cyto-

kines acting as inhibitors of osteoclastogenesis by blocking RANKL signaling, both directly and indirectly, thus reducing bone resorption. The proresorbtive cytokines are IL-1, 6, 7, 15 and 17, while the antiresorptive ones are IL-4, 5, 10, 12, 13, 18; however, interferon α, β and γ plays the same role.

Unlike cytokines, little is known nowadays about the role played by chemokines in regulating bone mass. Binder *et al* (11) showed that C-C chemokine receptor type 2 (CCR2) plays an important role in maintaining bone mass under physiological and pathological conditions. They demonstrated that CCR2 induces the expression of RANK on osteoclast precursor cells, thus increasing the susceptibility to RANKL-induced osteoclastogenesis.

The impact of inflammatory diseases on bone metabolism has been studied extensively, and a significant damage of osteoblast and osteoclast coupling has been noticed. Cytokines, including TNFα, are released in both local and systemic inflammation, which could lead to bone loss by the direct activation of osteoclast differentiation and activity.

Yoshitake *et al* (12) showed the important role played by IL-6 in osteoclast differentiation. It increases the expression of RANKL on osteoblasts and determines the suppression of the degradation of the kB factor and the activation of JNK, determining the direct stimulation of osteoclast differentiation by activating the RANK/RANKL/OPG pathway.

BONE LOSS IN RHEUMATOID ARTHRITIS

Both juxta articular osteoporosis and generalized osteoporosis can be found in rheumatoid arthritis, these patients being at high risk for fracture. It appears that periarticular osteoporosis is predominantly mediated by local mechanisms, related to those of rheumatoid synovitis. Identifying juxta articular osteoporosis is one of the earliest radiological signs in rheumatoid arthritis. Generalized osteoporosis is also detected in rheumatoid arthritis, with a high enough frequency, its etiology being considered multifactorial. T cell involvement, resulting in rheumatoid nodule formation, as well as B cell activation with autoantibody production, is encountered in this disease as well. The microscopic examination of the synovial tissue of inflamed joints highlighted a non-specific inflammatory infiltrate, containing T cells, B cells, neutrophil, macrophage, and mast cells. Raza *et al* (13) showed that inflammatory response is directed by a complex network of cytokines. The main role is held by the macrophages secreting proinflammatory cytokines in the inflamed synovial joints. As the disease progresses, the cellular basis of inflammatory response changes. In the early stages of the disease, the key role seems to be held by T cells.

Osteoporosis, both systemic and local, through periarticular bone loss adjacent to inflamed joints, is very common in patients with rheumatoid arthritis. These changes were due to an increased level of proinflammatory cytokines at local and circulatory levels. The activation of $CD4^+$ cells has been shown to occur in the inflamed synovium, the predominant immune cell population at this level, the stimulation of RANKL production supporting osteoclastogenesis thus taking place (14). In addition, the *in vivo* systemic activation of T cells has been shown to result in RANKL-mediated osteoclast differentiation and bone loss.

Kong *et al* (15) demonstrated that the administration of exogenous OPG in rats with adjuvant arthritis neutralizes RANKL and prevents bone loss and cartilage destruction. An increase in RANKL was noticed in patients with rheumatoid arthritis, which was associated with increased osteoclastogenesis. OPG levels are low in the synovial fluid of patients with rheumatoid arthritis, this being valid only for inflamed joints. These low OPG levels could limit the possibility to counterbalance the RANKL effects of stimulating osteoclastogenesis in join inflammation. Moreover, the same authors also discovered that the *in vitro* administration of exogenous OPG could inhibit IL-17, a proinflammatory cytokine with significant effects in stimulating osteoclastogenesis in rheumatoid arthritis. This could be a useful therapeutic option in severe forms of rheumatoid arthritis.

It appears that the loss of juxta-articular bone mass is based on a significant reduction of the cancellous bone at the level of the bone metaphysis adjacent to the inflamed joints. The bone geodes appearing alongside the inflamed joints are also part of the arthritic process of the disease.

The pathophysiological mechanisms involved in systemic osteoporosis in rheumatoid arthritis are multifactorial. The factors described to be involved in this process are: the systemic effect of the inflammation, drugs (such as corticosteroid therapy), DMARDs therapy, immobility, nutritional deficiencies, endocrine changes in menopause, weight loss. The activity of the diseases contributes independently to axial bone loss. This can be noticed especially at the level of the proximal femur. About the involvement of inflammatory cytokines, a significant increase in IL-1 and IL-6 serum levels has been noticed, many studies demonstrating that these cytokines determine the suppression of bone formation and accelerate bone resorption. The involvement of immobilization in bone loss has been demonstrated by many studies, showing a close link between functional capacity and axial BMD, the effects being more significant in the femur than in the vertebrae.

Approximately 25% of patients with rheumatoid arthritis suffer from osteopenic low bone mineral density, both at spine and hip-level, before the onset of the disease, and before initiating therapy, while 10% of such patients suffer from osteoporosis. Given that the prevalence of low bone mass is already present at the onset of the disease, this led to the conclusion that patients with rheumatoid arthritis have a high risk of developing complications determined by systemic bone loss. Another explanation is related to the presence of a low level of inflammation which can precede the onset of clinical symptoms in rheumatoid arthritis for a long period of time.

CONCLUSIONS

In our opinion, an impaired balance between bone resorption and bone formation mechanisms contributes to juxta-articular bone loss. This appears to be mediated by the increased cytokine and prostaglandin production in, by the synovium and bone marrow. Generalized bone loss is frequently encountered in patients with rheumatoid arthritis, resulting in increased risks of falling and fracture. Local bone destruction in rheumatoid arthritis could determine joint instability and can often lead to reconstruction surgery or even joint prosthesis.

About systemic bone loss in the context of rheumatoid arthritis, an increased risk of

fractures of the hip, spine and wrist has been noticed, the mechanisms involved in systemic osteoporosis in rheumatoid arthritis being multifactorial.

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A "RARE" CAUSE OF PERICARDIAL EFFUSION – CASE REPORT

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A "RARE" CAUSE OF PERICARDIAL EFFUSION–CASE REPORT (Abstract): Although tuberculosis classically affects the lungs, lymphatic and hematogenous dissemination, as well as direct invasion of the pericardium from the adjacent pleura make *Mycobacterium tuberculosis* the most frequent cause of acute pericardial effusion in developing countries. We present the case of a 68-year-old patient presenting with acute cardiac failure due to large pericardial effusion, a week after undergoing drainage of a facial subcutaneous abscess. Pericardiocentesis followed by video-assisted thoracoscopic pericardial window were performed. Blood cultures were repeatedly negative, but the biochemical analysis of the pericardial fluid showed low glucose concentration and high protein levels, elevated adenosine deaminase and lactate dehydrogenase levels. Although bacterioscopy and pericardial fluid cultures were negative, we made a presumptive diagnosis of tuberculous fibrinous pericarditis and initiated appropriate therapy with rifampin, isoniazid, pyrazinamide, and ethambutol, with favorable clinical outcome. Our diagnosis was confirmed 22 days later, when the Mycobacteria Growth Indicator Tube cultures were positive for *M. tuberculosis*. Although tuberculosis is a rare cause of pericarditis in the Western world, in a country endemic for tuberculosis (such as Romania), the tuberculous etiology of fibrinous pericarditis should not be overlooked. **Keywords:** EXTRAPULMONARY TUBERCULOSIS, FIBRINOUS PERICARDITIS, PERICARDIAL TAMPONADE, VIDEO-ASSISTED THORACOSCOPIC PERICARDIAL WINDOW, MYCOBACTERIA GROWTH INDICATOR TUBE.

Classic tuberculosis (TB) affects the lungs, but forms of extrapulmonary tuberculosis located in the genitourinary tract (1, 2), central nervous system (3), gastrointestinal tract (4), and skeletal system (5) have also been described. Tuberculous pericarditis is a severe form of extrapulmonary TB, being associated with a high 6-month mortality rate (17-40%) (6, 7). The incidence of TB pericarditis ranged from less than 4% in Western countries to 50-90% in developing regions, where tuberculosis is endemic (7, 8, 9). Although Romania remains endemic for tuberculosis, the number of extrapulmonary TB cases has gradually decreased during the past years, but 40-50 cases of TB pericarditis are still annually reported (10).

The most frequent clinical presentation of TB pericarditis is congestive heart failure (7). One of the most feared complications of TB pericarditis is constriction, which usually occurs within 6 months in 50% of cases in the absence of targeted

anti-TB therapy (6, 7). This rate has been reduced to 17-40% with the help of aggressive anti-TB treatment and corticosteroid therapy (6). A noteworthy limitation of TB chemotherapy is the poor penetration of antibiotics into the pericardium (11).

CASE REPORT

A 68-year-old male patient was admitted to an otorhinolaryngology clinic for drainage of a frontal abscess of unknown etiology. A week after discharge, the patient developed fever (38°C) accompanied by chills and was referred to the infectious diseases department. Despite aggressive antibiotic therapy, he developed dyspnea on minimal exertion, palpitations, chest pain and exercise-related liver pain and was transferred to the local cardiology clinic.

The clinical exam revealed tachypnea, tachyarrhythmia (113 bpm), normal blood pressure, and right-sided heart failure. Routine laboratory evaluation showed inflammation (erythrocyte sedimentation rate 100 mm/hr., fibrinogen 948 mg/dL, elevated C reactive protein), thrombocytosis (663,000/mm^3) and cardiac enzymes within normal range. Electrocardiogram revealed paroxysmal atrial fibrillation, managed with amiodarone and carvedilol. A throat swab culture revealed *Candida albicans* infection for which he received appropriate local treatment.

Echocardiography revealed circumferential pericardial effusion (34 mm) with fibrinous material and pericardial thickening (0.99 cm). Chest X-ray (radiography) showed pulmonary congestion and enlarged cardiac silhouette (fig. 1). The performed chest computed tomography (CT) (fig. 2, 3) detected multiple infracentimetric aortopulmonary lymph nodes with peripheral enhancement, but no active pulmonary or bone lesions. Although initial and repeated blood cultures were negative, the patient continued to present fever although he was on antibiotic therapy and nonsteroidal anti-inflammatory drugs.

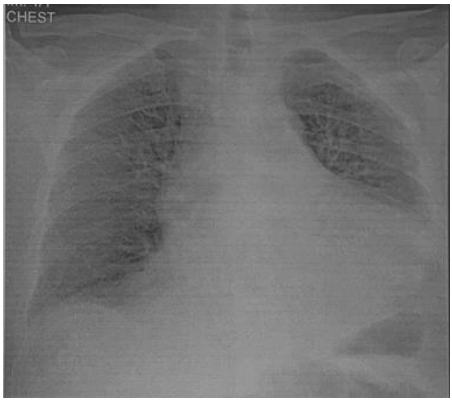

Fig. 1. Chest X-ray - enlarged cardiac silhouette, pulmonary congestion

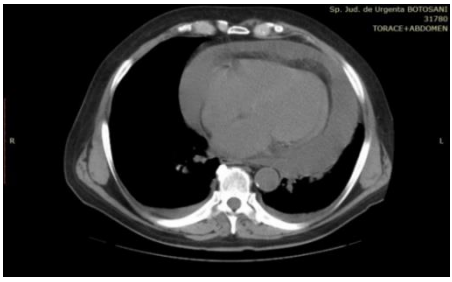

Fig. 2. Chest CT – pericardial effusion (maximum thickness 33 mm)

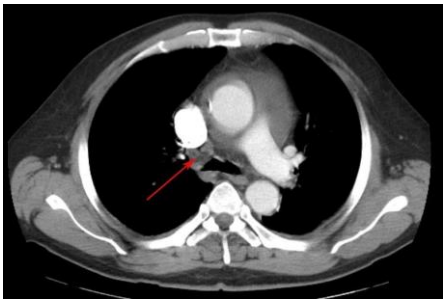

Fig. 3. Contrast enhanced chest CT – enlarged lymph nodes with peripheral enhancement

The patient began to show signs of incipient cardiac tamponade so he was transferred to the thoracic surgery department where pericardiocentesis and drainage of 100 mL serous fluid were performed. Pericardial fluid bacterioscopy and cultures were negative. Cytology was negative for tumor cells. Biochemical analysis revealed microscopic lymphocytic inflammatory reaction with high protein levels (46.8 g/L), high adenosine deaminase (ADA) - 71 U/L, high lactate dehydrogenase (LDH) - 849.2 U/L and low glucose (67.4 mg/dL) levels.

As the patient showed no clinical improvement after 2 days of conservative therapy he underwent a video-assisted thoracoscopic pericardial window performed on the left side. Pericardial biopsy confirmed our previous diagnosis of fibrinous pericarditis. A control chest X-ray was performed after the procedure (fig. 4).

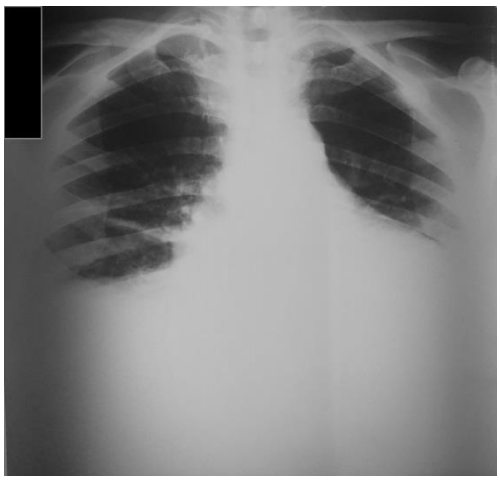

Fig. 4. Chest X-ray – bilateral pleural effusion, enlarged cardiac silhouette

We decided to initiate anti-TB chemotherapy with rifampin, isoniazid, pyrazinamide and ethambutol along with oral corticosteroids (prednisone). The diagnosis was confirmed after 22 days, when the *Mycobacteria Growth Indicator Tube* (MGIT) cultures detected *M. tuberculosis* susceptible to rifampin, isoniazid and ethambutol. The outcome was favorable with no evidence of constriction during the 4-month follow-up.

DISCUSSION

Our patient was admitted for rapidly progressive heart failure due to massive pericardial effusion. The following investigations were performed: chest X-ray (class I), inflammation marker panel (class I) and CT (class IIa). Although he did not meet the criteria for acute pericarditis (no pericarditic chest pain and friction rub, no ST elevation or PR depression), the current guidelines recommend managing pericardial effusions with positive inflammatory markers as pericarditis (7).

Our patient presented rapid clinical and biological decline after the drainage of a subcutaneous frontal abscess, so systemic staphylococcal or streptococcal infection with pericardial involvement was a possible cause of his current condition. Patient medical history was negative for TB and the CT scan did not detect any active pulmonary or bone lesions. However, he presented significant pericardial thickening, massive effusion with frond-like projections, and typical lymph node enlargement (hypodense center), all suggestive of TB etiology (7).

Since a bacterial etiology of the pericardial disease was suspected and our patient associated early signs of cardiac tamponade, pericardiocentesis (class I) (7) and drainage of 100 mL of serous fluid were performed.

Tuberculous pericardial effusions are usually cloudy and serosanguineous (12).

The detection of acid-fast bacilli during microscopic assessment is diagnostic for TB but reported in only 40-60% of cases (12). Although in our patient the fluid analysis was negative for acid-fast bacilli, it documented lymphocytic inflammatory reaction with very high protein levels, both recognized features of tuberculous pericardial effusions (12). Increased pericardial interferon-gamma and ADA levels are associated with TB pericarditis (7,12). In our case, pericardial ADA levels were >40 U/L (a cut off value with 93% sensitivity and 97% specificity in diagnosing TB pericarditis) (12).

Per current guidelines, subjects with relapsing cardiac tamponade, repeated accumulation of pericardial effusion and those who do not improve with drug therapy should undergo surgical drainage. Pericardial biopsy is a class IIb indication for suspected TB pericarditis (7). As our patient’s clinical condition did not improve despite optimal drug treatment, we decided to perform a video-assisted thoracoscopic pericardial window.

The definite diagnosis of TB pericarditis requires the detection of *M. tuberculosis* in pericardial fluid or tissue through polymerase chain reaction (PCR) or cultures (6,7). Microbiological diagnosis can be augmented through cultures from extracardiac sites (pleural fluid, lymph nodes and sputum) (13). A possible diagnosis is based on successful response to TB chemotherapy or high ADA, unstimulated interferon-gamma or lysozyme levels in the pericardial fluid (7). Only 17% of cases meet the criteria for definite TB pericarditis (6). A study reported interferon-gamma measurement in the pericardial fluid as the best diagnostic tool, especially combined with ADA and cytology of pericardial effusion (13). Our patient’s CT scan was negative for active pulmonary lesions and superficial adenopathies, suitable for biopsy. The tuberculin skin test is not recommended in adults and we did not perform it in our patient. The guidelines recommend the use of interferon-gamma release assays in patients with probable TB, but the results are less specific in countries where tuberculosis is endemic (7).

The initiation of empiric anti-TB treatment in endemic regions is allowed as soon as other causes of pericardial effusion have been excluded (trauma, autoimmunity, cancer, uremia, purulent pericarditis), but it is not justified in countries where TB is not endemic and there is no clear evidence supporting the tuberculous etiology. Prevention of pericardial constriction requires 6 months of standard combined anti-TB treatment (class I). Intrapericardial urokinase reduces the risk of constriction. High-dose prednisolone can reduce the risk of developing constriction and has a class IIb recommendation in immunodeficiency virus-negative human subjects (7).

Our diagnosis of tuberculosis was based on clinical suspicion (endemic area for TB), echocardiographic and CT findings (pericardial thickening, fibrinous material) and high pericardial ADA and protein levels. The diagnosis was later confirmed by a positive response to empirical anti-TB treatment and positive MGIT cultures. Our patient should have regular follow-up as part of the screening for constrictive pericarditis, although he showed no evidence of constriction after 4 months of anti-TB treatment.

CONCLUSIONS

This case report proves that in a country endemic for tuberculosis, the differential

diagnosis of pericarditis should include *Mycobacterium tuberculosis* as one of the most frequent causes of pericardial effusion. We consider video-assisted thoracoscopic pericardial window along with pericardial and pleural biopsies to be valuable diagnostic and therapeutic tools for tuberculous pericarditis. Because of the high mortality rates associated with this condition, we chose to initiate aggressive anti-TB chemotherapy even in the absence of a definite diagnosis.

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PROGNOSTIC VALUE OF RIGHT VENTRICULAR FUNCTION ASSESSED BY ECHOCARDIOGRAPHY IN PATIENTS PRESENTING WITH A FIRST ACUTE ST ELEVATION MYOCARDIAL INFARCTION TREATED BY PRIMARY PCI

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PROGNOSTIC VALUE OF RIGHT VENTRICULAR FUNCTION ASSESSED BY ECHOCARDIOGRAPHY IN PATIENTS PRESENTING WITH A FIRST ACUTE ST ELEVATION MYOCARDIAL INFARCTION TREATED BY PRIMARY PCI (Abstract) **Aims**: Informations regarding the prognostic value of right ventricular function changes in the setting of a first acute ST elevation myocardial infarction irrespective of the site of the necrosis and of the left ventricular systolic function are scarce. Purpose of the study was to assess the relation between parameters reflecting global and systolic right ventricular function assessed by conventional, speckle tracking and three-dimensional echocardiography and in hospital major cardiac events (MACE). **Material and methods:** We have prospectively analyzed a cohort of 44 consecutive patients (mean age 62,71 years, 70.5 % males) presenting with a first STEMI (2,3 % Topol 1, 38 ,6 % Topol 2, 20,6 % Topol 3, 31,8 % Topol 4, 6,8 % Topol5) treated by primary angioplasty. Patients with previous history of cardiac or pulmonary diseases were excluded. All patients underwent during hospitalization conventional 2D echocardiography and special techniques (2D speckle tracking echocardiography and also 3D echocardiography) RV global function was quantified by RV myocardial performance index (RV MPI) determined by PW Doppler ,whereas RV systolic function was studied using regional parameters like TAPSE , pulsed Doppler S wave and RV free wall 2D strain and global parameters like RV fractional area change (RV FAC) or RV ejection fraction (RVEF) determined by 3D echocardiography . LV systolic function was described by LV ejection fraction (LVEF). The combined endpoint of major adverse cardiovascular events **(**MACE**)** was defined by all cause mortality, reinfarction, need for revascularization and occurrence of heart failure during hospitalization. The association between MACE and RV functional parameters was assessed by bivariate correlation analysis followed by binary logistic regression. **Results**: Initially, regardless of the site of necrosis, the only RV functional parameter correlated with MACE was RV MPI (OR 9.17; 95% CI: 1.03 -83.7). After adjustment for LVEF all RV functional parameters were correlated with MACE: TAPSE (OR: 1.83; 95% CI : 0.41- 8.23), RV MPI (OR: 8.07; 95% CI : 0.9- 72.07), RVFAC (OR: 1.22; 95% CI : 0.25- 5.98) , RV free wall strain (OR : 1.04; 95% CI : 0.21- 5.08) , S wave (OR: 2.46 ; 95% CI : 0.14- 42.82), RVEF (OR: 0.83 ; 95% CI : 0.20- 3.43). **Conclusions:** Our study reveals that RV functional parameters are predictive for in hospital MACE beyond LV systolic function and regardless of the culprit coronary artery. Among these parameters, RV MPI seems to have the greatest predictive value for short term MACE in STEMI patients. **Keywords:** PRONOSTIC, RV FUNCTION, ECHOCARDIOGRAPHY.

The prognosis of patients presenting with acute myocardial infarction depends on many clinical, biological or imagistic factors. Parameters reflecting left ventricular systolic or diastolic function measured by echocardiography during hospitalization after an acute myocardial infarction are strongly related with the occurrence of adverse events in short and long term follow up studies (1-3). Accordingly, left ventricular functional noninvasive assessment has become indispensable at discharge in patients with acute myocardial infarction.

In clinical practice, the right ventricle has been initially called the forgotten chamber (4) because most studies have evaluated the prognostic importance of LV function. In the last five years, based on the increased number of papers describing RV functional assessment (5, 6) or the prognostic significance of RV function in many diseases (7-9), RV has been no more longer called the forgotten chamber but rather an incompletely understood cavity, still not a desirable state (10).

The importance of right ventricular function assessed in the setting of an acute myocardial infarction is not very good defined now. Echocardiographic assessment of right ventricular function, the most available imagistic method, consists of an integrative approach by measuring right ventricular size and function (5,6). In acute myocardial infarction with inferior localization, right ventricular involvement assessed through the fractional area change (RV-FAC), a 2D echocardiographic parameter describing RV global systolic function, has been defined as a strong predictor of complications and in hospital mortality (11). RV dysfunction is associated with adverse prognosis in post-acute myocardial infarction patients with moderate to severe LV dysfunction (12). Recent data suggests that RV functional involvement, evaluated through 2D and speckle tracking echocardiography in the first 48 hours after coronary angioplasty, is associated with strong prognostic informations regarding the occurrence of major cardiac events on long term follow up (13).

Informations regarding the prognostic value of right ventricular function changes in the setting of a first acute ST elevation myocardial infarction irrespective of the site of the necrosis and of the left ventricular systolic function continues to be scarce and have been less studied.

MATERIAL AND METHODS

From March 2015 to April 2016, 44 consecutive patients presenting with a first acute STEMI and treated with primary PCI within the first 12 hours were included and screened prospectively. Patients were treated per the national protocol, which is driven by the recommendations included in the most recent guidelines (14). This protocol provides that every patient with ST elevation myocardial infarction which presents in the first 12 hours should be transferred as soon as possible to a center capable of primary PCI. Patients are receiving thrombolysis if transfer time to the tertiary center exceeds 2 hours. ST elevation myocardial infarction was diagnosed on criteria included in the universal definition of myocardial infarction: new or presumably new ST segment elevation or new left bundle branch block on ECG in addition with rise and fall of cardiac biomarkers with or without symptoms of ischemia. In addition, all patients underwent conventional echocardiography and special echocardiographic techniques. Patients with previous history of cardiac and pulmonary diseases were excluded from the study.

The study was approved by the human

research committee of our hospital, and informed consent was obtained from all subjects.

Echocardiographic images were obtained with patients in the left lateral decubitus using Vivid Q and Vivid E9 machines (General Electric-Vingmed, Horton, Norway) with a 3.5 MHz transducer. Standard 2D echocardiography was performed in addition to 2D speckle echocardiography at discharge; also, all patients underwent three-dimensional echocardiography with a dedicated transducer. All images were obtained at a rate of 50-70 frames/s and three consecutive cardiac cycles were saved in digital format. Data analysis was performed offline by an experienced observer using a dedicated software (EchoPack BT12 GE Vingmed), to assess right and left ventricular function. The reference limits of all echocardiographic parameters were defined per the current adopted international guidelines (6).

LV functional assessment was defined by measuring LV ejection fraction through 2D echocardiography. The LV end-systolic volume (LVESV) and LV end –diastolic volume was determined in the apical 4 chamber and 2 chamber view and LVEF was calculated using the biplane Simpson method (6). RV functional assessment was made using the multiparametric approach as recommended by the current guidelines (5,6). RV global function was quantified by RV myocardial performance index (RV MPI) determined by PW Doppler, whereas RV systolic function was studied using regional parameters like tricuspid anular plane systolic excursion (TAPSE) , pulsed Doppler S wave velocity and RV free wall 2D strain and global parameters like RV fractional area change (RV FAC) or RV ejection fraction (RVEF) determined by 3D echocardiography.

The RVMPI has been obtained by the pulsed Doppler method, using the parasternal short axis view, where ejection time was measured at the RV outflow (time from the onset to the cessation of the flow) and the apical 4 chamber view where the tricuspid valve closure-opening time was obtained placing PW sample at the tips of the tricuspid valve. Results represent the average of measurements done in 5 cardiac cycles. Based on the idea that data is taken from different images, one most therefore attempt to use beats with similar R-R length for recording accurate data (fig. 1a, 1b). RV MPI represents the ratio between the difference tricuspid valve closure-opening time and the ejection time divided by the ejection time.

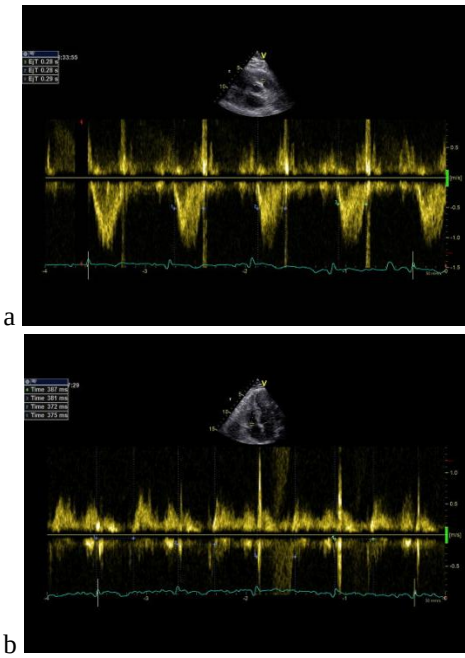

Fig. 1.
a. Measuring RV ejection time at the RV outflow; **b.** Measuring tricuspid valve closure –opening time in the apical 4 chamber view

RV systolic function was studied using regional parameters like TAPSE, tissue pulsed Doppler S wave velocity and RV free wall strain. TAPSE was measured in the 4-chamber view, placing the M mode cursor through the tricuspid annulus and the total displacement of the RV base from end- diastole to end-systole was measured. S wave velocity, representing the longitudinal velocity of the RV free wall, was measured in the 4-chamber apical view, using pulsed tissue Doppler, high lightening the RV free wall. The pulsed Doppler sample volume is placed on the tricuspid annulus or in the middle of the basal segment of RV free wall. Specific software generates velocity profiles over the cardiac cycle, S wave representing the highest systolic velocity after the iso volumic contraction period (fig. 2). Peak systolic longitudinal strain of the RV free wall was measured in the RV focused 4 chamber view, using speckle tracking analysis, with LV dedicated software, applied to RV. Strain analysis was performed offline by an experienced observer using Q-Analysis package. Briefly, the endocardium was manually traced at end-systole and the region of interest (ROI) was adjusted to include the whole myocardial wall but to exclude the pericardium. The reference point of each cardiac cycle was placed at the beginning of the QRS complex. Peak systolic longitudinal strain was determined as the average of the strain displayed by the system in the three segments of the RV free wall (basal, medial, apical). Strain analysis was feasible in all segments obtained from the 44 patients included in the study (fig. 3).

RVFAC was measured by tracking the RV end diastolic area and the RV end systolic area in the apical RV focused view, using the formula RVFAC=RVEDA-RVESA/RVEDAx100.

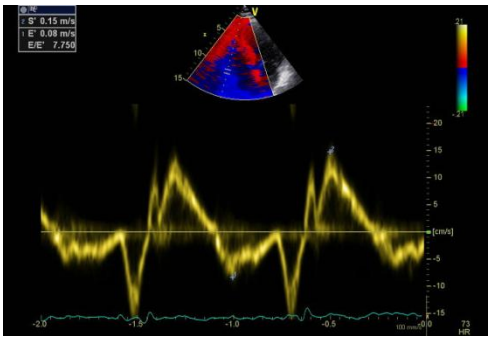

Fig. 2. Measuring S wave velocity using pulsed Doppler in the apical 4 chamber view

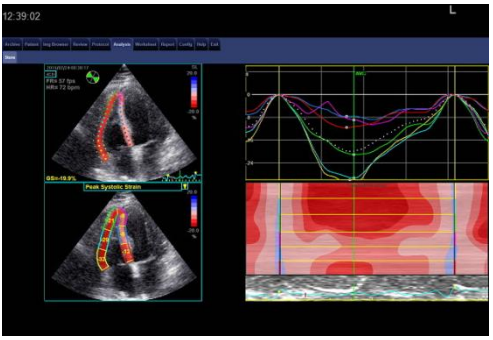

Fig. 3. Measuring free wall RV longitudinal strain by speckle tracking method

RVEF, as a measure of global systolic function was determined using three-dimensional echocardiography, requiring a dedicated multibeat 3D acquisition, with minimal depth and sector angle (for a temporal resolution > 20–25 volumes/sec) that encompasses entire RV cavity Automatically identified timing of end-diastole and end-systoles were carefully verified, whereas myocardial trabeculae and moderator band were included in the cavity. RVEF was measured offline by an experimented observer using dedicated TomTec software (fig. 4).

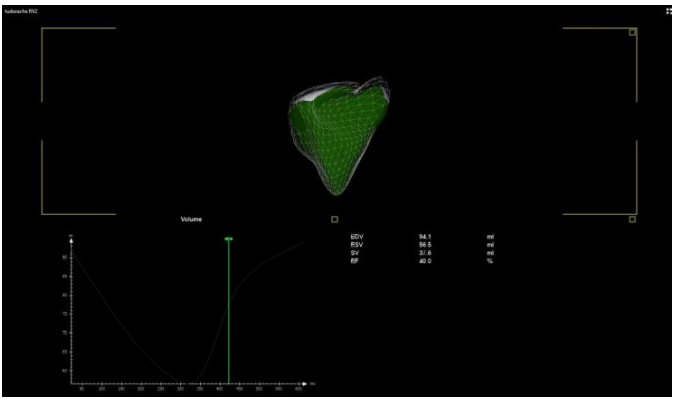

Fig. 4. Measuring RVEF by three-dimensional echocardiography, using TomTec software

Continuous data are presented as mean +/- standard deviation and categorical data are presented as frequencies and percentages. Chi-square and independent samples t test were used to validate the statistical significance between proportions and means respectively. The primary aim was to assess the association between RV function and adverse events. The combined endpoint of major adverse cardiovascular events **(**MACE**)** was defined by all-cause mortality, reinfarction, need for revascularization and occurrence of heart failure during hospitalization. The association between MACE and RV functional parameters was assessed by bivariate correlation analysis (Spearman correlation coefficient) followed by univariate binary logistic regression. Statistical analysis was performed using IBM *SPSS 20.0* at a statistical significance threshold of $p < 0.05$.

RESULTS

A total of 44 consecutive STEMI patients were studied. Mean age was 62,73+/- 13.42 years and most of the patients were men (70.5%). Regarding myocardial infarction localization on the electrocardiogram 2,3 % patients were Topol 1, 38 ,6 % patients were Topol 2, 20,6 % patients were Topol 3, 31,8 % patients were Topol 4 and 6,8 % patients were Topol 5. From the study population, 7 patients (15.9%) had right ventricular myocardial infarction based on the presence of ST elevation in the right precordial leads. A total of 27 patients (61.36%) suffered from anterior myocardial necrosis involving the left descending artery, whereas the remaining had non-anterior myocardial infarction with culprit lesions on the circumflex or on the right coronary artery. During in hospital follow up, major adverse cardiac events (MACE) defined as all cause mortality , reinfarction, need for revascularization or signs and symptoms of heart failure occurred in 12 patients (27.3 %).

Tables 1 and 2 summarize the clinical, electrocardiographic and echocardiographic characteristics and the differences between patients who reached the composite end and patients who remained event free. Average length of hospitalization was 8.7±3.77 days for patients with MACE respectively 7.18±2.19 days for patients without MACE. Simptom to balloon time was nonsignificant statistically between the two groups (MACE and MACE free)

467.14±245.473 minutes respectively 624.19 ± 906.064 minutes. The same situation was encountered regarding the door to balloon time 90±120 minutes for MACE patients and 148.32 ± 509.761 minutes for MACE free patients. Mean values of LVEF, TAPSE, S wave velocity, RV longitudinal strain, RV FAC, RVEF and RV MPI of the study group were: 44.49 %+/-8.67, 19.6 mm+/-4.03, 0.134 m/sec+/-0.027, 21.14 %+/-12.74, 42.1%+/-8.81, 44.98 %+/-9.31 and 0.55+/-0.18 respectively, also without statistically significance.

Regarding RV function and association with outcome, statistical analysis was performed for all echocardiographic measurements recorded during the in-hospital echocardiogram. Initially, regardless of the site of necrosis, the only RV functional parameter correlated with MACE was RV MPI (OR 9.17; 95% CI of OR: 1.03 -83.7; p=0.037). After adjustment for LVEF, all the studied echocardiographic parameters showed statistical significance: TAPSE (OR : 1.83; 95% CI : 0.41- 8.23; p<0.001), RVFAC (OR : 1.22; 95% CI : 0.25- 5.98; p<0.001) , RV free wall strain (OR : 1.04; 95% CI : 0.21- 5.08;p=0.024) , S wave (OR : 2.46 ; 95% CI : 0.14- 42.82 ;p=0.004; p=0.001) and RVEF (OR : 0.83 ; 95% CI : 0.20- 3.43) (tab. III).

TABLE I

Baseline clinical and electrocardiographic characteristics

	All patients (n=44)	MACE (n=12)	MACE free (n=32)	p (MACE *vs* MACE free)
Age (years)	62.73+/-13.43	65.92+/-11.35	61.53+/-14.12	NS
Sex **male** **female**	 31 13	 7(22.6%) 5(38.5%)	 24 (77.4%) 8 (61.5%)	NS
Smokers	26	19 (73.1 %)	7 (26.9%)	NS
Hypertension	27	7 (25.9%)	20 (74.1 %)	NS
Dyslipidemia	30	10 (33.3%)	20 (66.7%)	NS
Diabetes mellitus	13	3 (23.1%)	10 (76.9%)	NS
Symptom-to-balloon time (min)	44	467.14±245.473	624.19 ± 906.064	NS
Door-to-balloon time (min)	44	90 ± 120	148.32 ± 509.761	NS
Average length of hospitalization	44	8.7±3.77	7.18±2.19	NS
Infarct localisation				
Topol 1	1	1 (100%)	0	NS
Topol 2	17	2 (11.8%)	15 (88.2%)	NS
Topol 3	9	5 (55.6%)	4 (44.4%)	NS
Topol 4	14	3(21.4%)	11 (78.6%)	NS
Topol 5	3	1 (33.3%)	2 (66.7%)	NS
RV infarction	7	3 (42.9%	4 (57.1%)	NS

TABLE II.
Baseline echocardiographic characteristics

	All patients (n=44)	MACE	MACE free	p* (MACE vs MACE free)	p** (MACE vs MACE free)
LVEF	44.49+/-8.67	40.50+/-8.79	46.14+/-8.36		
TAPSE	19.6+/-4.03	19.33+/-4.71	19.71+/- 3.78	0.439	<0.001
S wave velocity	0.134+/-0.027	0.130+/-0.28	0.136+/-0.27	0.539	0.004
RV longitudinal strain	21.14+/-12.74	-20.83+/-12.26	-21.27+/-13.21	0.965	0.024
RV FAC	42.1+/-8.81	40.66+/-9.35	42.71+/-8.67	0.810	<0.001
RV EF	44.98+/-9.31	42.56+/-8.43	46+/-9.63	0.807	0.001
RVMPI	0.55+/-0.18	0.582+/-0.22	0.588+/-0.10	0.037	<0.001

*before adjustment for the LV function; **-after adjustment for LVEF

TABLE III.
RV functional echocardiographic parameters predictors for the composite endpoint

	OR	95% CI	p
TAPSE	1.83	0.41- 8.23	<0.001
S wave velocity	2.46	0.14- 42.82	0.004
RV longitudinal strain	1.04	0.21- 5.08	0.024
RV FAC	1.22	0.25- 5.98	<0.001
RV EF	0.83	0.20- 3.43	0.001
RVMPI	8.07	0.9- 72.07	<0.001

DISCUSSION

In the past, the clinical importance of RV function has been underestimated. The important finding of this ongoing study is that right ventricular function assessed during hospitalization in patients with acute myocardial infarction treated per the current guidelines could have additional prognostic information, in addition to the classic risk factors (15). Quantifying RV function is more difficult compared to the left ventricle and needs a multiparametrical approach with conventional 2D echocardiography and newer techniques like speckle tracking echocardiography or three-dimensional echocardiography. RV systolic and diastolic function can be expressed by variable echocardiographic measures including TAPSE, RVFAC, RV MPI, RV longitudinal strain, S wave velocity, or, the most complete parameter, RVEF. In our research, all these parameters were predictors of the combined endpoint of major adverse cardiovascular events, defined by in hospital all-cause mortality, reinfarction, need for revascularization and occurrence of heart failure. From the above-mentioned measurements, the one describing global RV function, the RVMPI, correlated with MACE irrespective of LV function. The other parameters quantified regional or global RV systolic function like TAPSE, S wave velocity or RVFAC or RVEF gained statistical significance after adjustment for LV function. RVMPI obtained by dividing the sum of the isovolumic contraction and

relaxation time to the RV ejection time represents o measure of global RV function. It is reproducible but is load dependent and unreliable when right atrial pressure is elevated like in cases of RV infarction, when it is inappropriately small or pseudo normalized (16). RVMPI has been studied in acute right ventricular myocardial infarction where it is used to quantify right ventricular dysfunction and assess improvements in RV function (17). Measurement of this parameter or determining its prognostic significance in the setting of a first acute myocardial infarction irrespective of localization hasn't been on our knowledge studied. In our study, the RVMPI was initially, regardless of the site of necrosis, the only RV functional parameter correlated with MACE (OR 9.17; 95% CI of OR: 1.03 -83.7).

TAPSE is maybe the most frequently used measurement to assess RV function and reflects the longitudinal systolic excursion of the lateral tricuspid valve annulus, which may not fully reflect RV contractility, as it is a measure of segmental systolic function. In the GISSI -3 echo sub study in acute myocardial infarction patients, TAPSE was significantly associated with LVEF and as LVEF recovered over time, RV function improvement was also noticed (18). In the study by Antoni et al (13), TAPSE proved strong association with adverse events on 24 months follow up in acute myocardial infarction treated by primary PCI. The S wave velocity determined by pulsed tissue Doppler S is easy to measure, reproducible, reflecting regional RV systolic function, but have not been studied like TAPSE in the setting of acute coronary syndromes. In our study both TAPSE and S wave velocity were associated with in hospital occurrence of adverse events after adjusting for LV function (OR: 1.83; 95% CI: 0.41- 8.23 respectively OR: 2.46; 95% CI : 0.14- 42.82).

Strain is a novel technique that permits angle independent measurement of longitudinal deformation of the RV , which represents the main mechanism of RV function, because 80% of the stroke volume is generated by the longitudinal shortening of the RV free wall (19) RV strain excluding the interventricular septum has been recently and for the first time demonstrated in the study by Antoni et al to have independent predictive value for adverse events in AMI patients, the same fact being demonstrated in our research but regarding short term follow up (13).

RVFAC is the most commonly used parameter to assess global systolic RV unction, but has inherent limitations because it is experience dependent and reproducibility is often poor. In AMI patients with LV dysfunction, characterized by LVEF <40% Anavekar et al (12) and Zornoff et al (11) highlighted that RV function, expressed through RVFAC is poor correlated with LV function and is independently associated with an increased risk of mortality and heart failure (OR: 1.22; 95% CI: 0.25-5.98) . In our research, RVFAC correlated with in hospital occurrence of MACE after adjustment for LVEF, but the study population consisted of post PCI patients with milder affected LV function (mean LVEF was 44.49+/-8.77%) than in the previous researches. RVEF represents a global measure of RV systolic performance and can be measured only with three-dimensional echocardiography, an expensive and not very available technique, yet. In our study, this is for the first time, by our knowledge, that RVEF assessed by three-dimensional echocardiography has

been demonstrated to correlate with in hospital MACE in AMI patients treated by primary PCI, after adjustment for LV function. (OR: 0.83 95% CI: 0.20- 3.43). The main limitations of this ongoing study are the small sample size and the length in evaluation. In future studies, we will evaluate larger sample of patients and if the RV functional echocardiographic parameters evaluated in acute state will have the same prognostic value on long term follow up as they had on short term follow up.

CONCLUSIONS

Our study reveals that RV functional parameters assessed by multiparametric echocardiography are predictive for in hospital MACE beyond LV systolic function and regardless of the localization of AMI or the culprit coronary artery. Among these parameters, RV MPI seems to have the greatest predictive value for short term all -cause mortality, reinfarction, need for revascularization or occurrence of heart failure in STEMI patients. This promising results requires validation in larger prospective studies. Consequently, more comprehensive echocardiographic evaluation including RV functional assessment should be implemented at hospital discharge in AMI patients for a better identification of patients at risk of adverse events. In addition, more precise characterization of RV function is warranted to improve our understanding of pathophysiological changes in RV physiology after reperfusion in acute STEMI patients.

ACKNOWLEDGEMENTS

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ATYPICAL SPITZ TUMOR - CASE REPORT

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ATYPICAL SPITZ TUMOR-CASE REPORT (Abstract): Atypical Spitzoid tumors represent a poorly defined and characterized category of melanocytic tumors with histologic features of both benign Spitz nevi and malignant melanomas. The group of atypical Spitz tumors represents a mixture of Spitz nevi with atypical features and Spitzoid melanomas. We report the case of an eight years old female patient, who was admitted in Dermatology Clinic Craiova for the presence of a skin tumor with 1/0.8 cm diameter, irregular edges, brown- black coloured with irregular pigment distribution. The skin tumor was located on the dorsal foot above the third metatarsal. The skin tumor appeared in the first two years of childhood and had a slowly increase. From about six months, the tumor had changed the colour becoming darker and had also a rapid increase in size. The patient had no comorbidities and had no family history of malignancy or other skin diseases. In our clinic we did the surgical excision of the skin tumor and sent the fragment for the histopathological examination and immunohistochemical tests. After the clinical examination, dermoscopic evaluation, histopathological and immunohistochemical examination, our diagnosis was Atypical Spitz Tumor. **Keywords:** ATYPICAL SPITZ TUMOR, SPITZ NEVUS, MELANOMA.

"Spitz nevus with atypia and metastasis" or "malignant Spitz nevus," was first described by Smith et al (1) in 1989 as a kind of lesion showing histopathologic features not enough for a diagnosis of malignancy, yet capable of nodal metastasis, usually with no further dissemination.

Barnhill then completes with the diagnostic category of "metastasizing Spitz tumor" (2), or "atypical Spitz nevus/tumor" (3).

Nowadays, some opinions are that there are only two diagnostic categories (nevus and melanoma) and that every "abnormal" behavior is simply a diagnostic mistake, and others suggest that Spitzoid lesions are indeed a "morpho-biologic spectrum" of lesions ranging from benignity to full-blown malignancy, and sharing a peculiar genetic profile, with chromosome rearrangements involving kinase fusions (4, 5, 6).

Intermediate lesions within such a spectrum have an equivocal histo-morphology, featuring a diagnostic agreement among

experts which is consistently lower than for "conventional" (non-Spitzoid) melanocytic neoplasms, but also peculiar clinical features and behavior with a relatively high incidence in prepubescent patients and a higher incidence of regional node involvement but a better prognosis than "conventional" melanoma of the same thickness/stage (possible low-grade malignancies) (7, 8, 9, 10).

CASE REPORT

We present the case of an 8-year-old female patient, who was admitted in Dermatology Clinic Craiova for the presence of a skin tumor with 1/0.8 cm diameter, irregular edges, brown-black colored with irregular pigment distribution. The skin tumor was located on the dorsal foot above the third metatarsal (fig. 1).

Fig. 1. Clinical aspect of the skin tumor

The skin tumor appeared in the first two years of childhood and had a slowly increase. From about six months, the tumor had changed, the color becoming darker and had also a rapid increase in size.

The patient had no comorbidities and had no family history of malignancy or other skin diseases.

After admission we performed dermoscopic examination which reveal nonspecific pattern with the presence of pigmented network, blue-white vale, white and black globules and white lines (fig. 2).

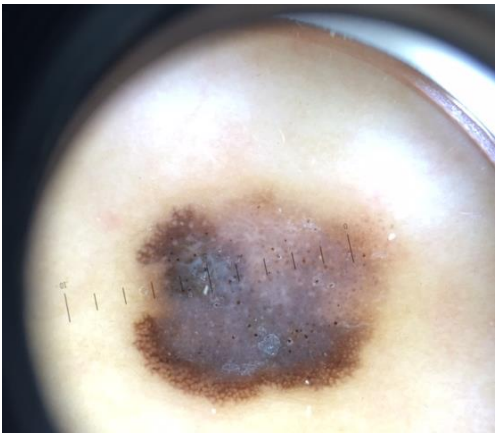

Fig. 2. Dermoscopic aspect of the skin tumor

In our clinic we did the surgical excision of the skin tumor and sent the fragment for the histopathological examination and immunohistochemical tests.

Histopathological examination revealed skin tumor with relatively symmetric architecture, histopathological aspect of proliferation of spindle cells, focal with intracytoplasmic accumulation of coarse melanin pigment arranged in large nests and isolated cells at the dermo-epidermal junction; numerous individual tumor cells and rare nesting with intraepidermic ascension and transepidermal elimination; extension in beams and isolated nests with minimum morphological maturation in the reticular dermis; tumoral cells have large nuclei, with 1-2 visible nucleoli, homogeneous eosinophilic cytoplasm; relatively frequent multinucleated tumor cells in surface; minimum interstitial fibrosis, rare mitosis in epidermal component; minimum lymphomonocyte inflammatory infiltrate with rare

melanofages peritumoral. The tumor proliferation has a tumor thickness of maximum 1.5 mm and extends laterally up to 2.5 mm from the closest lateral limit of fragments examined (fig. 3, 4).

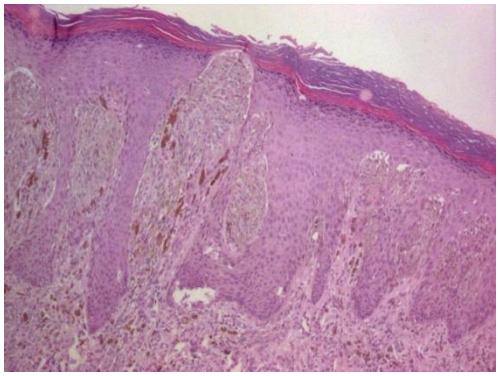

Fig. 3. Histopathological aspect of the skin tumor– HE col. X40

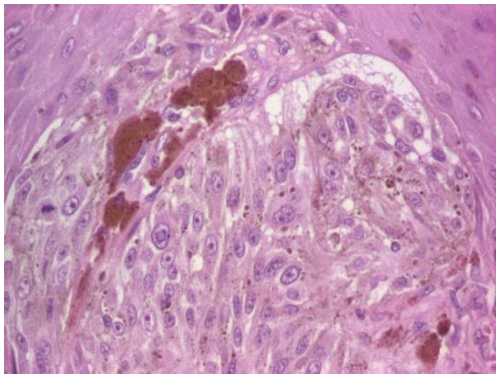

Fig. 4. Histopathological aspect of the skin tumor– HE col. X200

Immunohistochemical tests revealed HMB45- Positive tumor junctional component, positive in rare tumor cells in the dermis in the superficial part of the tumor (expression gradient compatible with the aging phenomenon); Tyrozinaza (T311)- positive junctional tumor component, positive in the dermis tumoral cells; p16- positive in the majority of tumor cells, small groups of tumor cells, predominantly those at intraepidermic level, negative; p21- positive in frequent nuclei of tumor cells; Cyclin D1- positive in tumor cells in both components (intraepidermic and dermal); Ki67- positive in about 1-2% of the tumor cells.

The test in fluorescence with co-location for Ki67 and protein S100 revealed two focuses were tumor cells shows relatively frequent nuclei Ki67-positive (index Ki67 4 positive nuclei/8 tumor cells and 3 Ki67 positive nuclei/7 tumor cells) without identified mitosis in dermal component. The test in fluorescence concluded histopathological and imunohistochimical aspects compatible with the diagnosis of Atypical Spitz Tumor.

After the clinical examination, dermoscopic evaluation, histopathological and immunohistochemical examination, our diagnosis was Atypical Spitz Tumor.

We indicated inguinal and popliteal lymph node ultrasound and the result was the absence of inguinal and popliteal lymph node metastases.

The patient was discharged with the indication of dermatological examination every 6 months.

DISCUSSION

Spitzoid tumors are estimated to be below 1% of melanocytic nevi in the first childhood but accurate data about incidence and prevalence are still not known. Atypical Spitz tumors (AST) associate minimal lethal potential, an increased melanoma risk, and a moderate risk of metastasis to regional nodes. Even today, there are no set of criteria to be used to predict the clinical outcome for atypical Spitz tumors with absolute assurance. AST occur more frequently in patients with Fitzpatrick Photo types I and II, affects both females and males and some authors describe a slight female predominance. About 50% of cases

occur in children under 10 years old and 70% of all cases are diagnosed in the first two decades of life (11).

From the clinical point of view atypical Spitz tumors present as a plaque or nodule, with variable size and localization. We can find a hypo pigmented reddish lesion or a brown-dark one. Regarding dermoscopic characteristics, nodularity, ulceration, linear vessels, polymorphic vessels, white lines, and blue-white veil are associated with atypical Spitz tumors (12).

The treatment recommended is surgical excision for all lesions with Spitzoid dermoscopic features detected after puberty. Before puberty, plaque-like or dome-shaped dermoscopic symmetric lesions with Spitzoid features can undergo dermoscopic digital monitoring (8). Large tumors over 1 cm, nodular or ulcerated tumors, rapidly growing or changing tumors, or otherwise atypical Spitz nevi of the childhood have to be surgical excised.

The management of patients with a histopathologic diagnosis of atypical Spitz nevus and Spitz tumor should be decided with a multidisciplinary approach. A re-excision can be considered for atypical Spitz nevi and has to be recommended for all incompletely excised lesions, as well as for Spitz tumors. The sentinel node biopsy for Spitz tumors should be evaluated case by case. A recent meta-analysis showed that 98–99% of young patients with Spitz tumors had no evidence of disease after a median follow-up of 59 months, regardless the sentinel node positivity. Therefore, the diagnostic and prognostic information given by a positive sentinel node in young patients seems to be negligible (10).

Echotomography of the regional nodes with an echotomography-guided fine needle aspiration biopsy cytology can replace sentinel node biopsy, because it allows to efficiently detect massive replacement of the lymph nodes by neoplasic cells, thereby addressing selected patients to election lymphadenectomy (13). This follow up protocol is probably the best choice in patients younger than 10 years, especially for lesions located in the head/neck area, a region in which surgical procedures have major aesthetically impact (14).

The management of the cases with atypical Spitz tumor depends also of patient age and the prognosis is also age-dependent (15, 16). Adult patients can be managed more aggressively.

The risk stratification for atypical Spitz melanocytic proliferations has been recently made with the use of a new four-probe fluorescence in-situ hybridization (FISH) assay for 6p25 (RREB1), 11q13 (CCND1), 9p21 (CDKN2a), and 8q24 (MYC). It resulted 4 categories: 1. Spitzoid melanoma with homozygous 9p21 deletion (high risk); 2. Spitzoid melanoma with 6p25 and/or11q13 gain (intermediate to high risk); 3. Atypical Spitz tumor with isolated 6q23 deletion (low risk); 4. Atypical Spitz tumor with no FISH abnormality (low to very low risk). But so far, the number of investigated cases is too low to validate this risk stratification system. However, the use of FISH techniques for prognostic purposes seems to be very promising (17).

Because of the lack of large studies of atypical Spitz tumor, no evidence-based treatment recommendations can be made.

Most current sources recommend complete excision to facilitate complete histologic evaluation and reduce risk of recurrence, but also they showed that very few sentinel lymph node biopsies are being performed for AST in clinical practice (18).

Atypical Spitzoid tumors represent a poorly defined and characterized category of melanocytic tumors with histologic fea-

tures of both benign Spitz nevi and malignant melanomas.

The group of AST represents a mixture of Spitz nevi with atypical features and Spitzoid melanomas (19).

CONCLUSIONS

This case report demonstrates that the diagnosis of pigmented tumors is difficult and it needs an experimental eye and performed investigations.

ACKNOWLEDGEMENTS

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NEWS

THE MOGE(S) CLASSIFICATION. A TNM-LIKE CLASSIFICATION FOR CARDIOMYOPATHIES

World Health Organization define cardiomyopathies as diseases of the myocardium associated with cardiac dysfunction. They were classified according to anatomy and physiology into the following types: dilated cardiomyopathy, restrictive cardiomyopathy (endocardial fibroelastosis), hypertrophic cardiomyopathy (athlete's heart), arrhythmogenic right ventricular cardiomyopathy, unclassified cardiomyopathies (left ventricular noncompaction, stress-induced cardiomyopathy, cirrhotic cardiomyopathy). Most cardiomyopathies are a familial disease and screening of the pedigree identifies asymptomatic family members for an early diagnosis cardiomyopathy. Genetic testing is increasingly becoming a part of the diagnostic process, and numerous new genes and mutations have been identified in the etiology of cardiomyopathies. Traditional definitions of cardiomyopathies, such as those by the American Heart Association and the European Society of Cardiology, do not consider the genetic basis of cardiomyopathies. In 2013, the World Heart Federation proposed a nosology that includes five simple attributes of a cardiomyopathy disorder: morphofunctional characteristic (M), organ involvement (O), genetic or familial inheritance pattern (G), an explicit etiological annotation (E) with details of genetic defects or underlying disease/cause, and information about the functional status (S) using the New York Heart Association (NYHA; I–IV) functional classes. The addition of (S) is optional, and should be used at the discretion. Using the description of the five attributes, the classification system has been designated as MOGE(S). The morphofunctional (M) notation provides a descriptive diagnosis of the phenotype: M_D = dilated cardiomyopathy; M_H = hypertrophic cardiomyopathy; M_A = arrhythmogenic right ventricular cardiomyopathy; M_R = restrictive cardiomyopathy; M_{LVNC} = LV noncompaction. Organ involvement (O) is defined as involvement of the heart only (O_H) or of other organs/systems. Healthy mutation carriers can be described as (O_0). The genetic/familial inheritance (G) notation gives information about autosomal dominant (G_{AD}), autosomal recessive (G_{AR}), X-linked (G_{XL}), X-linked recessive (G_{XLR}), X-linked dominant (G_{XLD}), or matrilineal (G_M) transmission. The etiological notation (E) adds to the information on the underlying cause. The MOGE(S) system resembles the TNM classification system for malignancy, and therefore it can be useful for the diagnosis, management, and treatment of cardiomyopathies in a similar manner to cancer management (E. Şahan, S. Şahan, M. Karamanlioğlu, M. Gul, O. Tufekcioğlu. The MOGE(S) classification. A TNM-like classification for cardiomyopathies. *Herz* 2016; 41(6): 503–506).

Ciprian Rezuş

THE "DARK SIDE" OF DEEP VEIN THROMBOSIS - CASE REPORT

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THE "DARK SIDE" OF DEEP VEIN THROMBOSIS - CASE REPORT (Abstract): Venous thromboembolism (VTE) is an increasingly common cause of morbidity and mortality in cancer patients. In various malignancies the incidence of thrombosis ranges from 5% to 60%, that is four times higher in cancer patients compared to the general population. Large retrospective studies have shown that in men the tumors which are most commonly associated with VTE are lung cancer and pancreatic cancer and, in women cancer of the genital area, pancreas, colon and rectum. Thromboembolic events may often occur before the cancer diagnosis. We present the case of a 41year-old female patient with a history of genital cancer which was surgically treated and who is now admitted for clinical signs of ileofemoral deep vein thrombosis (DVT) of the left leg. The diagnosis was confirmed by laboratory data and Doppler ultrasound and the patient received anticoagulant treatment. Given the history of the patient, abdominal and pelvic ultrasound and computer tomography (CT) were performed to detect the cause who predisposed to the thrombotic event. These confirmed the ovarian cancer, this time on the right side, and the presence of hepatic and pulmonary metastasis. This case highlights the importance of screening for a cause of the thromboembolic event in patients, especially in those who have a history of a neoplasia. **Keywords:** DEEP VEIN THROMBOSIS, MALIGNANCY, PARANEOPLASTIC SYNDROMES.

The association between malignancy and clinically manifest thrombosis in various sites has been recognized for almost 140 years. It was first described by Armand Trousseau in 1865 who noted that "in cancer patients a special condition of the blood predisposes to spontaneous coagulation" (1, 2). Hypercoagulable state is a characteristic of cancer patients and the spectrum of hemostatic disorders extends from abnormal coagulation tests in the absence of clinical signs, to massive thromboembolism at various sites (2 - 4). Thrombotic episodes may precede malignancy by months or days and can manifest in various forms: superficial migratory thrombophlebitis (Trousseau syndrome), nonbacterial thrombotic endocarditis, disseminated intravascular coagulation – DIC (malignancy is considered the third cause of DIC after infection and trauma), thrombotic microangiopathy. Arterial thrombosis can also occur (but less frequently than vein thrombosis), and is due to nonbacterial thrombotic endocarditis (2). The incidence of thrombosis ranges from 5% to 60% in vari-

ous malignancies, which is four times higher in cancer patients compared to the general population (3). In men, pancreatic and lung cancer are most commonly associated with VTE and in women - the genital area, pancreatic and colorectal cancer (5). It is worth remembering that not infrequently vein thrombosis may precede by a variable period of time the occurrence of the specific clinical picture of cancer (2).

CASE REPORT

We present the case of 41-year-old woman who was admitted to the Department of Cardiology, „Sf. Spiridon" Emergency Clinical Hospital Iaşi, for edema and pain in the left leg, occurring relatively quickly, in the last 48 hours, suggestive for the clinical diagnosis of iliofemoral thrombosis of the left leg.

The past medical history revealed that at the age of 37 years she underwent left oophorectomy and subtotal hysterectomy for ovarian tumor, the anatomopathological diagnosis findings being low-grade malignant stromal sarcoma, for which chemotherapy was not indicated but periodic checkups were recommended. The current diagnosis of deep vein thrombosis was confirmed by the presence of elevated raised D-dimers and Doppler ultrasound of the lower legs which showed incomplete thrombosis of the left external iliac, left common femoral and left popliteal veins (fig. 1).

Fig. 1. Doppler ultrasound of lower limbs showing thrombosis of the left common femoral vein

Laboratory data revealed: a mild inflammatory syndrome (ESR: 57 mm/h, fibrinogen: 5.65 g%), normal white blood cell and platelet counts, absence of anemia, negative result in antiphospholipid antibody test (the latter excluding a possible thrombophilia). The remaining laboratory data were within normal range.

Unfractionated heparin therapy via syringe pump monitored with activated partial thromboplastin time (APTT) was initiated, and laboratory tests were performed to elucidate the cause of DVT (fig. 2).

Given the history of genital cancer, abdominal and pelvic ultrasound scan was performed, which showed a cystic mass on the right ovary associated with liver metastases. Chest X-ray demonstrated pulmonary metastases, all these changes being confirmed by thoracoabdominal CT which in

its turn confirmed that the right ovarian cystic mass detected by ultrasound was in fact a right ovarian cystic neoplasm with liver and pulmonary metastases.

The final diagnosis was: paraneoplastic left iliofemoral vein thrombosis, right ovarian cystic cancer, liver and lung metastases.

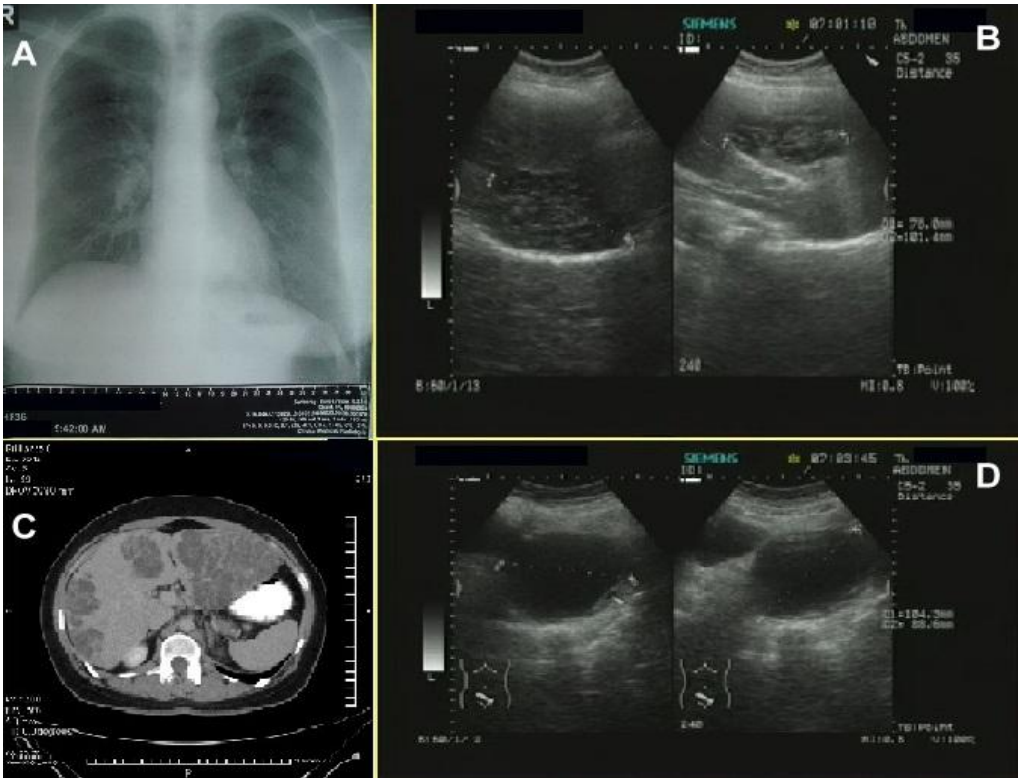

Fig. 2. A: Chest X-Ray showing left pulmonary metastases; B: Abdominal ultrasound showing liver metastases; C: Abdominal CT showing liver metastases; D: Pelvis ultrasound scan showing ovarian cysts with signs of malignancy

DISCUSSION

The occurrence of a prothrombotic status in cancer patients depends on the type, location and extent of the tumor, response of the host organism, as well as on therapeutic regimens used (2, 6, 7). The main mechanisms involved in the induction of prothrombotic states are:

- excessive thrombin production by an extrinsic pathway due to secretion by certain tumors of tissue-like factors (e.g.: gastric and pancreatic tumors) (2, 8, 9); these tumors frequently cause recurrent and migratory venous thrombosis (Trousseau) (2);

- production of thrombin directly by intact tumor cells that may express procoagulant activity; sometimes normal host tissues can express procoagulant activity in response to the tumor.

Malignant tumors can secrete substances with procoagulant activity mainly of two

types: tissue factor and cancer procoagulant. Tissue factor forms a complex with activated factor VII and factor IX and X leading to the initiation of coagulation and cancer procoagulant is a calcium-dependent cysteine protease which directly activates factor X, independent of tissue factor and activated factor VII (2). Monocytes, which normally do not have procoagulant activity can be stimulated, under certain conditions, to produce tissue factor or other direct activators of factor X by T lymphocytes, antigens, cytokines, lipoproteins, immune complexes, endotoxins (2). Also, the presence of a malignancy in the body produces an abnormal platelet activation which aggravates the hypercoagulable state. The endothelial cells may become procoagulant under the influence of pro-inflammatory cytokines or other peptides, and in particular due to TNF and IL-1 which increase the expression of leukocyte adhesion molecules, platelet activating factor and tissue factor. TNF also reduces the fibrinolytic activity of endothelial cells, increases endothelial production of IL-1 and expression of thrombomodulin which decreases the anticoagulant protein C activation (2, 8 - 10). Additional risk factors for VTE in patients with malignancies include: age, race, comorbidities, history of VTE, cancer site, metastases, recent surgeries, chemotherapy, central venous catheters and sepsis (2, 8 - 11). The period of 3-6 months after diagnosis is characterized by an increased incidence of VTE. Another mechanism by which malignant tumors can cause venous thrombosis, besides inducing hypercoagulable state, is extrinsic compression or direct vascular invasion (2, 11).

The literature review showed that different reported cases of VTE episodes were in the end diagnosed with occult malignancy (12 - 19). Each of them has its particularities: atypical location of venous thrombosis, bilateral involvement, or different types of cancer. As to the tumor markers, which were negative in our patient, cases reported in the literature presented either positive or negative tumor markers, depending on the histopathological type (13, 14, 20).

CONCLUSIONS

There are some particularities of this case: 1) Discovery of a cancer relapse in metastatic stage in the guise of DVT; 2) Paraneoplastic DVT usually occurs in adenocarcinomas (in our case the first tumor was low-grade malignant stromal sarcoma); 3) Absence of tumor markers even in the presence of metastases.

We believe that clinical history taking, routine laboratory findings, together with chest X-Ray and abdominal ultrasound are valuable screening tools for occult malignancy, especially in cases with atypical form or apparently idiopathic VTE.

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SHORT TERM EVALUATION OF PERIMETRIC PROGRESSION IN PATIENTS WITH OPEN ANGLE GLAUCOMA AND DIABETES

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SHORT TERM EVALUATION ON VISUAL DECLINE IN PATIENTS WITH OPEN ANGLE GLAUCOMA AND DIABETES (Abstract). **Aim of study:** progression rate in patients with glaucoma and diabetes. **Material and method:** cohort prospective study in Ophthalmology Clinic - "Sf. Spiridon" University Hospital – Iaş i. We recruited patients with positive history for diabetes and of open angle glaucoma (OAG). The control group included subjects with OAG (74 eyes from 74 patients) and the study group (44 eyes, from 44 patients) included subjects with OAG and diabetes. At enrollment all patients had a complete ophthalmologic evaluation along with full metabolic status assessment. There were included only incipient and moderate forms of glaucoma, with mild or no diabetic retinopathy changes. Perimetric progression was assessed at 24 months with automated methods. **Results**: globally, from 118 investigated eyes, 56.40% cases had primary open angle glaucoma, 41.03% normal tension glaucoma and 2.36% pseudoexfoliative glaucoma. Glaucoma severity classification showed early defects (mean deviation < -6 db) in the study group of 77.27% vs.83.78% in control group, whereas moderate defects (mean deviation > -6 db) were found in 22.63% in study group vs 16.21.0% in control group. Mean age of the patients was higher in absolute value in the open angle glaucoma group (64.31 +/- 1.66 years), vs diabetes + open angle glaucoma (62.69 +/- 1.8 years), with comparable visual acuities (0.91 +/-0.15 vs 0.89 +/-0.16), C/D ratios and other clinical parameters (p>0.05). Mean baseline IOP in the study group was 18.18 +/- 3.55 mmHg vs.17.08 +/-2.4 mmHg in controls (p>0.05). Analysis of visual field parameters at baseline showed a significant difference (p=0.0.48) between groups in MD levels -3.63 +/- 3.35 db (control group) vs -4.40 +/- 5.78 db (study group), but no difference (p>0.05) in PSD levels: 3.71 +/- 3.06 db (control group) vs 4.05 +/- 3.04 dB (study group). Perimetric progression was estimated at 24 months by Glaucoma Progression Analysis software (GPA –Humphrey Visual Field Analyzer II) using 6 reliable visual field (VF) exams. Progression rate was similar between groups - 0,19 ± 0,78 dB/year (OAG) vs. – 0.18 ± 0,05 dB/year (OAG+DM), p>0.05 and no other risk factor could have been linked to an increased progression rate, except visual field parameters at final and baseline evaluation, in both groups. **Conclusions:** on short term, functional deterioration in open angle glaucoma patients having early or moderate stages, occurs similarly in the presence or absence of diabetes. **Keywords**: SHORT TERM, PERIMETRIC PROGRESSION, OPEN ANGLE GLAUCOMA, DIABETES.

Glaucoma represents an optic neuropathy that can lead to optic nerve irrevesible damage and blindness. Among all glaucoma types, primary open angle glaucoma is

the most common form and a leading cause of visual loss wordwide (1).

Individual evolution is highly variable, despite adequate treatment (2,3). In consequence, visual field changes and progression rates differ greatly (4,5).

Recent literature data mention the contribution of risk factors in glaucoma progression such as older age, decreased corneal thickness, pseudoexfoliation, low ocular perfusion pressure, disk hemorhages, baseline visual field parameters or systemic diseases like diabetes, hypertension and cardiovascular events (3,6). Thus, the aim of our study was to assess if progression rate in patients with open angle glaucoma and diabetes is different on short term evaluation (24 months).

MATHERIAL AND METHOD

Our study was designed as cohort prospective clinical trial and included a total of 118 eyes from 118 patients (73% males, 27% females), recruited in the Ophthalmology Clinic - "Sf. Spiridon" University Hospital – Iaş i, between October 2012-March 2014. The patients were divided in two groups as following: 44 eyes, from 44 patients in the study group (diabetes + open angle glaucoma) and the control group with 74 eyes from 74 patients with open angle glaucoma (primary open angle glaucoma, normal tension glaucoma and pseudoexfoliative glaucoma). At enrollment all patients had a complete ophthalmologic evaluation and metabolic status assessment. We included only incipient and moderate forms of glaucoma, with mild or no diabetic retinopathy changes.

The diagnosis of glaucoma was defined in the presence of open anterior chamber angle on goniscopy, glaucomatous optic disc damage on clinical examination (focal or diffuse neuroretinal rim thinning, localized notching, or nerve fiber layer defect) and corresponding visual field (VF) defects. Glaucoma severity was graded according to Hodapp criteria (7). Diabetes was confirmed by previous tests in our patients in the Diabetology Department. We selected only those cases with no/mild form of diabetic retinopathy.

At baseline we recorded clinical parameters that included: age, gender, best corrected visual acuity (BCVA) by ETDRS chart, intraocular pressure (IOP) by Goldmann tonometer, CCT by ultrasonic pachymetrer (DGH-550, DGH Technology Inc., Exton, PA, USA) CD ratio (Volk 78D lens), number of topical medications, VF test parameters; at each follow up visit VA, IOP and VF tests were repeated. During the study the patients were followed each 3 months, when identical tests were performed. The last visit evaluated visual acuity, IOP, VF parameters. Majority of patients required topical therapy with 1-2-3 standard antiglaucoma medications. During monitoring, treatment was modified if the IOP was not efficiently controlled. Intermediary IOP was calculated by averaging all the IOPs taken during the 24 months follow up interval.

Exclusion criteria were related to significant lens opacities, other ophthalmic comorbidities, refractive errors >5D spherical, >3D cylinder. If both eyes were eligible, only one was chosed based on the worse mean deviation (MD) level at baseline

In Standard Achromatic Perimetry (24-2 SITA Standard SAP, Humphrey Field Analyzer II, Carl Zeiss Meditec Inc., Dublin, CA, USA) VF changes for glaucoma were defined if at least two of the three Anderson's criteria were fulfilled (three or more non-edged points in a cluster depressed to $P<5\%$ and one of which depressed to

P<1%, Glaucoma Hemifield Test outside normal limits and pattern standard deviation depressed to *P*<5%). Tests with fixation losses, false-positive or false-negative rates >20% were excluded from the analysis. 6 reliable visual fields were analyzed from each patient and the first two tests served as baseline landmark; progression was evaluated by Glaucoma Progression Analysis software. The event-based progression analysis, Glaucoma Progression Analysis (GPA) software used statistical criteria designed for the Early Manifest Glaucoma Trial to identify progression of VF defects (10). When significant (*P*<0.05) deterioration was evident on the pattern standard deviation (PSD) maps at the same three or more points on two consecutive follow-up tests, the GPA alarmed for 'possible progression'; if significant deterioration was seen at the same three or more points in three consecutive follow-up tests, GPA alarmed for 'likely progression'. When both the above criteria were not met, the software flagged as 'no progression detected'. For the purpose of this study, progression was quantitatively assessed by liniar regression analysis of the mean deviation (MD) changes over time; slopes of progression (decibels/ year) based on threshold maps and its level of significance (p-values) were calculated.

During the study the patients were followed each 3 months, when identical baseline tests were performed.

A SPSS 18.0 statistical software (SPSS Inc. Chicago, IL, USA) was used to process the data. Descriptive analysis was used on demographics, follow up time MD, PSD and IOP. Progression rate (MD slope) was calculated by linear regression analysis of MD values over time and expressed in dB/ year. Independent samples t tests were used for comparisons of continuous variables between groups. Also when paired groups were compared (baseline and final parameters) we used Wilcoxon test. Spearman's correlation coefficients (r) were calculated to assess the relationship between age, BCVA, MD, PSD, IOP and MD slope. Statistical significance was defined at the p <0.05 level.

RESULTS

From all the 118 eyes included in our study, 58.96% cases had primary open angle glaucoma (POAG), 41.03% cases had normal tension glaucoma (NTG) and 2.56% cases with pseudoexfoliative glaucoma (PXG), as shown in fig. 1. Sex ratio (F:M) was 1:2.9 (study group) and 1:1.5 (control group).

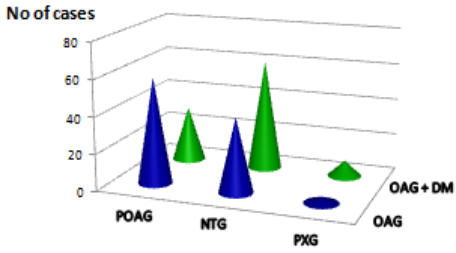

Fig. 1. Glaucoma type distribution in the two groups

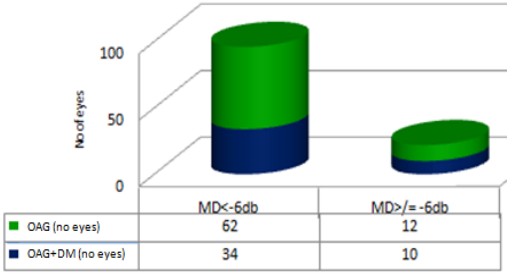

Fig. 2. Glaucoma severity classification (Hodapp criteria) in the 2 groups

Glaucoma severity classification showed early defects (MD < -6 db) in the study group of 77.27% vs. 83.78% in control group, whereas moderate defects (MD > -6 db) were found in 22.63% in the study group versus 16.21% in the control group, shown in fig. 2.

Baseline parameters and their evolution compared between the two groups were synthesized in table 1. The only statistically significant difference we found was related to the MD level at start, as the eyes with diabetes and OAG were more depressed in retinal sensitivity compared to the cases that had only OAG. All the other parameters were comparable and with no significant differences, meaning that we could compare homogenous groups, although the number of cases was not equal between them.

TABLE I

Parameters compared between groups at initial visit and during follow up

Baseline parameter	OAG+DM	OAG	T Student Test
Eyes (no)	44	74	nss
Age	62.69+/-1.8	64.31+/-1.66	nss-
Sex ratio (F:M)	1 : 2.9	1 : 1.5	nss
BCVA-ETRDS	0.91+/-0.15	0.89+/-0.16	nss
Sph. Eq	0.34+/-0.24	0.32+/-0.19	nss
CCT (μm)	538±35.9	528±62.8	nss
IOP (mmHg)	18.18±3.55	17.08±2.4	nss
MD (dB)	-4.40 ±5.78	-3.63+/3.35	p=0.02
PSD (dB)	4.05±3.04	3.71±3.06	nss
No. of medications	1.43±0.90	1.62+/-1.13	nss
Follow up parameters			
VF exams	6	6	-
BCVA-ETRDS	0.85±0.21	0.83±0.21	nss
IOP (mmHg)	16.49±2.81	16.62±2.90	nss
MD (dB)	-5.64±6.89	-5.3±6.25	nss
PSD (dB)	4.38±3.49	4.22±3.49	nss
MD slope (dB/ year)	-0.19 ± 0.78	-0.18± 0.05	nss

nss – not statistically significant

In evolution at 24 months we observed in the study group (OAG and diabetes) a statistically significant change in IOP level (18.18+/-3.55 mmHg vs. 16.49+/-2.81 mmHg, p=0.03) and in mean MD level (-4.40 ±5.78 vs. -5.64±6.89, p=0.04), aspect

shown in fig. 3,4.

Rate of glaucoma progression (MD slope) at 24 months was similar and did not differ statistically, but only in absolute value: -0.19 dB/year (OAG+DM) versus -0.18 dB/year (OAG), p=0.55 (t test).

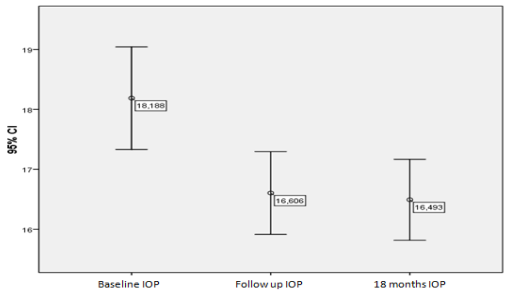

Fig. 3. IOP variation in the study group (OAG+DM): initial vs. follow up vs. final visit (24 months)

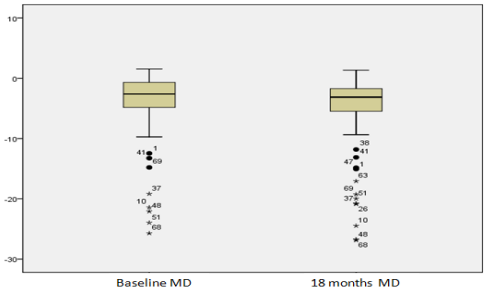

Fig. 4. MD mean values – baseline vs. 24 months in the study group (OAG+DM)

A more significant decrease in IOP level in the study group between baseline and after 24 months of follow up could be interpreted in the context of a more aggressive treatment applied initially to the patients with combined disease (OAG+DM) based on a hypothesis that all eyes are more prone to a more significant visual decay than the control group.

Spearman's coefficient detected significant correlations in the study group between MD slope and final/ initial MD levels (p=0.000), but also with baseline IOP (p=0.032). Intermediary IOP was correlated with final PSD (p=0.04), baseline IOP (p=0.000) and baseline MD (p=0.016). Both final and baseline visual field parameters were highly correlated, as expected at a p=0.000. Interestingly, MD slope in the study group was correlated (p=0.043) with the number of topical medications, but when age was controlled, the correlation became not significant any more.

In the control group we found the same type of correlations between MD slope and visual field parameters. Thus MD slope was correlated both with these parameters at baseline MD (p=0.039) PSD (p=0.011) and final MD level (p=0.011).

DISCUSSIONS

Progression rate and risk factors are among the most important aspects in glaucoma care because of their impact upon visual decay (8, 9, 10).

We found that visual field progression rate (MD slope) in both groups was dependent and correlated to both baseline VF parameters (MD/PSD) and also the final MD level ($p<0.05$). In this aspect, our results were similar to the OHTS (11) or EMGT study (12), where the initial MD level predicted progression rate in hypertensive glaucoma forms. Additionally in EMGT results, by reducing the IOP with 25%, progression occured later than in non-treated patients. This could also be the reason for our patients progressing at such a low rate compared to other studies, since medication was constantly changed/ added

to lower the IOP aiming for "the lower, the better" concept. No differences were detected in progression rate between patients with open angle glaucoma and diabetes compared to non-diabetic patients on short term evaluation. MD slope was similar in absolute values and no statistical significance could be attributed, p=0.55 (t test). Calculated MD slope at 24 months was -0.19 dB/year in OAG+DM group compared to -0.18 dB/year in OAG group.

In the study group we found correlations between MD slope and mean IOP at baseline; also there was a correlation between visual field decay and the number of topical medication, but when age was controlled the correlation was not significant anymore. Clinically this was associated with a more aggressive treatment from the beginning, in order to prevent a more rapid progression, as previously described by recent literature data where diabetes triggered faster glaucoma progression.

Preliminary analysis of progression at 24 months represented an important step in monitoring correctly both OAG patients and OAG+diabetes patients, as around this interval we could rule out fast progressing cases (MD slope >-2dB/year). Limitations of our study were basically the small number of patients and the short term evaluation of glaucoma progression, as all major clinical trials reported data after 4 years minimum (3,5); a clear image of glaucoma progression could have been obtained after a longer period of follow up, as it is known that in the first two years the investigators could only obtain a rough estimate. Still the reason for this evaluation was to rule out fast progressors (MD slope decay >-2dB/year).

CONCLUSIONS

In conclusion on short term evaluation (24 months), the visual field loss and functional deterioration in open angle glaucoma patients having early/ moderate stages, occurs similarly in the presence or absence of diabetes and no fast progressor was detected in our study during this interval.

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NEWS

THE INCIDENCE OF INVASIVE MRSA INFECTIONS IN GERMANY MAY BE DECLINING

As ECDC mentioned in the Annual epidemiological report for Antimicrobial resistance and healthcare-associated infections 2014 (data for 2012), while MRSA remains a public health priority as the percentage of MRSA isolates is still above 25% in seven of 29 reporting countries, mainly in Southern and Eastern Europe, including Romania (>50%), a recently published study showed that the incidence of invasive MRSA infections in Germany may be declining. MRSA infections in Germany are thought to be mainly healthcare-associated with only a small proportion, which are community-associated or livestock-associated. The notification of MRSA infections is mandatory in Germany since 2009 for microbiological laboratories. A team of researchers conducted a retrospective cohort study in the general population of Germany. The analysis was limited to the period from 2010 to 2014. Data of notifications of invasive MRSA infections for the above-mentioned period were extracted from the national surveillance database at the Robert Koch Institute as collected up to 1 March 2015 (Walter J, Haller S, Blank H et al.Incidence of invasive meticillin-resistant Staphylococcus aureus infections in Germany, 2010 to 2014. *EuroSurveill*. 2015; 20(46):pii=30067. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.46.30067).

Odette Popovici

PSYCHOTIC DISORDERS GENERATED BY AUTOIMMUNE ENCEPHALITIS (CLINICAL CASE)

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PSYCHOTIC DISORDERS GENERATED BY AUTOIMMUNE ENCEPHALITIS (CLINICAL CASE) (Abstract): Encephalitis is a brain inflammation, which could involve also the meninges. The etiology of encephalitis could be: viral, bacterial, fungal or autoimmune. Anti-NMDAR encephalitis is an immune disorder, easy to diagnose and is a treatable condition. Most patients with anti-NMDAR encephalitis develop a multistage illness that progresses from psychosis, memory deficits, seizures, to catatonic state and breathing instability. We present a case report of a 20-year old woman, who presented: amnesia, visual hallucination, illusions, seizures after that occurred following autoimmune encephalitis. The exact incidence of anti-NMDAR encephalitis is unknown, but it seems to be more frequent than any other known paraneoplastic encephalitis. The present case is important considering that autoimmune encephalitis is a rare frequency disorder in Romania, with patients presenting resounding psychiatric and neurological manifestations. **Keywords:** AUTOIMUNE ENCEPHALITIS, PSYCHOSIS, NMDA ANTI-RECEPTORS.

Infectious psychoses represents specific psychiatric manifestations that may appear during infectious diseases. The occurrence of psychic disorders is related to the type of infectious agent, the patient's constitution and the biological and psychological status of the affected organism. The infectious agent could be: a virus, bacteria, rickets, protozoa etc (1, 2). Since its discovery in 2007, the encephalitis associated with antibodies against the N-methyl-D-aspartate receptor (NMDAR) has entered the mainstream of neurology and other disciplines. The exact incidence of anti-NMDAR encephalitis is unknown (3).

In 2005, studies described a syndrome manifested by: memory deficits, impairment in consciousness and other psychiatric manifestations in young women diagnosed with ovary teratoma. In these patients specific anti-NMDA antibodies were detected. Two years later, in 2007, this syndrome was called anti-NMDA antibodies encephalitis.

The disorder occurs with or without tumor association, and responds to treatment but can relapse. The presence of a tumor (usually an ovarian teratoma) is dependent

on age, sex, and ethnicity. The disorder predominantly affects children and young adults. About 80% of patients with anti-NMDAR encephalitis are women (3, 4, 5). About 70% of patients have prodromal symptoms consisting of fever, nausea, vomiting, or upper respiratory-tract symptoms. Within a few days, usually less than 2 weeks, patients develop psychiatric symptoms (visual hallucination, illusions) and many are seen initially by psychiatrists. Patients respond well to immunotherapy, but psychiatric symptoms can be challenging to manage. Anxiety, insomnia, fear, grandiose delusions, hyper-religiosity, mania, and paranoia are frequent manifestations (3,6,7). After diagnosis, treatment focuses on immunotherapy and appropriate treatment of a tumor if it exists. Corticosteroids and intravenous immunoglobulin (Ig) or plasma exchange are recommended in managing the immune response (4).

In this case report we present a young woman that suffered an aggressive autoimmune encephalitis witch presents resounding psychiatric and neurological manifestations.

CASE REPORT

A 20-year-old woman, with no recorded medical history, presented in March 2015 with several medical complains, such as: headache, vertigo, tremor of the arms and balance deficits at the Infectious Diseases Hospital. Initially, the patient is hospitalized at the Infectious Diseases Hospital for 4 weeks. Further one, the patient was transferred to „Prof. Dr. Nicolae Oblu" Clinical Neurologic Hospital, for 2 weeks. During hospitalization at the Infectious Diseases Clinic (4 weeks) and at the Neurology Clinic (2 weeks), interdisciplinary examinations were made at the "Socola" Psychiatry Institute.

At this stage of the disease, the imagistic evaluation such as tomography, cranial-cerebral MRI (nuclear magnetic resonance imaging), MRI angiography, CIV were normal.

The disease progresses with new symptoms and aggravation of the older one with rapidly progressive confusion syndrome, psychiatric phenomena, tremor and bilateral extra-pyramidal rigidness. The psychiatric status is progressively altered with hallucinatory episodes, psycho-motor agitation, and disturbances of orientation and memory.

The neurological examination raises the suspicion of anti R NMDA autoimmune encephalitis and the patient is transferred at the Neurology Clinic for treatment. The examination revealed: confusion state, diminishing attention, speech and memory deficits, seizures, amnesia. Mini-Mental State Examination (MMSE) was initially 15 points. Also, during her hospital stay at the Neurology Clinic, the patient benefited from psychological consults, in witch an increase of MMSE from 15 points to 18 points was observed. The patient was examined by a gynecologist and neither ovary teratoma, nor other forms of tumors were found.

Further one, the patient developed generalized tonic seizures, and oral and tracheal intubations were performed and ICU (Intensive Care Unit) stabilization was mandatory for 41 hours.

Our patient, cerebrospinal fluid (CSF) was examined and the results indicated anti-NMDAR encephalitis. Patient electroencephalograms (EEG) presented increased incidence of theta rhythm on bilateral hemispheres with rare sharp, asynchronous left TPO (temporal-parietal-occipital) waves.

Without any psychiatric history, the pa-

tient presented into the ambulatory of the "Socola" Psychiatry Institute in March 2015, with confusion, visual hallucinations, unsteady gait and extra-pyramidal syndrome. At her second psychiatric exam, in April 2015 the patient was confused, partially oriented to space and time, and presented generalized tremor of the extremities. A month later, in May 2015, the patient had the next psychiatric consultation in ambulatory setting. Following the psychological exam a MMSE (Mini Mental State Examination) of 23 points is noted. The patient is treated with a combination of antipsychotic and benzodiazepines.

During Infectious Diseases Clinic and Neurological Clinic hospitalization, the patient received treatment with corticosteroids, cerebral anti-edematous medication, liver-protective medication, hydro-electrolytic support. The evolution is slowly favorable under a high dosage corticoid treatment.

DISCUSSION

We also discuss several confounding factors that often delay the recognition of this disorder, and propose an algorithmic strategy to guide treatment. Many symptoms that a patient presents (bizarre or stereotypical behaviors, insomnia, and memory deficits) cannot be classified as cortical (3).

The current knowledge about autoimmune encephalitis is that the disorder evolves progressively, with psychotic symptoms, convulsions, memory impairment, and speech disturbances up to a comatose state. In majority of cases, this disorder could be associated with tumors, such as ovary teratoma in approximately 90% of the total cases. In only 2% of the cases may include other types of tumors such as: Hodgkin's lymphoma, or mammary tumors. At our patient, neither ovary teratoma, nor other forms of tumors were found. The autoimmune encephalitis responds well to adequate treatment, but the risk of future relapses is also present (1, 5). About 70% of patients have prodomal symptoms consisting of headache, fever, nausea, vomiting, diarrhea, or upper respiratory-tract symptoms. Within a few days, usually less than 2 weeks, patients develop psychiatric symptoms and many are treated initially by psychiatrists. Short-term memory loss is common but underestimated (3).

Electroencephalograms (EEG) are abnormal in most patients, usually showing disorganized activity, sometimes with electrographic seizures. Slow, continuous, rhythmic activity in the delta-theta range predominates in the catatonic stage (3). Electroencephalograms (EEG) are abnormal at our patient, with predominant theta waves. The cerebrospinal fluid (CSF) is initially normal in 80% of patients and becomes abnormal later. At our patient, the results indicated anti-NMDAR encephalitis.

In our case we did not find teratomas, but the clinical evolution with: seizures, impaired memory, speech and comatose state and the laboratory exams sustain the diagnostic mentioned above. Almost 75% of the patients with severe anti-NMDAR encephalitis are fully recovered or remain with mild sequels (2, 3, 7). The present case received high-dose immunosuppressive therapy, anticonvulsants and antipsychotic to reduce symptoms. The patient remains under close medical supervision with mild sequels (amnesia) (4, 5, 6).

In patients without a tumor (in whom relapse is more common), continued im-

munosuppressive therapy is recommended for at least 1 year and periodic screening for an ovarian teratoma over 2 years (4).

CONCLUSIONS

In the present clinical case, the patient diagnosed with autoimmune encephalitis remains with mild symptoms such as a persistence of amnesia 2 months after the illness occurred. Also, high risk of relapse is considered in case of this patient.

This complex disorder requires sustained management and coordination of care between multiple medical specialties. Future clinical work will need to examine, in depth, the psychiatric manifestations of the disorder and how to provide optimal care, not only during acute faze hospitalization but also in the prolonged recovery process. Future studies should clarify the best treatment approach, and clinical strategies to accelerate the process of recovery.

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VALUE OF DOPPLER ULTRASOUND IN DECISION MAKING FOR CESAREAN SECTION

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VALUE OF DOPPLER ULTRASOUND IN DECISION MAKING FOR CESAREAN SECTION DECISION (Abstract). Based on the Doppler phenomenon, several tools were developed that are able to measure the blood flows in various vessels. The authors aimed at determining whether in some cases such an examination is sufficient to decide if a cesarean section is indicated. According to the literature and authors' own professional experience, the answer to this question is yes, as the most advanced method measures diastolic flows in the umbilical artery. Fetuses with intrauterine growth restriction are the most suited for this assessment. In less clear-cut cases, doctors may resort to middle cerebral artery, ductus venosus, uterine artery and umbilical vein assessments, or to biophysical score calculations. Any obstetrical approach should consider the fetal gestational age, condition and weight, and the corroborated results of the various flow and velocimetry measurements. The authors suggest a continuation of this research, the ultimate goal being the development of an overall score including several types of Doppler examinations, which would improve assessment accuracy. **Keywords**: DOPPLER ULTRASOUND, CESAREAN SECTION, INTRAUTERINE GROWTH RESTRICTION, FETAL MONITORING.

Doppler ultrasound scanning of fetal circulation has already become a current method for the assessment of prebirth fetal condition. Our paper aims to determine whether in some cases such an examination is sufficient to decide whether a cesarean section is indicated. The answer to this question is yes, and intrauterine growth restriction (IUGR) is the best application for this method.

History. Christian Andreas Doppler (1803-1853), an Austrian mathematician and physicist, discovered the wavelength alteration phenomenon depending on the relative speed between the source and the receptor. The piezoelectric effect was discovered by Pierre and Jacques Curie, in 1880.

The Doppler Effect started to be used for medical purposes towards the end of the 1950's. The pulsatile Doppler dates back to 1966 (1).

Umbilical artery (UA) velocimetry. Vasoconstriction of the tertiary villi is considered responsible for the velocimetric alterations in the umbilical artery blood flow, consisting in a decrease in diastolic velocities and an increase in the resistance and impedance indices. Evidence of any histological placental alterations due to

absent or reversed diastolic flow is still lacking. Currently it is believed that reversed blood flow is the last stage of the spectrum of placental insufficiency, with a high risk of perinatal death. It seems that over 50% of the placental vessels have to be obstructed before absent or reversed diastolic flow in the umbilical artery to occur in (2).

Studies in which IUGR fetuses were longitudinally monitored showed that up to 80% of them had abnormal umbilical indices two weeks before the fetal acute deterioration. End-diastolic velocities are present approximately one week before acute deterioration. Up to 40% of the fetuses with acidosis have this abnormal umbilical flow pattern.

In severely compromised IUGR fetuses, this pattern may predict perinatal morbidity and mortality, with a relative risk of 4 and 10.6, respectively, in babies with absent or reversed end-diastolic blood flow. These babies also have a higher risk of long-term abnormal neurological development (2).

According to the Growth Restriction Intervention Trial (GRIT), an emergency cesarean section performed on account of reversed end-diastolic blood flow does not significantly improve immediate and late prognosis and supports the need for a 24-48 hour interval to allow corticosteroid administration (2).

In a high risk population, the use of Doppler ultrasound scanning of the umbilical artery reduces perinatal morbidity and mortality. Umbilical artery Doppler should be the first-line choice for monitoring the SGA (small-for-gestational-age) fetus. If the umbilical artery Doppler is normal, the pregnancy may be monitored every two weeks (3).

When the values of umbilical artery Doppler velocimetry are abnormal and the delivery is not indicated, the fetus should be monitored twice a week to check the diastolic blood flow. Fetuses with absent or reversed end-diastolic blood flow should be examined daily (4).

The delivery of a premature SGA fetus with absent or reversed umbilical artery blood flow is recommended before 32 gestational weeks if pulsations in the umbilical vein are detected, the fetus is considered viable and steroids were administered (4).

If the SGA diagnosis was made after 32 weeks gestation and UA abnormalities were detected, the delivery should occur not later than at 37weeks gestation.

Delivery through cesarean section is recommended for SGA fetuses with absent or reversed diastolic blood flow. SGA fetuses with normal UA blood flow or abnormal UA blood flow and present diastolic blood flow may be delivered naturally, yet the rate of emergency cesarean section is high in these fetuses (4).

SGA fetuses with abnormal umbilical artery Doppler are smaller. There is a significant amount of data supporting the assumption that SGA fetuses with low umbilical artery blood flow are at higher risk of adverse perinatal outcome than normal blood flow fetuses (2).

There is a significant association between abnormal umbilical artery blood flow and perinatal outcome. Moreover, perinatal death is very rare if the umbilical blood flow is normal. Consequently, umbilical artery Doppler may be considered a tool allowing risk level differentiation in fetuses with low birth weight. Fetuses with normal Doppler ultrasound should be monitored more rarely. They may be considered normal SGA fetuses, and represent the lowest spectrum of healthy fetuses. Their

monitoring should be done accordingly (2).

There are few studies conducted during birth which compare the efficacy of CTG (cardiotocography) and Doppler, as a method able to reveal fetal distress.

Poor perinatal outcome is unusual in fetuses with IUGR and normal UA Doppler. Usually, associated diseases are present. Generally, IUGR and normal UA Doppler is a benign combination. A UA Doppler ultrasound of IUGR fetuses reduces the incidence of such obstetrical procedures as cesarean section and perinatal mortality. These fetuses are born at older gestational age and have higher average birth weight than fetuses with abnormal UA Doppler.

According to the 2014 Irish practical guidelines, IUGR fetuses with normal UA Doppler ultrasound should be monitored every two weeks and delivered between the 37 and 40 weeks gestation. These guidelines show that abnormal UA Doppler fetuses run a higher risk of adverse perinatal outcomes (5).

Middle cerebral artery (MCA) velocimety. In a premature SGA fetus, the MCA Doppler has limited accuracy for predicting acidemia and adverse outcome and should not be used to determine the time of delivery. In a term SGA fetus with normal UA Doppler, if the MCA PI value does not exceed the 5th percentile, the predictive value is moderate, but it may be considered when determining the time of fetus delivery (3).

If the MCA Doppler is abnormal, the delivery is recommended not later than 37 weeks of gestation (4).

The umbilical and cerebral flow relationship is thought to be more sensitive tool when it comes to discriminating between constitutional SGA and IUGR fetuses. Animal studies showed that this rate is rather related to the degree of hypoxia than to individual components (2).

For the IUGR fetuses, the most important decision is whether their extraction from a hostile uterine environment is more beneficial than the risks brought about by prematurity. Fetal well-being tests can be classified as chronic or acute. The former tests become progressively abnormal as hypoxia increases, and the latter are related to the acute changes that occur in the advanced stages of fetal compromise and usually precede fetal death by a few days. The test results should be correlated.

The value of cerebral Doppler for predicting adverse outcome in a general population of SGA fetuses is limited and has low sensitivity (2).

It has been suggested that near term MCA Doppler may be useful in the prediction of adverse outcome independent of UA Doppler (2).

The abnormal cerebroplacental ratio in IUGR fetuses is a useful marker able to predict severe preeclampsia and increased cesarean section rate. In late-onset IUGR this ratio is even more efficient. Both vascular tests need to be conducted in the umbilical and middle cerebral arteries (6).

The Doppler velocimetric parameters for MCA assessment are not an independent factor. Prematurity and placental insufficiency play a major role in newborn survival prognosis. The degree of cerebral circulation vasodilation does not influence survival, which seems dependent on the degree of impairment of fetoplacental circulation. Placental insufficiency is a complication involving high neonatal mortality rates, especially if the fetus is premature.

The degree of placental circulation impairment, analyzed by UA PI, influences prognosis on an independent basis. Doppler velocimetry of cerebral vascularization is

also associated with neonatal mortality. Of all the multivariate tests, prematurity and the degree of impairment of placental circulation are independent factors of the final outcome, in placental insufficiency pregnancies (7).

***Uterine artery (UtA) velocimetry*.** According to some protocols, uterine artery Doppler may be an independent IUGR criterion unlike other Doppler tests. Further research seems to be necessary before its inclusion in routine medical practice (2). We suggest performing UtA Doppler velocimetry in addition to basic ultrasonography during the 30 – 32 weeks gestation, in order to identify newborns with severe SGA (8).

***Umbilical vein (UV) velocimetry*.** A decrease in umbilical vein blood flow was also detected in an asymptomatic stage of the disease related to a decrease in placental volume (2).

***Ductus venosus (DV) velocimetry*.** Ductus venosus Doppler has moderate predictive value for acidemia and adverse outcome. It should be used to monitor premature SGA fetuses with umbilical artery abnormalities and to determine the time of delivery (4).

Umbilical blood tests are considered the gold standard for the assessment of newborn condition. The acid-base balance at delivery is more accurate than the Apgar score for the detection of intrapartum asphyxiation. There are undeniable connections between acidosis at birth and venous Doppler alterations, which account for the use of this method when making decisions to induce labor.

In conclusion, abnormal DV velocimetry is more common in high risk pregnancies than UA or MCA velocimetry. DV RI seems to be a better perinatal outcome predictor than the MCA S/D ratio (9).

Umbilical venous blood flow could be considered a more direct and physiological measurement of placental vascular function than that of umbilical artery Doppler indices because it indirectly reflects the amount of oxygen and nutrients reaching the fetus. Intrapartum UA Doppler is a poor predictor of the obstetrical outcome.

In normal circumstances, 20-30% of the umbilical vein blood passes the liver and is shunted through the ductus venosus to supply blood to the fetal heart and brain. In cases of chronic hypoxia, the ductus venosus is dilated and the amount of shunted blood increases to maintain adequate vital organ oxygenation. Consequently, diastolic blood flow velocimetry in the ductus venosus is the first parameter to be impaired by fetal hypoxia, followed by pulsations in the intra-abdominal part of the umbilical vein.

In conclusion, intrapartum CTG and UV Doppler monitoring may provide additional information in high risk pregnancies about the fetal condition and any suspected intrapartum fetal hypoxia (10).

Fetuses delivered through emergency cesarean section for suspected fetal distress had the lowest umbilical venous blood flow rates. If this blood flow is diminished, the cesarean section rate is higher and the relative risk is 2.83 times higher than in those with normal flows. Poorer outcome is also noted after birth. Measuring the umbilical venous flow may contribute to a hierarchical classification of pregnancy risk before delivery. The flow is calculated by multiplying blood velocity with vein diameter (11).

Biophysical score (BPS). Doppler abnormalities occurring in several vessels before 34 weeks gestation foresee an abnormal biophysical score and, hence, intra-

uterine death. After 34 weeks gestation death after abnormal MCA occurs faster than death predicted by the biophysical score. Fetal compromise progresses from placental to cerebral abnormalities, then to venous circulation abnormalities and only later to an abnormal biophysical score (12).

The median interval between a low MCA PI and death is 5 days. Fetus follow-up two times a week seems reasonable. The BPS becomes abnormal too late and it is not useful for indicating follow-up intervals. The BPS is slightly more useful before 34 weeks gestation f or determining the follow-up intervals in SGA fetuses in order to prevent fetal death. After 34 weeks gestation, MCA Doppler is the only parameter employed to set the fetal follow-up intervals (12).

Obstetric management. Unless other problems occur, normal SGA fetuses, the average umbilical and cerebral artery Doppler values of whom are normal, may be delivered naturally, under surveillance, yet before 40 weeks gestation.

Normal IUGR fetuses with abnormal cerebroplacental ratio (CPR) but without uterine artery Doppler changes are recommended a Doppler ultrasound two times a week and natural delivery after 37weeks gestation.

The obstetric management in the IUGR fetuses with abnormal cerebroplacental ratio or abnormal uterine artery Doppler and absent end-diastolic blood flow in the umbilical artery depends on their gestational age.

After 34 weeks gestation they may be delivered naturally, but in case of absent diastolic flow by cesarean section. Between 32 and 34 gestational weeks, reversed end-diastolic flow – corticosteroids and cesarean section within 24-48 hours; absent end-diastolic flow, steroids, daily Doppler ultrasound and delivery after 34 weeks gestation. Before 32 weeks – steroids and daily Doppler ultrasound until 34 weeks (2).

The IUGR fetuses with abnormal cerebroplacental ratio or uterine artery Doppler flow, with persistent ductus venosus pulsatility and abnormal biophysical profile: beyond 32 weeks – cesarean section, bellow 32 weeks – steroids, admissio, daily Doppler ultrasound until 32 weeka.

The IUGR fetuses with abnormal cerebral and uterine Doppler ultrasounds and decompensation – cesarean section at a tertiary care center. In those under 28 weeks, each case should be assessed by a multidisciplinary team. The family has also to be consulted (2).

Babies' outcome. The fetoplacental relationship is most strongly impaired during the intrapartum period. Uterine contractions may reduce by up to 60% the blood flow in the uterine arteries, and reduced placental perfusion may cause fetal distress. About 14.5% of the cases of neonatal cerebral palsy and encephalopathy are associated with intrapartum hypoxia. In developed countries, hypoxic-ischemic encephalopathy occurs in 2-3 out of 1000 live newborns, whereas in developing countries, the incidence is 10 times higher. Up to 63% of intrapartum hypoxia cases occur in pregnancies with no antenatal risk factors. The cerebroplacental (C/U) ratio analysis in term babies from low risk pregnancies before active delivery may predict a diagnosis of intrapartum fetal distress and the need for emergency cesarean section (13).

Correlations. In a study conducted in Ireland and published in AJOG in 2014, 76% of the 1200 IUGR pregnancies required active decision making regarding the mode and timing of delivery. Forty

percent required pre-labor cesarean section and 11% of them had abnormal Doppler velocimetry as delivery indication (14).

The cesarean section rate for suspected fetal distress was higher in normal weight and low cerebroplacental ratio fetuses than in SGA and normal CPR fetuses, which proves that CPR is more commonly associated with fetal distress than birth weight. Only SGA fetuses with abnormal CPR were admitted to a neonatal emergency unit (NNU) more frequently. Neonatologists are more prone to admit babies to NNU if they are younger (15).

Stratification of birth outcomes is more accurate using Doppler indices than newborn weight. The study supports the assumption according to which normal weight babies may also be compromised due to placental insufficiency and recommends Doppler ultrasounds in all of them. Most national guidelines recommend Doppler ultrasound only in complicated SGA pregnancies and not in normal weight babies (15).

The C/U ratio was often used in SGA studies. It seems it was the first time when it was used in normal weight and term babies. All those in the 90th percentile had no problems at birth and did not require emergency cesarean section.

The effect is independent of parity, gestation, birthweight and mother BMI. The technique should be generalized in order to classify pregnancies before delivery (13).

CONCLUSIONS

In our obstetrical experience, Doppler follow-up of IUGR babies is a good indicator of their health and may help the decision making for a caesarian section.

According to a particular protocol this method slightly reduces the cesarean section rate and decreases premature birth frequency (16): the absent or reversed blood flow in the umbilical artery is the most efficient cesarean section indicator; the MCA flow is more reliable in near-term pregnancy; the biophysical score is more reliable at younger gestational ages; ductus venosus examination often helps; some authors suggest that umbilical vein blood flow should be measured in all pregnancies; sometimes, uterine artery velocimetry is also necessary; measurements of various vascular systems should be correlated and an overall integrative score should be developed.

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NEWS

IMPACT OF BACTERIAL CELLS AND SECRETOMES ON WOUND HEALING

According to Brothers KM *et al.*, bacterial cells and secretomes from *Serratia marcescens (*95%), *Pseudomonas aeruginosa* (71%) and *Staphylococcus aureus* (29%) strains inhibit human corneal epithelial cell migration *in vitro* and *ex vivo*, thus affecting the process of wound healing. Lipopolysaccharide (LPS) seems to be a key factor in this inhibitory mechanism, since LPS core biosynthetic genes are required to inhibit corneal epithelial cell migration. Purified LPS from *S. marcescens* inhibits epithelial cell migration *in vitro* and wound healing *ex vivo*, while after LPS depletion of *S. marcescens* secretomes, migration of epithelial cells was no longer inhibited, suggesting that *S. marcescens* LPS is sufficient for inhibition of epithelial wound healing (Brothers KM, Stella NA, Hunt KM, Romanowski EG, Liu X, Klarlund JK, et al. Putting on the brakes: Bacterial impediment of wound healing. Sci Rep. 2015;5:14003. doi: 10.1038/srep14003).

Teodora Vremera

ATYPICAL PRIMARY HYPERPARATHYROIDISM DUE TO HYPOVITAMINOSIS D

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ATYPICAL PRIMARY HYPERPARATHYROIDISM DUE TO HYPOVITAMINOSIS D (Abstract): Vitamin D deficiency is nowadays very common in the general population and also in patients with primary hyperparathyroidism. Hypovitaminosis D may modify the clinical features and the severity of primary hyperparathyroidism. We present the case of a 75-year-old woman with a 10 year history of nephrolithiasis and severe osteoporosis, with multiple fragility fractures. Her bone and kidney status required a more thorough metabolic assessment. Despite minimal changes in serum calcium and phosphate levels, parathyroid hormone (PTH) level was markedly elevated. Ultrasound and specific Sesta-MIBI scintigraphy diagnosed and localized a left parathyroid adenoma. Vitamin D assessment showed levels in the range of hypovitaminosis. Vitamin D deficiency may mask hypercalcemia despite high serum PTH levels, and does not seem to diminish but on the contrary increases the risk of kidney lithiasis, as well as the deleterious effects of hyperparathyroidism on bone. **Keywords:** PRIMARY HYPERPARATHYROIDISM, VITAMIN D, PARATHYROID HORMONE.

Primary hyperparathyroidism (hPTH) is currently considered one of the most common endocrine diseases, with a prevalence reaching 1-2% among women aged over 50 (1). Excessive parathyroid hormone (PTH) secretion may be deleterious due to massive bone demineralization and severe hypercalcemia accompanied by a higher risk of kidney lithiasis and soft tissue calcification. However, the spectrum of severity of hPTH has widely changed in recent decades, especially since milder forms are diagnosed by routine calcium measurement, cervical ultrasound and bone mineral density assessed by Dual X-ray absorptiometry (DXA).

PTH and vitamin D are known to be the two major regulators of calcium and bone metabolism, the two hormones having direct interferences. PTH activates vitamin D to 1,25(OH)2D3 in the kidney by stimulating 1-alpha hydroxylase. Active intestinal calcium absorption is directly stimulated by 1,25(OH)2D3. At its turn, 1,25(OH)2D3 downregulates PTH secretion by direct effects on the parathyroid glands (2).

The increase of life span and the decrease of sunlight exposure due to modern

life caused a vitamin D deficiency epidemic in the general population. Interestingly, vitamin D deficiency was found by several authors to be more common in patients with primary hPTH than in the general population (3,4,5). The consequences of vitamin D deficiency in these patients and its appropriate management are unclear. We present the case of a 75-year-old woman diagnosed with primary hPTH and vitamin D deficiency.

CASE REPORT

BG, a 75-year-old woman was admitted to the Endocrinology Department of "St. Spiridon" Hospital for the metabolic investigation of severe osteoporosis with multiple pathological fractures. Our third age patient had a body weight in the lean range (BMI = 21 kg/m2), therefore the presence of osteoporosis was not a surprise. However, the patient had a profoundly decreased bone mineral density (BMD) evaluated by DXA in all locations, with the lowest T-score found at the distal 1/3 of the radius (Table I), highly suggestive of primary hPTH. Anamnesis revealed not only a 10-year history of multiple fractures, but also relapsing nephrolithiasis for which she underwent several sessions of extracorporeal shockwave lithotripsy, increasing our suspicion of primary hPTH. However, serum calcium and phosphate were only mildly out of the normal range (Table II).

TABLE I

Bone mineral density assessed by Dual X-ray absorptiometry

	T-score	BMD (g/cm^2)
LUMBAR	-4.4	0.635
hip	-3.7	0.491
forearm	-5.4	0.371

The association of "stones and bones" complications raised the suspicion of primary hPTH. In contrast with the important skeletal and kidney impact, the metabolic profile of our patient showed only mild hypercalcemia (10.78 mg/dl) and hypophosphatemia (2.25 mg/dl), albeit significant hypercalciuria (408 mg/24h). The measurement of PTH revealed an elevated level (228 pg/ml). Renal function of our patient was intact: urea 36 mg/dl (reference range 21-43 mg/dl), creatinine 0.82 mg/dl (reference range 0.57-1.11 mg/dl). The discrepancy between the mild changes in serum calcium and phosphate and the marked increase in serum PTH level made us evaluate vitamin D levels that were found decreased (10.59 ng/ml) (tab. II).

TABLE II

Comparison between metabolic parameters

Normal ranges	2013	2014
Calcium 8.8-10.2 mg/dl	10.78	10.89
Phosphate 2.5-4.7 mg/dl	2.25	2.2
Calciuria 100-300 mg/24h	408	445
PTH 15-65pg/ml	228	242
25-OH-D >30 ng/ml	10.59	-

Neck ultrasound revealed a hypoechogenic nodule, posterior to the left thyroid lobe, measuring 1.6x0.8cm, with peripheral vascularization (fig. 1). The specific scintigraphic investigation showed increased uptake of Technetium (Tc) 99m Sestamibi (MIBI) in the suspected nodule, localizing a left parathyroid adenoma (fig. 2).

The patient was advised to undergo a minimally invasive approach to remove the

abnormal parathyroid gland, under protection by bisphosphonate therapy (alendronate, 70 mg/week) and vitamin D supplementation. The patient did not present, however, to the Surgery Department as scheduled.

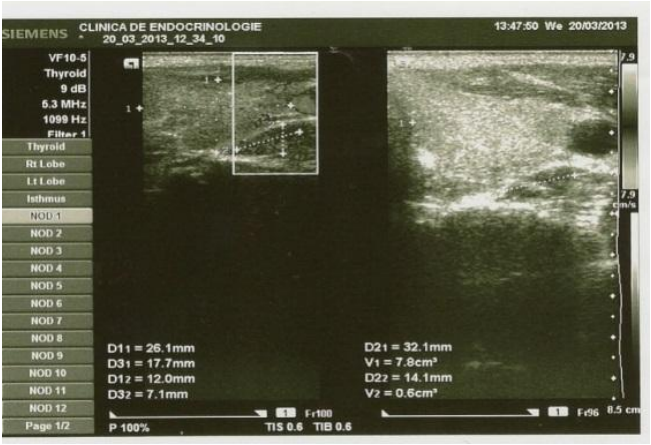

Fig. 1. Cervical ultrasound showing a left parathyroid adenoma

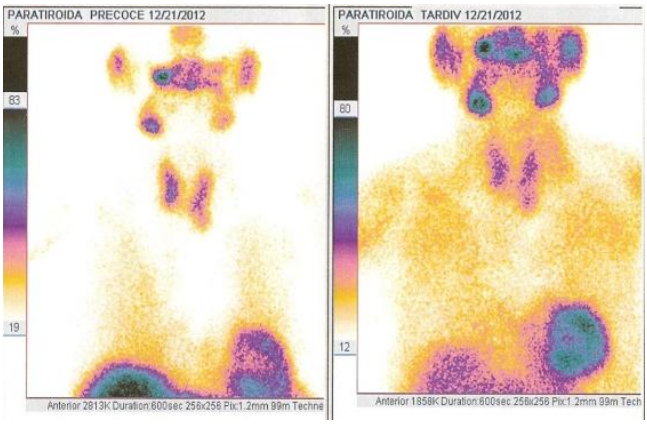

Fig. 2. Scintigraphic investigation, localization of a nodule posterior to the left thyroid gland

The patient presented to our Department for another check-up in January 2014, with a somewhat aggravated metabolic profile (tab. II). 25-OH-D level was not reassessed, but vitamin D supplements were not taken. Despite persistence of primary hPTH, DXA revealed a general increase in BMD following antiresorptive treatment (Lumbar T-score -3.1; Hip T-score -2.7; Forearm T-score -5.1) (fig. 3).

The patient underwent a left inferior parathyroidectomy with the excision of a parathyroid adenoma with a maximum diameter of 1.6 cm (fig. 3). Immediately after excision, PTH dropped to a normal level of 22 pg/ml, confirming an excessive PTH secretion.

The patient had a favorable outcome af-

ter surgery, with phosphocalcic parameters within normal range after just one day (calcium 9.03 mg/dl, phosphate 3.46 mg/dl, PTH 13.11 pg/ml).

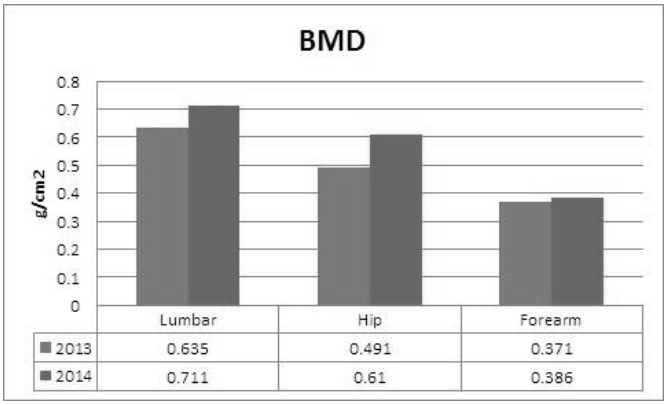

Fig. 3. BMD improvement after one year of treatment

Fig. 3. Left inferior parathyroid adenoma

DISCUSSION

Primary hPTH is usually accompanied by hypercalcemia, hypophosphatemia and increased_vitamin D level due to excessive 1-alpha hydroxylase activation by PTH. Co-existence of hypovitaminosis D in hPTH may decrease the active intestinal calcium absorption, thereby diminishing serum electrolyte changes (3, 5). Hypovitaminosis D may also contribute to a further tumor growth and increase in PTH secretion due to lower calcium and vitamin D levels and to a consecutive decrease of the negative feedback exerted on the parathyroid adenoma

(3,6-9). This leads to a vicious circle, worsening the renal and bone changes (9, 10, 11).

The higher incidence of hypovitaminosis D in hyperparathyroidism could be explained by several hypotheses. Due to lack of negative feedback, low vitamin D levels may favor the development of an autonomous parathyroid gland, with subsequent occurrence of hyperplasia and adenoma transformation, or accelerated course of a preexisting adenoma (12). PTH-mediated increase of $1,25(OH)_2D$ may alternately reduce 25(OH)D levels by inhibiting the hepatic synthesis of vitamin D (3) .

In our case, hypovitaminosis D partially masked the typical serum metabolic profile of primary hyperparathyroidism, but did not impede the deleterious impact of hPTH on bone loss and kidney stone formation. Delay in diagnosis was also influenced by the additional risk factors for osteoporosis in our patient (age, sex, body mass). PTH tends to be, however, more catabolic at cortical sites, especially at the distal 1/3 of the radius, and this particular modification was also observed in our patient. The association of severe osteoporosis and relapsing renal lithiasis with hypercalciuria increased the suspicion of primary hyperparathyroidism. Despite the persistence of primary hyperparathyroidism, therapy with bisphosphonates had protective effects on bone, but did not diminish PTH levels or improve the metabolic profile of our patient.

CONCLUSIONS

Our case report highlights that when hypovitaminosis D is associated with hyperparathyroidism it masks hypercalcemia, but may aggravate the deleterious effects of the disease on bone loss and on kidney stone formation, so any patient with relapsing lithiasis and/or disproportionately severe osteoporosis should be suspected of primary hyperparathyroidism; if the metabolic parameters are only slightly modified, vitamin D deficiency should be suspected. Vitamin D supplementation in these patients remains a question of debate.

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NEWS

TOPICAL METHOTREXATE FORMULATION FOR PSORIASIS TREATMENT

Psoriasis, a chronic inflammatory skin disease, affects 1 to 3% of the world population. The therapeutic agents used in the treatment of this disease are corticosteroids, dithranol, salicylic acid and vitamin D analogues. The nanostructured lipid carriers (NLCs) play an important role in targeting the drug to the skin. In order to obtain new topical formulations for the treatment of psoriasis, NLCs were used for incorporation of methotrexate. The formulations contained methotrexate, Witepsol S51, oleic acid, Polysorbate 60 (MTX_NLC-P60) or Polysorbate 80 (MTX_NLC-P80). The formulations were tested for their stability by measuring the particle size, the surface charge and the amount of MTX after storage at 25°C for 28 days. Scanning electron microscopy was used to study surface morphology. All formulations showed a high physical stability. *In vitro* skin permeation was evaluated using a Franz cell. The results revealed an increased penetration of MTX_NLC-P60 (5.8 ± 0.2%) and MTX_NLC-P80 (4.2 ± 0.1%) as compared to free MTX (3.6 ± 0.2%). *In vitro* evaluation of drug release from both formulations in a simulated physiological environment showed a quick initial phase of drug release (64%) in the first 2 h, followed by a sustained release until 8 h. The study revealed the potential use of NLCs in the development of topical formulations for psoriasis treatment (Pinto MF, Moura CC, Nunes C, Segundo M *et al*. A new topical formulation for psoriasis: development of methotrexate-loaded nanostructured lipid carriers. *Int J Pharm* 2014; 477 (1-2): 519-526).

Cristina Lungu

COEXISTENCE OF CELIAC DISEASE AND SYSTEMIC LUPUS ERYTHEMATOSUS IN A 6-YEAR-OLD GIRL-CASE REPORT

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COEXISTENCE OF CELIAC DISEASE AND SYSTEMIC LUPUS ERYTHEMATOSUS IN A 6-YEAR-OLD GIRL (A CASE REPORT) (Abstract): Systemic lupus erythematosus (SLE) is a serious and potentially fatal syndrome characterized by an autoimmune assault on various organs and systems that may include the skin, joints, central nervous system, heart and kidneys. Recent research shows that gluten sensitivity causes more than just celiac disease and gluten has been linked to numerous autoimmune conditions. We report here a 6-year-old girl presenting with malaise, abdominal pain, loss of appetite, abdominal distension. After three weeks she developed other symptoms such as arthralgias, malar rash, being finally diagnosed with SLE and possible autoimmune hepatitis. The suspicion of celiac disease was based on a combination of symptoms (poor growth, iron deficiency anemia, chronic abdominal pain, abdominal distension, constipation, "sad child"), IgA deficiency, in the presence of SLE diagnosis. Positive diagnosis of celiac disease was confirmed by the presence of an anti IgG anti-transglutaminase antibodies titer of 120 EU/ml (normally less than 20 EU/ml). Small bowel biopsy showed a IIIB1 stage according to the Marsh classification. Three methylprednisolone pulses were promptly administered, followed by oral prednisone (2 mg/kgbw/day) with a good outcome.
Keywords: SYSTEMIC LUPUS ERYTHEMATOSUS, PEDIATRIC CELIAC DISEASE, AUTOIMMUNE HEPATITIS, OVERLAP SYNDROME

Systemic lupus erythematosus is an autoimmune disease characterized by auto antibodies directed against self antigens, immune complex formation and immune disturbances which may affect joints, skin, kidneys, heart, lungs, the nervous system and the immune system (1). Children generally have a more severe disease onset and progression than adults (2, 3).

The disease is more common in girls and is quite rare under the age of 6 years. Diagnosing SLE in children is usually problematic. It was previously believed that the association between celiac disease (CD) and SLE is rare and uncertain (4). However their coexistence was proved by showing that both diseases have an autoimmune origin and share human leukocyte antigen (HLA)-B8 and histocompatibility antigen (HLA)-DR3 (5).

The manifestation of Celiac disease can occur before or after the diagnosis of lupus (6). The description of overlap syndromes

in terms of auto antibodies is based on the assumption that the pattern of autoimmunity reflects the underlying cause for the disease (7).

We present a severe case of a young patient who was diagnosed with overlap syndrome: SLE, celiac disease and hepatitis.

CASE REPORT

We report the case of a 6-year-old girl who presented with malaise and abdominal pain. She had no chronic medical conditions and no significant family history of medical illnesses. Before the onset of symptoms she was in good health but her mother complained about poor weight gain and constipation. She also had stated that her daughter was always a "sad child".

Current disease history stands ten days before admission with malaise, fatigue, pale skin, abdominal pain and loss of appetite. On physical examination, the patient was in obvious discomfort, her body temperature was 37°C, her skin was pale with discrete jaundice, nutritional status revealed a low weight for age (2 standard deviations for normal weight), her blood pressure was normal but a systolic murmur was detected. Further on, full clinical exam revealed hepatosplenomegaly, no palpable lymphadenopathies or abdominal bloating.

The laboratory testing revealed normal white blood cell (WBC) count of 6.88 × 103/μL, moderate anemia with red blood cells (RBC) count of 3.05 × 106/μL, a hematocrit of 24.9%, hemoglobin level of 7.6 g/dL and platelet counts of 320× 103/μL. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated (AST=1641 U/L, ALT=1554 U/L), indirect hyperbilirubinemia at 2.03 mg/dL was also detected. Liver functional involvement syndromes revealed low fibrinogen values of 179 mg/dL (normal between 200-400 mg/dL), low albumin values of 37% (normal between 53.5-66.5%), elongated TQ of 21.7 seconds (normal between 13-17 seconds), hypergammaglobulinemia of 42% and no abnormal bilioexcretory syndrome. Acute and chronic liver diseases were ruled out by negative serologies for viral hepatitis A, B, C, cytomegalovirus (CMV) and Epstein-Barr virus (EBV); also human immunodeficiency virus (HIV) infection was ruled out by negative serologies. An abdominal ultrasound was performed and it showed hepato-spleno-megaly without deep lymphadenopathy.

She promptly received arginine (10mL/kgbw/day) as hepatoprotective perfusions. Meanwhile we excluded metabolic hepatitis, alpha-1-antitrypsin deficiency, Wilson's disease (ceruloplasmin, cupruria and cupremia with normal values), cystic fibrosis (iontophoresis with values of 25 mmol/L NaCl). She had a very high erythrocyte sedimentation rate (ESR) of 138mm/h and a positive C-reactive protein (CRP).

Anemia associated with indirect hyperbilirubinemia imposed investigations for a possible hemolytic anemia; reticulocytes were increased (5%), but globular resistance, autohemolisis and alkali-resistant hemoglobin were all within normal ranges. The Coombs test was positive, confirming autoimmune hemolytic anemia. We also evaluated serum iron with low values of 25 μmol/L, total iron-binding capacity (TIBC) of 455 μg/dL (normal 228-428 μg/dL), and serum ferritin of 20 ng/mL (normal values between 13-150 ng/mL). Bone marrow aspiration and biopsy showed all three cell line elements without evidence of malignancy.

As a result of these investigations we

suspected an autoimmune disorder. Immunological profile revealed a positive value of antinuclear antibodies (AAN) 1 index (negative <0.7 index), the antideoxyribonucleic acid antibodies (dsDNA) positive titers, with high values of 120.4 IU/mL (negative <10), a normal complement C'3 fraction 99 mg/dL (normal between 90-180 mg/dL), a negative titer of anti-LKM 1 antibodies (liver-kidney microsome) and anti-smooth muscle antibodies and absence of SLE cells and cryo-globulins.

Other immunological investigations have revealed immunoglobulin A (IgA) deficiency of 20.37mg/dL(normal 91-255 mg/dL), hyper IgG of 6824.15 mg/dL (normal 850-1300 mg/dL) and normal levels of IgM.

The result of renal function tests were in the normal range (serum urea 30 mg/dL, serum creatinine 0.72 mg/dL). No microscopic hematuria or proteinuria was detected. Also serum glucose, amylases and cholesterol were on normal range but triglycerides are elevated values of 165 mg/dL (normal 40-135 mg/dL).

During her three-week hospitalization her symptoms gradually worsened and other important elements occurred: malar rash and arthralgia in the small joints of the hands, without local inflammatory phenomena. We performed a chest X-ray for exclusion of TB infection and an echocardiography who revealed normal structure and function of the heart with minimal presence of pericardial reaction.

Finally positive lupus diagnosis was based on 6 of the 11 required ACR criteria: "butterfly rash", arthritis, serositis (pericarditis), hematological disorder: autoimmune anemia, immunological changes as SLE cells, antideoxyribonucleic acid antibodies (dsDNA) positive titers and positive antinuclear antibodies (ANA).

Because the patient maintained unexplained abdominal distension in the context of the existence of an autoimmune disease we considered necessary to perform further investigations for other autoimmune disorder. We detected an elevated level of antitransglutaminase IgG antibody titer of 120 EU/ml (normal less than 20 EU/ml); IgA antritransglutaminase antibodies were negative. The small bowel biopsy result revealed a IIIB1 stage according to the Marsh classification.

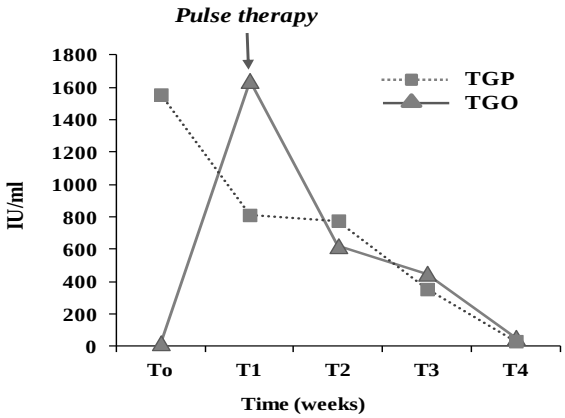

Fig. 1. Evolution of transaminases; TGO and TGP liver enzymes decreased significantly after initiating pulse therapy.

We initiated the treatment with corticosteroids, three methylprednisolone pulses (1 g/1.73 m2 per day) and gluten free diet. Patient's response to steroid along with gluten-free diet was excellent, with a significant decrease of liver enzymes (TGO and TGP) (Fig. 1), ESR (26 mm/h) and improvement of Hb to 8.8 g/dL. After that she was treated with oral Prednisone (2 mg/kgbw/day).

The particularity of this case consists in the complexity onset, with accompanying hepatitis, young age and the association of other autoimmune disorders: celiac disease and hepatitis

DISCUSSION

SLE is one of the most diverse autoimmune diseases as it may affect more organs in the body. It has a broad spectrum of clinical and immunological manifestations (1, 8).

Nowadays, among people with lupus "overlapping" autoimmune conditions, including celiac disease, are common. On the other hand, according to statistics, patients diagnosed with celiac disease will be diagnosed with other autoimmune diseases in their lifetime: collagen vascular disease, type 1 diabetes, rheumatoid arthritis, Sjögren's syndrome, liver disease (2, 9).

People with celiac disease face three-times higher risk of SLE compared to the general population (10).

Celiac disease is a multifactorial, autoimmune disorder that occurs in genetically susceptible individuals. It is triggered by an environmental factor-gluten (11). Testing for CD should be performed in children with unexplained poor growth, iron deficiency anemia, chronic abdominal pain, constipation, and abnormal liver biochemistry, symptoms that our patient has showed (12).

Serological diagnostic based on determination of autoantibodies against transglutaminase type 2 (TG2) and endomysial antibodies (EMA) and can be confirmed by result of small bowel biopsy and HLA serotyping (11). HLA testing should be performed in cases where there is an uncertain diagnosis of CD (cases with negative CD specific antibodies and mild changes on proximal small intestinal biopsy) (4).

In our case the suspicion of celiac disease was based on combination of symptoms (poor growth, iron deficiency anemia, chronic abdominal pain, abdominal distension, constipation, "sad child"), immunological changes (IgA deficiency) and the presence of other autoimmune diseases.

It is also important to test total serum IgA to be able to interpret the IgA anti-TG2 result (as IgA deficiency is more frequent in patients with CD) and, in those individuals with IgA deficiency, IgG TG2 antibodies may be used (although these are less specific).

In our case with IgA deficiency was necessary to use IgG TG2 antibodies; it was not necessary to carry out HLA serotyping because the biopsy was conclusive.

In practice, the diagnosis of an overlap syndrome makes little difference to treatment although the detection of an autoantibody does help the clinician to anticipate particular complications (13).

Management of SLE in children is challenging (9). The unpredictability of the disease, with remissions and relapses, can be difficult for the family and child and for doctors. Corticosteroids are the mainstay of treatment (14).

Most of the children will also require additional medications depending on organ involvement. However, investigations and treatment should not be delayed until these criteria are fulfilled. Our patient requires careful, long-term follow-up and should adopt a gluten-free diet to avoid future complications of the condition.

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CIRCULATING TUMOR CELLS IN RECTAL CANCER PATIENTS – A REVIEW OF THE LITERATURE

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CIRCULATING TUMOR CELLS IN RECTAL CANCER PATIENTS-A REVIEW OF THE LITERATURE (Abstract): Rectal cancer treatment has been continuously improving in the past decades and research has rightly been directed for this purpose, because this is a major public health issue. On the other hand, circulating tumor cells have emerged in the past two decades as an important element in oncology, because of the large potential they offer. They have been studied mostly in breast cancer and have already been implemented as a tool in certain strategies in this field. Thus, circulating tumor cells in rectal cancer may have the same important applicability, with a wide range of usages, from screening to diagnostics, guidance of the treatment and even follow-up. This article aims to review the literature and exhibit data about the pathophysiology, utility and methods of identification of circulating tumor cells with focus on their link to rectal cancer. **Keywords:** RECTAL CANCER, CIRCULATING TUMOR CELLS.

The importance of rectal cancer resides both in its high incidence, as well as in the complexity of the treatment, that relies in most cases on a multidisciplinary team, being a multimodal approach.

Circulating tumor cells (CTCs) are an innovative field in oncology. This subject is widely investigated in the recent years and is of great interest in the research field due to the various advantages of studying the tumor cells in the blood stream. These cells can derive either from the primary tumor, or from its metastases, thus being an important marker in evaluating the prognostic of the disease, monitoring the response to certain therapies, or selecting patients as targets for certain therapies (1).

Rectal cancer, an important public health issue and its connection to circulating tumor cells

According to the Globocan 2012 study, colorectal cancer is the second neoplasia in incidence in both sexes, both in Romania and within the European Union. On a global scale, it is situated in incidence after pulmonary and breast cancer. The incidence of 13.1%, the 5-year prevalence of 13.3%, as well as a 11.9% rate of mortality make it an important public health issue, especially in developed countries.

With all these aspects aside, the mortality through this type of cancer is on a

descending trend in most developed regions. All this thanks to the increase in national and local screening programs, due to the increase in the efficiency of the diagnostic methods, and because of the improvements brought to treatment methods (2).

Screening in rectal cancer is one method of decreasing mortality, through an early diagnostic and the opportunity yielded by precocious identification of potentially malignant lesions or preneoplastic polyps. The clinical practice guidelines elaborated by the National Comprehensive Cancer Network (NCCN) serve as tools to direct the clinician towards this endpoint. Although not applied on a national level now in Romania, these guidelines setup a consensus between colorectal cancer specialists, that can be taken into consideration when aiming for a precocious diagnosis and a suitable treatment (3).

Bearing this aspect in mind, two categories of screening tests arise: structural tests and stool-based tests.

Structural tests give the possibility of identifying incipient lesions or even polyps that have the potential of malignant transformation. These tests are based on imaging-colonoscopy and radiology. Rigid rectoscopy, rectosigmoidoscopy, colonoscopy, barium enema and computed-tomography (which gives the possibility of obtaining a virtual colonoscopy) are the methods included in this category, which are, of course, invasive, with risks that increase with age and associated morbidities (4).

On the other hand, stool-based tests, consisting of the fecal occult blood test, the fecal immunochemical test and the detection of altered DNA, have the advantage of non-invasiveness, but are, on the other hand, poor in means of specificity; the probability of detection of adenomatous polyps is low. The immunochemical test brings an improvement in the matter of sensitivity (5, 6) and the DNA testing is the best of the non-invasive methods in means of sensitivity but lacking in the mater of cost-effectiveness (7).

All in all, the gold standard for colorectal cancer screening is the colonoscopy, with great advantage of providing a biopsy in given cases. Moreover, a positive non-invasive test will lead to a colonoscopy evaluation (3).

Another factor that aims to improve mortality rates is the treatment and its continuous developments. The multidisciplinary approach, based on the decisions taken by a complex team of specialists aims to individualize the treatment based on the characteristics of each patient. On this subject, a sequential treatment plan is taken into discussion; the decision to apply a neoadjuvant treatment or opt for upfront surgery is based on the extent of the tumor, set up by imaging means. The efficiency of neoadjuvant radio chemotherapy on rectal cancer has been demonstrated through various trials, becoming routine in locally advanced, non-metastatic tumors (8, 9). However, some tumors respond well to neoadjuvant treatment, whereas others do not, with a stationary or even progressive status after the long-term scheme of neoadjuvant radiotherapy, with a considerable amount of time lost in the detriment of the patient. Therefore, additional predictive factors for the neoadjuvant treatment must be developed to reduce this setback. CTCs may play an important role in this matter, providing the examiners with important information.

The adjuvant treatment in rectal cancer is based on the prognostic value of the

tumor staging, along with additional factors, such as microsatellite instability, tumor budding, circumferential resection margins, perineural invasion, tumor regression grade, and K-ras genotype. However, bearing in mind the actual tendency of treatment individualization, other factors are studied, to provide access to the best therapeutic option. CTCs might have an important role in this aspect as well.

Circulating tumor cells and their presence in the body

CTCs are cells of tumor origin that are released into the blood stream. Even if they were identified and described over one century ago, in 1869, they became a domain of interest approximately one decade ago, when studies performed on CTCs, methods of identifying and counting them began to flourish in oncology (10). They are considered the main mechanism of metastasis, thus studying them contributing to a better understanding of the metastasis cascade and helping better the treatment of the oncologic disease (11). Moreover, there is the notion of disseminated tumor cells (DTCs), that result from the embedding of CTCs in a metastasis site (12).

CTCs are rare in the patient's bloodstream - 1 to every $10^6/10^7$ circulating mononuclear cells. Studies from 1975 mention a quantity of one million tumor cells released per each gram of the tumor (13). However, this number is overrated, bearing in mind the conditions in which CTCs are released into the bloodstream and the half-life of these cells within the bloodstream.

The modality in which they leave the tumor site varies: either single or in clusters - clusters can be homotypic or heterotypic (14, 15). Depending on their properties, there are different characteristics specific to each category. Thus, single CTCs are more frequent in the bloodstream, with a good chance of penetrating small vessels, but have the downside of having a high risk of apoptosis, with low chances of metastasis formation. On the other hand, clusters are rarer, but have higher chances of metastasizing due to the lower risk of apoptosis and the fact that they already have their own formed microclimate. Although many aspects about CTCs and DTCs are yet unknown, one particularity is clear: the phenotypic plasticity, manifested in the high possibility of phenotypic changes to "survive" (15).

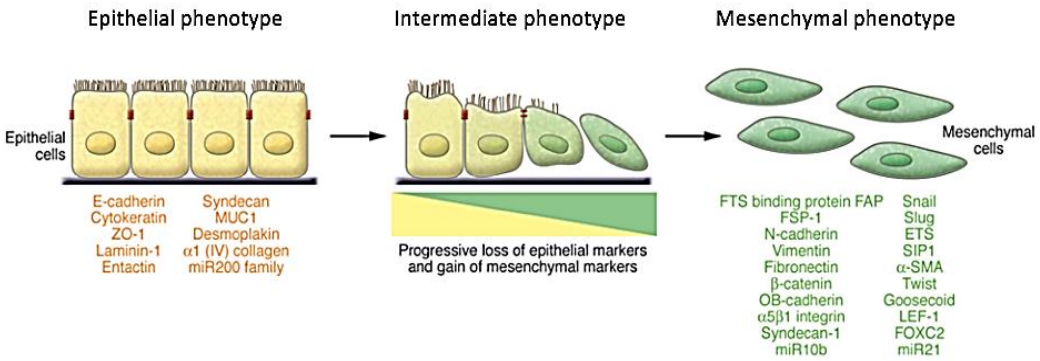

Fig. 1. Epithelial-mesenchymal transition (reproduction after Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *JCI* 2009; 119(6): 1420-1428)

The efficiency of a CTC or DTC is given by its capacity to metastasize. For this event to take place, these cells need to undergo changes that increase their invasive capacity (16, 17).

Carcinomas are the most frequent type of neoplasia, deriving from epithelial cells from different organs. Under certain mutagen factors, the cells comprising the normal tissue lose their apico-basal polarity along other phenotypes, such as growth control and become adenomas. In time, these abnormal cells become in situ carcinoma, with properties that make it malignant (16).

Up next, these malignant cells undergo changes that are essential for obtaining the ability of invasion and metastasizing. Normally, cells are linked to the basement membrane by molecules such as E-cadherin, plakoglobin and integrins. In special situations, such as organogenesis and tissue repair, these cells obtain the capability of detaching from the basement membrane. Oncogenesis and metastasis also imply the occurrence of this event, in which the above-mentioned proteins are replaced by vimentin and N-cadherin; it is this change that determines the exposure of detached cells to the stromal fibroblasts, leucocytes, extracellular matrix and neoformation vessels. This event is called epithelial-mesenchymal transition (EMT) (fig. 4) (18).

The primary tumor may contain cells in different steps of this transition, thus expressing different characteristics; they may be included in two categories (12):

1. A mesenchymal-like form, with receptors to adhere to the extracellular matrix. These cells produce enzymes such as metalo-proteinase that permit the progression of the tumor through: degradation of the extracellular matrix and of the intercellular junctions, stimulation of cell motility and EMT. These cells may be thought as the promoters of cellular invasion, by creating an adequate microenvironment for this event.

2. An amoeboid form, with cells with high plasticity, that can easily pass through the gaps in the basal membrane made by the above-mentioned cells.

On the other hand, once released in the bloodstream, the tumor cells can have three types of evolution (19):

1. The first situation is when cells are released into the bloodstream, once detached from the extracellular matrix and, in the absence of a propitious microenvironment, they either suffer anoikis, apoptosis or necrosis, being annihilated, finally, by the immune system. Obviously, this is unproductive prim the metastasis point of view.

2. The second type of evolution is represented by cells that have reached a metastatic target and remain in this site in a G0 phase of the cell cycle, dormant, with an equilibrium between proliferation and apoptosis, thus being a temporary status, that is partially unproductive from a metastatic point of view.

3. The third category is that of cells that have reached the metastatic site and launch an efficient proliferation process, that allows the cells to grow in number and to invade the metastatic site. These cells can be either from the bloodstream, or dormant cells, that have been locked in the G0 phase for a certain amount of time.

The phenomenon of “dormancy” of cells situated in metastatic sites has been studied in patients with breast cancer, where CTCs have been identified if 22

years distance from the radical intervention (20). However, the clinical implications of this event are still unclear. Moreover, these sites with dormant tumor cells have been found to be capable of releasing CTCs in the bloodstream, which is why it is important to identify and characterize these cells in the patient's circulation (21).

The utility of identifying and counting the CTCs

Circulating tumor cells may be identified and counted in the patient's blood, with methods that have seen great improvements over the last couple of decades. The utility of examining CTCs in rectal cancer patients may be seen from several points of view:

1. as a screening instrument
2. for diagnostics
3. as predictive factors for orienting the treatment
4. as prognostic factors
5. for guiding and assessing the treatment the treatment
6. as a follow-up tool.

1. CTCs could become primary or complimentary identification markers for rectal cancer, with the possibility of detecting tumor cells that are in the patient's bloodstream.

Studies from 2008 on breast cancer patients (22) and from 2012 on pancreatic cancer (23) support the idea that tumor cells enter the circulation even before the formation of the main tumor, thus concluding that the identification of CTCs in peripheral blood may be used for screening purposes.

2. Liquid biopsy is a popular subject in recent years. CTCs represent an important source of information in advanced neoplasia, in which they are more abundant in blood. However, the main goals are to identify them when they are less abundant in the patient's bloodstream, in early-stage neoplasia (which hinders them from being an adequate screening tool now) and to study them in their own tumor microenvironment (which may be solved by examining the heterotypical clusters of CTCs (24).

3. Prediction of the response to treatment for breast cancer has been studied in a phase III clinical trial – the SWOG S0500 trial, that was based on evaluating the number of CTCs at the beginning of the treatment and at 21 days after commencing chemotherapy; it aimed to observe the opportuneness of changing the line of chemotherapy after 21 days of chemotherapy based on the variation of CTCs. The trial demonstrates that a large number of cells at the beginning and at 21 days is a negative prognostic factor for disease free survival and overall survival. All in all, no clinical predictive utility of CTCs in adjuvant therapy was demonstrated through this trial (25).

However, this domain is promising and could show of great utility in locally advanced rectal cancer patients that must receive neoadjuvant therapy and develop metastases during this period of at least 12 weeks between diagnosis and the surgical sequence. CTC dosage during neoadjuvant therapy may prove to be of interest in this eventuality. No studies can be found on this subject now in literature.

4. An accepted and validated clinical usage of the CTCs is the relationship between the number of CTCs at the beginning of the treatment and the overall and disease-free survival. Thus, it has been

demonstrated that a number greater than 5 cells per 7.5 mL of peripheral blood is correlated with a worse survival (11). Moreover, this important aspect has been included, for breast cancer for the time being, in the last TNM classification (8th edition-2017): CTCs present in blood classify the disease as cM0(i+) (26).

This aspect has been initially studied in patients with breast cancer (27), but afterwards other types of cancer have been studied; in colorectal cancer, the cut-off value for CTC number per 7.5 mL of blood is 3, which is significant both for overall, as well as disease free survival (28).

5. The term of liquid biopsy has been widely discussed in the past few years and is a significant step forward for oncology. CTCs may prove to be an important marker both to be taken into consideration within the multidisciplinary team to decide upon the treatment, and for obtaining information about the tumor cell's phenotype and using this information to decide upon the elected treatment by each healthcare professional in part (29).

6. CTCs may be a precocious marker for recurrence or metastatic disease in rectal neoplasia, which is why a sensitive and accessible, cheap identification would be an important follow up tool.

All in all, CTCs are useful in all phases of the neoplastic disease, from screening to follow up. Nevertheless, their usage is subject to the refinement of identification and count methods. This domain is under development, but more trials are necessary to find and apply utilities of this resource in cancer treatment.

Methods of identification of CTCs

Because they are rare cells in the circulation, these cells' separation from leukocytes, red blood cells and other circulating cells is necessary. There are multiple methods of identifying these cells in the patient's bloodstream. A classification of these methods taking into consideration the modality used for selection divides them into (30, 31):

1. Affinity-based evaluation
2. Non-affinity-based evaluation (based on physical properties)
3. Molecular techniques of evaluation.

1. Affinity-based techniques are the most widely used modalities of evaluation of CTCs. These techniques are based on ligation to different antigens expressed by tumor cells, that differentiate them from other cells in the bloodstream. One of these molecules is EpCAM (Epithelial Cell Adhesion Molecule), that is expressed by tumor cells originating in epithelial tissue but is absent from other cells in the bloodstream. Thus, identification and ligation of this glycoprotein permits the selection of the targeted cells.

CellSearch is the most utilized method and is considered gold standard for the identification of CTCs. It is the only method approved by the US FDA for identifying and counting CTCs in breast, prostate and colorectal cancer. This method is based on ligation of EpCAM by iron-fluid nanoparticles and attachment of these functionalized cells in a magnetic field. This platform has been validated through multiple studies (30).

Other methods include Mag Sweeper (an automatic immunomagnetic separation technology), CTC-Chip (a chip that uses functionalized micro posts with anti-EpCAM antibodies), GEDI (geometrically

enhanced differential immunocapture). More recently, there have been described technologies that combine affinity-based methods with other physical techniques, thus emerging: Nano-Velcro (a device with a patterned silicon nanowire substrate coated with anti-EpCAM antibodies), Graphene Oxide Nanosheets with EpCAM antibodies, Immuno-microbubbles (perfluorocarbon gas filled microbubbles conjugated with anti-EpCAM antibodies) and GILUPI Cell Collector (a functionalized medical wire that detects CTCs in circulation, being inserted for 30 minutes in a peripheral vein) (30).

The disadvantages posted by these methods are the fact that tumor cells may present phenotypic variations, which may impede their detection by affinity alone, with less cells identified. This setback can be addressed by identifying and ligation of more than one type of CTC receptor.

2. These disadvantages led to the development of other methods of identifying and counting CTCs: methods based on size and electrical properties of CTCs. This is because tumor cells differ from other blood cells by size, density and polarity, alongside the molecules expressed on their surface.

The simplest, but the least effective method is centrifugation, which uses the cells' density to separate them from other blood components. Through centrifugation, CTCs are separated, along other nucleated cells in the blood, thus being identified afterwards by microscopic examination.

Based on the larger dimension of CTCs, compared to other cells in the blood, micro-filtering may be used, alone or in combination with other affinity-based or non-affinity-based methods. The filtrating material may be obtained through different modalities.

There are other methods to separate CTCs, such as microfluidics or Nano roughened surfaces, but these are still in the testing stage to evaluate their efficiency.

Dielectrophoresis is toe modality of separating CTCs in an electrical field. This technique has been patented by Herbert Pohl in 1950 and was observed as a modality of moving particles using their volume and form. Several studies have used dielectrophoresis in separating CTCs for studying them (31).

All in all, we can see that the methods are somewhat complimentary, and a method that combines them would be the most preferred, provided that is cost-efficient as well. GEDI (geometrically enhanced differential immunocapture) has been proven by Gleghorn to do this but has so far been used only in prostate cancer (32).

CONCLUSIONS

The emergence of the subject of CTCs has been, throughout history, a tough one, because in 1869, when they were first described, the medical world was not evolved enough to see the advantages and begin research; only after more than a century were CTCs seen as a domain of great potential.

We can conclude that the subject of CTCs is an emerging one, of great possibilities, with utilities that are reachable through research and refinement of the isolation techniques. The fact that CTCs are useful both in the field of oncology and in surgery is one more argument in favor of the studies and extensive research that are being put into this field.

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COMPUTERIZED CALCULATION OF BREAST DENSITY: OUR EXPERIENCE FROM ARCADIA MEDICAL IMAGING CENTER

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COMPUTERIZED CALCULATION OF BREAST DENSITY: OUR EXPERIENCE FROM ARCADIA MEDICAL IMAGING CENTER (Abstract): The **aim** of this study was to implement a spatial fuzzy C-means algorithm for image segmentation and breast tissue density quantification and compare it with BI-RADS breast density classes determined by radiologists. **Material and methods**: The analysis was based on 206 mammograms performed in 111 women with various breast abnormalities. Digitized mammographic films were independently double read by radiologists certified in breast diagnosis, followed by consensus with arbitration agreement (radiological ground truth). Reporting was done using the BI-RADS mammography lexicon. Using an algorithm based on a combination of spatial fuzzy C-means clustering and binary thresholding, percent mammographic density was computed in digitized mammograms. The BI-RADS breast density readings were compared with percent breast density measurements determined by computer algorithm. **Results**: The algorithm was found to match the BI-RADS density classification in 90% of the cases, with an excellent agreement ($\kappa = 0.88$) between the radiological ground truth versus the algorithm breast tissue density estimates. **Conclusions**: Our study proposed an algorithm that can be applied both to digitized and digital mammograms, which proved to be effective in breast density estimates. The method can accurately determine the percentage density removing the human observer variability. The proposed method showed an excellent agreement with radiological ground truth. **Keywords:** MAMMOGRAPHIC DENSITY, SPATIAL FUZZY C-MEANS, BREAST SEGMENTATION, BINARY MASKS

Mammographic density is a risk factor for breast cancer, and it has been shown that women with high mammographic density have four to five-fold increased risk of developing breast cancer (1). Assessment of breast density may be carried out using qualitative or quantitative estimates. The most widely used clinical density estimate is the qualitative evaluation based on the Breast Imaging Reporting and Data System (BI-RADS) classes developed by the American College of Radiology (ACR) (2). However, this classification scheme is still limited by its considerable intra- and inter-reader variability.

As an alternative to qualitative evaluation, different quantitative approaches have been developed. A quantitative method for measuring breast density is supposed to be simple, accurate, reproducible, and ready to be implemented with a minimum level of operator dependency. In the assessment of

breast density from mammograms, the breast is usually segmented into distinct regions (3). There have been various approaches proposed to the task of segmenting the breast profile region in mammograms. Some of these have focused on using threshold (4), fuzzy C-means approaches (5), Gaussian mixture models (6) and statistical methods (7).

In this paper, we investigated the feasibility of quantifying breast density with an automatic approach, employing spatial fuzzy C-means (SFCM) clustering segmentation and binary thresholding.

MATERIAL AND METHODS

The mammographic records of 117 consecutive women (mean age, 56 years) who underwent diagnostic mammography at our facility was included in the present study. Standard cranio caudal view (CC) and medio lateral oblique (MLO) image projections of each breast were obtained. Subjects who had very poor quality mammograms were excluded. A data set of 95 cases of two-view bilateral mammograms and 16 two- view unilateral examinations (patients who had undergone mastectomy) was used in this study. No informed consent was required, as patient identification information was removed. This research protocol has been conducted according to the ethical guidelines and principles of the Declaration of Helsinki.

All mammographic examinations were performed by using a Senographe DMR unit (GE Healthcare, Milwaukee, Wisconsin, USA) along with a screen-film technique (Kodak Min-RS Film; Carestream Health Medical Imaging, Rochester, NY). All film-screen mammograms were digitized by using Diagnostic PRO Advantage film digitizer (VIDAR Systems, Herndon, Virginia, USA) with a dedicated software that convert images in Digital Imaging and Communications in Medicine (DICOM) format at a high resolution (570 dpi) of 44.5 microns and a 12-bit grayscale imaging output.

Image segmentation. To further process the digitized film mammograms we used public domain image-processing software, FIJI/ Image J (8), with a SFCM algorithm implemented as a plug-in.

The first step of our approach consisted in breast image segmentation. The SFCM method relies on classifying pixels into distinct regions containing a group of connected pixels (cluster) with similar properties within each group (9).

First, let $X = (x_1(1\ ), x_1 2, \ldots, x_1 N\ )$ be an image with N pixels to be partitioned into c clusters, where x_j is an image pixel. The identification of clusters in the image depends on a cost function that exploits the feature-based similarities between pixels, defined as:

$$J = \sum_{j=1}^{N}\sum_{i=1}^{c} u_{ij}^{m} \|x_j - v_i\|^2 ,$$

where u_{ij} is the membership function of pixel x_j in the ith cluster, v_i is the ith cluster center, $\|.\|$ is the Euclidean distance, and m is a constant which determines the amount of fuzziness of the resulting classification. The SFCM algorithm takes into account the local spatial relationship between adjacent pixels by introducing the spatial information into the membership function for clustering. SFCM algorithm can be summarized as follows: a set of integers as a parameter of the number of clusters to be allocated to the algorithm, c $=\{3, 4, 5, 6\}$.

A weighting exponent (fuzzifier) m $=\{1.1, 2.5\}$, and a parameter p that deter-

mines the influence of the weights, p ∈ [1…∞] . The spatial function it is defined as the probability that pixel x_j belongs to i^{th} clustering:

$$h_{ij} = \sum_{k \in NB_{(xj)}} u_{ik} \quad ,$$

where $NB_{(xj)}$ is a square window centered on pixel x_j in the spatial domain. The spatial function is incorporated into membership function as follows:

$$u_{ij}^{*} = \frac{u_{ij}^{p} h_{ij}^{q}}{\sum_{k=1}^{c} u_{kj}^{p} h_{kj}^{q}} ,$$

where p and q are parameters to control the relative importance of both functions, as described in detail elsewhere (10).

The SFCM method generated images of dense, adipose and whole breast tissue each one representing a cluster whose membership function u_{ij}^{*} indicates that the pixels are toward that cluster (fig. 1).

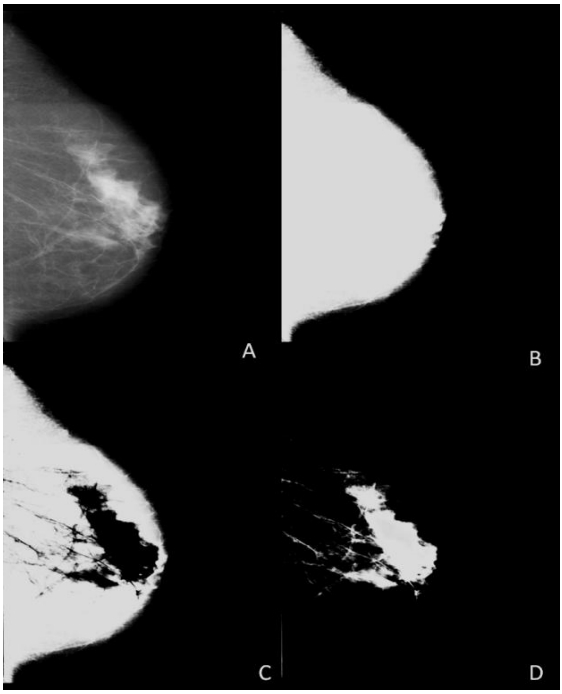

Fig.1. Steps for the segmentation of the fibro glandular breast tissue using SFCM: (A) Original digitized mammogram; (B) Segmented image of the whole breast; (C) Segmented image of the fatty regions; (D) Segmented image of the fibro glandular regions.

Estimation of mammographic density. For breast tissue density quantification only the CC projections were used, as it has been previously reported that density estimates from CC and MLO views showed very strong correlation (11). Images corresponding to dense and whole breast region were transformed into binary images using the isodata thresholding algorithm implemented in Image J. The method separates the image into breast tissue and background by automatically choosing a global threshold

that is equidistant from the average intensity of pixels below and above it:

$$Threshold = \frac{(average\ background + average\ tissue)}{2}.$$

The area-based measurements of mammographic density were computed by recording the number of pixels that lie within the defined region (fig. 2). The percent mammographic density (PD) was then calculated as the ratio of dense area to the total area of the breast multiplied by 100.

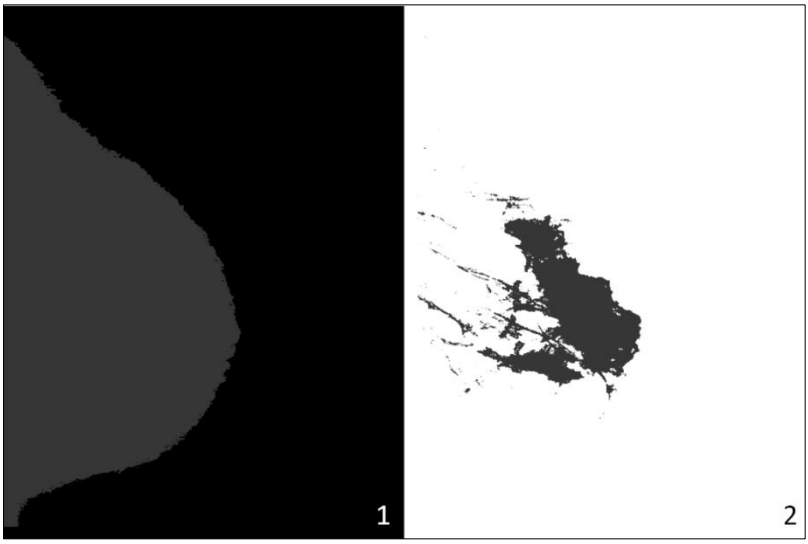

Fig. 2. The whole breast area delineation in CC views (1);
The fibro glandular area delineation in CC view (2)

Visual interpretation of digitized mammograms.

Each mammogram was initially independently reviewed by two trained breast imaging radiologists using the corresponding digitized screen-film mammograms. The CC and MLO views were displayed side-by-side on dedicated image displays (Totoku, Electric Co Ltd, Japan). The observers evaluated each case according to the BI-RADS mammography lexicon published by the ACR (2). After scoring the mammographic breast density in one of the four quantitative BI-RADS categories, the observers assessed the presence of associated findings such as masses, architectural distortion, focal asymmetry and calcifications. All lesions were described by using the terminology of the fourth edition of the BI-RADS lexicon and assigned to a final BI-RADS category. For cases with discordant results, a consensus reading with arbitration by a third expert radiologist was carried out, and the observers were asked to make the final assessment considered the ground truth.

Statistics. In order to facilitate convenient evaluation of the interobserver agreement, data readouts from the left and right breasts were pooled. Weighted kappa values were calculated to assess the level of agreement between observers. The interpretation of the kappa values was based on the criteria proposed by Landis and Koch (12): 0.20 or less indicated poor agreement; 0.21–0.40, fair agreement; 0.41-0.60, moderate

agreement; 0.61–0.80, substantial agreement; and 0.81–1.00, excellent agreement. The Wilcoxon signed-rank test was used for comparisons between the right and left breast. The Pearson's correlation coefficient was used to test the association between the PD area and patients age. All P values less than 0.05 were interpreted as statistically significant. All statistical analyses were performed by using *MEDCALC* software, *version 12.5.0* **(**Mariakerke**,** Belgium**).**

RESULTS

Visual interpretation of digitized mammograms.

Of 206 breasts analyzed, 95 (46%) were classified as fatty, 62 (30%) as scattered fibro glandular densities, 29 (14%) as heterogeneously dense, and 20 (10%) as extremely dense. There was a total of 53 (26%) breast masses, of which 11 (5%) histologically proved malignant, and 42 (21%) presumed benign. Calcifications within breast tissue were found in 88 (43%) breasts, of which 6 (3%) were associated with malignancy. Architectural distortion and focal asymmetric densities were found in 3 (1%) and 26 (13%) breasts, respectively. A total of 69 breasts were assigned to BI-RADS class 1, 79 to class 2, 39 to class 3, seven to class 4, and nine to class 5. Interobserver agreement at the first reading varied from substantial ($\kappa = 0.67$ for breast composition, $\kappa = 0.75$ for masses, $\kappa = 0.65$ for calcifications and $\kappa = 0.62$ for BI-RADS class) to fair ($\kappa = 0.36$ for focal asymmetry and $\kappa = 0.33$, for architectural distortion).

Breast density evaluation. The measured mammographic density was 36 ± 20% for the right breast and 34 ± 20% for the left breast (P=0.45). Breast density showed a statistically significant inverse relationship with age (Pearson product-moment correlation coefficient, $r = -0.32$, $P<0.001$). Based on the PD values (<25%, 25%–50%, 51%–75%, and ≥75%), each breast was assigned to the corresponding BI-RADS density class. When compared with the results of the consensus reading, overestimation occurred in 6% of the cases and underestimation in 4%. Agreement between the consensus reading versus algorithm PD estimates was excellent, with weighted kappa values of 0.88 (tab. I).

TABLE I

Visual classification and the software analysis of the breast composition categories (N=206 breasts).

	Consensus reading	**Algorithm estimates**	**Observer 1**	**Observer 2**
Almost entirely fat	95 (46%)	94 (46%)	77 (37%)	110 (53%)
Scattered fibro glandular densities	62 (30%)	63 (31%)	73 (36%)	49 (24%)
Heterogeneously dense	29 (14%)	29 (14%)	36 (17%)	25 (12%)
Extremely dense	20 (10%)	20 (10%)	20 (10%)	22 (11%)
Correct classification	-	186 (90%)	175 (85%)	169 (82%)
Classification overestimated	-	12 (6%)	27 (13%)	14 (7%)
Classification underestimated	-	8 (4%)	4 (2%)	23 (11%)
Weighted kappa- κ	-	0.88	0.84	0.80

DISCUSSION

In this study we propose an automatic method for breast density estimates that is fast, accurate and easy to implement at minimal costs. We used open-source software, Fiji/Image J distribution, which can be downloaded for free. All of the image-processing algorithms used in this work are available as plug-in that can be tailored to user's specific needs. Using only open-source software, we have tried to make our results reproducible and therefore easy to replicate.

Quantitative analysis of mammographic density would provide an objective assessment of breast cancer risk (13, 14) associated with varying mammographic densities. The main advantages of the proposed method are the completely automated breast segmentation and automatic thresholding technique. The algorithm was found to be superior to single-observer visual assessments in breast density measurements, matching the ground truth density classification in 90% of the cases.

The method has its disadvantages, because it is more time consuming than the visual assessment and require digitized images, but can accurately determine the percentage density removing the human observer variability. Additionally, an important limitation is that the area measurement from the 2D projection image ignores the variations in breast thickness.

Regarding algorithm optimization, a model for breast compression and thickness should be implemented and multiple comparisons between different devices (mammography systems, film scanning systems) are required, so further work is needed to implement this algorithm in routine practice.

CONCLUSIONS

Our study proposed an algorithm which proved to be effective in breast image segmentation and density estimates, with potential impact on diagnostic performance. The method is more time consuming than visual assessment, but can accurately determine the percentage density removing human observer variability. The main advantages of the proposed method are completely automated breast segmentation and automatic thresholding technique. The algorithm was found to be superior to single-observer visual assessments in breast density measurements. The proposed method showed an excellent agreement with radiological ground truth.

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NEWS

ELASTIN-LIKE POLYPEPTIDE -COLLAGEN HYDROGELS

In order to minimize post-surgical infections and support wound healing, Anderson *et al.* studied the preparation of hydrogels with effective antibiotic release profile and better mechanical properties. The team prepared elastin-like polypeptide (ELP)-collagen composite hydrogels that showed a higher elastic modulus in comparison to the collagen hydrogels. The release behavior of the collagen and ELP-collagen hydrogels loaded with varying dosages of a doxycycline hyclate was characterized. Both types of hydrogels showed a gradual time dependent release of doxycycline over a period of 5 days. The ELP-collagen hydrogels had a slower doxycycline release compared to the collagen hydrogels. The released doxycycline was effective in a dose dependent manner against strains of *E. coli, P. aeruginosa, S. sanguinis* and MRSA. The study concluded that ELP-collagen hydrogels have improved mechanical properties, effective drug release and may be beneficial for tissue engineering applications and drug delivery (Anderson TR1, Marquart ME, Janorkar AV. Effective Release of a Broad Spectrum Antibiotic from Elastin-Like Polypeptide-Collagen Composite. *J Biomed Mater Res A*. 2014. doi: 10.1002/jbm.a.35219. [Epub ahead of print]).

Teodora Vremeră

GLAUCOMA EVOLUTION IN PATIENTS WITH DIABETES

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GLAUCOMA EVOLUTION IN PATIENTS WITH DIABETES (Abstract): Glaucoma and diabetes are two chronic diseases with a long suspected pathogenic relationship. **Purpose**: Screening for glaucoma in patients with diabetes. **Material and methods**: A retrospective study on 92 eyes from 46 patients with primitive open angle glaucoma (POAG) (normal and hypertensive) and intraocular hypertension (OHT) receiving medication and / or surgery associated with diabetes mellitus (DM) (type I, type II, mixed) is presented. Participants were divided into two groups as following: 16 eyes with glaucoma and diabetic retinopathy changes (group1) and 76 eyes with glaucoma and without diabetic retinopathy changes (group 2). The following parameters were analysed: ocular pressure (Goldmann aplanotonometry), perimeter development (computerized perimetry) and fundus condition (absence, presence or progression of diabetic retinopathy). **Results**: In patients with glaucoma and diabetic retinopathy (8 patients) we found a mean difference between treated intraocular pressure (IOP) and IOP last untreated control of 4.95 mmHg; a depreciation of the MD by 4.18 dB and an average number of glaucoma medications used of 0.889 ± 1.054. Predominant changes in proliferative diabetic retinopathy were mild. In patients with glaucoma in the absence of diabetic retinopathy, the average difference between untreated IOP and IOP under treatment at the last check-up was 1.63 mmHg, the MD depreciation was by 0.65 dB and the average number of glaucoma medications used was 0.795 ± 0.978. **Conclusions**: No statistically significant differences in terms of initial and final pressure were found. No statistically significant differences in the evolution of changes in perimeter between the two groups were observed. The presence of non-proliferating diabetic retinopathy influenced (only marginally statistically) the glaucomatous disease progression. Large comparative prospective studies are needed for the long-term follow up. **Keywords**: POAG, DIABETES, PIO, MEAN DEVIATION, DIABETIC RETINOPATHY

Glaucoma and diabetes are two chronic diseases that affect the population for over 40 years, views on its relationship are considered pathogenic (1). Numerous studies have attempted to find an association between diabetes and primary open-angle glaucoma (POAG), some successfully, some not. MO Gordon and colleagues presented a study of 1637 subjects in 1999, which included a large number of risk factors including diabetes. The study concluded that 3.1% of the population does develop glaucoma, and that diabetes acts as a protective factor to decrease the probability of developing POAG (2). This early conclusion was in part due to a relatively small

number of people with diabetes excluding those with diabetic retinopathy changes. Several studies have tried and have failed to demonstrate an association between diabetes and glaucoma. In an article by Benkes (3) in 1967 suggested for the first time the relationship between glaucoma and diabetes, "diabetes occurs more frequently in those with POAG than in the population without glaucoma claiming, glaucoma is more prevalent in patients with diabetes than without". The Rotherham Study concluded, "newly diagnosed diabetes and high blood glucose levels are associated with elevated IOP and glaucoma with high pressure" (4). In a recently published article entitled "Diabetes - risk factor for glaucoma?", the authors showed that both diseases have a vascular component and retrobulbar blood flow abnormalities in retinal microcirculation were found in both diabetes and glaucoma (5). Our study objective was to follow the development of glaucoma specifically in patients with diabetes.

MATERIAL AND METHODS

We performed a retrospective study on 92 eyes from 46 patients recorded with normal or hypertensive POAG, an OHT treated with drugs and/or surgery both associated with diabetes (type I, II and mixed). All patients were selected from „Sf. Spiridon" University Hospital of Iasi between January 2009 – May 2012. The inclusion criteria were: normal or hypertensive glaucoma, OHT associated with diabetes. Patients with other types of glaucoma were excluded (pseudoexfoliation, pigment, cortisone, and primitive neovascular angle closure) as well as those without associated diabetes. Participants were divided into two groups as following: group 1 represented by patients with glaucoma and changes of diabetic retinopathy (DR) and group 2 patients with glaucoma and without DR.

All patients were monitored under the following parameters: the clearance pressure (with Goldmann applanotonometer), the perimetrical progression (by automated perimetry with Humphrey Field Analyzer II) and fundus change evaluated with the Volk lens 78D (absence, presence and progression of DR). It was also recorded the duration of glaucoma, diabetes and number of glaucoma medications used.

Diagnosis criteria for normal or hypertensive POAG were: age >35 years, normal IOP or >21mmHg without treatment, open angle gonioscopy, the presence of optic nerve damage (ratio c/d > 0.5), abnormal visual field (Humphrey Field Analyzer by perimetry) and normal or diffuse or localized defects in the retinal nerve fiber layer (optical Coherence tomography Cirrus HD-OCT Zeiss) (6).

Evaluations of the changes in diabetic retinopathy were performed with Volk lens and retinal photography with Fundus Camera Zeiss. Retinal changes in dilated pupils were monitored. The criteria for classification as DR changes (ETDRS) were the following: unchanged or absence of signs of DR, mild nonproliferative diabetic retinopathy (presence of only microaneurysms), moderate DR (microaneurysms, haemorrhages in 2-3 quadrants, venous dilatation and exudates), severe DR (microaneurysms, hemorrhages in all quadrants, dilated veins in 2-3 quadrants, exudates) and proliferative diabetic retinopathy (neovascularization disk and retina in different quadrants). It was considered progression of DR any new clinical item appearing on subsequent checks that led to their classification as severe nonproliferative or proliferative diabetic retinopathy (7). Sigma Plot was used for the statistical analysis of the results (student t test).

RESULTS

In our study all the 92 eyes from 46 patients met the eligibility criteria. The mean age was 62.87 ± 8.4 years (between 39 and 80 years old). Among patients with DM 36 had DM type II, 5 had DM type I and 5 with insulin-dependent DM type II. From all studied eyes, 72 eyes (78.26%) had POAG, 10 eyes (10.87%) had normal tension glaucoma (NTG) and 10 eyes (10.87%) OHT (fig.1). Regarding the changes in diabetic retinopathy, of the 8 patients, 5 had mild changes in diabetic retinopathy (15%), 3 had proliferative diabetic retinopathy (7%) and the rest (78%) did not show any change (fig.2).

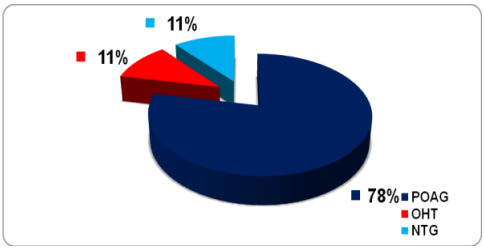

Fig. 1. Distribution of patients with glaucoma POAG = primitive open angle glaucoma, OHT = ocular hypertension, NTG = normal tension glaucoma

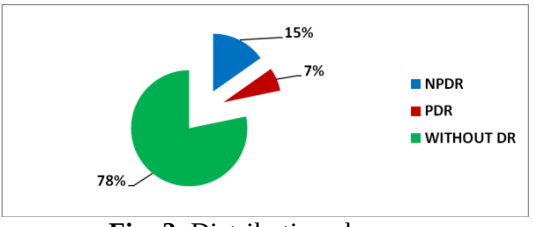

Fig. 2. Distribution changes of diabetic retinopathy

NPDR = diabetic retinopathy mild form, PDR = proliferative diabetic retinopathy, Without DR = no modification of diabetic retinopathy

The average duration in years of glaucoma in group 1 was 1.7 ± 1.16 (range 0-3) and 4.33 ± 4.602 (range 0-19) in group 2. No statistically significant differences were found between the two groups (P <0.05 for significant relevant statistics) (tab. I).

TABLE I

Statistical distribution of glaucoma

	group1	group2
Glaucoma duration (years)	1.7 ± 1.16	4.33 ± 4.602
	(0-3)	(0-19)
t student	(P=0.818)	

The average duration of diabetes in years was in group 1 with 9 ± 10.029 (range 0-26) and 4.59 ± 4.284 (range 0-17) in group 2, no statistically significant differences existed between the two groups (tab. II).

TABLE II

Statistical distribution diabetes

	group1	group2
Diabetes duration (years)	9 ± 10.029	4.59 ± 4.284
	(0-26)	(0-17)
t student	(P=0.081)	

The mean number of glaucoma medications used was similar in the two groups, respectively 0.889 ± 1.054 (range 0-3) in group 1 (glaucoma + DR) and 0.795 ± 0.978 (range 0-3) in group 2 (glaucoma without DR) as there were no statistically significant differences in student t-test between the two groups (tab. III). In group 1 the glaucoma and DR average difference between the initial untreated IOP and the final treated IOP at the last check-up was 4.9 mmHg. Patients whose IOP was less than 23 mmHg after one single medication did not require any further intervention to reduce the IOP (fig. 3).

In group 2 (glaucoma and without DR) we found a mean difference of 1.63 mmHg between the initial untreated IOP and final treated IOP at the last check-up. The initial pressure was also lower compared to the group 1. As a consequence the majority of cases did require a small number of drugs and only 2 cases with IOP >30 mmHg required three drugs to reduce pressure (fig. 4).

By comparing the mean IOP of the two groups using Student t test a p value of 0.062 was obtained. This value is however statistically insignificant since p <0.05 is considered statistically significant (fig. 5).

Analyzing the characteristic parameters of standard perimeters, particularly of Mean Deviation (MD) an impairment of the MD's of 4.18 dB was found in group 1 (glaucoma and DR) (fig. 6). In group 2 (glaucoma and without DR) a MD's depreciation of 0.65 db was found (fig. 7).

TABLE III.

Statistical distribution of number of glaucoma medication

	group1	group2
Mean no. of glaucoma medications	0.889 ± 1.054	0.795 ± 0.978
	(0-3)	(0-3)
T student	(p=0.847)	

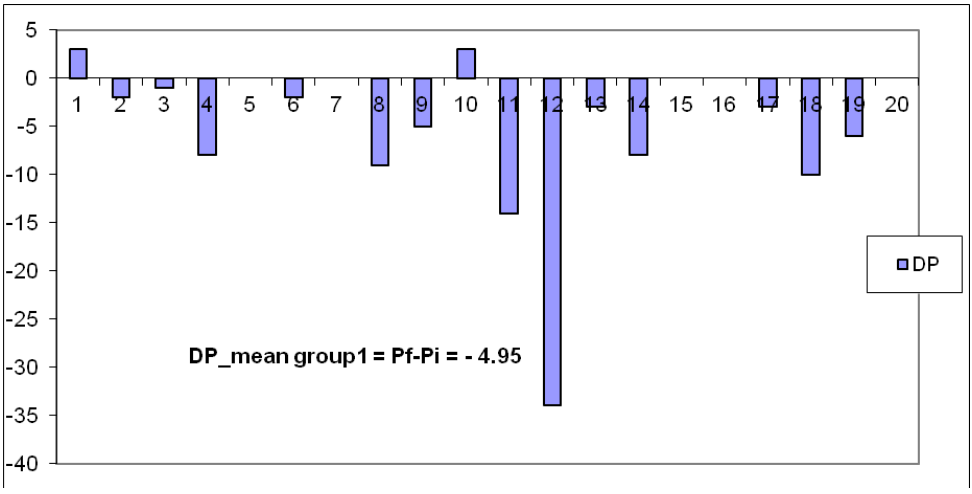

Fig. 3. Statistical description of the mean pressure difference outcomes in group 1
Pf = final pressure therapy, pi = initial pressure therapy,
DP_mean = average difference between the final and initial pressure

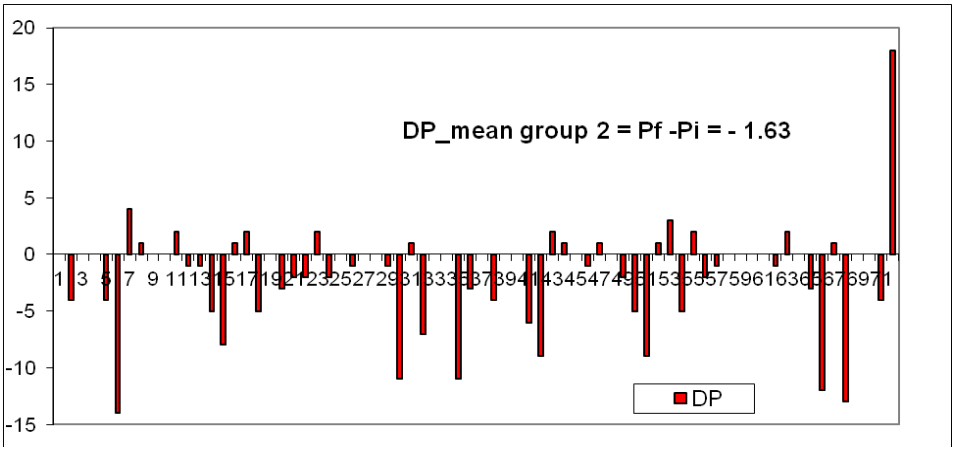

Fig. 4. Statistical description of the mean pressure difference outcomes in group 2
Pf = final pressure therapy, pi = initial pressure therapy,
DP_mean = average difference between the final and initial pressure

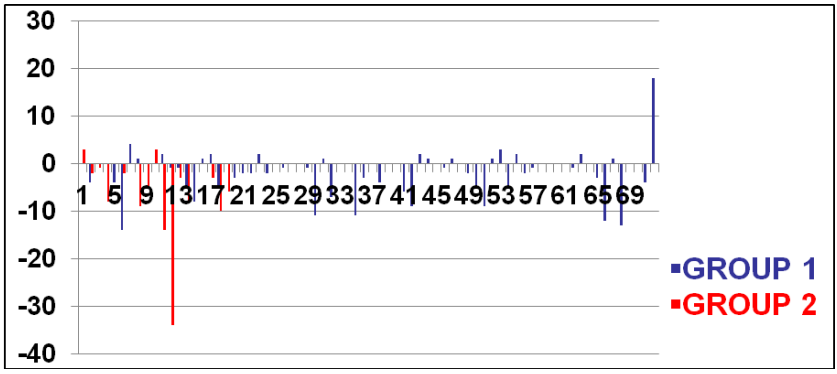

Fig. 5. Statistical significance average initial and final pressure between the two groups

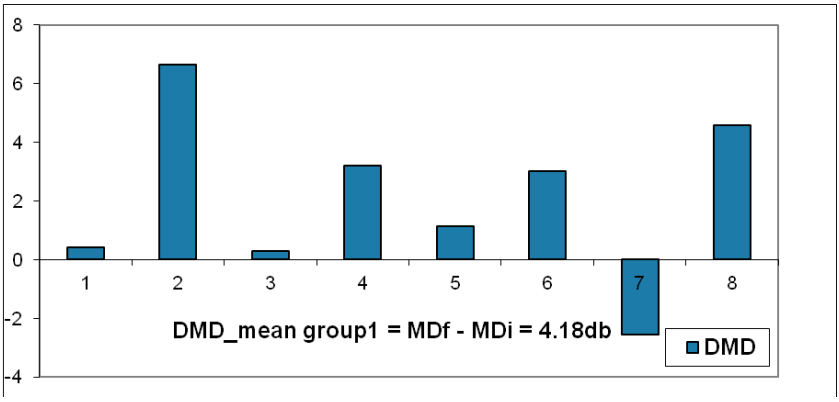

Fig. 6. Statistical description of the results - MD mean value group 1
MDf = mean deviation final, MDi = mean deviation initial from the first control

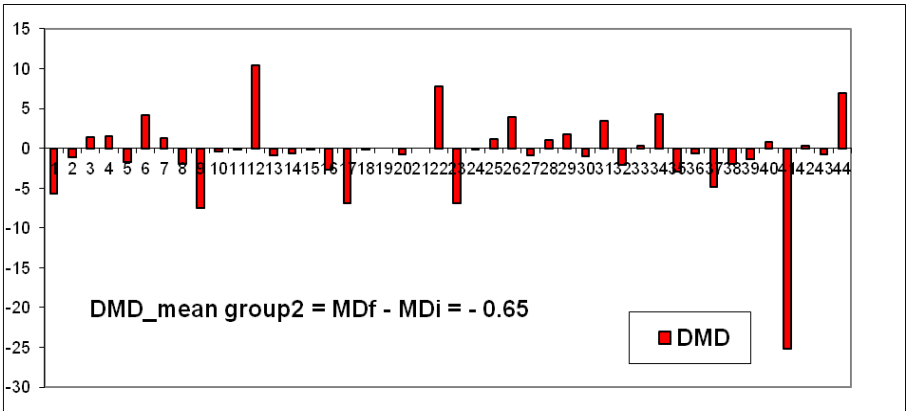

Fig. 7. Statistical description of the results - MD mean value group 2
MDf = mean deviation final, MDi = mean deviation initial from the first control

However when comparing the average MD of the two groups using Student t test a p-value = 0.044 was obtained which is a statistically significant value although only marginal ($p<0.05$ to be statistically significant) (fig.8). The statistical analysis of the data showed higher initial value of the IOP in the group 1 (glaucoma+DR), which required a greater number of drugs to reduce it, while long standing diabetes (0-28 years) correlated positively with the presence of diabetic retinopathy and the MD's important depreciation (4.18 db) vs group 2 (glaucoma and without modifications RD). All these results reveal the statistically significant difference in terms of the average MD and confirm that this influenced the diabetic retinopathy (although marginally statistically) and the glaucomatous disease progression.

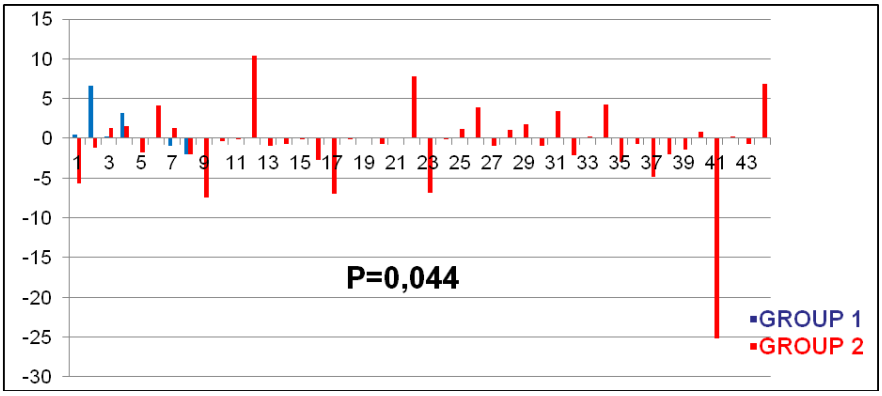

Fig. 8. Mean statistical significance initial and final MD between the two groups

DISCUSSION

Secondary conditions such as vascular degeneration related to diabetes cause some severe risks to the eye such as diabetic retinopathy, crystalline opacities, increased intraocular pressure, rubeosis iridis and open-angle glaucoma. Furthermore IOP has a role in the development of diabetic reti-

nopathy and influences the development progression of glaucoma. Eyes with proliferative diabetic retinopathy had IOP lower than those with non-proliferative diabetic retinopathy. Increased IOP may protect by delaying DR changes. The statement " Increased IOP may protect by delaying DR changes" is controversial; low IOP associated with worsening diabetes as a logical consequence of the Starling law (on the balance between the intravascular and extravascular fluid transfer) (8). Many studies on patients with glaucoma and diabetes are clinical and retrospective and population-based. However obtaining sufficient information from any systematic retrospective study is difficult. A large number of studies have been conducted over time, some have found an association between glaucoma and diabetes, others have shown the opposite (2), or failed (Baltimore Eye Survey, The Rotherham Study) (9, 10, 11). The controversial Ocular Hypertension Treatment Study conducted in 2002 (2) does not find a correlation between the two disorders further supporting that diabetes is a protective factor for glaucoma (glaucoma develops only 3.1% of those with diabetes). It should be noted that compared to our study they did not include patients with diabetic retinopathy, which could change the results of the study. The 22% of those included in our study with diabetic retinopathy, glaucoma influenced the evolution (depreciation MD's with 4.91 dB 0.65 dB in group 1 vs group 2) even if marginal ($p = 0.044$, $p < 0.05$ to be statistically significant). The age group 1 diabetes in years (range 0 - 29 years old) also may play a role in the changes of diabetic retinopathy and glaucomatous disease evolution. Of those that have found an association between the two disorders are groups for which their group resembles the selection of cases and results of our study (10, 11, 12). Barbara Klein and colleagues published in BJO 1997 a prospective study at 10-year in which she examined the incidence of glaucoma in patients with DM. In her study she found that glaucoma is more prevalent in those with long-term diabetes and treated with insulin (12). Los Angeles Latino Eyes Study, a population-based study that included patients with glaucoma DM type II and demonstrated that this DM type II and its long-evolution (duration > 15 years) had a higher prevalence (40%) of developing glaucoma ($p<0.0001$) (13). The difference from our study is that it included only patients with type II diabetes ($a < 30$ years treated with oral agents or by diet) while diagnosis of glaucoma was based on the optic nerve appearance, regardless of the IOP. We found that in our group, most participants had DM type II (78%), a small percentage being with DM type I and mixed. Similar Blue Mountain Study, study of the population range that includes 40% black participants, argues that there is a significant and consistent association between diabetes and glaucoma (14).

Despite all the contradictions in the literature, our study shows similar results to recent studies, however we believe a prospective, general study on a larger number of patients and with a longer follow-up period should be conducted.

CONCLUSIONS

This study demonstrated an association and support that diabetes influences the evolution of the open-angle glaucoma in our patients. We have also found statistically significant differences in the evolution perimeter changes (Mean Deviation) between the two groups ($p = 0.044$). In group

1 represented by patients with glaucoma and diabetic retinopathy depreciation, MD was 4.91 dB (glaucoma progression) compared with group 2 (glaucoma + without RD) which was only 0.65, this allowed us to say that the presence of diabetic retinopathy changes even if statistically marginal influenced by the evolution of glaucomatous disease. Regarding the evolution of initial and final IOP no statistically significant differences between the two groups were found. This study has however several limitations: it is a retrospective study, the number of patients is small, high group heterogeneity (smaller number of patients with glaucoma and RD) and short duration of follow-up. Future prospective studies containing comparative larger batches of patients are necessary for a more accurate idea of the influences of the two diseases.

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THE INCIDENCE OF SUPRAVENTRICULAR ARRHYTHMIAS IN PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION - CLINICAL AND EVOLUTIVE CORRELATIONS

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SUPRAVENTRICULAR ARRHYTHMIAS IN PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION - CLINICAL-EVOLUTIVE CORRELATIONS. (Abstract): **Aim of the study**: The analysis of supraventricular rhythm disorders encountered in hypertensive patients and the establishment of correlations with other associated risk factors, duration of hypertension, the evolution and prognosis. **Material and methods**: The study included a group of 110 patients , 80 men and 30 women, aged between 40 and 85, admitted in the 1st Cardiology Clinic during 2010-2013, diagnosed with essential arterial hypertension second and third degree. For the detection of arrhythmic events surface 12-lead ECG and 24-hour Holter monitoring were used. **Results and discussion**: supraventricular arrhythmias encountered in the patients of the group were: ESA (mostly isolated, but also doublets, atrial bigeminy), atrial fibrillation, atrial flutter, sinus tachycardia, sinus bradycardia. Some arrhythmias may be an expression of the excessive activation of the sympathetic nervous system or on the contrary, vagal predominance, (ex. sinus bradycardia). Part of the arrhythmias occurring in hypertensive patients are hypertensive heart disease expressions, others are due to associated risk factors (smoking, alcohol) and even medication administered. In some cases, rhythm disturbances disappear with the normalization of the blood pressure; however, most of the times, the specific combination of anti-arrhythmic medication with antihypertensive medication is required. **Conclusions**: supraventricular arrhythmias are frequently found in hypertensive patients, especially in those with long term, uncontrolled hypertension, with impact on the evolution, prognosis and therapeutic management. **Keywords**: HYPERTENSION, SUPRAVENTRICULAR ARRYTHMIAS.

Essential high blood pressure (HBP) continues to be an extremely important health problem, on the one hand being a major risk factor for ischemic heart disease, and on the other, due to the complications that affect the targeted organs (1, 2, 3). One of the manifestations of hypertensive heart disease are the arrhythmias (supraventricular as well as ventricular) which, when they appear, alter the course and prognosis of the hypertensive patient and at the same time require treatment particularities (4, 5, 6, 8). Left ventricular hypertrophy caused by uncontrolled arterial hypertension is considered a major risk factor for the development of subsequent arrhythmias (4, 5, 7, 8).

Aim of the study**:** The analysis of the supraventricular rhythm disorders encoun-

tered in hypertensive patients and the establishing of correlations with associated risk factors, duration of hypertension, and the development and prognosis.

MATERIAL AND METHODS

The study included a group of 110 patients, 80 (72.72%) men and 30 (27.27%) women, with ages between 40 and 85, hospitalized in the 1st Clinic Cardiology during 2010-2013, with a diagnosis of essential hypertension of the second and third degree. The diagnosis of arterial hypertension was based on the classical criteria: blood pressure (BP) values above 140/90 mmHg at rest, in the absence of antihypertensive drugs on repeated measurements. All the patients were asked about the duration of hypertension, hypertension-related symptoms, and treatments followed up to the time of admission.

All the patients underwent a complete functional balance of hypertension which consisted of:

- Complete clinical examination of the cardiovascular system: auscultation of the heart, carotid arteries, abdominal aorta and renal arteries;
- Assessment of associated risk factors (smoking, diabetes, obesity, alcohol consumption, stress);
- Measuring the BP in supine and standing position on both arms;
- Complete 12 lead ECG;
- 2D echocardiogram, M mode and Doppler for the assessment of LV systolic and diastolic function and any murmurs;
- Chest X-ray - for the assessment of cardiomegaly and pulmonary stasis;
- Fundus;
- Renal function and serum electrolytes;
- Urinalysis examination;
- Complete lipid profile, blood glucose and uric acid;
- In some patients, BP monitoring 24 hours (ABPM) and 24-hour Holter monitoring for arrhythmias was used ambulatory.

The functional balance was completed in some cases with a minimum etiology balance to rule out a possible cause of secondary hypertension. In the detection of arrhythmias surface electrocardiogram and/or 24-hour Holter monitoring was used and 24 -hour Holter monitoring was performed on 82 patients (74.5 %).

RESULTS AND DISCUSSION

A group of 110 patients has been studied, 80 men and 30 women, with ages between 40 and 85, with the essential hypertension diagnosis.

Depending on the BP values the lot division was as follows:

- Hypertension 1st degree - 20 (18.18%) patients, 12 men, 8 women.
- Hypertension 2nd degree - 40 (36.36%) patients, 30 men, 10 women.
- Hypertension 3rd degree - 50 (45.45%) patients, 38 men, 12 women.

The risk factors analysis in the studied group has shown the following:

- Smoking - 60 (54.54%) patients, 50 men, 10 women.
- Obesity, varying degrees 1-4: 75 (68.18%) patients, 25men, 50 women.
- Dyslipidemia - 85 (77.27%) patients, 75 men, 10 women.
- Diabetes type 2 - 30 (27.27%) patients, 20 men, 10 women.
- Diabetes type 1 - 1 case.
- Alcohol - 70 (63.63%) patients, 65 men, 5 women.
- Stress - 31 (28.18%) patients, 20 men, 11 women.
- In most cases, risk factors have been associated.

Supraventricular arrhythmias (detected on surface ECG or by 24-hour Holter moni-

toring) encountered in patients of the group were:

- Atrial extrasystole - 45 cases (40.9%) (26 men, 19 women) - mostly isolated, but also doublets, atrial bigeminy;
- Atrial fibrillation (AF) - 35 cases (31.81%) (23 men, 12 women);
- Atrial flutter - 5 cases (4.5%) (4 men, 1 woman);
- Sinus tachycardia -18 cases (16.36%) (10 men, 8 women);
- Sinus bradycardia - 13 cases (11.81 %) (7 men, 6 women);

Regarding the medication that the patients have received, it was found that:

- 44 (40%) patients received a diuretic (indapamide) associated with angiotensin converting enzyme inhibitors (enalapril, quinapril, perindopril, ramipril);
- 34 (30.9%) patients received a diuretic (indapamide) associated with angiotensin converting enzyme inhibitors (enalapril, perindopril, ramipril), and a beta-blocker (metoprolol or nebivolol);
- 10 (9.09%) patients received a diuretic (indapamide) associated with angiotensin converting enzyme inhibitors (enalapril, perindopril, ramipril) and anticalcic (amlodipine or slow release nifedipine);
- 15 (13.6%) patients received ARBs (telmisartan, candesartan) associated with diuretic (indapamide);
- 7 (6.36%) patients received a quadruple combination: diuretic, ACE inhibitor, beta blocker, calcium channel blocker);

Of the entire group, digoxin was used for 10 (9.09%) patients as antiarrhythmic; for 24 (21.81%) patients, propafenone was used for the control of atrial and/or ventricular extrasystolic arrhythmias.

Most of the patients in the group have received antiplatelet therapy (aspirin or clopidogrel), and, for 25 (22.72%) of the patients, oral anticoagulants were considered necessary (the patients with atrial fibrillation and dilated left atrium seen at echocardiography).

In the group of patients with atrial extrasystole, the majority were men, smoking and consuming alcohol. A correlation between the duration of hypertension and the presence of atrial extrasystole could not be made, but atrial extrasystole presence could be correlated with the systolic blood pressure value. Thus, patients who had values above 190 mmHg SBP had a greater incidence of atrial extrasystole. A positive correlation between the size of the left atrium and the presence of atrial arrhythmias has also been found.

In patients with AF the following findings were made:

- AF was more frequent in male hypertensive patients who associated as risk factors smoking and alcohol consumption;
- AF appearance correlated with the duration of hypertension (over 5 years), with the presence of left ventricular hypertrophy changes on the surface ECG and on the echocardiogram, and also with the size LA (at echocardiography);
- All patients in this group had varying degrees of left ventricular hypertrophy and LA dilated (over 50/50 mm);
- In 12 (10.9%) patients from this group the presence (both as ultrasound and clinical) of aortic and mitral regurgitation was highlighted.

In patients with atrial flutter, four patients were male and one, female. All male patients had uncontrolled hypertension until the time of admission and were also drinkers (declarative and positive markers). Female patients were also old, hypertensive, with BP neglected and consuming alcohol.

From the group of patients with AF and atrial flutter, 15 (13.6%) patients, 12 men, and 3 women, presented the clinical features of angina pectoris associated with the

presence of ischemic electrocardiographic changes. In all these patients were objectified elevated serum cholesterol, triglycerides and LDL cholesterol, dyslipidemia being an additional risk factor for ischemic heart disease.

From our group, 18 (16.36%) patients (10 men, 8 women) had constant sinus tachycardia, defined as a heart rate above 100/min in sinus rhythm. Of these, 4 patients (3 men, 1 woman) were diabetic, one under insulin therapy, and 5 were previously treated with calcium channel blocker (nifedipine or amlodipine).

Sinus bradycardia was encountered in 13 (11.8%) cases (7 men, 6 women), heart rate was between 48-55/minute. In 5 cases drug etiology was taken into account; patients were previously treated with beta-blocker.

CONCLUSIONS

The presence of supraventricular rhythm disorders in hypertensive patients has the following implications:

- Some arrhythmias may be an expression of an excessive sympathetic nervous system activation or, on the contrary, of vagal predominance, if sinus bradycardia;
- A part of arrhythmias occurring in hypertensive patients are hypertensive cardiomyopathy expression (following a sustained, uncontrolled hypertension);
- A large percentage of these arrhythmias are due to associated risk factors (smoking, alcohol) and even medication management (e.g. frequent sinus tachycardia in patients who received treatment with the dihydropyridine group anticalcic or sinus bradycardia more frequently in those receiving beta-blockers);
- In some situations rhythm disturbances disappear with the normalization of the BP values, however, that often requires the specific combination of anti-arrhythmic medication with antihypertensive;
- Depending on the severity of certain arrhythmias (e.g. atrial fibrillation), they require combining anticoagulant and antiplatelet therapy.

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PACT WITH THE DEVIL: ALEMTUZUMAB THERAPY, IMMUNE SUPPRESSION AND INFECTIOUS COMPLICATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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PACT WITH THE DEVIL: ALEMTUZUMAB THERAPY, IMMUNE SUPPRESSION AND INFECTIOUS COMPLICATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA (Abstract). Infectious complications are an important cause of hospitalization in patients diagnosed with chronic lymphocytic leukemia. The pathogenesis of infection is complex, involving both disease-induced and treatment-related immune depression. During the last decade, the management of chronic lymphocytic leukemia (CLL) has been redefined by the approval of monoclonal antibody-based treatment, which resulted in improved therapeutic responses. Nonetheless, the profound lymphopenia induced by monoclonal agents was accompanied by increased incidence of infections caused by a new spectrum of opportunistic microorganisms. We report the case of a patient with hypercellular CLL who received Alemtuzumab as first line therapy and obtained a satisfactory therapeutic response, but developed subsequent atypical infectious complications. **Keywords:** CHRONIC LYMPHOCYTIC LEUKEMIA, ALEMTUZUMAB, IMMUNE SUPPRESSION, MYCOBACTERIUM TUBERCULOSIS, ATYPICAL INFECTION.

Infectious complications represent a significant cause of morbidity and mortality in patients diagnosed with chronic lymphocytic leukemia (CLL), infection-related mortality ranging between 30-50% (1).

The pathogenesis of infection is complex, involving both disease-induced and treatment- related immune depression. Immune depression determined by the natural course of the disease features hypogammaglobulinemia, T-cell subpopulations abnormalities, B-cell and neutrophil defects, as well as neutropenia through bone marrow involvement (2, 3). In addition to these preexisting abnormalities, specific therapy further increases the risk of infection by agent-specific effects on the immune system. Monoclonal antibodies represent a novel and innovative treatment approach in CLL, in combination with standard chemotherapy or in single-agent regimens. Alemtuzumab is a monoclonal antibody directed against CD 52, expressed on tumor cells, and normal B and T cells, and macrophages (4). Its introduction in CLL therapy has been linked to an in-

creased risk of viral infection, notably cytomegalovirus (CMV), opportunistic infections, prolonged lymphopenia and neutropenia, especially in pretreated patients or refractory disease (5,6). We report the case of a patient with hypercellular CLL who received Alemtuzumab as first line therapy with a satisfactory therapeutic response, but developed subsequent atypical infectious complications.

CASE REPORT

We present the case of a 41-year-old male patient with low socioeconomic status admitted to our hospital for general fatigue, night sweats and abdominal swelling experienced over the previous 3 months. Family and personal history were not significant. Physical examination revealed ECOG (Eastern Cooperative Oncology Group) performance status 2, splenomegaly 25 cm below the left costal margin, hepatomegaly 9 cm, and generalized adenopathy of 3 cm maximum diameter. The complete blood count revealed extreme hyperleukocytosis 934.000/mm^3, lymphocytes 919.000/mm^3, Hb 7.5 g/dl, and platelets 30.000/mm^3. Biochemical analysis showed elevated LDH, uric acid, and erythrocyte sedimentation rate, with the rest of the parameters within normal range. Pulmonary radiography was normal. Flow cytometry identified a lymphoid population with specific CLL phenotype, CD 38 negative. Risk assessment at diagnosis was based on hyperleukocytosis, large tumor mass, elevated LDH, CD 38 negativity, and absence of deletion 17 p. Due to the large circulating tumor mass, the patient was considered high risk. Alemtuzumab was started in standard regimen of weekly administration for 12 weeks, inducing a partial response: normalized lymphocyte count, persistent splenomegaly (24cm), hepatomegaly (8 cm), anemia and thrombocytopenia. In the eleventh week of treatment the patient developed a prolonged febrile syndrome. Alemtuzumab therapy was discontinued. The etiologic spectrum of febrile splenomegaly in this case included infection or CLL transformation to aggressive B-cell proliferation (Richter syndrome). Alemtuzumab therapy included the patient in the highest risk class for infection (grade 4 neutropenia (250/mm^3) and lymphopenia). Cytomegalovirus (CMV) infection was ruled out by successive PCR determinations. Hepatitis viruses were absent, and empirical antiviral therapy with acyclovir and valaciclovir was ineffective. Repeated blood cultures remained negative throughout the 2 months of persistent fever. Broad spectrum antibiotics were unsuccessful. Invasive hepatosplenic fungal disease was highly unlikely due to normal ultrasound appearance, normal liver function tests and unresponsiveness to empiric antifungal therapy. Serology for leishmaniosis was negative. We considered the possibility of a mycobacterial infection. Diagnostic splenectomy and hepatic biopsy were performed to rule out the probability of Richter syndrome. Histopathologic examination and tissue cultures certified the presence of Mycobacterium tuberculosis. The general health status of the patient declined immediately after splenectomy, with persistence of the febrile syndrome and cholestasis. The pulmonary radiography revealed features of disseminated miliary tuberculosis. The patient was referred to the pneumology department where standard tuberculostatic therapy was initiated (isoniazid, pyrazinamide, ethambutol, rifampicin) with prompt resolution of the febrile syndrome. Tuberculostatic treatment was continued for 9 months. The patient maintained the partial

therapeutic response for 2 years before disease recurrence through hyperleukocytosis and thrombocytopenia.

DISCUSSION

The major determinants of infection in CLL patients are tumor-associated immune deficiency and immunochemotherapy-related immunsuppression (3).

CLL specific immune deficiency features defects in humoral and cellular effector mechanisms, complement and neutrophil function. Hipogammaglobulinemia is a common finding (7).

Defects in cell-mediated immunity are mainly linked to T-cell dysfunction. Apart from purine analogue and alemtuzumab therapy, CD4+ and CD8+ T-cell defects are the direct effect of the malignant clone or cytokine secretion and include impaired intracellular signaling and cytoskeleton dysfunction reflected in modified motility, chemotaxis and deficient immune synapses (8, 9). Aberrant populations of suppressor T cells and Th2 responses have also been reported (3).

NK cell and monocyte dysfunction are being cited (10). Finally, neutrophyls exhibit migration and phagocytic defects, while neutropenia becomes more prominent in the late stages of the disease through extensive bone marrow infiltration (2,3,7).

During the last decade the management of CLL has been redefined by the approval of monoclonal antibody-based treatment, which resulted in improved therapeutic responses. Nonetheless, the profound lymphopenia induced by monoclonal agents was accompanied by a corresponding increase in the incidence of infections caused by a new spectrum of opportunistic microorganisms (5).

Alemtuzumab is a humanized monoclonal antibody directed against the CD52 antigen expressed on both normal and malignant B CLL lymphocytes, NK cells, monocytes, macrophages and eosynophils. It is currently indicated as first line therapy in high risk patients associating 17p deletion and as salvage therapy for fludarabine-resistant disease (5, 6). Its mechanism of action is both complement and cellular-dependent. Alemtuzumab treated patients develop profound and prolonged (up to a year) lymphopenia, resulting in the increasing incidence of low CD4 count-associated infections. The degree of therapy-induced immunosuppression is also determined by CLL stage, previous chemotherapy regimens and treatment response. The spectrum of Alemtuzumab-associated infections ranges from banal bacterial infections, CMV reactivation (10-25%), varicella zoster virus, parvovirus, herpes simplex infection, *Pneumocystis carinii* pneumonia to more atypical pathogens: *Cryptococcus, Histoplasma*, invasive fungal infections (*Aspergillus,Candida,Rhizopus, Fusarium), Rhodococcus* and mycobacterial infection (7). Mycobacterial infection in CLL patients has been documented less frequently, with an incidence of 88/10000 cases, and is correlated with steroid use and autoimmune phenomena (11).

We present a case of splenic mycobacterial infection developed 12 weeks after Alemtuzumab therapy, presenting as a prolonged febrile syndrome associating treatment-resistant splenomegaly. Alemtuzumab-associated immunosuppression raised a high suspicion of CMV or atypical infection but, as the febrile syndrome persisted, the presence of a large tumor mass was considered suggestive for transformation to a higher grade lymphoproliferation. The cardinal diagnostic procedure that offered

the correct diagnosis was splenectomy. It is debatable whether splenic tuberculosis was already present at the time of diagnosis and progressed with the onset of Alemtuzumab-induced lymphopenia or developed during treatment. We hypothesize that the defects in CD8+ mediated immunity and the predominant Th2 response determined the activation of a preexisting infection, while subsequent treatment-induced T-cell depletion permitted its progression and extension.

The high incidence of infection in Alemtuzumab treated CLL patients resulted in the development of guidelines for antiviral, Pneumocystis and antifungal prophylaxis. There are no current recommendations for preemptive mycobacterial therapy in high risk febrile patients.

CONCLUSION

We presented an atypical and rare case of splenic tuberculosis developed under mixed Alemtuzumab and CLL-related immune deficiency. Although Alemtuzumab treated patients are at high risk for CMV reactivation, the clinician should consider atypical micobacterial infection in the presence of prolonged febrile syndrome, especially in patients with poor socioeconomic status. It remains to be seen whether the low incidence of mycobacterial infection in high risk febrile patients will result in specific guidelines for diagnosis and monitoring.

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THE CORRELATION BETWEEN ANTERIOR ISCHEMIC OPTIC NEUROPATHY THE NON-ARTHRITIC FORM, AND THE TREATMENT INVOLVING α -INTERFERON

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THE CORRELATION BETWEEN ANTERIOR ISCHEMIC OPTIC NEUROPATHY THE NON-ARTHRITIC FORM, AND THE TREATMENT INVOLVING α-INTERFERON (Abstract). The Anterior Ischemic Optic Neuropathy is an uncommon complication of α-Interferon treatment, characterized by the either temporary or permanent diminishing in visual acuity. We are hereby presenting the clinical case of an Anterior Ischemic Optic Neuropathy (non-arteritic form) complicating interferon therapy for Chronic Hepatitis C. In such cases we recommend ophthalmological examinations and if severe ophthalmologic complications occur antiviral treatment should be stopped immediately. **Keywords:** ANTERIOR ISCHEMIC OPTIC NEUROPATHY, α- INTERFERON, VIRUS C PERSISTENT CHRONIC TOXIC HEPATITIS

Interferons are glycoproteins that have multiple antiviral, antiproliferative and immunomodulatory activities and can be effective in the treatment of hepatitis B and C, malignant melanoma, follicular lymphoma, *condyloma acuminata* (1,2).

Ophthalmologic complications with interferon therapy are rare, generally mild and transient(3).Optic neuropathy, retinopathy with cotton wool spots, hemorrhages, macular edema, trombotic microangiopathy occur in less than 1% of the patients (3).

The ocular complications are reported at the individuals with predisposing risk factors: diabetes, arterial hypertension, dyslipidemia(4).Systemic side effects due to interferon use are: flu-like syndrome, myalgia, rash, hypotension, thrombocytopenia, depression, anemia, peripheral neuropathy(1,3).

CASE

We've examined patient B.E, aged 64, a retiree, living in Iaşi. The patient was admitted on March 23rd, 2013, for the following symptoms: a progressive diminishing in visual acuity at R.E. for approximately two weeks, and the peripheral narrowing in her visual field at the O.U. The patient men-

tioned that she had been diagnosed some 36 months prior to her admission, with virus C persistent chronic toxic hepatitis, and then she had been under treatment with α- Interferon (IFN-PEG 2α, 180 mcg/ml/ weekly) for 2 months and with Ribavirin (1.200 mg/ daily). Her general pathological history was: Virus C Persistent Chronic Toxic Hepatitis, High Blood Pressure Stage III, Chronic Vestibular Syndrome, Multiple Lacunar Cerebral Infarctions. The patient had no previous visual problem. The biomicroscopic exam: normal. Visual acuity R.E. 1/3, L.E.1. Cromatic sense: R.E. red-green axis dyscromatopsia, L.E. normal. R.E. relative afferent pupillary defect, L.E. normal. The ophthalmoscopic exam: R.E. diffuse hyperemic optic nerve papilla, indefinite contour, edematous, peripapillary hemorrhages, "flaming", of small dimensions in the inferior temporal zone, narrowed arteries, veins dilated, Salus-Gunn sign, testing positive for foveolar reflex macula; L.E. optic nerve papilla of clear contour, temporally pale, narrowed arteries, veins dilated, Salus-Gunn sign, testing positive for foveolar reflex macula. The intraocular pressure: P.R.E. 14 mmHg; P.L.E. 16 mmHg. The anamnesis data, the general and the overall ophthalmologic clinical exam are pointing to the following probable diagnosis: O.U. Anterior Ischemic Optic Neuropathy Non-Arteritic Form, Secondary α-Interferon.

Other complementary explorations were: visual field, M.R.I., carotid and temporal arteries Doppler ultrasonography.

At the M.R.I. native exam (M.R.I. Nr.725/30.05.2013), no suggestive M.R.I. modifications were shown, on either M.A.U, P.E.I.C, A.V.C. or cerebral edema. The orbit, the optic chiasm, the optic strips were found to be within normal limits. Some cerebral atrophy, more prominent in the frontal-temporal area, bilateral, asymmetric.

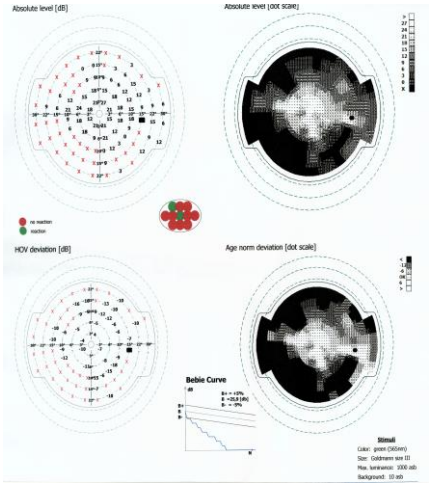

Fig. 1a. V.F.R.E. lower and upper arcuate scotoma, with a narrowing in the visual field fixed at 10^0 in the nasal sector, and 15^0 temporal ocular fixations

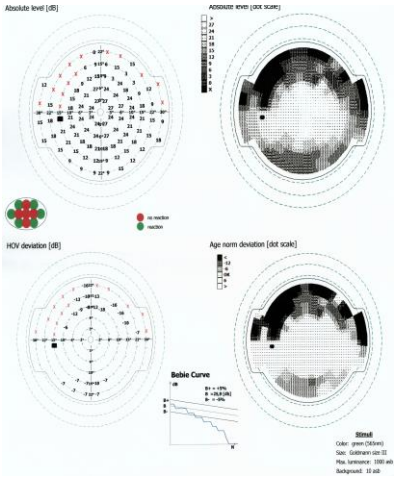

Fig.1 b. V.F.L.E. lower paracentral scotoma, upper arcuate scotoma

Laboratory investigations: VSH 90 mm/h, CRP 64 mg/l, Erythrocytes

$3.08.10^3.10^3/\mu L$, Hemoglobin 10,2 g/dl, Haematocrit 31,5 %, Average Erythrocytes volume 102/fl., Average Erythrocyte Hemoglobin 33/pg, Lymphocyte 1 x $10^3/\mu L$, mean Platelet volume 7,6/fl, Lymphocyte 23,8%, Urea 75,8 mg/dl. The internal medicine exam established the following diagnoses: C Virus Chronic Persistent and Toxic Hepatitis, Hypertension in the 3rd degree with great additional risk, Macrocytic Hypochromic Anemia, Multiple Lacunar Cerebral Infarctions, 1st degree Obesity, Sequel Pulmonary Tuberculosis. It was recommended: low in lipids and low in soda diet, treatment with Metoprolol 100 mg/dayly, Indapamide 1.5 mg/dayly, Ribavirin 1.200 mg/dayly, α-Interferon 180 mcg/ml/weekly, Acetylsalicylic Acid 75 mg/daily. The patient refused the temporal artery biopsy (no clinical sign of irregulary enlarged and ropey appearance of the temporal arteries was noticed).

The carotid and temporal arteries Doppler ultrasonography did not highlight any obstructive modifications at the carotid and temporal artery levels, indicative of a carotid obstructive syndrome and for the Anterior Ischemic Optic Neuropathy the Arteritic Form. The Otorhinolaryngology exam did not highlight neither acute nor chronic inflammatory process in the vicinity.

Following every investigation, the visual loss was decided to be due to α-Interferon therapy and the positive diagnosis takes shape: O.U. Anterior Ischemic Optic Neuropathy Non-Arteritic Form, Secondary α-Interferon; C Virus Persistent Toxic Chronic Hepatitis; Macrocytic Hypochromic Anemia; Multiple Lacunar Cerebral Infarctions, Atherosclerotic Hypertension in the 3rd degree, of very high risk; 1st degree Obesity; Sequel Pulmonary Tuberculosis. The diagnosis is being backed up by: the visual loss, the ophthalmoscopic exam (hyperemic disc edema), the visual field changes, the chronic treatment with α-Interferon, the risk factors (arterial hypertension, obesity), M.R.I., carotid and temporal Doppler ultrasonography.

The differential diagnosis

The papillary edema differential diagnosis is by the ischemic optic neuropathy arteritic form, non-arteritic form, toxic, optic neuritis, pseudo-syndrome Foster Kennedy.

Treatment

Throughout hospitalization, the patient was administered the following: systemic corticosteroids and antibiotics, vitamin therapy (B_1, B_6), aspirin, neurotrophics.

EVOLUTION

One month later, the evolution under treatment was favorable: V.A.R.E. 2/3, V.A.L.E.1, the optic disc edema partially resolved, resorption of the peripapillary hemorrhages, but the visual field did not improve. The prognostic under treatment: cautious. It was recommended a treatment with peripheral vasodilators, neurotrophic, aspirin, antihypertensive and the interruption of α-Interferon treatment immediately.

The antiviral treatment (α-Interferon 180 mcg/ml/weekly and Ribavirin 600 mg/daily) was not stopped and the patient experienced seven months later a relapse of optic neuropathy of controlateral eye. Ophthalmologic examination showed: the visual acuity L.E. 1/500, R.E. 2/3; L.E., hyperemic optic disk edema with peripapillary hemorrhages, R.E. optic nerve papilla of clear contour, temporally pale, L.E. relative afferent pupillary defect, O.U. red green dyschromatopsia. Laboratory investigations: VSH= 48 mm/1h, RBC=

3,68/$10^{3x}10^3$/µl, TGP= 93 U/L, TGO= 42 U/L, Fibrinogen= 351µg/dl, CRP = 1,1 mg/L. IgM, IgG for *Borrelia, Toxoplasma, Toxocara* was negative. Complementary explorations were: L.E. constricted visual field at 5^0 ocular fixations, R.E. constricted visual field at 5^0 in the nasal sector and at 10^0 in the temporal ocular fixation; fluorescein angiography (fig. 2).

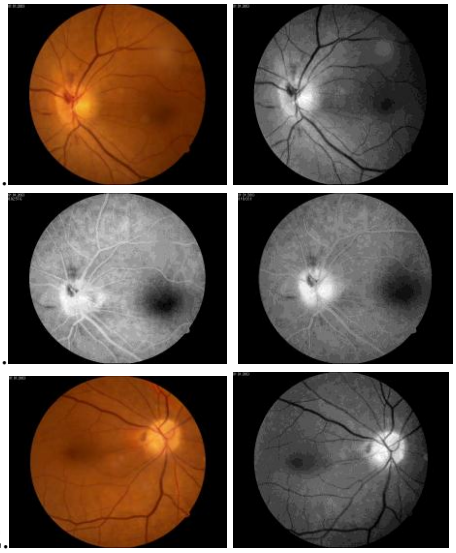

Fig. 2. Flourescein angiography revealed: L.E. blocking retinal flouerescence flame-shaped hemorrhages; O.U. hiperflourescent papillary growing in intensity until the late phase

The treatment was: intravenously metilprednisolone therapy, followed by oral metilprednisolone one mg per kilogram body weight for ten days, followed by tapering, vitamin therapy (B1,B6), aspirin, neurotrophics. The recommendation was the rapid cessation of α-Interferon therapy. One month later, the ophthalmologic examination showed: visual acuity L.E. 1/8, R.E. 2/3, L.E. optic disk edema partially resolved, O.U. red-green dyschromatopsia.

DISCUSSION

The Anterior Ischemic Optic Neuropathy is either a idiopatic or secondary ocular condition. The World Health Organization criteria recommends that AION (non-arteritic form) associated with α-Interferon therapy is "possible" (1).

The literature reports few cases of AION (non-arteritic form) during antiviral treatment for chronic hepatitis C (2 cases), cancer (2 cases), treatment of primary trombocytemia (1 case), malignant melanoma (1 case), amyotrophic lateral sclerosis (5).

The mechanism for how α-Interferon could cause AION (non-arteritic form) is unknown(1); the underlying immunologic mechanism has been suggested. It has been proposed that interferon is able to produce auto antibodies leading to the depositions of immune complexes in the small retinal or optic arteries (6).

The α-Interferon is causing the formation of cryoglobulin, rheumatoid factor, anticardiolipins antibodies (7).

Interferon has immunomodulatory properties capable of stimulating the production of various interleukins that can cause an inflammatory response affecting the blood vessels, leading to ischemia (8).

CONCLUSIONS

AION (non-arteritic form) secondary α-Interferon therapy may occur in rare cases, but the clinicians should be aware of the possible association and recommend interruption of the antiviral treatment.

Our patient received corticosteroids, with favorable course of visual function; the interferon therapy was stopped immediately after the relapse.

We suggest that the patients who would receive interferon therapy should have ophthalmologic examinations before starting and during the treatment and to discontinue or stop the α-Interferon treatment, if serious ocular implications occur.

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NEWS

LASER TREATMENT OF ONYCHOMYCOSIS

The efficacy of 1064nm Nd:YAG laser for the treatment of onychomycosis was investigated by Noguchi *et al*. The study included 12 patients with onychomycosis caused by *Trichophyton rubrum* and *T. mentagrophytes*, confirmed by fungal culture and/or real-time PCR. The laser therapy was applied in 3 sessions at 4-week intervals, to a single hallux nail, with thickness at baseline <3mm and turbidity affecting <75% of the surface of the nail. The efficacy of the therapy was evaluated 6 months after the first treatment: the nail turbidity significantly improved in 3 cases (>70%), while in 2 cases turbidity improved 50-70%, in one case it was slightly improved (30-50%) and in 5 cases there was no change in turbidity (<30% improvement) ; in one case, the turbidity was worse. Overall, there was a decrease in the turbidity of the total lesion area from 664.4mmto 481.0mm and a 27.6% improvement. The study concluded that the 1064nm Nd:YAG laser therapy is well tolerated and can be used as an alternative for treatment of mild onychomycosis (Noguchi H, Miyata K, Sugita T, Hiruma M et al. Treatment of Onychomycosis Using a 1064nm Nd : YAG Laser. *Med Mycol J*. 2013;54(4):333-9).

Teodora Vremera

APPROACHING FRAILTY AS THE NEW GERIATRIC SYNDROME

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APPROACHING FRAILTY AS THE NEW GERIATRIC SYNDROME (Abstract): Ageing is inevitably associated with a decline in physiologic reserves. Frailty results from reaching a threshold of decline across multiple organ systems. By consequence, it is associated with a high vulnerability and reduced ability to maintain homeostasis. This vulnerability is not only age-related, but also related to disability and comorbidity, as illustrated by three clinical cases. Sarcopenia, which is defined as age-related loss of muscle mass, is considered to be a central manifestation of frailty. In addition to being highly prevalent in elderly population, frailty also exerts a substantial impact on quality of life. As it is extremely challenging by defying conventional medication and involving new therapeutically approaches, frailty fully qualifies as a new geriatric syndrome. **Keywords**: GERIATRIC SYNDROME, SARCOPENIA, FRAILTY SYNDROME

Just a word in the dictionary thirty years ago, frailty has become one of the most researched fields in the medical world. Over the years, it has had many definitions and different screening methods; nonetheless, all authors considered that frailty should have a multi-dimensional approach, involving a variety of psychological, social, emotional and spiritual elements, in addition to the physical ones (1-5).

Frailty is a clinical state of high vulnerability and reduced ability to maintain homeostasis, which is correlated with age and characterized by a decline of functional reserves which involve multiple organs and systems (6, 7). This vulnerability is not only age-related, but also related to disability and comorbidity. If disability is the result of the dysfunction of one or more systems, frailty is always an accumulation of multi-systemic dysfunctions. Disability is not always associated with instability, whereas frailty is always accompanied by this (8). All these three notions, though, often intertwine. Each of them has different prognosis on long term and needs a different therapeutic approach, this emphasizing the importance of their discovery and individual approach.

The cornerstone of frailty is sarcopenia, the loss of muscle mass and strength in aging population. Physiologic age-dependent changes (drop in growth hormone (GH), IGF-1, menopause/andropause) explain the impaired protein synthesis, the decline of muscle mass, strength, and bone density. Harmful consequences of sarcopenia in old age are loss of muscle

strength, inducing itself loss of mobility, neuromuscular impairment, and homeostatic balance failure syndrome with gait and balance disorders (9, 10). Sarcopenia increases the risk of falls and fractures – immobilization and its dreaded consequences, interferes with the nutrition skills (empty refrigerator) and malnutrition and favors the decline of the protein reserve of the body with diminished capacity to meet the extra demand of protein synthesis associated with disease and injury (11). Frailty is a part of all these different "vicious loops" including sarcopenia, neuromuscular impairment, falls and fractures, immobilization, malnutrition, impaired protein synthesis, and decreased protein reserve of the body (9).

In addition to being highly prevalent in elderly population, frailty also exert a substantial impact on quality of life. It is a multifactorial condition in which many risk factors interact and affect different organ systems, influencing clinical presentation, course of disease(s) and outcome of elderly patient. As it is extremely challenging by defying conventional medical wisdom and crossing traditional clinical boundaries, frailty fully qualifies as a new geriatric syndrome (7).

Lang PO, et col. (12) describes 3 stages of frailty. The preclinical stage encompasses the situations when the organism has enough physiological reserves to correctly respond to stressors (acute onset of a disease or aggravation of a previous one, physical or psychological trauma); in this stage recovery should be complete. In the stage of clinical frailty, the functional reserves are insufficient and the recovery is incomplete. The final stage is that of frailty complications, resulting from physiological vulnerability and reduction of functional reserves. This last stage leads to a high risk of falls, disability, polimedication, long-term hospitalizations, severe infections, institutionalization and death.

The clinical diagnosis of frailty is based on a multitude of signs and symptoms, such as weakness, fatigue, ponderal loss, decreased capacity to maintain balance, low level of physical activity, loss of motor performance, social withdrawal, mild cognitive disorder and growth of vulnerability to stress. There were several approaches to identify frailty. One was developed by Rockwood K, et col. (13) and consists of measuring deficits across many different types of health conditions: functional, clinical, and physiological. A second approach considers that frailty has a distinct pathophysiology and its own clinical presentation (14). Fried LP, et col. (15) defined a phenotype of frailty with the following features:

- unintended loss of weight (4-5 kg in a year);
- self-reported exhaustion;
- decrease of muscle strength (reduced grip strength, less than 20% in dominant hand);
- slowing down of walking speed (less than 20% for the walking time of 4,5 m);
- reduction of physical activity (less than 20% of caloric expenditure).

Meeting three out of five criteria define the frail elderly. There also has been identified a sub-clinical form, pre-frail, characterized by meeting one or two out of five criteria, this state having a high risk to progress to manifested frailty.

This phenotype, being the basis of screening methods and diagnosis in clinical practice, includes only the physical component of the syndrome, though neglecting other domains, such as physical and social

components. Thus, numerous new diagnosis scales of frailty have been developed which contain all components, such as the Canadian Study of Health and Aging (CSHA) Frailty Index, Edmonton Frail Scale, Groningen Frailty Indicator (16, 17).

A new system of biomarkers has been identified which could provide the early diagnosis of frailty: IL-6, TNFα, PCR, Ciliary Neutrophic Factor (CNTF), etc. However, recent studies (18, 19, 20) agree that the best evaluation and classification of frailty is made by the complete geriatric assessment.

To exemplify frailty as a geriatric syndrome, we have chosen 3 representative clinical cases, of patients hospitalized in the Geriatric Clinic of „Dr C. I. Parhon" Hospital, Iași.

Patient 1 is a 81 years old female who has been recently diagnosed by the general practitioner with pneumonia and treated at home with antibiotics. The evolution was not favorable and she was admitted in our clinic. She had dyspnea, cough with mucopurulent sputum, sweating, nausea, weight loss (approximately 5 kg in the last year), and fatigue. She had a history of hypertension, angina with left bundle branch block, and stroke with secondary right hemiparesis. She lives in the countryside with her family and she benefits from family support. The MRC (Medical Research Council) Scale for Muscle Strength for lower limbs and right upper limb = 4/5. The Groningen Frailty Indicator (GFI) = 8/15 points. Comprehensive geriatric assessment showed a high risk of malnutrition (MNA = 16/30 points) and an almost complete need for care for daily activities (ADL) and for instrumental daily activities (IADL). Need for assistance has increased from the moment of aggravation of respiratory disease and the 3-days bed rest. The patient has geriatric frailty syndrome neglected in the last year and aggravated progressively in the last 3-4 days.

Due to the multidisciplinary approach (correct and complete medical treatment, correct diet, physical therapy and adequate family support) she did very well during hospitalization: she acquired autonomy for ADL and decreases the level of assistance for IADL, she started to eat on her own and had a weight gain of 2 kg. She started to walk, first with the support of the family members and with a walker when discharged home. Later reevaluations showed that she maintained a good functional and nutritional baseline.

Patient 2 is a 77 years old female from an urban area. She was referred to our clinic by ambulance for fatigue, excessive sleepiness, temporal-spatial disorientation, intense pallor, and weight loss of approximately 10 kg in the last year. She has been living alone in a flat since her husband died 4 years earlier but she has a caregiver who comes every day. The family noticed depression and loss of appetite, refusing food for the last couple of weeks. She had a history of diabetes, hypertension and anemia but without any medical records. The very first investigations showed severe macrocytic anemia which requested several blood transfusions.

Comprehensive geriatric assessment was performed after the management of the acute episode. She had a mild cognitive impairment (MMSE = 23/30 points), malnutrition (MNA = 17/30 points) and clinical frailty (GFI = 9/15) but no depression. She did well during hospitalization with the help of the multidisciplinary team: she resumed feeding, initially with the help of the family and caregivers, later on her own. She started to

walk under the supervision of the kinetotherapeut, initially for short distances with bilateral support, then longer distances with the help of a cane. She gained weight, approximately 3 kg. In 6 months time she surpassed her previous functional and psychological baseline, mostly due to correct multidisciplinary approach and increased family support that motivated her to fight frailty and win the battle.

Patient 3 is an 85 years old female from the rural area. She was admitted for intense fatigue, weight loss of approximately 10-12 kg in the last year and edema. She had a history of atrial fibrillation and osteoarthritis. She had no family and lived alone. GFI = 6/15 points. Comprehensive geriatric assessment showed: the MRC for lower limbs is 3/5 (can walk but prefer to stay in bed), malnourishment (MNA = 15/30 points), needs assistance for IADL, mild cognitive impairment (MMSE = 24/30 points). We started a complex therapy, including nutritional assistance, physical therapy, adequate medication and psychological support but she deteriorated progressively, developed multiple organ insufficiency probably secondary to urinary sepsis and she died after 11 days of hospitalization.

DISCUSSION

We consider that these 3 cases are illustrative for the frailty syndrome. Even if the three patients had different pathologies, they still had several common features: weight loss, no physical activity, fatigue, different degrees of depression and mild cognitive impairment. All these represent the frailty phenotype observed as being more or less pregnant with the three patients described earlier.

Our first patient is a clinically frail lady with important cardiovascular pathology hospitalized for an acute infectious episode (pneumonia). She recovers well due to simultaneous treatment of both acute episode and frailty. The constant and active family support during recovery was definitely a very important factor in regaining patient's autonomy. The evolution of the acute pulmonary infection has been influenced more by the degree of frailty than by the preexistent cardiovascular condition.

The second patient had a more severe class of frailty so her recovery was not as good as in the previous case. It is to be noticed that frailty is a negative predictor in the evolution of geriatric patient and leads to comorbidity aggravation.

The third case represents an obvious frail elderly lady with a series of adverse events (lives alone, has no family) but no major comorbidities. Still, she didn't cope with this new stressor (urinary infection) despite the correct, multidisciplinary treatment applied.

These cases suggest that frailty is a continuum, ranging from latent physiological alterations to clinically manifest syndrome. This phenotype plays an important role in the outcome of elderly patients. Applying frailty scores systematically to elderly population could lead to early discovery and identifying the stage of frailty and early interventions to this syndrome, which can make a difference between autonomy and dependence, between being active and confined in bed and, finally, between an optimal quality of life and 'the burden of old age'.

Comprehensive geriatric evaluation is considered 'golden standard' for identifying frail, vulnerable individuals, preferably before the adverse outcomes of frailty itself occur. If frailty appears to be the primary

condition, the goal would be: prevention of muscle loss and improvement in strength and balance, preservation of fat-free mass, prevention of sarcopenia and balanced nutritional intake. The easiest way to prevent or to delay frailty is to prescribe regular physical exercises associated with additional nutritional supplementation (21, 22). In our experience, family support is of outmost importance in maintaining a good quality of life for the elderly persons is a strong motivation to keep them independent and is a strong impulse to fight all adverse outcomes of aging: disability, dependency, falls, acute illness, hospitalization. Frailty is a geriatric syndrome and it became the cornerstone of modern geriatric medicine; identifying and establishing a personalized preventive strategy for frailty is not only a need but also a responsibility.

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NEWS

THE RELATIONSHIP BETWEEN CARDIOVASCULAR DISEASES AND PERIODONTITIS

A recent study conducted by Khosravi Samani M and collaborators from the Department of Periodontics - Babol University of Medical Sciences - Iran aimed to investigate a possible relationship between cardiovascular diseases and periodontitis. In the study were included 60 patients with myocardial infarction as study group and 63 subjects with periodontitis without myocardial infarction as control group. The mean ages of patients in study and control groups were 54.97±9.68 and 55.89±11.9 years, respectively. Periodontitis was evaluated according to Ramfjord periodontal diseases index and also according to the number of missing teeth. The classic risk factors of myocardial infarction were recorded. The obtained data revealed that the patients who lost more than 10 teeth presented more risk of myocardial infarction (odds ratio of 2.73). A significant relationship between mean attachment loss and myocardial infarction (p=0.0001) was established. A relation between attachment loss more than 3 mm and myocardial infarction with odds ratio of 4 was established. Significant difference between mean periodontal disease index was seen in both groups (p=0.0001). Subjects with periodontal disease index more than 4 were at more risk of periodontal diseases (odds ratio of 7.87). The results obtained by the authors revealed the presence of significant relation between periodontitis and myocardial infarction which could serve as an alarm signal to treat periodontitis with great care. (Khosravi Samani M, Jalali F, Seyyed Ahadi SM, Hoseini SR, Dabbagh Sattari F. The relationship between acute myocardial infarction and periodontitis. *Caspian J Intern Med*, 2013; 4 (2): 667-671).

Irina Grădinaru

PULMONARY REHABILITATION - FROM GUIDELINES TO PRACTICE

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PULMONARY REHABILITATION – FROM GUIDELINES TO PRACTICE (Abstract): Pulmonary rehabilitation is an integral part of clinical management and health maintenance of patients with chronic respiratory disease who remain symptomatic or continue to have decreased function despite standard medical therapy. It is a spectrum of evidence-based, multidisciplinary, and comprehensive intervention for patients with chronic respiratory problems. Most evidence for the efficacy of respiratory rehabilitation is for patients with chronic obstructive pulmonary disease but there are encouraging results for extending its indications. Current programs of pulmonary rehabilitation are based on guidelines which formally grade the level of scientific evidence and on practical recommendations developed by several prestigious societies. The main components of a pulmonary rehabilitation program are patient selection and assessment, exercise training, education and self-management of the disease, psychosocial and behavioral intervention, nutritional support, outcome assessment and enhancement of long-term adherence to the rehabilitation recommendations. Exercise training is the central component of respiratory rehabilitation, aiming at physical reconditioning, and upper and lower limb muscle training, using several strategies to optimize training. Outcome assessment is an integral part of the program including the measurement of several valid, interpretable and easy to administer parameters. The beneficial effects of pulmonary rehabilitation are the improvement of dyspnea and degree of physical and social disability, less hospitalizations and exacerbations, better overall quality of life by increasing independence.
Keywords: GUIDELINES, PULMONARY REHABILITATION, PROGRAM.

According to the current definition, pulmonary rehabilitation is "an evidence-based, multidisciplinary, and comprehensive intervention for patients with chronic respiratory diseases who are symptomatic and often have decreased daily life activities. Integrated into the individualized treatment of the patient, pulmonary rehabilitation is designed to reduce symptoms, optimize functional status, increase participation, and reduce health-care costs by stabilizing or reversing systemic manifestations of the disease" (1,2).

Pulmonary rehabilitation has become a standard of care for patients with chronic lung diseases. The current evidence-based guidelines for diagnosis, management and prevention of these diseases (3) reffer to pulmonary rehabilitation patients with chronic obstructive pulmonary disease

(COPD). In the last years was assessed the role of rehabilitation programs in chronic lung diseases other than COPD: pulmonary fibrosis (4), primary pulmonary hypertension (5), before and after pulmonary surgery (lung transplantation) (6), the results being encouraging for expanding the indications of such programs.

The practical need for pulmonary rehabilitation occurred because patients with a chronic respiratory disease are highly symptomatic, need frequent hospitalizations, are physical deconditioning, and have a significant psycho-social dysfunction, all of which are accompanied by a significant decrease in their quality of life. From these observations it also came out the particularity of rehabilitation intervention, namely that it primarily addresses the subjective aspects of the disease, represented by the persistence of symptoms and the degree of disability (7).

In 2006 was published a joint statement on pulmonary rehabilitation of the European Respiratory Society (ERS) and American Thoracic Society (ATS) (2) this being complementary to other two major documents: the evidence-based guidelines of the American College of Chest Physicians (ACCP) and American Association of Cardiovascular & Pulmonary Rehabilitation (AACVPR) (8), which formally graded the level of scientific evidence, and AACVPR guidelines for pulmonary rehabilitation programs that offer practical recommendations (9). Updated evidence-based clinical practice guidelines for pulmonary rehabilitation, published in Chest in 2007 (10), AACVPR guidelines in 2010 (11) and the Canadian Thoracic Society guidelines (12) are added. These guidelines should be implemented by a multidisciplinary team that covers all the aspects of a comprehensive rehabilitation program and could be performed in all settings, including hospital inpatient, outpatient or even home in order to maintain the favorable results of respiratory rehabilitation (13).

COMPONENTS OF PULMONARY REHABILITATION PROGRAM

According to the guidelines, the main components of a pulmonary rehabilitation program are patient selection and assessment, exercise training, education and self-management of the disease, psycho-social and behavioral intervention, nutritional support, outcome assessment, and enhancement of long-term adherence to the pulmonary rehabilitation recommendations (2, 10, 11).

❖ Patient selection and assessment

Based on current evidence pulmonary rehabilitation should be considered primarily for the symptomatic COPD patients, presenting with reduced exercise capacity and decreased daily life activities but other patients with chronic respiratory disease and impairment can also be referred for achievement of some beneficial effects (14) (tab. I).

Concurrent diseases or conditions such as severe cardiovascular diseases, disabling neurological or orthopedic conditions may negatively interfere with physical training program while cognitive dysfunction and severe psychiatric disorders prohibit cooperation and understanding, being considered exclusion criteria.

Before entering a pulmonary rehabilitation program, patients should be complexly assessed in order to develop a strictly individualized program tailored to the patient's needs for maximal benefits (14) (fig. 1).

TABLE I

Pulmonary diseases appropriate for pulmonary rehabilitation

OBSTRUCTIVE LUNG DISEASE	RESTRICTIVE LUNG DISEASE
Chronic obstructive lung disease	*Interstitial diseases (interstitial fibrosis, sarcoidosis, occupational lung disease)*
Asthma	*Neuromuscular diseases (Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis)*
Bronchiectasis	*Chest wall diseases (ankylosing spondylitis, kyphoscoliosis)*
Cystic fibrosis	
OTHER PULMONARY CONDITIONS	
Lung cancer	
Primary pulmonary hypertension	
Obesity-related respiratory disease	
Before and after thoracic and abdominal surgery	

Clinical elements such as orthopnea, behavioral signs associated with pain (antalgic position, grimacing), dyspnea scales (mMRC scale - *modified British Medical Research Council*, modified Borg scale) and pain scales (visual analogue, verbal numerical scales) are used in order to assess these two symptoms that may directly affect patient's ability to follow the physical training program, thus reducing the benefits of the rehabilitation program (14).

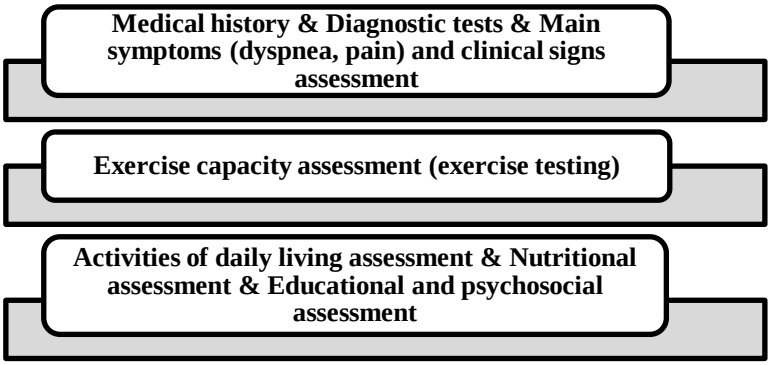

Fig. 1. Main steps in the assessment of patients included in pulmonary rehabilitation programs (14)

Exercise capacity is determined through incremental exercise tests on cycle ergometer or treadmill or through field tests, particularly the 6-minute walk test, and the guidelines for its application reducing the variability in administration of the test (15). This stage of assessment is crucial for the correct prescription of physical training level in the setting of respiratory rehabilitation program.

Initial assessment of activities of daily living (ADL) allows determining the degree of dependence of chronic pulmonary patients on family members or caregivers.

In recent years were developed questionnaires extremely easy to use by physicians and patients having a small number of items that cover all key elements with impact on the quality of life of patients with lung diseases, such as CAT (*COPD Assessment Test*) (16,17) and ACT (Asthma Control Test) (18), the Romanian version of these questionnaires being validated.

❖ **Exercise training**

Exercise training is the central component of the comprehensive respiratory rehabilitation program and has three practical goals: physical reconditioning, respiratory muscle training and upper and lower extremities exercise.

The program is gradual and individualized, tailored to the patient's exercise performance under the supervision of a physician. Its benefits are increased exercise capacity, reduced ventilatory effort at submaximal work loads, and improved perception of dyspnea and fatigue, so common in patients with chronic lung diseases. In addition, sequences of high-intensity training result in an anabolic stimulus and improve oxidative capacity of the skeletal muscles, their dysfunction being improved gradually (19).

Physical reconditioning aims at achieving a sensation of „well-being" and improving the chances of survival. Physiologically, this is achieved by increased maximal oxygen uptake, improved tissue oxygen utilization, increased skeletal muscle strength and endurance, improved motor coordination and change in body composition with loss of adipose tissue in favor of muscle mass (19).

These benefits are achieved by selecting the adequate intensity, frequency and duration of training sessions, and by maintenance strategies following pulmonary rehabilitation in order to slow benefits' decline after stopping the program.

There is still controversy over the best parameters for training sessions, but the evidence-based recommendations suggest an intensity of 50% -70% of the predicted maximal heart rate or peak oxygen uptake, session duration of 30 minutes, and a frequency of 3-4 sessions /week for 6-12 weeks (1, 7, 10, 12, 19).

Given the resting tachycardia and severe skeletal muscle dysfunction in patients with chronic respiratory diseases some authors have proposed the use of dyspnea ratings instead of heart rate to guide exercise training intensity, and a two-phase training, intensive training followed by maintenance training, in order the benefits to last longer (19,20). Also, there are several strategies to optimize physical training of these patients: use of optimal bronchodilator therapy, oxygen supplements whether desaturation is present or not, administration of helium gas mixtures, application of noninvasive mechanical ventilation, respiratory muscle training, neuromuscular electrical stimulation, extension of the training program beyond 12 weeks, and combined resistance and aerobic exercise (1, 12, 19, 21).

Lower-limb muscle training leads to increased walking distance, improved peak oxygen consumption, and even reduces the severity of dyspnea when performed at an intensity of 60-80% of maximal work capacity as determined by an exercise test, with close monitoring of heart rate and dyspnea. Exercise intensity is progressively increased during a 24-session training cy-

cle. Studies on this issue of physical training in patients with chronic pulmonary disease had conflicting results, some showing benefits across all stages of disease, others only in those with mild to moderate impairment (22).

Upper-limb muscle training is beneficial in strengthening scapular belt and upper chest wall muscles, thus improving the traction force applied on the thoracic cage during inspiration in this patient in which diaphragm functions in ventilatory mechanics are affected. The best results are obtained with supported arm ergometry training, performed 20 minutes/session, at 60% of maximal work capacity as assessed by an exercise test. Also beneficial is the method for unsupported arm training, consisting in lifting dowels (weight = 750 g) to shoulder level in the rhythm of breathing rate and progressively increasing its weight by 250 g/week. Each session dyspnea and heart rate should be monitored (11, 14).

❖ **Education and self-management**

Education delivery in pulmonary rehabilitation is performed individually or in small groups and is focused on behavioral modification of both the patient and his/her family members in order to achieve active involvement.

Any educational intervention should begin with a basic simple to understand overview of the main physical and psychological changes occurring in chronic lung diseases and their evolvement over time. The patient should be advised to advanced planning, simplify and prioritize his daily activities so that in the early stages of the disease to be able to perform his job-related tasks and in the advanced stages to carry out activities of daily living (23).

For the purpose of energy conservation essential is the assisted acquisition of breathing, cough, and postural drainage techniques, particularly important in patients with hyperinflation (COPD patients) and/or in those with bronchial hypersecretion (bronchiectasis or cystic fibrosis). Relaxation and stretching techniques (yoga, Pilates) help to improve the patient's psychological distress and increase ventilatory coordination (24).

Essential in chronic pulmonary patient education is counseling and therapeutic support for quitting active smoking and avoiding exposure to secondhand smoke or other respiratory hazards. Also, the patient has to know how to self-administer the inhaled medication properly, how to use auxiliary devices (spacers), to be aware of the possible side effects and of the effects of drug abuse, concerning especially sympathomimetics, theophylline or antibiotics (tachyarrhythmias, elevated blood pressure, precipitation of angina episodes, microbial multiresistance). Proper use of long-term oxygen therapy and noninvasive positive pressure devices should also be explained to the patients with chronic respiratory disease.

❖ **Nutritional assessment and counseling**

Various studies have shown that weight loss in chronic respiratory patient is a poor prognostic indicator, especially in those with COPD. Alarming weight loss refers to the loss of more than 10% of body weight in the last six months or more than 5% in the last month and is particularly dramatic in disease exacerbations when food intake decreases, systemic inflammation is markedly increased, and energy balance becomes negative. On the other hand, obesity adversely affects respiratory function by increasing the load on the already compromised respiratory system (2, 14). Anthro-

pometric measurements of nutritional status are body mass index (BMI) and estimation of lean body weight by bioimpedance, a simple and noninvasive method (25, 26).

Nutritional counseling is aimed at achieving an adequate alimentary plan for achieving and maintaining an optimal weight including, if necessary, supplements in liquid form.

❖ **Psychosocial support**

Patients with chronic pulmonary disease commonly have psychiatric co morbidities, primarily anxiety and depression, generated by the difficulty of accepting their illness as well as coping with the associated physical and neuropsychological impairments having major adverse consequences on the quality of life (27). Assessment is done by clinically validated questionnaires and scales (Beck depression inventory and depression scale for geriatric patients) and the results are integrated into the multidisciplinary program of pulmonary rehabilitation (28).

The practical way of providing psychosocial support is to organize support groups focused on specific issues, group therapy and education, individual psychotherapy, stress reduction and panic control techniques with the permanent involvement of family members and caregivers (14, 21).

❖ **Outcome assessment**

Outcome assessment is an integral part of a comprehensive respiratory rehabilitation program and aims to quantify its beneficial effects (fig. 2).

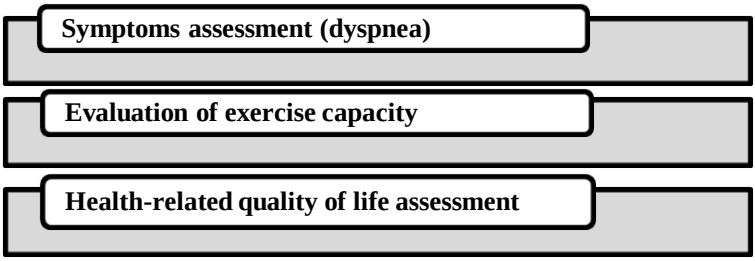

Fig. 2. The main objectives for outcome assessment in pulmonary rehabilitation program

The clinical practice use several types of measurements, validated in studies, easy to interpret and manage. To evaluate the dyspnea, the most severe symptom of patients with chronic respiratory impairment, are used dyspnea scales (Borg, mMRC), whichever is subjective improvement, which correlate with decreased ventilatory burden during exercise as a result of training (1, 2, 10).

Exercise capacity and its improvement after aerobic or resistance exercise training is assessed by conventional exercise testing, cardiorespiratory testing - which is more complex, or by the pragmatic option of field tests, the 6-minute walk test being the most commonly used and recording the increase in the walking distance – the estimated minimal clinically important difference being 54 meters (2, 10, 29). Pedometers have been introduced recently, being simple and portable devices that measure the number of steps and are useful in self-monitoring, progress assessment, and in enhancing compliance with the training program and activities of daily living (30).

Health-related quality of life is assessed using generic or disease-specific questionnaires, being commonly used in COPD. *St*

George's Respiratory Questionnaire (SGRQ) is one of the most frequently used questionnaires being designed for long-term studies and having well represented psychometric properties, the Romanian version being validated. In studies, SGQR score improvement was maintained even 1 year after the end of respiratory rehabilitation program (2, 10, 21).

CONCLUSION

Pulmonary rehabilitation is a dynamic evidence-based field of pneumology, which is an integral part of the current treatment of chronic respiratory diseases.

Respiratory rehabilitation programs follow the current guidelines, being delivered in inpatient or outpatient settings, their success depending on the skill and experience of the multidisciplinary team, socio-economic conditions, and adherence of patients and their families.

The most important benefits of pulmonary rehabilitation are the relief of symptoms, particularly dyspnea, improvement of complications, less and shorter exacerbations and hospitalizations, with significant cost-reduction, decreased physical and social disability, improved muscle deconditioning and increased exercise capacity, reduced anxiety and depression and improved emotional well-being, all these resulting in an improved overall quality of life for patients with chronic lung disease.

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EPIDEMIOLOGICAL CHARACTERISTICS OF PERIPHERAL ARTERY DISEASE: RETROSPECTIVE STUDY

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EPIDEMIOLOGICAL CHARACTERISTICS OF PERIPHERAL ARTERY DISEASE: A RETROSPECTIVE STUDY (Abstract): Peripheral artery disease (PAD) is a common condition. Over the past 20 years the risk factors for PAD have changed. **Aims:** To identify the regional characteristics of the patients with PAD and the sex- and urban/rural-related differences. **Material and methods:** Retrospective study on patients admitted to the Iasi "St. Spiridon" University Hospital in the interval 2007- 2012. The dataset was prepared for t student analysis in in view of determining statistical significance. **Results:** Of the 361,248 patients admitted to the 20 units of the hospital, 2,623 patients were diagnosed with advanced stages of PAD (Leriche - Fontain III and IV). Significant statistical differences were found between men and women, and between rural and urban men (p=0.001). 20.5% of the patients underwent amputations. **Conclusions:** This is one of the first studies that has identified urban/rural differences. The characteristics specific to PAD patients from North-Eastern Romania are presented. **Keywords:** PERIPHERAL ARTERY DISEASE, EPIDEMIOLOGY, RISK FACTORS.

Peripheral artery disease (PAD) is a major cause of disability, morbidity and mortality with a negative effect on the quality of life (1, 2). PAD is chronic, ischemic, with slowly-progressive. The patient adapts to the compromised arterial system by reducing oxygen demand and indirectly physical activity.

Standard therapy for PAD includes the administration of antiplatelet drugs (3,4) and a good control of the risk factors (smoking, dyslipidemia, diabetes, hypertension, etc.) (5) Antiplatelet therapy is usually combined with physical exercise that may help prevent or delay the onset of atherosclerosis. In advanced cases a wide variety of interventional surgical procedures are used to address specific findings in each individual case (5).

The prevalence of PAD increases with age, several epidemiological studies finding it in the range of 3% to 10 % (6), and 15% to 20% in persons over 70 years (7).

Many risk factors are involved in the development of PAD, their prevalence being higher in the male population. The most frequently involved risks are: sex, smoking, diabetes, hypertension and hyper-

lipidemia (8).

MATERIAL AND METHODS

We conducted a retrospective study including a total of 2623 subjects diagnosed with various forms and stages of PAD. The data were extracted from the database of "St. Spiridon" University Hospital (Iasi, Romania), covering the interval January 1, 2007 – January 1, 2012.

The data were statistically analyzed using *Microsoft Excel* and *IBM® SPSS® Statistics version 17.0*, and *Graph Pad Prism* for graphics.

The obtained data were organized into groups according to age, sex and urban/rural area to facilitate the analysis of treatment and laboratory data, by writing a Visual Basic macro in MS Excel. A descriptive statistical analysis was performed for the various subgroups. Student t test was used to compare the groups. This type of analysis is the best choice for analysis of small data sets specific to medical studies, for comparison with 95% confidence interval and α value of 0.05.

RESULTS

During the study interval a total of 361,248 patients were admitted to the 20 units of the hospital, Vascular Surgery, Cardiology, Internal Medicine included. Of these, 2,623 patients were diagnosed with PAD, totalizing 4869 readmissions during the study interval.

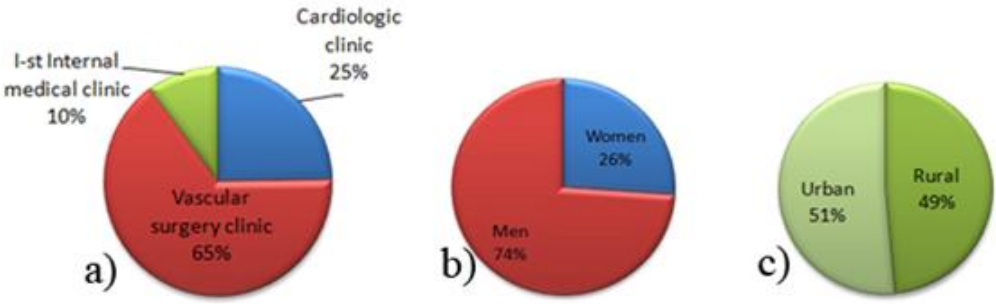

Fig. 1. Distribution of the PAD patients according to a) hospital unit b) sex, and c) urban/rural area

Of all PAD patients, 685 were women and 1938 men (fig. 1b.). All patients were caucasians and presented the classical urban/rural characteristics (fig. 1c).

The mean age of the study group was 64.5 years, and median age 66 years. Age distribution was bimodal (about 57 years and about 69 years) (fig. 2a). This trend suggests the presence of two different groups that need further analysis and separation (fig. 2b, c, and d).

Fig. 2b shows the age distribution by sex. Bimodal age distribution with a first peak at 57 years seen in the male population is almost absent in women (fig. 2b). Further, urban/rural distribution of women showed bimodal distribution in rural women while in the urban women the distribution is lognormal (fig. 2c).

In the male patients there was a clear rural/urban difference (fig. 2c). Urban patients had an obvious bimodal distribution

of the age at onset (57 and 70 years) while in the rural male patients the first peak was almost absent, as in urban women. Furthermore, in the urban patients the onset was at slightly older age.

The prevalence of PAD in men from rural areas was higher than in urban men, fact also confirmed by Student T test *(Paired Samples T Test)*, statistical significance p = 0.001, the same being true for women (p <0.05).

Smoking is one of the most important risk factors for peripheral arterial disease. We evaluated only the patients admitted to internal medicine units, a total of 1028 patients, of which 857 (83.4%) self-declared smoking or former smoking status. There is a clear male prevalence of smoking (p=0.001), while no rural/urban difference in smoking status was noticed (p = 0.753).

Laboratory tests showed an average fibrinogen level of 413 mg/dl, above the normal upper limit (200-400 mg/dl). Also average total cholesterol was 200 mg/dl, the normal upper limit. HDL-cholesterol was lower (average 38 mg/dl) than normal level (normal > 40 mg/dl).

Of the 2623 study patients 537 (20.5%) underwent amputation (fig. 4).

Surgical revascularization techniques were used in 782 (53.7%) patients, 424 (54.2%) requiring multiple revascularization techniques. A total of 1457 surgical procedures were performed in patients with PAD.

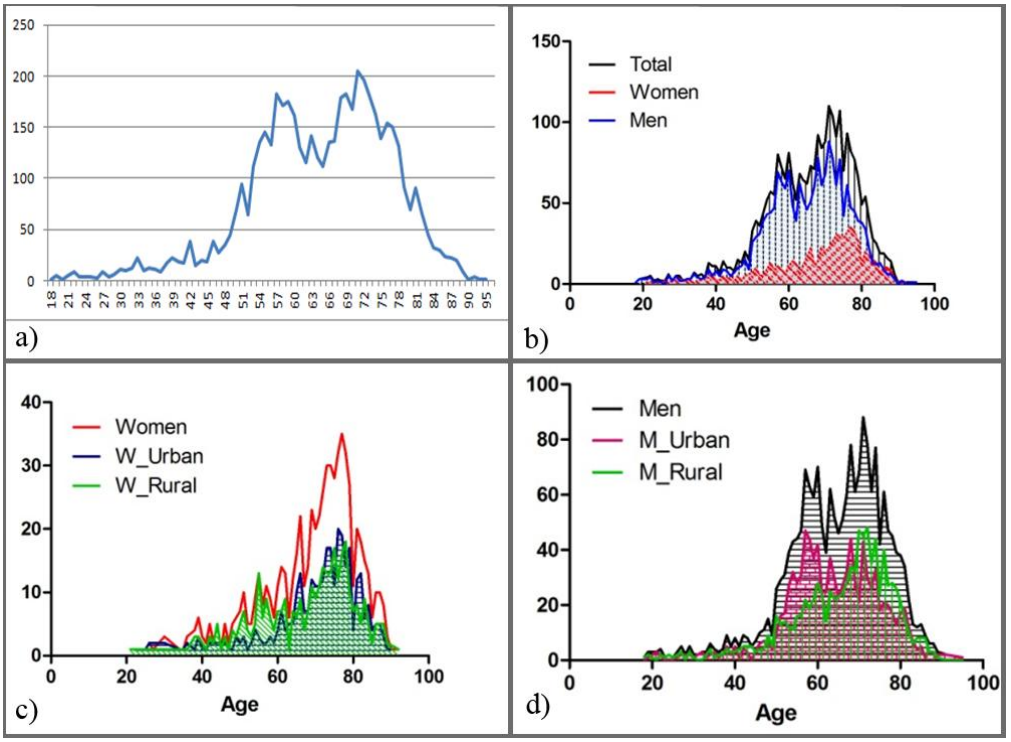

Fig. 2. Distributions of patients according to: a) age, b) sex vs. age, c) women age vs. urban/rural, and d) men age vs. urban/rural. The graphs are presented as age vs. patient frequency.

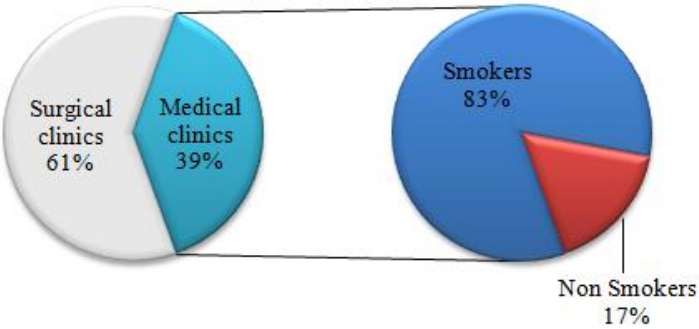

Fig. 3. Distribution of smoking patients

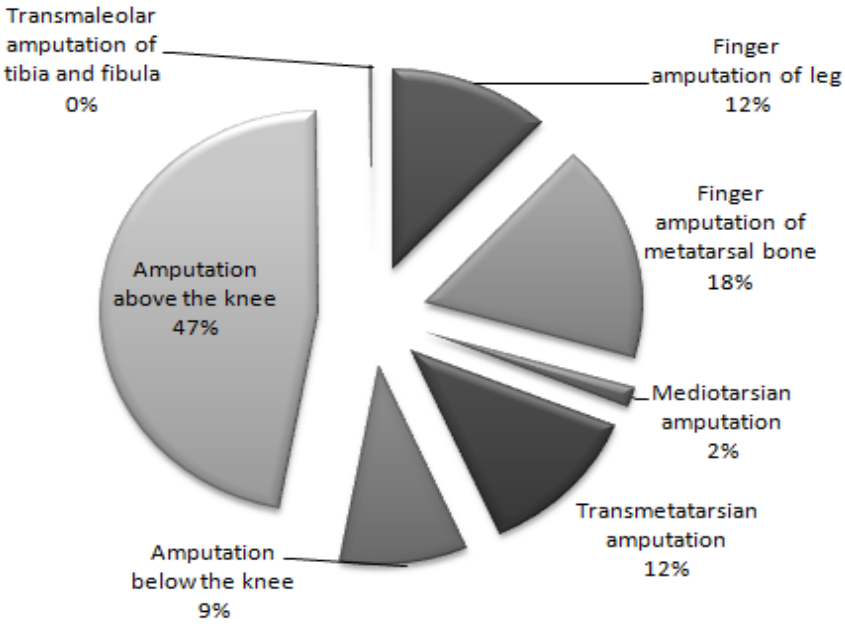

Fig. 4. Distribution of amputations performed in patients with PAD

In 602 surgical revascularizations (41.3%) synthetic or composite grafts were used, that required the association of anticoagulant therapy. In 338 (56.1%) amputations of the lower limb segments, and in 100 (16.6%) major amputations (thigh or calf) were performed.

DISCUSSION

Peripheral artery disease is known to be a disease affecting small and medium-sized arteries of the body, with a prevalence varying according to age and sex. In our study the prevalence PAD in the patients admitted to the Iasi "St. Spiridon" University Hospital Iasi was 1.35%; mean age of our patients was 64.5 years, very close to that reported by other studies (7).

The incidence of the disease in our study is lower than in other studies (9).

This difference may be explained by the late-stage presentation of most of our patients. All our patients were stage III or IV according to Leriche-Fontain classification.

The prevalence of PAD is higher in men than in women, fact not reported by previous studies. This can be explained by the socioeconomic status of our patients as demonstrated by the subgroups comparison. There are no statistically significant differences between women and men from urban areas. The high statistically significant difference between rural women and men areas ($p<0.001$) can be explained mostly by the fact that rural communities are traditional and religious, and smoking among women is rare.

A statistically significant difference ($p=0.001$) was found between rural and urban men. Thus, the onset of PAD is at a younger age in rural than urban males. This is mainly related to the rural life style and also very likely to socioeconomic status (10). It is confirmed by an obvious bimodal distribution (fig. 2d) with a first peak at a younger age (about 57 years) and an almost similar frequency at about 70 years. In the urban male population the onset at a younger age is much less obvious, while the second onset peak is at a slightly older age (about 72 years). Student T test showed that these data are statistically significant ($p = 0.001$), thus being a second characteristic never described by other studies.

Laboratory tests showed no differences between study groups except for hemo leukogram that detected higher red blood counts and hemoglobin concentrations in the smokers.

Several studies have demonstrated that amputation rate is 1-7% in patients with 5 to 10 years of persistent claudication. And only one patient out of four will complain of worsening symptoms (11).

There is no evidence that shows that patients with symptomatic PAD have a more rapid progression as compared with asymptomatic patients, and it was found that variations in disease symptoms are related to patient activity level.

CONCLUSIONS

Chronic ischemia of lower limbs has a high prevalence, and it is often under diagnosed. Its prevalence increases with age, and in the Moldavian rural population the onset is at a younger age. In this study we found that of the total number of patients admitted for vascular conditions, 1.4% had chronic ischemia. In these patients the disease is in advanced stages, and 60% of them require revascularization surgery or amputation.

In Iasi County, an estimated 5% of the total population (723,553 according to the 2011 census) has peripheral artery disease (over 36000 persons).

Another observation based on our study is the increased prevalence of PAD in men as compared to women, finding reported by few studies. This may be accounted for by the fact that women have later onset of PAD and a different lifestyle than men.

Under these circumstances, primary prevention of PAD is ineffective. It is a consequence of the fact that asymptomatic patients with critical ischemia do not present to a physician, and thus do not benefit from an early treatment and avoidance of complications in later stages.

The question why PAD has a younger age onset in urban men and rural women needs to be answered. This may require collaboration with the National Institute of Statistics for identifying: (i) the possible relationship between age of onset and under-

development (ii) relationship between geographical areas and regional infrastructure, (iii) relationship with unemployment, etc.

In further studies we plan to map geographically PAD prevalence in order to identify the regions where maximum attention should be given to prevention and screening.

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PARTICULARITIES IN DIAGNOSIS AND TREATMENT FOR INFECTIOUS ENDOCARDITIS IN CHILDREN

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PARTICULARITIES IN DIAGNOSIS AND TREATMENT FOR INFECTIOUS ENDOCARDITIS IN CHILDREN (Abstract): Infectious endocarditis (IE) represents a rare pathology in children, but with lethal potential. The goal of the therapy is fast and total eradication of the infection. **Aims**: To study particularities in diagnosis and treatment for infectious endocarditis in children. **Material and methods:** Children with infectious endocarditis hospitalized between January 2007 - February 2012 in the Cardiology Department of the „Sfânta Maria" Children Emergency Hospital of Iasi have been included in the study. **Results**: The patients are aged between 23 days and 16 years, the average age being 4 years old. At approximately 88% of the patients (14 cases), the endocardial damage appeared in the pre-existent valvular lesions, specially mitral and aortal. As associated congenital malformations, the patients prevailingly presented ventricular septal defect, mitral valve prolapse, arterial canal persistence, aortic stenosis, coarctation of the aorta. Blood cultures were collected and the most frequent identified etiological agents were: *Staphylococcus coagulase-positive, Streptococcus mitis, Staphylococcus speciae coagulase-negative, Staphylococcus haemolyticus, Streptococcus bovis, Escherichia coli***,** for which the antibiogram showed sensitivity for beta-lactam, cephalosporins, glycopeptides, trimethoprim-sulfamethoxazole, rifampicin, quinolone, lincosamides, oxazolidinones, and thus specific treatment was set up according to the antibiogram. **Conclusions**: The infectious endocarditis is a serious disease that affects young age too, leading towards exitus in some cases. Diagnostic imaging and early blood cultures are of relevance in order to intervene promptly. The treatment must be targeted and applied as fast as possible. **Keywords:** ENDOCARDITIS, HEMOCULTURES, CONGENITAL HEART MALFORMATIONS.

Infectious endocarditis is defined as the microbial infection of the endocardial surface (1). Infectious endocarditis represents "the infection of the endocardium with the presence of microorganisms at the level of the lesion" indicating that it may be the valvular, parietal endocardium, of some septal defects or a persistent arterial canal. This is a descriptive definition that stresses out the initial endocardial modifications, more frequent when addressed from a cardiologic perspective (2).

Over the last 50 years, major changes occurred in infectious endocarditis. There was a steep decrease in mortality, the etiological spectrum modified, new antibiotics and new lab techniques appeared, echocardiography developed and the susceptible population modified, the rate of the immune-compromised individuals and of the ones with prosthetic valves or pacemaker growing over those with rheumatic valvular disease (1).

Almost 10% of the endocarditis cases include pediatric population around the age of 10 (3, 4).

The incidence of the infectious endocarditis varies according to the location of the world population, thus 17 to 145 cases in a million persons being recorded per year. Despite new sophisticated cardiac procedures and early intervention, they still led to an increase of the endocarditis incidence in children with congenital heart defects. Pre-existent heart malformations are discovered in approximately 75-90% of children with infectious endocarditis (5). This increasing incidence noted in the last period is due to the growing survival rate in MCC, and to the more frequent use of central venous catheters, artificial valves and prosthetic material, as well as intravenous infections (including the drug users) (6).

At present, the mortality rate in pediatric patients with infectious endocarditis is of approximately 16-25% (5).

The etiological agents most frequently involved are: *Streptococcus viridans* (alpha-hemolytic) *Staphylococcus aureus*, *Streptococcus* species, *Enterococcus* species (more than 80% of EI), Gram-negative bacteria of HACEK group (responsible of 1-4% of EI), *Candida* and other fungi (< 10% of EI) (1).

Group B streptococcus and pneumococcus can also be met in the new-born. The gram-negative bacteria, the anaerobes and fungi appear especially in postoperative IE. IE is rare in nurslings, where it can appear only in association with open-heart interventions or with central venous catheters use (6).

The risk factors are represented mostly by the alteration of the structural integrity of the heart, respectively congenital heart malformations, especially the cyanotic ones, heart catheterization and procedures of vascular surgery, infections and interventions in the field of dentistry, ENT. The gastrointestinal and urogenital surgical interventions are not to be neglected, lately contested as being risk factors.

Specialized literature describes different localizations, the valve most frequently affected is the mitral (45-50% of cases) followed by the aortic (35-39%) and the tricuspid (19%). IE on pulmonary valve is less met (1.5-2%), or extravalvular, on the atrial septum, the ventricular wall, the cordage tendon (1).

The clinical diagnosis implies, in association, major criteria (positive blood cultures, highlighting the valvular damage at the echocardiographic examination) and minor criteria (fever, glomerulonephritis manifestations, Osler nodules, predisposing cardiac factors, suggestive echocardiographic images but not conclusive enough to be considered a major criteria, splenomegaly, increased VSH, increased CRP, the presence of the venous catheters, microscopic hematuria).

The bacteriologic diagnosis consists in highlighting the etiologic agent in blood cultures (3 samples are recommended), and the transthoracic and/or transesophageal echocardiography performed in dynamics allow certain affirmation of the valvular changes (7, 8, 9).

Taking into consideration that the infectious endocarditis remains a problem to consider at a young age, and the involved factors are more and more often met, this study aims to highlight issues commonly encountered, for a better knowledge of the disorder and a faster intervention.

MATERIAL AND METHODS

We have performed a retrospective study using epidemiological, clinical, off-clinical

and therapeutic data of 16 pediatric patients diagnosed with infectious endocarditis and hospitalized in the Cardiology Department, Clinic I of Pediatrics of the „Sf. Maria" Children Emergency Hospital of Iasi, during January 2007 – February 2011.

RESULTS AND DISCUSSION

During January 2007 – February 2011, in the Pediatric Cardiology Department of the „Sfânta Maria" Children Emergency Hospital of Iaşi, 16 children were diagnosed with infectious endocarditis (tab. I).

TABLE I

Epidemiological and social demographic data

Infectious endocarditis	2007	2008	2009	2010	2011	Total
No. of cases	2	3	5	4	2	16

The patients were aged 23 days to 16 years, most of the cases being recorded at the age group of 5 months – 8 years (9 cases), the average age being 4 years. The patients were coming mostly from urban locations (10 cases), the masculine gender being the most affected (10 cases). Regarding symptoms and clinical signs on admission in all 16 cases was observed occurrence of heart murmurs new fever was present in 81.25% of cases, and fatigue was noted in 93.75% of cases (tab. II).

TABLE II

Clinical aspects at hospitalization

Symptoms and clinical signs	Cases	%
Heart murmurs	16	100.00
Physical and mental fatigue	15	93.75
Fever, shiver	13	81.25
Little effort dyspnea	11	68.75
Chest pains	8	50.00
Cough	8	50.00
Headache	6	37.50
Loss of appetite	7	43.75
Dizziness, paralysis, paresthesia	5	31.25
Weight loss	5	31.25
Myalgia / arthralgia	3	18.75

At approximately 88% of the patients (14 cases), the endocardial damage appeared in the preexistent valvular lesions, especially mitral and aortic.

As associated congenital malformations, the patients predominantly presented ventricular septum defect, mitral valve prolapse, arterial canal persistence, aortic stenosis, coarctation of the aorta.

In all patients, the echocardiographic aspect represented a major criterion for the diagnosis of infectious endocarditis. As localization, six cases of IE on the pulmonary valve, two on the mitral valve, three localizations at the level of the aortic one, and three on the tricuspid valve. The imagistic description that came to our attention was of one of the patients (diagnosed previously with oper-

ated optical glioma) that presented several localizations (5 in number) that is on the aortic, pulmonary, mitral, tricuspid valve, and at the tip of the left ventricle (tab. III).

TABLE III
Echocardiographic distribution of the vegetations

Echocardiographic localizations of the vegetations	Cases	%
Mitral valve	6	37.50
Aortic valve	4	25.00
Tricuspid calve	3	18.75
Pulmonary valve	2	12.50
Normal	1	6.25

Five cases presented in association pulmonary hypertension.

Regarding the character of the vegetations, 81.25% had dimensions < 10 mm and only in 12.5% of cases the vegetations were > 10 mm (tab. IV).

TABLE IV
The character of the vegetations reported to the number of cases

The character of the vegetations	Cases	%
Mobile vegetations	14	87.50
Vegetations <10 mm	13	81.25
Big vegetations (>10 mm)	2	12.50
Multiple localizations of the vegetations	1	6.25

Aortic regurgitation was met in 6 cases (37.5%), the mitral regurgitation in 3 cases (18.75%), and the tricuspid one in 7 cases (43.75%).

The diagnosis associated was often present and affected patient's evolution: mitral insufficiency, aortic insufficiency, tricuspid insufficiency, aortic stenosis, severe pulmonary hypertension.

Blood cultures were collected and the most frequent identified etiological agents were: *Staphylococcus coagulase-positive, Streptococcus mitis, Staphylococcus speciae coagulase-negative, Staphylococcus haemolyticus, Streptococcus bovis, Escherichia coli***,** for which the antibiogram showed the sensitivity for betalactamine, cephalosporin, glycopeptide, trimethoprim-sulfamethoxazole, rifampicin, quinolone, lincosamides, oxazolidinone, and thus treatment according to the antibiogram was given.

Antibiotherapy with wide spectrum was mainly of first intention, monotherapy with aminopenicilin that is quinolone was administered in each case, cephalosporine of 3rd generation + quinolone in 4 cases, amonopenicilin + glycopeptides in 4 patients, carbapenemes + oxazolidinones in 2 patients, carbapenemes in 2 patients, glycopeptides + rifampicin 1 case, fluoro-quinolone + lincosamide 1 case, beta-lactamin + lincosamide 1 case.

The given treatment led to negativity of the blood cultures in the first 4 - 7 days, but it was necessary to extend the antibiotherapy for sterilization of the vegetations that were present echocardio-graphically.

The echocardiographic reevaluation as well as the cardiologic consultation in dynamics allowed an adequate and permanent monitoring of the therapeutic behavior.

The complications of cardiac type appeared despite the applied treatment and of the performed reevaluations (tab. V) .

TABLE V

Complications that appeared during the evolution of the pediatric patients

Complications	Cases
Heart failure	11
Pericarditis	6
Thromboembolism	4

Approximately 50% of the patients evolved favorably, in 5 cases (approx. 30% of the patients) it has been surgically intervened with an as complete as possible resection of the vegetations, restoring of valvular and ventricular function and correction of the risk factor (subjacent cardiopathy), and for 2 of the patients (approx. 15%) this problem was lethal.

CONCLUSIONS

Infectious endocarditis is a serious injury that reaches young age too, leading to death in some cases.

The different associated cardiac malformations - present more often in pediatric clinical reality- are an important predisposing factor for IE.

The imaging diagnosis and early blood cultures are of particular importance in order to intervene promptly.

The applied treatment must be fast and targeted.

The pediatric patients with surgery indication must be monitored after surgery, in order to rule out any complications.

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TUBERCULOUS MENINGITIS – CLINICAL AND EPIDEMIOLOGICAL CONSIDERATIONS (A RETROSPECTIVE STUDY 2008 - 2011)

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TUBERCULOUS MENINGITIS – CLINICAL AND EPIDEMIOLOGICAL CONSIDERATIONS (A RETROSPECTIVE STUDY 2008 - 2011) (Abstract): Tuberculous meningitis represents one of the most severe forms of tuberculosis and is often a difficult diagnostic and therapeutic problem. The objective of this study is to analyze the cases of tuberculous meningitis in our region. **Material and Methods** We retrospectively analyzed 76 observation sheets of patients aged 4 months to 84 years, diagnosed with tuberculous meningitis, admitted to Infectious Diseases Hospital Iaşi during 2008 to 2011. **Results:** Tuberculous meningitis has affected mostly males (65.7%). Only a small proportion of patients (23.68%) had tuberculosis in their personal history. 26.2% were diagnosed also with other localization of tuberculosis (mostly pulmonary). Fever was identified in only 43.4% of cases; 40% of patients had an altered conscience at admission. The outcome was favorable in all cases. **Conclusions:** Tuberculous meningitis predominantly affects males, almost equally affects patients with other focuses of TB and those with a history of tuberculosis disease. **Key words**: QUANTIFERON, CEREBROSPINAL FLUID, MENINGITIS

Tuberculosis remains a frequent disease in our country (1, 2). Tuberculous meningitis represents one of the most severe forms of tuberculosis (3).

MATERIAL AND METHODS

We evaluated 76 cases of tuberculous meningitis admitted in the Hospital of Infectious Diseases Iaşi between 2008 and 2011, taking into account the clinical and biological aspects. We studied the incidence of new cases and the distribution of these cases according to age, gender, the way they were diagnosed and according to evolution under specific treatment.

We have taken into account the following aspects (as they result from the admission paper) - age, sex, a personal history of tuberculosis, a diagnosis of tuberculosis at other site than CNS, the presence of other diseases (cancer, diabetes, other conditions that can cause immunosupression), the clinical presentation, laboratory exams – the CSF direct exam, culture results, the Quantiferon test.

We also evaluated the treatment – time elapsed between admission and the initiation of specific therapy, the evolution of CSF parameters with treatment and the adverse effects.

RESULTS

Tuberculous meningitis afected mostly male patients (50 cases / 65.7%).

The distribution according to age groups was as follows- 9 patients (11.84%) with ages between 0 and 20 years, 18 patiens (23.68%) with ages between 21 and 30 years, 12 cases with ages between 31 and 40 years (15.78%), 7 cases with ages between 41 – 50 years (9.21%), 12 patients with ages between 51 and 60 years (15.78%), 6 cases (7.89%) with ages between 61 and 70 years and 12 cases (15.78%) with ages over 71 years.

We notice a higher incidence of tuberculous meningitis among teenagers and young adults.18 patients (23.68%) had a personal history of pulmonary tuberculosis (but not active pulmonary disease). 20 patients had simultaneous tuberculous with other localization (different from CNS), as follows – 16 patients (21.05%) with pulmonary tuberculosis, 3 patients (3.94%) with pleural tuberculosis, one patient with both pulmonary and pleural disease and one patient with tuberculous arthritis of the left knee.

Three patients had a personal history of neoplastic disorders (multiple myeloma, melanoma, Hodgkin's disease); 3 patients had a personal history of diabetes mellitus.

The time elapsed between the onset of symptoms that could be related to meningitis and admission varied, but for the majority of patients (53 - 69.73%), this period had an average duration of 14 days. The period of time between the suspicion of tuberculous meningitis and the moment when the specific therapy was initiated was usually one or two days.

Fever was present in 33 (43.42%) patients. In 35 (40.50%) cases, we identified an altered conscience. 59 (77.63%) had meningeal signs. The degree of neurological involvement was variable 40 (52.63%) were admitted with no neurological disturbances, 24 (31.57%) had minor neurological signs (cranial nerve involvement) and 12 (15.80%) had major neurological disturbances - seizures, major motor deficits (paresis or plegia).

The exam of the cerebrospinal fluid (CSF) has an important diagnostical value. In 6 (7.89%) patients, at the direct exam of the CSF was noticed the presence of *Mycobacterium tubeculosis* (using the Ziehl Neelsen stain). Furthermore, CSF cultures were positive in 37 patients (46.68%). The plasmatic Quantiferon test was positive in 16 patients (21.05%) (tab. I).

TABLE I
The number of CSF elements at admission

No. elements/mmc	No. cases	%
1 – 200	37	48.68
201 – 500	27	35.52
>500	12	15.78

At the time of the first control evaluation (by spinal tap) the number of elements in CSF varied as follows in Table II.

TABLE II
The dynamic of CSF elements

No.elements / mmc	No. cases	%
1 – 200	46	60.52
201 – 500	15	19.74
>500	15	19.74

Etiological treatment – all patients received the quadruple association of antituberculous drugs Isoniazide - 50 mg/kg b.w./day, Rimfapicin - 10 mg/kg b.w./day, Etambutol – 15 to 25 mg/kg b.w./day, Pirazinamid - 20 to 25 mg/kg b.w./day, for the duration of hospitalization (a average duration of 21 days), with the recommendation of continuing the treatment up to 1 year. The pathogenic treatment - with in-

travenous corticosteroid therapy (Dexamethasone) in all cases, with an average duration of 8 days.

DISCUSSION

The outcome is influenced by age (more severe in extreme age groups), the evolutional state of meningitis at admission, the neurological status of the patient, the progression of CNS involvement and the co morbidities (4).

Most of the patients in this study had ages between 20 and 60 years, which could explain the favorable evolution. Furthermore, most of the patients were admitted with no major neurological involvement.

In this study there were no deaths (although two patients were transferred in ICU wards). Because The Clinical Hospital of Infectious Diseases Iaşi receives patients from the entire North-East region of the country, the discharged patients do not always come back for check-up, so we cannot have a clear picture of their evolution.

Tuberculous meningitis is the most severe form of *M. tuberculosis* infection. It is also the most common form of CNS tuberculosis (3).

An early diagnosis and a rapid specific treatment can reduce mortality and associated morbidity (5, 6). The treatment is usually no less than 9 months. The pathogenic treatment with dexamethasone, although still controversial, is successful in reducing morbidity (7), especially in HIV-positive patients.

An increased mortality can be seen in patients with other major diseases, in those patients with severe neurological involvement at the time of the admission, in patient with a rapid evolution and in extreme age groups (8).

CONCLUSIONS

The incidence of tuberculous meningitis is influenced by the tuberculosis epidemics. In our study, the disease was most common at the young adult, affecting more frequently the male sex.

Only a small proportion of patients (23.68%) had tuberculosis in their personal history.

Although the tuberculous meningitis does not evolve as fast as other bacterial meningitis, the antituberculous therapy must be initiated at the first suspicion of *M. tuberculosis* infection. Any delay can influence the outcome. The suspicion of tuberculous meningitis appears in the presence of clinical signs (meningeal signs, gradual involvement), laboratory findings (the CSF exam, with a number of elements usually between a few hundreds and one thousand, with increased proteins and decreased glucose) end epidemiological data (a personal history of tuberculosis or contact with a known case of pulmonary tuberculosis).

A rapid diagnostic and an early treatment are essential in reducing the mortality and morbidity of this formidable disease.

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NEWS

PLANTS USED IN AYURVEDIC AND TRADITIONAL CHINESE MEDICINE MODULATE AMYLOID PRECURSOR PROTEIN

Alzheimer's disease, the most common form of dementia, is characterized by memory and cognitive impairment, speech loss and personality changes. The main pathological event is the accumulation of neuritic plaques composed of β-amyloid peptide (Aβ). This peptide is generated through the proteolysis of a transmembrane glycoprotein named amyloid-β-precursor protein (APP). The non-amyloidogenic cleavage of APP leads to α soluble APP (sAPPα), p3 peptide and APP intracellular domain (AICD) while the amyloidogenic cleavage generates soluble APPβ (sAPPβ), Aβ and AICD. As it is estimated that 115.4 million people will suffer from this disease by 2050, numerous efforts are done to discover new therapeutic agents that reduce Aβ accumulation. This study reports on *in vitro* screening on APP modulation of 15 herbs used in Ayurvedic and Traditional Chinese medicine for the treatment of cognitive disorders. N2a-SweAPP cells (mouse neuroblastoma N2a cells that have been stably transfected with human Swedish mutant APP) were treated with different doses of ethanolic extracts of tested herbs; the Aβ levels in the supernatant were determined by ELISA. The extracts showing a significant inhibition on Aβ production, namely extracts of *Polygonum multiflorum* Thunb. roots and *Convolvulus pluricaulis* Choisy. leaves, were further subjected to an APP modulation assay. In this respect, their effects on sAPPα and sAPPβ were studied through western blot analysis. Only the extract of *Polygonum multiflorum* Thunb. roots showed APP modulation activity by up-regulating sAPPα and down-regulating sAPPβ. This result justifies not only the traditional uses of the plant but also further investigations on the therapeutic potential of this species. (Liu L-F, Durairajan SSK, Lu J-H, Koo I, Li M. In vitro screening on amyloid precursor protein modulation of plants used in Ayurvedic and Traditional Chinese medicine for memory improvement. *J Ethnopharmacol* 2012; 141: 754-760).

Anca Miron

DATE PRELIMINARE PRIVIND DETERMINAREA NIVELULUI SERIC A VEGF-A ÎN RÂNDUL PACIENȚILOR CU CANCER GASTRIC. STUDIU CLINIC

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PRELIMINARY RESULTS REGARDING VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF-A) LEVELS IN THE SERUM OF GASTRIC CANCER PATIENTS (Abstract): The most studied VEGF molecule is VEGF-A (Vascular Endothelial Growth Factor). Its involvement in various neoplastic processes represents an intensely controversial hypothesis. **Material and methods:** This is a prospective study extending over a period of 7 months, and including 38 hospitalized patients who underwent surgery for gastric cancer between 01/01/2011 – 07/01/2011, at the Surgery Departments of the Emergency Clinical County Hospital Mureş. Survival rate was determined based on age and gender of the patients, the macroscopic and microscopic appearance of the tumor, and pT, N, M parameters of the resected specimens. All these tumor parameters were correlated with preoperative VEGF-A levels, measured at the Central Laboratory of the Emergency Clinical County Hospital Mureş, using plasma samples and a human VEGF ELISA kit with cross-reactivity with VEGF 165 (*BioLife Group*), according to the instructions provided by the manufacturer. No enrolled patient had any preoperative treatment. In order to establish the reference value for VEGF-A in serum, we tested a group of 14 apparently healthy persons, and we calculated a mean value. **Results:** The reference value for VEGF-A in serum was 157.3 pg/ml. In gastric cancer patients the preoperative VEGF-A levels were 376, 188±247.11, showing significant elevation vs. the control group ($p<0.001$), but it did not correlate with any of the tested tumor parameters. Survival rate displayed statistical correlation with histological type of the tumor, and VEGF-A serum levels, so that patients with intestinal type gastric cancer showed a superior survival vs. those with diffuse type ($p=0.0043$). A better survival was noted in patients with VEGF-A serum levels over the threshold value of 173 pg/ml. **Discussion and conclusions:** The VEGF-A levels in serum are significantly increased preoperatively in gastric cancer patients, compared to apparently healthy persons, but they dot show correlation with tumor parameters. As a result it cannot be used as prognostic factor, but it may be an evolution marker. Survival rate is significantly higher in intestinal type gastric cancers, ads compared to the diffuse type, as well as in patients with serum VEGF-A values over the threshold value. **Key words:** GASTRIC CANCER, VASCULAR ENDOTHELIAL GROWTH FACTOR, SERUM LEVELS, SURVIVAL, TUMOR PARAMETERS, CORRELATION

Semnificația prognostică a nivelului seric a factorului de creştere a endoteliului vascular (VEGF-A), este în atenția numeroaselor

cercetări recente dar rezultatele sunt controversate (1,2,3,4) şi asocierea numeroaselor efecte secundare terapiei antiangiogenice fac din angiogeneza tumorală un subiect de mare actualitate în literatură.

În ceea ce priveşte cancerul gastric, sunt autori care susţin că nivelul seric crescut a VEGF-A se corelează cu, profunzimea invaziei tumorale şi stadiul tumorii, prezenţa metastazelor la distanţă constituind un factor prognostic independent (5, 6, 7).

MATERIAL ŞI METODĂ

În studiul de faţă am analizat nivelul seric a VEGF la pacienţii cu cancer gastric, în raport cu factorii clinico-patologici.

Studiul prospectiv a fost efectuat pe o durată de 7 luni, şi a inclus 38 de pacienţi internaţi şi operaţi cu cancer gastric în perioada 01.01.2011-01.07. 2011, în cadrul secţiilor de chirurgie ale Spitalului Clinic Judeţean de Urgenţă Mureş. Nici un pacient inclus în studiu nu a beneficiat de tratament preoperator, iar pentru determinarea nivelului seric a VEGF-A, la toţi pacienţii incluşi li s-a prelevat sânge preoperator. În vederea determinării valorii serice de referinţă pentru VEGF-A, s-a recoltat sânge şi de la un lot de 14 persoane aparent sănătoase.

Pentru recoltarea probelor s-a obţinut consimţământul pacienţilor şi menţionez că studiul a primit aprobarea Comitetului de Etică al Universităţii de Medicină şi Farmacie Tg. Mureş.

Determinarea serică a nivelului VEGF-A s-a realizat în cadrul Laboratorului Central al Spitalului Clinic Judeţean de Urgenţă Mureş, şi s-au utilizat probele de plasmă şi kitul ELISA VEGF human cu reactivitate încrucişată pentru VEGF 165 (*BioLife Group*) conform instrucţiunilor date de producător.

Datele au fost obţinute din foile de observaţii, buletinele histopatologice, şi în urma anamnezei pacienţilor, iar parametrii analizaţi au fost: vârsta, sexul pacienţilor, aspectul macroscopic şi microscopic al tumorii, stadializarea conform sistemului pTNM, nivelul seric al VEGF, supravieţuirea pe termen scurt a pacienţilor cu cancer gastric.

Pentru clasificarea pTNM s-a utilizat cea de a 7-a stadializare AJCC intrată în uz în 2010. Pentru studiul statistic am utilizat programele *GraphPad* şi *Microsoft Excel*.

REZULTATE

1. Determinarea valorii serice de referinţă a VEGF-A

Valoarea medie de referinţă la persoanele aparent sănătoase a fost de 157,3 pg/ml.

2. Parametrii clinico-patologici ai pacienţilor cu cancer gastric

Din cei 38 de pacienţi incluşi în studiu, 7 cazuri au fost limfoame gastrice (18,43 %), şi 31 de pacienţi au prezentat carcinoame gastrice (81,57%). În studiul de faţă am analizat doar cazurile de carcinoame gastrice, limfoamele fiind excluse.

S-a observat predominanţa tipului masculin totalizând 20 de cazuri (64,52%), în defavoarea celui feminin reprezentat de 11 cazuri (35,48%) şi un raport bărbaţi/femei de 1,8:1.

În ceea ce priveşte vârsta 18 pacienţi aveau peste 65 de ani (58,06%) şi 13 sub 65 de ani (41,94%). Din cele 31 de carcinoame, 16 (51,62%) au fost localizate antral şi 15 (48,38%) la nivelul corpului gastric. Conform clasificării Lauren, 25 de cazuri au fost carcinoame de tip intestinal (80,65%) şi 6 carcinoame difuze (19,35%).

Majoritatea cazurilor au fost diagnosticate în stadiul pN3, respectiv 18 cazuri (58,06%), şi doar 5 cazuri (16,14%) au fost în stadiul pN2, respectiv 8 cazuri (25,80%)

în pN-1.

64,52% din cazuri au fost încadrate în stadiul pT4. Nu s-a înregistrat nici un caz în pT1.

3. Determinarea valorii serice a VEGF la pacienții cu cancer gastric

Media valorilor serice a VEGF-A înregistrată în rândul pacienților cu cancer gastric este semnificativ mai mare față de media obținută la lotul control, ($p<0,001$) după cum se poate observa în tabelul I.

Nu s-a înregistrat o diferență semnificativă între valorile serice ale VEGF în raport cu vârsta şi sexul pacienților, localizarea tumorii, tipul histologic, gradul invaziei tumorale, numărul de grupuri limfatice invadate. Datele obținute sunt prezentate în tabelul II.

TABELUL I

Valorice serice pentru VEGF

Media valorilor VEGF pentru lotul control	Media valorilor VEGF preoperator, pentru lotul de studiu	Valoarea lui p
157,39±68.5,	376, 188±247,11	p<0,001

TABELUL II

Analiza nivelului seric de VEGF în raport cu factorii clinico-patologici studiați

Factori clinico-patologici		Media valorilor VEGF	Valoarea lui p
Sex	Masculin	393,41±235,91,	Testul t student p=0,6091
	Feminin	344,86±275,27	
Vârstă	>65 ani	370,83±266,38	Test Mann Whitney p=0,7793
	<65 ani	383,59±228,11	
Localizarea tumorii	Antru	379,14±264,94	p=0,9466
	Corp	373,03±255,91	
Tipul histologic	Intestinal	365,12±208,75	Test Mann Whitney p=0,8265
	Difuz	421,770±392,58	
Descriptorul N	N0-N1	296,17±201,30	Test ANOVA p=0,5652
	N2	378,61±262,98	
	N3	411,076±265,19	
Descriptorul T	T1-T2	330,43±250,12	Testul ANOVA, p=0,8411
	T3	349,22±108,16	
	T4	395,73±281,03	
T1-T2/T3-T4	T1-T2	330±249,86	p=0,5374
	T3-T4	411,68±269,77	

Rata supraviețuirii

În lotul studiat am înregistrat 7decese (22,58%). Am considerat mortalitatea intraspitalicească, în primele 30 de zile postoperator. La 1,5 luni de la operație s-au înregistrat 2 decese, la 2 luni tot 3 decese, iar la 3 şi respectiv 4 luni câte un deces.

Pentru a studia supraviețuirea pe termen

scurt a pacienților în funcție de vârstă am împărțit lotul studiat în 2 subloturi: 13 pacienți cu vârste sub 65 de ani (41,94%) şi 18 de pacienți cu vârsta peste 65 de ani (58,06%). Rata supraviețuirii nu diferă semnificativ în raport cu vârsta (p=0,3942).

Nu am obținut o diferență semnificativă privind rata de supraviețuire în funcție de sex. (p=0,7046), şi nici în ceea ce priveşte localizarea tumorii primare (p=0,6648).

Am înregistrat o diferență semnificativă statistic privind tipul histopatologic al tumorii, astfel rata de supraviețuire în rândul pacienților cu cancer gastric de tip intestinal a fost mai mare comparativ cu rata de supraviețuire pentru tipul difuz (p=0,0043)

Am considerat nivelul prag VEGF(cut-off), ca fiind valoarea ce depăşeşte 95% din valorile înregistrate pentru lotul martor. Pentru studiul nostru această valoare a fost de 173pg/ml. Pacienții la care s-au înregistrat valori serice de VEGF-A peste valoarea prag de 173 pg/ml au fost considerați VEGF-A pozitivi (+), iar cei cu valori sub 173 pg/ml, VEGF-A negativi (-). Astfel din 31 de cazuri incluse în studiu, în 24 de cazuri (77,42%) valoarea serică VEGF-A a fost peste valoarea prag şi în 7 cazuri (22,58%) sub această valoare.

Rata de supraviețuire pentru pacienții VEGF + a fost mai mare (75%), comparativ cu rata de supraviețuire înregistrată la pacienții cu VEGF - (85,71%) În urma analizei seriei de factori clinico-patologici clasici, s-a observat predominanța carcinoamelor de tip intestinal, şi a cazurilor încadrate ca pT4 şi pN3, acest aspect constituind un semnal de alarmă în ceea ce priveşte capacitatea de diagnosticare precoce a cancerului gastric.

În privința aspectelor referitoare la nivelul seric a VEGF-A, o serie de autori au evaluat nivelul seric a VEGF-A, ca factor prognostic în diferite procese neoplazice, iar o parte din valorile serice de referință obținute de aceştia pentru lotul control sunt redate în tabelul III.

TABELUL III

Valoarea serică a VEGF la lotul control

Valoare de referință lot control VEGF seric		**Autor**
178,4 pg/ml (110,3-159,5 pg/ml)	Circulating levels of VEGF family and their receptors in hepatocelullar carcinoma (9)	Kemic O ş i colab, 2010
141 pg/ml (76-209 pg/ml).	Factori predictive în evoluția sindromului de detresă respiratorie (10)	Solomon SR, 2011
346.9 pg/ml (31.1-630.6 pg/ml)	Measurement of circulating levels of VEGF-A, -C, and –D and their receptors, VEGF-1 and -2 in gastric adenocarcinoma (11)	Mansour S Al-Moundhri şi colab, 2008
186 (101–266) pg/ml	Circulating VEGF levels in the serum of gastric cancer patients (6)	Karayiannakis AJ., 2002
167.5 pg/ml (101.5–245.3) pg/ml	Serum Vascular Endothelial Growth Factor in Breast Cancer. Its Relation with Cancer Type and Estrogen Receptor Status (12)	Heer K. şi colab, 2001
157.3 pg/ml (75-274) pg/ml		

Valoarea serică de referinţa pentru VEGF-A înregistrată în studiul nostru a fost raportată la literatura de specialitate, însă în vederea unei interpretării obiective am utilizat în analiza statistică valorile proprii.

Menţionez că s-a evidenţiat o diferenţă semnificativă între nivelul seric a VEGF înregistrat la pacienţii cu cancer gastric, care este mult crescut, comparativ cu valoarea de referinţă a lotului control, aspect confirmat de mai mulţi autori (7,13,14).

Datele din literatură demonstrează un prognostic favorabil pentru tumorile bine diferenţiate, care înregistrează o rată de supravieţuire semnificativ mai bună comparativ cu cele slab diferenţiate (15,16).

Sung Y Oh şi colaboratorii într-un studiu în 2008 au arătat că nivelul seric preoperator a VEGF se corelează cu mărimea tumorii, şi prezenţa invaziei limfatice (17).

Karayiannakis AJ şi colaboratorii într-un studiu realizat în anul 2002 au raportat că nivelul seric pentru VEGF se corelează direct cu gradul infiltrării tumorale, stadiul TNM şi prezenţa metastazelor la distanţă (6).

Printr-o analiză multivariată au demonstrat ca nivelul seric VEGF reprezintă un factor prognostic nefavorabil alături de factorii clinico-patologici clasici (localizarea tumorii, gradul tumoral de infiltrate, stadiul TNM, prezenţa invaziei limfatice, prezenţa metastazelor la distanţă).

FDA (*US Food and Drug Administration*) a stopat tratamentul cu Avastin (Bevacizubam) în cancerul mamar, având în vedere faptul că nu s-au înregistrat îmbunătăţiri privind rata de supravieţuire mai mult decât atât s-au înregistrat şi o serie de efecte adverse

În ceea ce priveşte cancerul gastric Atsushi Ohtsu şi colaboratorii într-un studiu realizat în anul 2011, au arătat că rata supravieţuirii pentru pacienţii cărora li s-a administrat tratament chimioterapic asociat cu Avastin este semnificativ mai mare comparativ cu pacienţii la care s-a administrat doar tratament chimioterapic (18).

CONCLUZII

Nivelul seric de VEGF-A este semnificativ crescut în rândul pacienţilor cu cancer gastric comparativ cu lotul control, însă acesta nu se corelează cu nici unul dintre parametrii clinico-patologici clasici analizaţi.

Pacienţii cu carcinoame de tip intestinal şi niveluri serice crescute ale VEGF-A prezintă supravieţuire mai bună.

Nivelul seric de VEGF nu poate fi utilizat ca factor prognostic în cancerul gastric, însă poate fi utilizat ca factor evolutiv.

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VARIAȚIILE STATUSULUI PONDERAL ŞI PARAMETRII METABOLISMULUI LIPIDIC ÎN TIMPUL DIETEI DE EXCLUDERE A GLUTENULUI

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EVOLUTION OF NUTRITIONAL STATUS AND LIPID METABOLISM PARAMETERS IN GLUTEN FREE DIET (Abstract): Celiac disease is one of the most common forms of food intolerance in children, being an autoimmune enteropathy occurring in genetically susceptible individuals. The only treatment of celiac disease consists in a lifelong strict gluten-free diet. After gluten free diet patients with normal weight or growth failure risk become obese on the one hand because of recovery intestinal absorption and, on the other hand, nutritionally unbalanced diet, high in fat and protein. **Aim**: the aim of this study was to assess the nutritional status and the presence and prevalence of various changes dyslipidemia in gluten free diet. **Material and methods:** the trial was formed by 92 patients (mean age at diagnosis 88,5 months) diagnosed with CD between January 2008 – December 2010 in 2nd and 3rd Pediatric Clinics of "Sf. Maria" Hospital Iaşi. Patients were divided into two groups: group I included 36 patients younger than 2 years and group II included 52 patients aged over 2 years at time of diagnosis of celiac disease. All patients received gluten free diet. Were evaluated periodically anthropometric indices (weight, height, body mass index and Z scores in children over 2 years and weight and nutritional index in children under 2 years) and lipid profile (total lipids, total cholesterol, HDL cholesterol, triglycerides). Assessing food intake was achieved by interviewing parents and children on dietary habits and daily food ration. **Results:** Assessment of nutritional status: group I: - after 24 months of gluten free diet 5 cases presented overweight; group II: after 24 months of gluten free diet obesity (BMI> 30) was found in 16.07% of cases (9 patients) and overweight (BMI> 25) was found in 28.57% of cases (16 patients). Food intake: in all cases was observed increased calorie intake by an excess of lipids and carbohydrates in the diet. Investigation of lipid metabolism: in group II high values of cholesterol were found in 12 cases (21,42 of cases). In conclusions, in celiac disease gluten free diet should be balanced caloric, fat and protein to ensure an optimal nutritional status and prevent long-term complications. **Key words**: CELIAC DISEASE, GLUTEN FREE DIET, OBESITY, DYSLIPIDEMIA, CHILDREN.

Boala celiacă (BC) reprezintă una din cele mai frecvente forme de intoleranță alimentară la copil, fiind definită ca o enteropatie permanentă, imunomediată, declanşată la indivizii susceptibili genetic de prezența glutenului în alimentație. Singurul tratament al BC este reprezentat de excluderea glutenului pe toată durata vieții din

alimentație (1).

După inițierea regimului fără gluten (RFG) pacienții cu greutate normală sau cu falimentul creşterii în momentul diagnosticării riscă să devină obezi pe de o parte datorită refacerii absorbției intestinale şi, pe de lată parte, datorită dietei necorespunzătoare, bogată în lipide şi proteine (2).

Scopul studiului a fost de a evalua statusul nutrițional precum şi prezența şi prevalența diferitelor modificări dislipidemice la copiii diagnosticați cu BC în timpul RFG.

MATERIAL ŞI METODE

Am realizat un studiu prospectiv în care au fost incluşi 92 de pacienți diagnosticați cu boală celiacă în Clinicile a II-a şi a III-a Pediatrie a Spitalului de Copii „Sfânta Maria" din Iaşi în perioada 2005-2010. Vârsta pacienților a fost cuprinsă între 19 luni şi 127 luni (vârsta medie 88,5 luni). Diagnosticul de BC s-a stabilit pe baza criteriilor revizuite ale *Societății Europene de Gastroenterologie, Hepatologie şi Nutriție Pediatrică*. Pacienții au fost împărțiți în două loturi: lotul I a cuprins 36 de pacienți cu vârsta sub 2 ani şi lotul II în care au fost incluşi 56 de pacienți cu vârsta peste 2 ani în momentul stabilirii diagnosticului de BC. Toți pacienții au primit RFG şi tratamentul carențelor nutriționale secundare malabsorbției intestinale. S-au evaluat periodic (la intervale de 6 luni) indicii antropometrici (greutatea, talia, indicele de masă corporală şi scorul Z la copiii peste 2 ani şi indicele ponderal şi nutrițional la copiii cu vârsta sub 2 ani), precum şi profilul lipidic (lipide totale, colesterol total, HDL colesterol, trigliceride). Interpretarea rezultatelor s-a efectuat prin raportarea la valorile normale pentru vârstă şi sex. Aprecierea aportului alimentar s-a realizat prin chestionarea părinților şi a copiilor privind obiceiurile alimentare şi rația alimentară zinică în care s-a urmărit aportul de calorii, lipide, proteine, vitamine.

Lotul martor a cuprins un număr egal de subiecți cu vârstă şi sex corespunzătoare lotului studiat, IMC calculat fiind între percentila 25-75 pentru vârstă şi sex.

REZULTATE

1. Evaluarea statusului nutrițional Lotul I: la pacienții cu vârsta sub 2 ani în momentul stabilirii diagnosticului de BC a predominat malnutriția protein-calorică (MPC) de gradul II în 58,33% din cazuri (fig. 1).

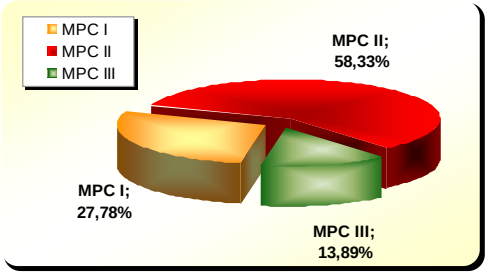

Fig. 1. Deficitul ponderal la pacienții cu vârsta sub 2 ani

În timpul RFG curba ponderală a fost ascendentă: după 1 lună de RFG pacienții cu MPC gradul I şi-au reluat creşterea în greutate, curba ponderală devenind ascendentă după 2-6 săptămâni, pentru ca după 6-12 luni greutatea corpului să ajungă la valori corespunzătoare vârstei în 55.83% din cazuri (fig.2).

În 5 cazuri cu MPC gradul III deficitul ponderal s-a menținut după 6 luni, la aceştia pe lângă deficitul proteic asociindu-se şi carențele vitaminice, minerale, hormonale, recuperarea nutrițională necesitând o perioadă mai lungă de timp.

După 24 de luni de RFG excesul ponderal (IP>1) a fost întâlnit în 5 cazuri.

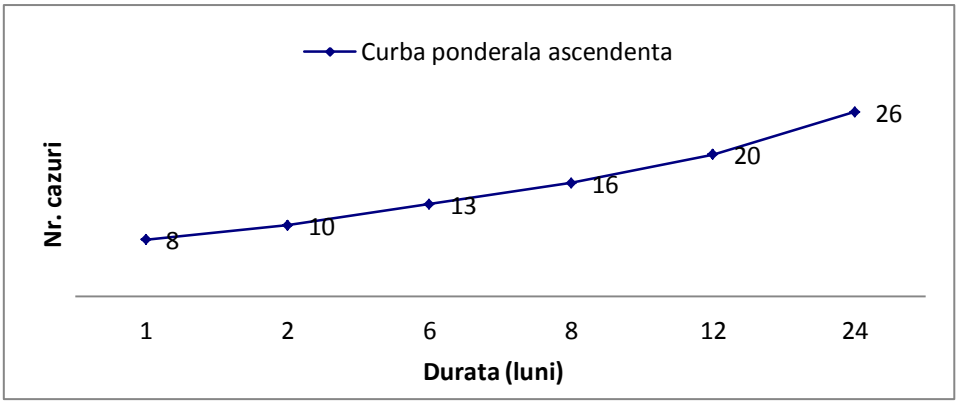

Fig. 2. Aspectul curbei ponderale la pacienții cu vârsta sub 2 ani în timpul RFG

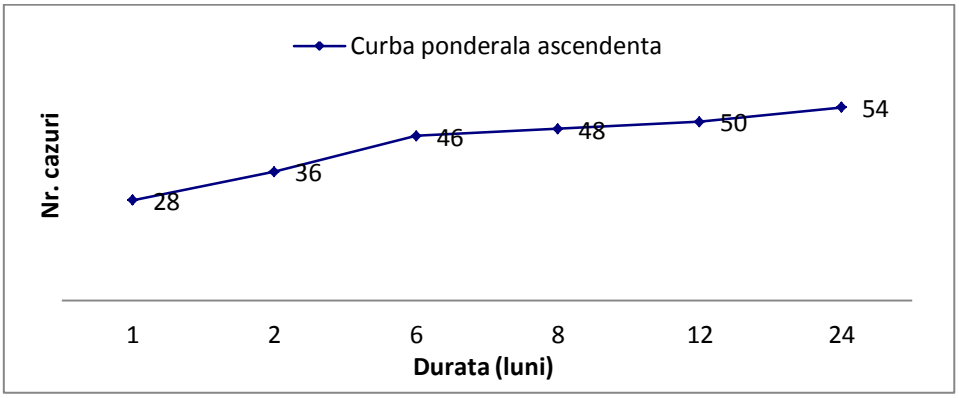

Fig. 3. Evoluția curbei ponderale sub RFG la pacienții cu vârsta peste 2 ani

Lotul II: în momentul diagnosticării BC hipotrofia ponderală a fost întâlnită la 81,82% din pacienți. După 8 luni de dietă fără gluten deficitul ponderal s-a menținut la 8 pacienți (14,63% din pacienți) (fig. 3).

În lotul II, după 24 luni de RFG deficitul ponderal a fost întâlnit în 7,14% din cazuri (4 pacienți), reluarea anamnezei evidențiind faptul că principala cauză a deficitului ponderal a fost nerespectarea RFG. Urmărind evoluția curbei ponderale după 24 luni s-a observat că obezitatea (BMI>30) a fost întâlnită în 16,07% din cazuri (9 pacienți), iar excesul ponderal (BMI>25) la 28,57% din cazuri (16 pacienți).

2. Ancheta alimentară. Indiferent de vârsta pacienților ancheta alimentară a evidențiat creşterea aportului caloric printr-un exces de lipide şi carbohidrați în dietă. Mai mult, în lotul II s-a remarcat un aport scăzut al fibrelor, calciului, fierului în special în dieta adolescenților cu BC. Aceste dezechilibre nutriționale au fost observate în special la copiii de vârstă şcolară şi adolescenți. Astfel, la copiii de vârstă şcolară excesul ponderal şi obezitatea au fost mult mai frecvent întâlnite decât la copiii de vârstă mică.

3. **Investigarea metabolismului lipidic.** În lotul I: la debutul bolii hipocolesterolemia a fost întâlnită în 3 cazuri, hipercolesterolemia în 2 cazuri iar lipidele au

prezentat valori normale la toţi pacienţii cu vârsta sub 2 ani. După 2 ani de RFG 3 pacienţi au prezentat hipercolesterolemie cu valori normale ale lipidelor totale. Efectuarea lipidogramei la părinţi a evidenţiat hipercolesterolemia familială în 2 cazuri. În lotul II s-au remarcat valori moderat scăzute ale lipidelor doar în 11,59% din cazuri, iar hipocolesterolemia a fost întâlnită în 6 cazuri. Probabil absenţa modificărilor semnificative ale metabolismului lipidic datorită malabsorbţiei intestinale s-a datorat diagnosticului precoce a BC la copiii incluşi în studiu. Ulterior, monitorizarea periodică a parametrilor metabolismului lipidic a evidenţiat hipercolesterolemia la 12 pacienţi cu vârsta peste 10 ani (21,4% din cazuri). Toţi pacienţii cu hipercolesterolemie prezentau exces ponderal (4 cazuri) şi obezitate (8 cazuri). Anamneza a evidenţiat hipercolesterolemia familială (cel puţin unul dintre părinţi prezenta hipercolesterolemie) în 6 cazuri. Hipertrigliceridemia a fost întâlnită la 2 pacienţi, fiind familială în ambele cazuri. Doi dintre pacienţi erau cunoscuţi cu diabet zaharat tip I diagnosticat anterior stabilirii diagnosticului de BC.

DISCUŢII

La copiii diagnosticaţi cu BC RFG pe viaţă reprezintă singura opţiune terapeutică care permite rafacerea absorbţiei intestinale, precum şi prevenirea complicaţiilor. Odată cu refacerea vilozităţilor intestinale, refacerea absorbţiei principiilor nutritive determină creşterea în greutate, uneori până la exces ponderal şi obezitate în condiţiile nerespectării unei diete echilibrate în carbohidraţi, proteine şi lipide.

Menţinerea dietei fără gluten nu este uşoară, mai ales pentru pacienţii de vârstă şcolară. În funcţie de tipul de produse fără gluten consumate pot apărea efecte secundare cum ar fi excesul ponderal şi obezitatea datorită consumului excesiv de carbohidraţi şi lipide. Multe dintre produsele fără gluten au tendinţa de a fi bogate în carbohidraţi, lipide şi calorii şi un conţinut scăzut în fibre, vitamine şi fier (3). De exemplu, pastele cu gluten conţin 41 grame de carbohidraţi comparativ cu pastele fără gluten care conţin 46 grame carbohidraţi la 100 grame de paste. Astfel, de multe ori adoptarea RFG duce la creşterea în greutate şi perturbarea metabolismului lipidic pe termen lung la copil şi adolescent.

La pacienţii incluşi în studiu am observat că raţia alimentară a fost hiperproteică, hiperlipidică şi hipercalorică, probabil şi datorită grijei excesive a mamelor de a oferi copiilor diagnosticaţi cu BC o raţie alimentară zilnică care să asigure o creştere şi dezvoltare optimă, în condiţiile în care nu a fost posibilă consilierea acestor părinţi şi copii de către un dietetician specializat pe probleme de nutriţie pediatrică. În studiul realizat în Italia de către Mariani et al (4) s-a observat că 72% dintre adolescenţii cu BC care au aderat strict la RFG au prezentat exces ponderal secundar unei diete bogată în lipide şi proteine şi săracă în carbohidraţi, fier, calciu şi fibre.

În prezent, obezitatea reprezintă o importantă problemă de sănătate publică la nivel mondial, prin prevalenţa în continuă creştere şi prin complicaţiile sale pe termen lung (dislipidemia, diabetul zaharat de tip II, hipertensiunea arterială) (5). Astfel, în studiul realizat de Venkatasubramani N. et al (2) s-a arătat că prevalenţa obezităţii la copiii şi adolescenţii din Statele Unite a crescut îngrijorător, 1 din 3 copiii fiind cu exces ponderal, iar 15% dintre aceştia vor deveni obezi (5).

În studiul de faţă excesul ponderal şi obezitatea în timpul dietei fără gluten au

predominat la pacienții de vârstă şcolară şi adolescenți şi au fost întâlnite la 28,57%, respectiv 16,07% dintre pacienții cu BC. De asemenea, este binecunoscut în prezent faptul că obezitatea poate fi una din formele atipice de debut a BC, de aceea screeningul imunologic trebuie efectuat inclusiv la copiii obezi (2).

Evaluarea profilului lipidic a arătat că la pacienții cu hipercolesterolemie familială valorile colesterolului au crescut mult mai repede comparativ cu cei fără antecedente familiale de dislipidemie în timpul RFG, indiferent de vârsta pacientului. 12 pacienți din cei cu obezitate şi exces ponderal au prezentat hipercolesterolemie. Ancheta nutrițională la aceşti pacienți a demonstrat o dietă inadecvată vârstei, datorată pe de o parte hiperprotecției materne (dorința mamelor de a corecta deficitele nutriționale prin diete hipercalorice, hiperlipidice) şi lipsei de consiliere nutrițională specializate şi, pe de altă parte, consumului de către pacienți a produselor fără gluten care sunt hipercalorice. Refacerea structurii şi funcțiilor enterocitului în timpul RFG determină creşterea absorbției trigliceridelor şi producția de lipo- şi apolipo-proteine, crescând riscul apariției dislipidemiei în condițiile unui aport nutrițional inadecvat.

O atenție deosebită trebuie acordată regimului fără gluten la pacienții cu diabet zaharat de tip I în vederea asigurării unui status nutrițional optim şi prevenirii complicațiilor pe termen lung (6,7,8).

CONCLUZII

Ancheta alimentară a copiilor cu BC incluşi în studiu a evidențiat că 44,64% dintre aceştia primeau o dietă săracă în fibre alimentare şi carbohidrați. Excesul ponderal şi obezitatea în timpul RFG au predominat la pacienții de vârstă şcolară şi adolescenți. RFG reprezintă singura metodă de tratament a BC şi trebuie să asigure un aport zilnic echilibrat caloric, lipidic şi proteic pentru a asigura un status nutrițional optim şi a preveni complicațiile pe termen lung.

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TUBERCULOSIS PLEURO-PERICARDITIS: SO MUCH FIBRIN! CASE REPORT

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TUBERCULOSIS PLEURO-PERICARDITIS: SO MUCH FIBRIN! CASE REPORT (Abstract): A young man with thoracic pain and progressive dyspnea was hospitalized for a pleuro-pericarditis. Ultrasonography confirmed the presence of left pleural and pericardial effusion with a high amount of fibrin. With exudative characters and without macroscopic appearance of empyema, the left pleural effusion has reoccurred two days later during the diagnosis period. Bacteriological exams were repetitively negative. A malign etiology was excluded as no atypical cells in the pleural fluid were found. Adenosine deaminase level was not helpful for the diagnosis. The diagnosis was established through video assisted thoracoscopy pleural biopsy which identified pleural granulomas. This case illustrates the difficulty of a pleuro-pericarditis tuberculosis diagnosis. **Keywords**: PLEURISY, PERICARDITIS, TUBERCULOSIS, FIBRIN.

Tuberculosis remains a global health threat with more than 1.5 million victims each year (1). Tuberculous pleurisy is the main cause of pleural effusion in many countries and the most common form of extrapulmonary tuberculosis as well as (2). According to the World Health Organization Global Tuberculosis Report 2013, an estimated 8.6 million people developed tuberculosis (3). Tuberculous pleuropericarditis is more likely to occur in younger patients who are more immunocompetent (3). Although other disorders including fungal diseases, sarcoidosis, tularemia and rheumatoid pleuritis may produce granulomatous pleuritis, more than 95% of patient with granulomatous pleuritis have tuberculosis (3). A high amount of fibrin on thoracic ultrasound can be a highly suggestive imagistic diagnostic clue. It seems that tissue factor, an initiator of coagulation, is important for the generation of fibrin, which supports granuloma formation in tuberculosis (4).

CASE REPORT

A 43-year-old man with a history of chronic alcohol consumption and peptic ulcer was referred due to thoracic pain and progressive dyspnea. Chest radiography revealed a massive left pleural effusion (fig. 1A). The electrocardiogram raised the suspicion of coexistent pericardial effusion (fig. 1B).

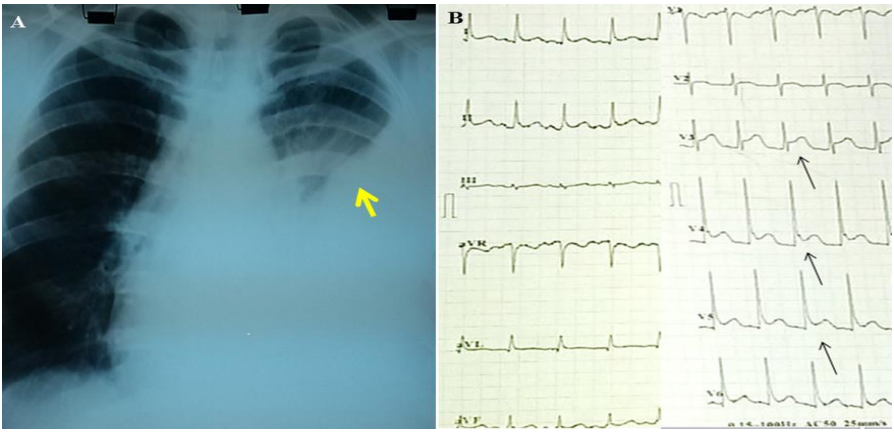

Fig. 1. The chest radiography and the electrocardiogram. Chest radiography demonstrating massive left pleural effusion (image A). The electrocardiogram shows diffuse ST segment elevation (image B).

Transthoracic echocardiography confirmed the presence of a moderate pericarditis, without hemodynamic consequences (fig. 2A). A high amount of fibrin was seen on the visceral pericardium. The echocardiogram showed also that the left visceral and parietal pleura were linked by many thick strings of fibrin (fig. 2 B). Thoracic ultrasound confirms that left visceral and parietal pleura are related by thick strings of fibrin (image B).

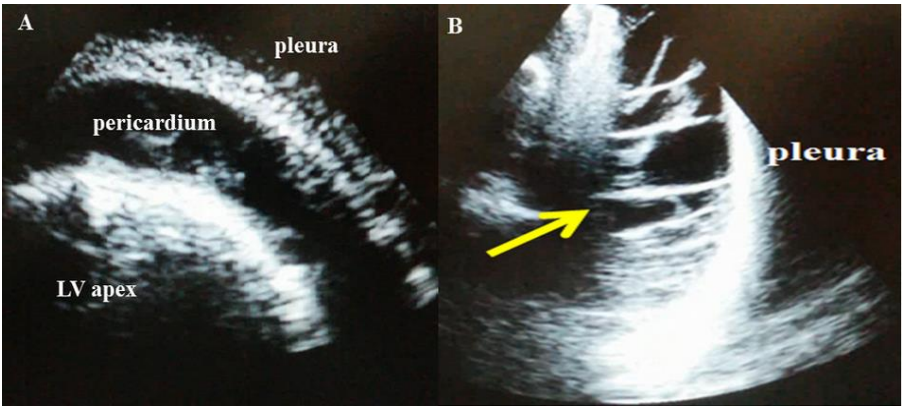

Fig. 2. Transthoracic echocardiography on apical four chambers view shows the presence of high amount of fibrin on the visceral pericardium (image A) and visceral pleura (image B).

Two liters of pleural effusion with exudate character were evacuated. After two days the pleural effusion has reoccurred. No atypical cells in the pleural fluid were found and bacteriological exams were negative. Human immunodeficiency virus test was negative. Adenosine deaminase level (57 U/L) was not helpful for the

diagnosis; usually a value of >70U/L means probably tuberculous pleurisy. Macroscopic examination of pleura by video assisted thoracoscopy confirmed thick fibrin strings between the visceral and parietal pleura (fig. 3A) and visualized multiple granulomas on the visceral pleura (fig. 3B).

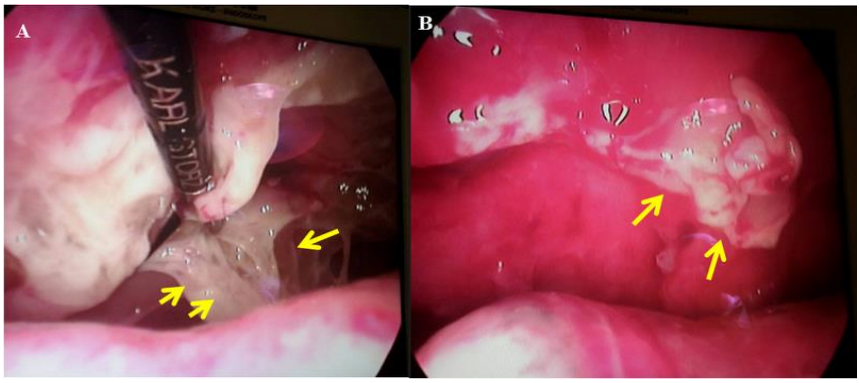

Fig. 3. Video assisted thoracoscopy shows thick fibrin formations between the left visceral and parietal pleura (image A) and the presence of granulomas on visceral pleura (image B).

Empiric tuberculosis treatment with chemotherapy was started after the second evacuation of pleural effusion, without reoccurrence of the pleural effusion in the following three days. Resected pleural granulomas (fig. 4A) were analyzed by pathologist and histologic examination confirmed their tubercular etiology. On histopathological investigation there were caseous granuloma and multinucleated Langhans giant cells with nuclei arranged in a horseshoe (fig. 4B). Thus, the etiology of pleuro-pericarditis was tuberculosis. The patient was discharged after 10 days in a good clinical condition. One year later the patient had no sign of relapse.

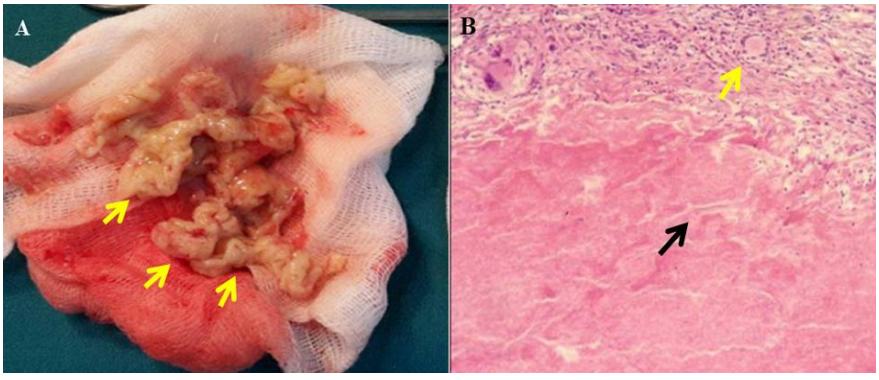

Fig. 4. Macroscopic and histopathology aspects of tuberculous granulomas. Macroscopic aspect after thoracoscopic excision (image A). Histopathology exam shows caseous granuloma (hematoxylin- eosin staining; 4x magnification) – black arrow; multinucleated Langhans giant cells with nuclei arranged in a horseshoe (hematoxylin - eosin staining; 40x magnification) – yellow arrow (image B).

DISCUSSION

Despite the introduction almost a century ago of a vaccine against *Mycobacterium tuberculosis*, tuberculosis remains a global health threat with more than 1.5 million victims each year (1). Tuberculous pleuro-pericarditis is more likely to occur in younger patients who are more immunocompetent (2). Mycobacterial antigens enter the pleural/pericardial space and interact with T-cells previously sensitized to mycobacteria, then results in a delayed hypersensitivity reaction. Although other disorders including fungal diseases, sarcoidosis, tularemia and rheumatoid pleuritis may produce granulomatous pleuritis, more than 95% of patient with granulomatous pleuritis have tuberculosis (2). A high amount of fibrin on thoracic ultrasound can be a highly suggestive imagistic diagnostic clue. It seems that tissue factor, an initiator of coagulation, is important for the generation of fibrin, which supports granuloma formation in tuberculosis (3). Tuberculous pleuro-pericarditis' diagnosis is not so easy. Multimodality imaging including thoracic ultrasound is very important. Algorithmic approach for tuberculous pleurisy diagnosing proposed by Doosoo J could be helpful (2). Nowadays it seems that SERS spectra and chemometric methods are new tool for tuberculosis determination (5).

CONCLUSIONS

This case illustrates that tuberculous pleuro-pericarditis' diagnosis sometimes is not so easy. Multimodality imaging including thoracic and cardiac ultrasound and video assisted thoracoscopy is very important. In this case histopathologic examination of resected pleural granulomas established the diagnosis.

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URGENȚE ONCOLOGICE ÎN DEPARTAMENTUL DE URGENȚĂ

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ONCOLOGICAL EMERGENCIES IN THE EMERGENCY DEPARTMENT (Abstract): **Aims**: to assess the profile and the characteristic of oncological patients, establishing management in patients with neoplasia presented in the emergency department (ED), the analysis of short-term movements in patients with neoplasia whilst in the ED. **Material and Methods**: we performed a retrospective study on non-randomized consecutive cases. The lot analysis included 1315 oncological patients admitted in the Emergency Department of the Clinical Emergency Hospital "St. Spiridon" Iasi, in the period June 1st, 2009 - May 31st, 2010. 23.12% of the patients had high suspicion of neoplasia at the first visit to the ED. **Results**: 67.07% of patients were in metastatic stage disease located as follows: liver metastasis 37.59%, lung metastasis 18.36%, lymph node metastasis 13, 29%. After processing the data there were found statistically significant correlations between the age distribution of patients and the documented / suspected diagnosis of neoplasia ($p = 0.01$) in the sense that a neoplasia diagnosis in emergency was more frequent on people of young age. It should be mentioned that other studies rarely mention first diagnosis of neoplasia in emergency department with presence of complications. **Conclusion**: 1315 oncological patients presented in ED, almost a quarter of which presented high suspicion of neoplasia (still without histopathological confirmation) when in ED (23.12%). Most of them were aged male patients (over 65 years old), with tumors in the digestive system. A significant proportion (almost 60%) of these patients ended up in emergency due to complications and the therapy intended life support and pain management. Some of these patients were directed to further exploring and emergency outpatient therapy while 75% of patients were hospitalized after stabilization. Although we expected that the frequency of complications to be higher in patients previously diagnosed with cancer, data analysis showed no statistically significant differences ($p = NS$) between the rate of complications in patients previously diagnosed with neoplasia and those with high suspicion neoplasia first diagnosed in the ED.
Key words: ONCOLOGICAL EMERGENCIES, NEOPLASIA, EMERGENCY DEPARTMENT, METASTATIC STAGE DISEASE

Urgenţele oncologice sunt condiţii comune asociate cu o morbiditate şi mortalitate semnificative. Acestea pot fi definite ca şi condiţie acută cauzată de neoplazie sau tratamentul implicat, care necesită intervenţie rapidă pentru a evita apariţia complicaţiilor sau decesul (1).

În decursul anilor terapia antineoplazică a îmbunătăţit supravieţuirea pacienţilor cu neoplazii, diagnosticul prompt şi controlul urgenţelor oncologice ocupând un loc important. Din fericire, multe din aceste urgenţe (în special cele metabolice) pot fi prevenite cu succes dacă sunt suspicionate pe baza evoluţiei pacientului postterapie oncologică (2).

Îngrijirea pacienţilor cu urgenţe oncologice reprezintă o provocare nu numai pentru medicii oncologi, dar, de asemenea şi pentru medicii de medicină de urgenţă, existând mai multe tipuri de probleme medicale pentru care pacienţii cu neoplazie necesită asistenţă imediată în departamentul de urgenţă (insuficienţă respiratorie acută, sindroame ocluzive, hemoragie, anemie, durere, icter) (1).

O evaluare corespunzătoare este esenţială pentru majoritatea pacienţilor cu urgenţe de natură oncologică, important fiind de recunoscut şi tratat complicaţiile asociate cu neoplazia. Atenţia trebuie îndreptată pe acele probleme care ameninţă organele vitale sau compromit calitatea vieţii pe termen lung. Deseori, înainte de terapia definitivă prescrisă de oncolog, medicul de urgenţă trebuie sa stabilizeze aceşti pacienţi (3,4).

Pacienţii se pot prezenta în departamentul de urgenţă pentru efectele locale ale tumorii, efectele tratamentului oncologic sau pentru o problemă survenită independent de diagnosticul oncologic (1).

Urgenţele oncologice sunt clasificate astfel (1,3,4):

1. Urgenţe determinate de formaţiunile tumorale expansive;
2. Urgenţe metabolice;
3. Urgenţe determinate de tulburări sangvine şi de coagulare.

Având în vedere creşterea numărului de pacienţi prezentaţi zilnic în departamentul de urgenţă pentru diverse complicaţii ale unei patologii oncologice sau cu o simptomatologie de urgenţă (durere, greaţă, vărsături, febră, astenie marcată, pierderea stării de conştienţă) care ulterior se dovedeşte a fi determinată de un proces neoplazic nediagnosticat anterior, ne-am propus să realizăm un studiu referitor la urgenţele oncologice în Unitatea de Primire Urgenţe.

OBIECTIVE

Obiectivele studiului au fost:

- evaluarea profilului şi caracteristicilor pacienţilor oncologici,
- stabilirea managementului la pacienţii cu neoplazie prezentaţi în departamentul de urgenţă,
- analiza evoluţiei pe termen scurt a pacienţilor cu neoplazii în timpul staţionării în urgenţă.

MATERIAL ŞI METODĂ

Am realizat un studiu retrospectiv, pe cazuri consecutive, nerandomizat. Studiul a fost avizat de comisia de etică a spitalului.

Metoda de selecţie

Lotul analizat a inclus 1315 pacienţi oncologici primiţi în Departamentul de Urgenţă (Unitatea Primire Urgenţe) a Spitalului Clinic Judeţean de Urgenţă „Sf. Spiridon” din Iaşi în perioada 01 iunie 2009 – 31 mai 2010.

Criteriile de selecţie pentru pacienţii incluşi în studiu au fost următoarele:

- pacienți cu vârsta peste 18 ani, admişi în Unitatea Primire Urgențe a Spitalului "Sf. Spiridon" în perioada 1 iunie 2009 – 31 mai 2010.

- diagnostic cunoscut de neoplazie, aflat în evidența Clinicii Oncologice a Spitalului "Sf. Spiridon" Iaşi.

- suspiciune diagnostică de neoplazie pe criterii clinice sau paraclinice in Unitatea Primire Urgențe.

Metoda de achiziție a informațiilor

Am realizat analiza fişelor de urgență la pacienții incluşi în studiu, pacienți cu vârsta medie 63,5+/-13,81 ani. Datele au fost înscrise inițial într-un tabel *Microsoft Excel* şi analizate cu ajutorul programului *SPSS 18.0*. Etapele analizei statistice au fost: 1. Statistica descriptivă, 2. Corelațiile statistice.

REZULTATE ŞI DISCUȚII

Dintr-un total de 40322 prezentări în departamentul de urgență în perioada iunie 2009 – mai 2010, 1315 pacienți s-au prezentat pentru o patologie de natură oncologică.

Media de vârstă în lotul de studiu a fost de 63,5+/-13,81 ani, grupa de vârstă cea mai frecventă în cadrul pacienților incluşi în studiu a fost cea de peste 60 ani, respectiv 61,98 %, urmată de grupa pacienților cu vârsta cuprinsă între 40 – 60 ani, (33,40 %). Un procent de 5,62 % dintre pacienții incluşi în studiu au reprezentat grupa de vârstă tânără, respectiv 18 – 40 ani (fig. 1, fig. 2).

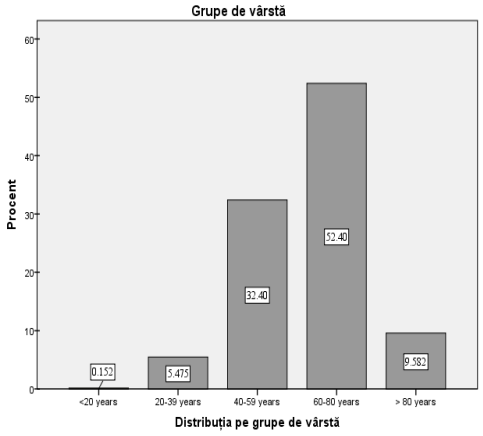

Fig. 1. Distribuția pacienților pe grupe de vârstă

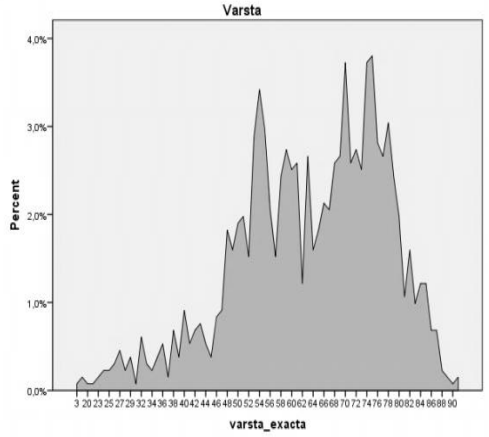

Fig. 2. Distribuția pacienților în funcție de vârstă

Trebuie să reținem că în lotul de studiu a existat o predispoziție a sexului masculin indiferent de grupa de vârstă (fig. 3). În perioada studiului 76,80 % dintre pacienți aveau precizat anterior diagnosticul de neoplazie, în timp ce 23,12 % dintre pacienți au prezentat suspiciune înaltă de neoplazie la prima vizită în departamentul de urgență (fig. 4).

Cele mai frecvente localizări ale neoplaziei în cadrul pacienților incluşi în studiu au fost reprezentate de: tub digestiv 29,03 %, leucemie/limfom 17,80 %, aparat genito-urinar 11,23 % şi sân 9,01 % (fig. 5).

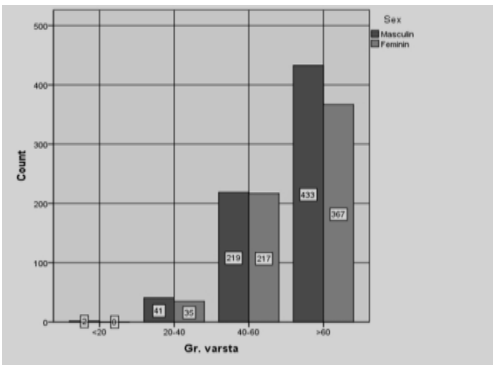

Fig. 3. Distribuția cazurilor funcție de sex şi grupă de vârstă

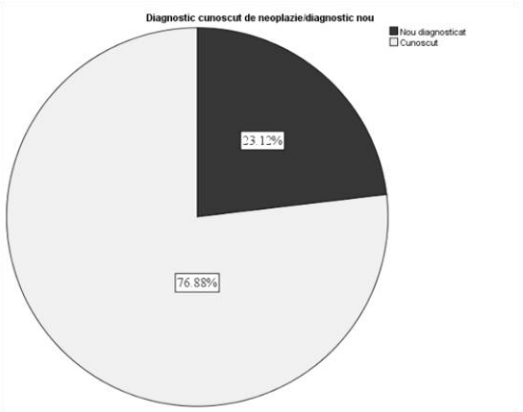

Fig. 4. Diagnostic cunoscut de neoplazie/diagnostic nou

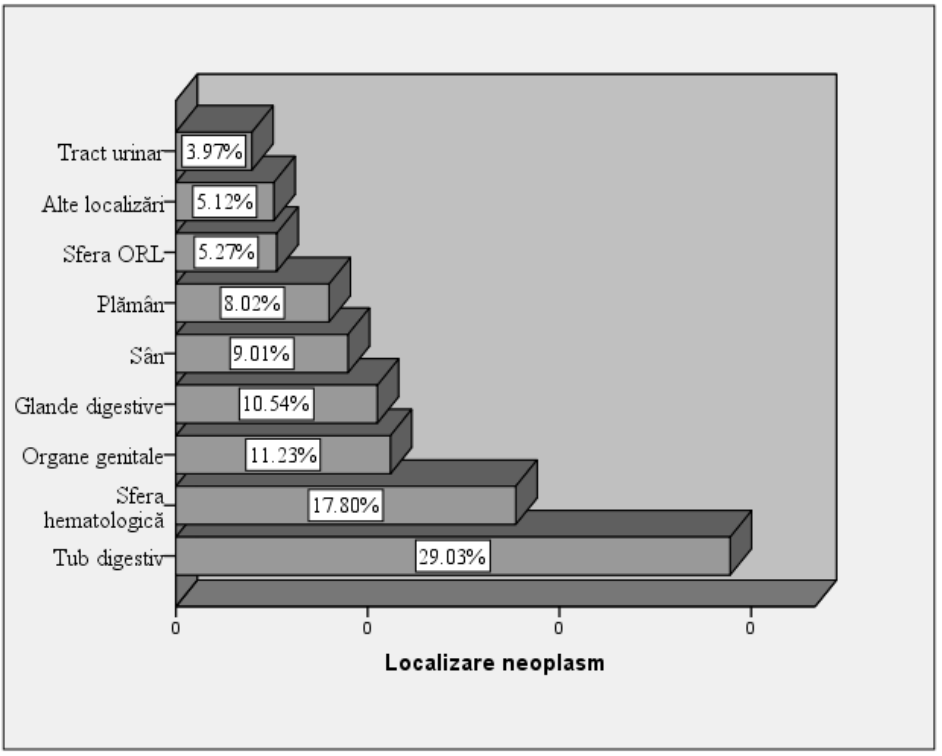

Fig. 5. Localizarea tumorilor

În lotul de studiu, 67,07 % dintre pacienți se aflau în stadiul de metastazare a bolii, cele mai frecvente localizări fiind reprezentate de: metastaze hepatice 37,59 %, metastaze pulmonare 18,36 %, metastaze ganglionare 13,29 % (fig. 6).

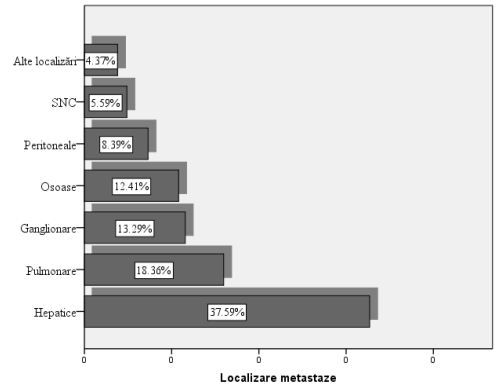

Fig. 6. Localizare metastaze

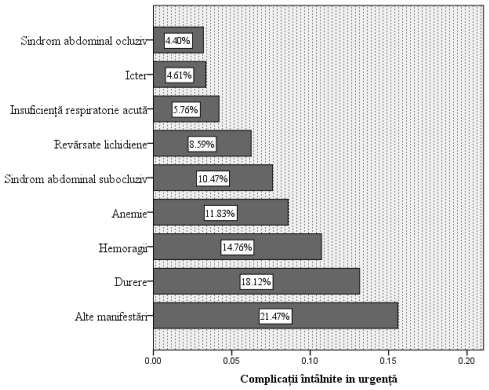

Fig. 7. Tipul complicațiilor în urgență

Din totalul pacienților prezentați în departamentul de urgență pentru o patologie oncologică, 59,92 % s-au prezentat pentru complicații date, fie de neoplazie, fie de tratamentul antineoplazic administrat. Cele mai frecvente complicații specifice au fost reprezentate de: sindroame hiperalgice (18,12 %), sindroame hemoragice (14,76 % - hemoragie digestivă superioară), sindroame anemice (11,83 %), sindroame subocluzive (10,47 %) (fig. 7).

Mortalitatea pacienților în departamentul de urgență (ceea ce semnifică un interval de maximum 24 h) este foarte redusă, respectiv 1,06 %, majoritatea pacienților fiind monitorizați şi tratați în urgență şi apoi internați în secțiile clinice de profil: oncologie şi hematologie (54,19 %), gastroenterologie (15,53 %) şi chirurgie generală (6,85 %) sau au putut merge la domiciliu cu recomandări terapeutice (23,44 %) (fig. 8).

Măsurile terapeutice în Unitatea Primire Urgențe au fost centrate pe susținerea funcțiilor vitale: oxigenoterapie, hidratare, montarea cateterelor venoase centrale, sonde urinare, sonde de aspirație naso-gastrică sau clisme evacuatorii. 23,44 %, respectiv 308 dintre pacienți au beneficiat de tratament activ în departamentul de urgență, principala acuză fiind durerea acută (6,4 % dintre pacienți au necesitat tratament analgetic de tip opioid).

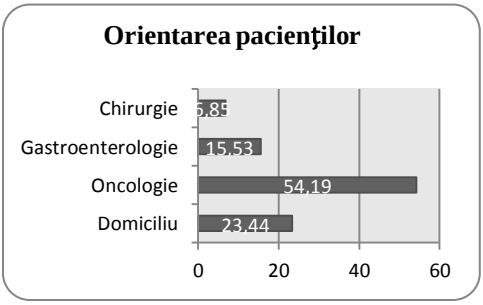

Fig. 8. Orientarea pacienților cu neoplazii din Unitatea Primire Urgențe

După prelucrarea datelor, au rezultat corelații statistic semnificative în ceea ce privește grupa de vârstă a pacienților din lotul de studiu şi diagnosticul cunoscut/suspectat de neoplazie (p= 0,01) (fig. 9) ceea ce semnifică diagnosticarea unei neoplazii în urgență mai frecvent la persoanele de vârstă tânără.

Deşi ne-am fi aşteptat ca frecvența complicațiilor să fie mai mare la pacienții diagnosticați anterior cu neoplazii, analiza

datelor nu arată diferenţe semnificative statistic (p=NS) între rata de apariţie a complicaţiilor la pacienţii diagnosticaţi anterior cu neoplazie faţă de cei cu suspiciune înaltă de neoplazie diagnosticaţi pentru prima dată în departamentul de urgenţă (fig.10). Este o realitate întâlnită în puţine alte studii realizate în acest domeniu, aceea că primul diagnostic de neoplazie este precizat în departamentul de urgenţă în prezenţa complicaţiilor, în condiţiile numărului redus al programelor de *screening*.

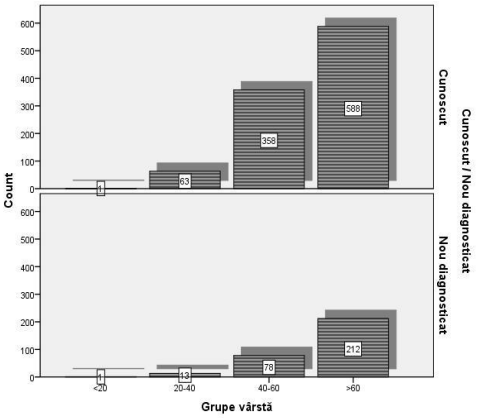

Fig. 9. Corelaţia grupă de vârstă - diagnostic

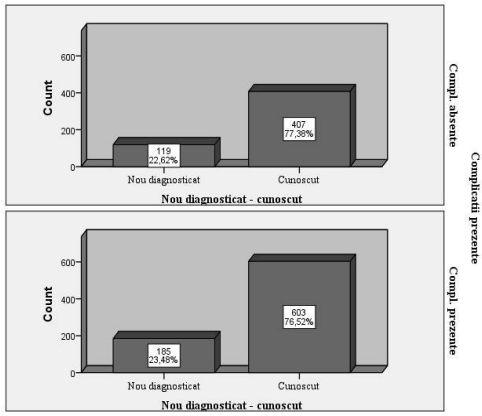

Fig.10. Corelaţia diagnostic nou/cunoscut - complicaţii

Pornind de la rezultatele studiului retrospectiv de faţă ne propunem aplicarea în practică a conceptului de *terapie de paleaţie în medicina de urgenţă după modelul din unele state din SUA şi Marea Britanie (Division of Palliative Emergency Medicine) (5), cu scopul îmbunătăţirii calităţii îngrijirilor acordate pacienţilor cu neoplazii prezentaţi în urgenţă, odată cu scăderea mortalităţii pe termen scurt şi îmbunătăţirea calităţii vieţii acestor pacienţi.*

CONCLUZII

Am inclus în studiul de faţă 1315 pacienţi oncologici prezentaţi în Unitatea Primire Urgenţe în decursul unui an, dintre care 23,12 % cu suspiciune înaltă de neoplazie (fără a avea încă confirmarea histopatologică) la prezentarea în urgenţă. Predomină pacienţii vârstnici (peste 65 de ani), de sex masculin, cu tumori la nivelul aparatului digestiv. Un procent important (aproape 60 %) dintre aceşti pacienţi ajunge în urgenţă datorită complicaţiilor, iar terapia vizează rezolvarea acestora, susţinerea funcţiilor vitale şi tratarea durerii. O parte dintre aceşti pacienţi sunt rezolvaţi în urgenţă, continuînd explorările şi terapia în ambulatoriu, în timp ce 75 % dintre pacienţi se internează după stabilizare. Rolul Departamentului de Urgenţă în evaluarea şi tratarea pacienţilor cu neoplazii este unul puţin evidenţiat de literatura de specialitate, dar totuşi trebuie recunoscută importanţa lui atât în diagnosticul iniţial cât mai ales pentru rezolvarea complicaţiilor şi susţinerea funcţiilor vitale.

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NOUTĂȚI

DEMONSTRAREA ROLULUI LEUCOCIDINEI PANTON VALENTINE ÎN EVOLUȚIA INFECȚIILOR CUTANATE DETERMINATE DE *S. AUREUS*

În ciuda datelor epidemiologice care asociază infecțiile cutanate necrotizante cu producerea leucocidinei Panton Valentine (PVL), implicarea acestei toxine în virulența *S. aureus* a fost mult disputată datorită rezultatelor neconcludente ale studiilor *in vivo*. Cu toate acestea, majoritatea rezultatelor anterioare proveneau din experimente pe şoarece, specie la care neutrofilele, principalele ținte celulare ale PVL, sunt lipsite de sensibilitate la acțiunea acestei leucocidine. În contrast, neutrofilele de iepure prezintă aceeaşi sensibilitate la acțiunea PVL ca şi celulele umane. Studiul realizat de Lipinska U *et al.* a utilizat iepurele drept model pentru infecția cutanată, în scopul evidențierii implicării PVL în patogenitatea *S. aureus*. Experimentul a arătat că iepurii care au fost injectați intradermic cu suspensii ale unor tulpini comunitare PVL pozitive de *S. aureus* au dezvoltat leziuni cutanate mai severe şi un grad mai mare de necroză tisulară comparativ cu tulpinile PVL negative şi au prezentat o creştere a titrului anticorpilor anti-LukS-PV. Acest studiu dovedeşte că iepurele este un model animal mai potrivit pentru investigarea rolului PVL în infecțiile stafilococice şi susține legătura stabilită pe criterii epidemiologice între tulpinile stafilococice PVL pozitive şi infecțiile cutanate necrotizante (Lipinska U, Hermans K, Meulemans L, Dumitrescu O, Badiou C, Duchateau L *et al*. Panton-Valentine Leukocidin Does Play a Role in the Early Stage of *Staphylococcus aureus* Skin Infections: A Rabbit Model. *PLoS One*. 2011; 6(8):22864).

Teodora Vremeră

DETECŢIA GENELOR DE FUZIUNE - PARTE INTEGRANTĂ A EVALUĂRII COPIILOR CU LEUCEMIE ACUTĂ

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DETECTION OF FUSION GENE - INTEGRANT PART OF THE ASSESSMENT OF CHILDREN WITH ACUTE LEUKEMIA (Abstract): Acute leukemia is the most common malignancy in children, being mostly produced by such chromosomal abnormalities as translocations or inversions causing gene fusion. Different clinical studies showed that translocations identified in ALL (acute lymphoblastic leukemia) and AML (acute myeloblastic leukemia) may be used to classify patients into risk groups. **Aim:** To detect three fusion genes that have been proven very important in patient classification: t(9:22)p190, t(4:11)and t(12:21). **Material and method**: We conducted a prospective study on 30 patients with acute leukemia diagnosed in the interval September 2009 - September 2010 at the Iasi Hemato-Oncology Unit of Saint Mary Hospital for Children. **Results**: We found t(9:22)p190 in two patients, t(4:11) in two patients and t(12:21) in one patient. From the total of 30 patients, 7 were considered at high-risk, 3 were diagnosed with AML, and 20 were considered at standard-risk. **Conclusions**: Day 33 can still be considered the reference time in the evaluation of treatment response; patients with BCR-ABL seem to have a worse prognosis than those who do not have this translocation. The detection of fusion genes is very important in patient classification. **Key words**: LEUKEMIA, CHILD, FUSION GENES

Leucemia acută este cea mai frecventă cauză de malignitate la copil, reprezentând 30% din cazurile de cancer în copilărie. O mare parte din leucemiile acute sunt produse de anomalii cromosomiale de tipul translocaţiilor sau inversiilor care produc gene de fuziune (1,2,3,4). Cele mai frecvente anomalii întâlnite în leucemiile acute la copii sunt t(9:22), t(4:11), t(15:17) şi inversia(16) (5, 6, 7). Numeroase studii clinice au arătat că translocaţiile din LAL (leucemia acută limfoblastică) şi LAM (leucemia acută mieloblastică) pot fi utilizate pentru ierarhizarea pacienţilor pe grupe de risc. De exemplu t(9:22) şi t(4:11) în LAL sunt asociate cu un prognostic nefavorabil iar t(12:21) în LAL şi t(8:21), t(15:17), inversia(16) în LAM sunt asociate cu prognostic favorabil (1,7,8,9). Punerea în evidenţă a acestor anomalii cromosomiale se poate realiza prin tehnici diferite, dintre care *nested RT-PCR* are una din cele mai bune rezoluţii(1).

MATERIAL ŞI METODĂ

Studiul de faţă a urmărit detecţia prezenţei genelor de fuziune la pacienţii inter-

naţi în Secţia Hemato-Oncologie a Spitalului Clinic de Urgenţe pentru Copii "Sf. Maria", Iaşi şi s-a desfăşurat pe o perioadă de un an (octombrie 2009-septembrie 2010), fiind un studiu de tip prospectiv.

Criteriile de includere în studiu au fost:

- pacienţi cu vârsta sub 18 ani
- pacienţi cu diagnosticul de leucemie acută confirmat prin medulogramă şi imunofenotipare
- pacienţi care au acceptat intrarea în studiu şi au semnat consimţământul informat (consimţământul a fost semnat de aparţinători, iar în cazul pacienţilor adolescenţi, aceştia au semnat şi ei un consimţământ în afara celui semnat de părinţi)

Obiectivele au constat în identificarea prezenţei genelor de fuziune la aceşti pacienţi şi realizarea de corelaţii între tipul genei de fuziune, imunofenotip, vârsta pacienţilor şi răspunsul la tratament.

În perioada 1 octombrie 2009 - 1 septembrie 2010 au fost diagnosticaţi cu leucemie acută, în secţia de Hemato-Oncologie a Spitalului "Sf. Maria", Iaşi, un număr de 30 de copii cu vârste cuprinse între 27 de zile şi 16 ani. Diagnosticul a fost stabilit pe baza manifestărilor clinice şi a rezultatelor analizelor paraclinice (hemogramă, medulogramă, imunofenotipare). Ulterior, pacienţii confirmaţi cu diagnosticul de leucemie acută au fost testaţi pentru prezenţa genelor de fuziune prin RT-PCR. Testarea a fost efectuată la diagnostic pentru toţi pacienţii, în ziua 33 de tratament pentru 10 pacienţi şi în ziua 56 pentru 2 pacienţi.

REZULTATE ŞI DISCUŢII

Împărţirea pacienţilor pe grupe de risc este de o deosebită importanţă pentru instituirea tratamentului deoarece în funcţie de acestea pacienţilor respectivi li se aplică un anumit tip de protocol chimioterapic mai simplu sau mai complex. Ierarhizarea pacienţilor se face pe baza unor criterii clinice şi paraclinice: vârstă, hemograma la diagnostic, imunofenotip, prezenţa sau absenţa unei translocaţii, răspunsul la corticoterapie (ziua 8) şi la chimioterapie (8).

Pacienţii din studiu au fost împărţiţi pe grupe de vârstă astfel: copii sub 2 ani (4 pacienţi), copii cu vârsta între 2 şi 10 ani (12 pacienţi), copii peste 10 ani (11 pacienţi). Prima grupă de vârstă este considerată a fi de risc crescut cu un prognostic mult nefavorabil pentru nou-născuţi, a II-a grupă are prognostic bun din acest punct de vedere, iar copiii cu vârsta peste 10 ani au, de asemeni un prognostic nefavorabil. Cele mai frecvente manifestări clinice au fost: astenia fizică, inapetenţa şi durerile osoase. Adenopatiile au fost obiectivate la 19 pacienţi, hepatosplenomegalia la 23, paloarea la 25 dintre pacienţi, scăderea ponderală la 22 dintre pacienţi.

Au fost diagnosticaţi 2 pacienţi care prezintă şi Sindrom Down, însă asocierea acestei patologii nu implică un prognostic nefavorabil pentru răspunsul la tratament.

Numărul de leucocite la diagnostic permite încadrarea leucemiilor acute în 3 forme – leucemică (număr de leucocite în limite normale), aleucemică (număr scăzut de globule albe), forma hiperleucocitară (globule albe>20.000 în hemograma la diagnostic).

Au prezentat formă hiperleucocitară considerată cu risc crescut, 7 pacienţi.

Medulograma a confirmat prezenţa de blaşti limfoizi sau mieloizi în măduva osoasă la toţi pacienţii incluşi în studiu.

În funcţie de imunofenotip, pacienţii au fost împărţiţi astfel: leucemie acută limfoblastică cu celule precursoare B 27 pacienţi, leucemie acută mieloblastică 3 paci-

enţi. Nici unul din pacienţi nu prezintă imunofenotip de celulă B matură considerat a avea prognostic rău. Imunofenotipul de celulă precursoare B este considerat a avea prognostic bun, cu atât mai bun cu cât stadiul de maturare al celulelor care formează clona malignă este mai precoce.

Răspunsul la tratament este un factor de prognostic foarte important în leucemiile acute. Acesta este cuantificat la ziua 8 – răspunsul la corticoterapie, pacientul fiind considerat că prezintă corticosensibilitate dacă nu mai prezintă blaşti în sângele periferic sau dacă numărul lor a scăzut şi corticorezistent dacă numărul de blaşti este acelaşi cu cel de la diagnostic sau mai mare.

Răspunsul la chimioterapie este cuantificat în ziua 33 (sfârşitul tratamentului de inducţie) prin medulogramă, chimiosensibilitatea fiind apreciată prin gradul remisiunii.

În lotul nostru 7 pacienţi au fost corticorezistenţi şi doar unul singur a fost şi chimiorezistent.

Încă de la diagnostic s-a urmărit identificarea prezenţei genelor de fuziune prin RT-PCR pentru o încadrare mai exactă a pacienţilor pe grupe de risc. Studiul de faţă şi-a propus detectarea a 3 gene de fuziune: t(9:22), t(4:11), t(12:21).

MLL-AF4 (*mixed lineage leukemia*) (t(4:11)) este întâlnită la 50-70% din leucemiile acute limfoblastice neonatale şi aproximativ 5% din cazurile de LAL la copilul mare şi adulţi. În cazul MLL-AF4 au fost identificate cel puţin 10 puncte de ruptură în interiorul genelor. La copilul mare prezenţa acestei gene se asociază cu prognostic nefavorabil şi reprezintă o indicaţie pentru transplantul medular. La nou-născuţii cu LAL este prezentă aproape obişnuit şi nu reprezintă o indicaţie de transplant. Studii recente au pus în evidenţă faptul că unele variante ale acestei gene se găsesc şi în populaţia normală ceea ce ar putea fi punctul de plecare pentru noi cercetări în domeniu (9,10,11).

În lotul nostru a fost identificată această genă la un singur caz - un nou născut diagnosticat la 27 de zile cu LAL.

BCR-ABL (*breackpoint cluster region*), care este o marcă pentru Leucemia mieloidă cronică, se întâlneşte şi în 5% din LAL la copil şi 20-50% din LAL la adulţi, incidenţa crescând cu vârsta. În LMC ruptura în BCR se face în "*major breackpoint cluster region*"(M-BCR), iar a 2-a zonă de ruptură în BCR (m-BCR) se găseşte aproape exclusiv în LAL cu cromosom Philadelphia+. Prezenţa cromosomului Philadephia în LAL este asociată cu un prognostic nefavorabil şi este, de asemeni, o indicaţie pentru transplant medular.

În lotul nostru au fost identificaţi doi pacienţi cu m-BCR. Unul din pacienţi prezenta o formă hipercelulară de LAL (GA= 250000/mmc) şi a decedat la 3 zile de la stabilirea diagnosticului. Celălalt pacient prezintă o formă rară de LAL cu debut prin tumefacţie osoasă dureroasă (rezultatul de la examenul anatomo-patologic al biopsiei osoase a sugerat diagnosticul). Acest copil a prezentat corticorezistenţă în ziua 8 de tratament şi chimiorezistenţă în ziua 33, prognosticul fiind extrem de rezervat.

TEL-AML1 (ETV6-RUNX1) este cea mai frecventă anomalie din LAL la copil, incidenţa fiind de 25%. Majoritatea pacienţilor sunt încadraţi în grupul de vârstă 1-12 ani cu un vârf între 2-5 ani. Unele studii au raportat prognostice favorabile pentru pacienţii pozitivi pentru TEL-AML1.

În lotul nostru a fost identificat un singur pacient cu această translocaţie, acesta având vârsta de 4 ani.

Pacienţii la care nu s-a detectat prezenţa nici unei gene de fuziune au fost încadraţi

pe grupe de risc în funcţie de celelalte criterii: vârsta la diagnostic, numărul iniţial de leucocite, imunofenotip şi răspunsul la tratament (corticoterapie şi chimioterapie) (14, 15, 16). Astfel pacienţii cu BCR-ABL pozitiv au fost încadraţi în grupul de risc înalt, la fel pacienţii cu forme hiperleucocitare şi cei care au prezentat corticorezistenţă în ziua 8. În total 7 pacienţi au fost încadraţi în grupul cu risc înalt, 3 pacienţi au fost diagnosticaţi cu LAM, 20 pacienţi au fost încadraţi in grupul de risc standard. Din numărul total de 30 de pacienţi diagnosticaţi 2 pacienţi au decedat, iar 10 sunt pierduţi din evidenţă, 2 pacienţi au plecat chiar după momentul diagnosticului, ceilalţi la finalul tratamentului de inducţie, fie pentru tratament în centre din străinătate, fie au renunţat la tratament.

Pacienţii urmează tratament conform protocolului ALL-IC-BFM 2002, urmându-se aceleaşi momente de investigare a răspunsului la tratament: ziua 33 şi ziua 56.

CONCLUZII

Ziua 33 rămâne un moment de referinţă în evaluarea răspunsului la tratament cu posibilitatea emiterii unor previziuni prognostice.

S-a confirmat valoarea de factor prognostic de risc înalt a prezenţei genei de fuziune BCR-ABL, evoluţia pacienţilor fiind nefavorabilă soldându-se într-unul din cazuri cu deces.

Detecţia genelor de fuziune este foarte importantă în stratificarea pacienţilor pe grupe de risc.

Mulţumiri

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NOUTĂȚI

MARKERI DE ACTIVITATEA A NEFRITEI LUPICE LA COPIL

Schemele terapeutice curente folosite pentru copiii cu nefrită lupică (LN) sunt asociate cu efecte secundare şi toxocitate semnificative, in perioade vulnerabile ale creşterii şi dezvoltării. Numărul redus de biomarkeri, în special pentru copii, împiedică o îngrijire corespunzătoare şi dezvoltarea de noi strategii terapeutice. Autorii au analizat markerii sistemului imun (B-cell acitvating factor BAFF cunoscut şi ca TNF, RANTES), complementul (Bb,C1q, C3d-CIC, C5a) şi activarea celulelor endoteliale (sVCAM-1) la copiii cu LN (n=22, vârsta medie 14,8±4,7 ani), sindrom nefrotic (n= 13) şi un grup de control de vârstă comparabilă (n=20) pentru a identifica parametrii care corelează cu activitatea LN. Fragmentele de complement ale căii alternative (Bb, $p<0,0004$), clasice -C3d-CIC $p<0,0001$) şi a căii comune (C5a, $p < 0,0001$) sunt semnificativ crescute]n perioada de activitate comparativ cu cea de remisiune a LN. Activarea complementului a fost asociată cu apariţia de anticorpi anti-C1q şi scăderea complementului C1q. Fragmentele activatoarea a complementului se corelează foarte strâns cu markerii sistemului imun şi activarea celulelor endoteliale. Ansamblul acestor parametri pot avea o mare valoare în identificarea semnelor precoce de activitate sau de remisiune a LN la copii, ba mai mult, pot proba utilitatea în evalaurea noilor tratamente şi determinarea optimizării folosirii acestora. (Edelbauer M, Kshirsagar S, Riedl M, Haffner D et al, Markers of childhood lupus nephritis indicating disease activity, *Ped Nephrol*, 2011; 3(26): 401-410).

Mihela Munteanu

INFLUENȚA ASCARIDIOZEI ASUPRA SEVERITĂȚII ASTMULUI BRONŞIC LA COPIL

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SENSITIZATION TO ASCARIS LUMBRICOIDES AND ASTHMA SEVERITY IN CHILDREN (Abstract)**:** There is little information concerning asthma severity and sensitization to *Ascaris lumbricoides* in pediatric patients. **Material and methods:** The relationship between sensitization to *Ascaris lumbricoides* and asthma severity in 219 patients with a high prevalence of parasitic exposure (IgE antibodies) was studied by using linear, multivariate and logistic regression. **Results**: Sensitization to *Ascaris lumbricoides* was associated with positive skin tests for aeroallergens, increased total serum IgE, increased eosinophils, reductions in FEV1 and FEV1/FVC ratio, increased airway Methacholine, bronchodilator responsiveness, and hospitalizations for asthma in the past year. **Conclusions:** Sensitization to *Ascaris lumbricoides* is associated with increased severity of asthma among Romanian children. This association is mediated by a high degree of atopy among the asthma children sensitized to *Ascaris lumbricoides* and belonging to a population with a low prevalence of helminthiasis.
Key words: *ASCARARIS LUMBRICOIDES*, ASTHMA, HELMINTHIASIS, ATOPY

Infecția cu *Ascaris lumbricoides* afectează la nivel mondial, aproximativ 1,4 miliarde de persoane, cu o distribuție preponderentă la populațiile cu un nivel socio-economic scăzut. Prevalența ascaridiozei în România a scăzut între 1953-1956 pe fondul ameliorării condițiilor igienico-sanitare şi accesibilității la tratamentul antihelmitic, iar începând cu 1997 înregistrează o creştere lentă, probabil, datorită alterării statusului socio-economic (1).

Relația dintre infestarea cu *Ascaris lumbricoides* şi astm, sau atopie este controversată, deşi este dovedit că totuşi se asociază cu un risc de atopie şi simptome astmatice în zonele cu prevalență ridicată a infestațiilor (2,3,4).

Aceste date susțin relația complexă dintre helmintiază, atopie şi astm, relație mediată de factori dependenți de gazdă, durata şi intensitatea infestației şi expunerea concomitentă la acțiunea altor paraziți (5,6,7).

În literatura de specialitate există puține informații privind corelația dintre *A. lumbricoides* şi severitatea astmului, în special

în zonele cu o prevalenţă scăzută a infestării cu helminţi (8,9).

În cadrul prezentului studiu am investigat asocierea dintre sensibilizarea la *A. lumbricoides* şi severitatea astmului pe un lot de 219 copii diagnosticaţi cu astm bronşic.

MATERIAL ŞI METODĂ

Studiul a fost efectuat pe un lot de 219 copii cu vârsta cuprinsă între 6-16 ani, diagnosticaţi cu astm, în perioada 2008-2010, în Spitalul Clinic de Urgenţă pentru copii "Sf. Maria" din Iaşi. Criteriile de includere în lot au fost reprezentate de prezenţa diagnosticului de astm sau istoric de crize de astm în cursul ultimelor 12 luni.

A fost determinată prezenţa anticorpilor IgE, numărul de eozinofile şi reactivitatea căilor respiratorii la Metacolină şi bronhodilatatoare. La 137 (62,5%) pacienţi au fost examinate materiile fecale pentru evidenţierea prezenţei unor paraziţi.

Probele ventilatorii şi răspunsul la bronhodilatatoare. Pacienţii au fost instruiţi să evite utilizarea de bronhodilatatoare cu durată scurtă de acţiune, timp de cel puţin 4 ore înaintea testării iar spirometria a fost efectuată cu pacientul în poziţie şezândă, după aplicarea unui clips nazal.

Cele mai mari valori au fost obţinute în cazul FEV_1 şi a capacităţii vitale forţate (FVC). După realizarea testării spirometrice în condiţii bazale, pacienţilor li s-au administrat două pufuri de Albuterol (200µg), iar testarea a fost repetată după 15 minute. Răspunsul la bronhodilatatoare a fost considerat prezent în cazul unei creşteri cu cel puţin 12% (200ml) a FEV_1 după administrarea de Albuterol.

Inducerea bronhoconstricţiei prin administrarea de Metacolină. După realizarea spirometriei bazale subiecţii a căror FEV_1 a fost de cel puţin 65% au fost supuşi testului de provocare cu Metacolină conform protocolului Chatham (5 inhalări de soluţie salină urmate de o inhalare de soluţie de Metacolină 1mg/ml, 1-4 inhalări de soluţie de Metacolină 5mg/ml şi o inhalare de soluţie de Metacolină 25 mg/ml). Fiecare inhalare a durat 6 secunde şi a fost urmată de 2 secunde de apnee. Testele spirometrice au fost repetate la 180, 210 şi 240 secunde după fiecare etapă de inhalare. Testarea a fost întreruptă în cazul scăderii FEV_1 cu cel puţin 20% din valoarea înregistrată după inhalarea soluţiei saline. Responsivitatea căilor respiratorii la inhalarea de Metacolină a fost considerată pozitivă în cazul scăderii cu 20% a FEV_1 la o doză de Metacolină $\leq$ 1,98 µmol.

Testarea cutanată a fost efectuată pe faţa ventrală a antebraţului folosind antigenele *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blatella germanica, Periplaneta americana, Alternaria tenuis*, păr de pisică şi de câine, polen mixt de ierburi şi de copaci. Testarea cutanată a fost considerată pozitivă în cazul apariţiei unei papule cu diametrul $\geq$ 3mm după stabilirea diametrului maxim al controlului negativ.

Concomitent s-a realizat şi examenul coproparazitologic în cadrul laboratorului Spitalului Clinic de Urgenţă pentru copii "Sf. Maria'' Iaşi.

Datele obţinute au fost analizate şi exprimate numeric, logaritmic şi binar, iar asocierile dintre diferiţi factori au fost interpretate cu ajutorul testelor de corelaţie. Prelucrarea statistică a fost realizată prin intermediul programului *Statistica 8.0*.

REZULTATE

Din totalul celor 219 pacienţi incluşi în studiu, 85 (38,8%) au prezentat sensibilizare la antigenele de *A. lumbricoides*. Majoritatea pacienţilor sensibilizaţi au fost de sex

masculin, au fost spitalizaţi pentru astm în timpul ultimului an, au prezentat cel puţin un test cutanat specific pozitiv, responsivitate crescută a căilor respiratorii la testarea cu Metacolină şi bronhodilatatoare comparativ cu pacienţii nesensibilizaţi (tab. I).

TABELUL I
Caracteristicile pacienţilor incluşi în studiu

Parametri evaluaţi	Sensibilizare la *A. lumbricoides*				p
	Nr	%	Nr	%	
Sex masculin	60	69,6	77	58,6	0,2
Nivelul educaţional al părinţilor					
Primare sau gimnaziale	44	50,9	58	43,7	0,33
Liceu	19	22,8	36	27,2	
Studii superioare	23	26,3	39	29,1	
Expunerea la fum de ţigară	22	25,7	35	26,3	0,91
Mamă astmatică	25	28,7	45	33,7	0,29
Tată astmatic	19	22,5	27	20,8	0,72
Teste cutanate pozitive la ≥ 1 alergen	81	95,3	106	79,8	<0,001
Responsivitate la testarea cu metacolină ≤ 1,98 μmol	54	70,4	72	58,3	0,02
Responsivitate la bronhodilatatoare	16	19,1	10	8,3	0,002
Spitalizare pentru astm în ultimul an	7	7,6	4	3,4	0,07
Prezentare în urgenţă pentru crize astmatice în ultimul an	67	84,2	106	79,1	0,21

În cazul pacienţilor sensibilizaţi la *A. lumbricoides* s-au înregistrat valori mai mari ale vârstei medii, ale IgE seric total, a numărului de eozinofile, a responsivităţii căilor respiratorii la bronhodilatatoare (exprimată sub forma diferenţei dintre valorile FEV_1 înainte şi după administrarea de bronhodilatatoare), a responsivităţii la testarea cu Metacolină şi valori mai mici ale raportului FEV_1/FVC comparativ cu pacienţii nesensibilizaţi. Nu au fost înregistrate diferenţe statistic semnificative în ceea ce priveşte nivelul educaţional al părinţilor şi istoricul matern sau patern de astm la pacienţii cu şi fără sensibilizare la antigenele de *A. lumbricoides*.

Reactivitatea cutanată la alergeni, IgE total, IgE specific şi numărul de eozinofile s-a evidenţiat prin rezultatele analizei bi şi multivariate ale relaţiei dintre sensibilizarea la *A. lumbricoides,* eozinofilie şi markerii alergiei. Sensibilizarea la *A .lumbricoides* a fost asociată cu o creştere de 5 ori a şanselor de a prezenta mai mult de un test cutanat pozitiv la alergeni şi de 3 ori a riscului de prezentare în urgenţă pentru crize astmatice. De asemenea, sensibilizarea la *A. lumbricoides* a antrenat o creştere moderată a riscului de hipereozinofilie şi a numărului total de teste cutanate pozitive.

Determinările spirometrice, responsivitatea căilor respiratorii la testarea cu bronhodilatatoare şi metacolină. Rezultatele analizei bi şi multivariate ale relaţiei dintre sensibilizarea la *A.lumbricoides,* indicatorii funcţiei pulmonare şi responsivitatea căilor aeriene la bronhodilatatoare şi metacolină a dovedit că sensibilizarea la *A. lumbricoides* a antrenat o creştere de 1,6 ori a şanselor de bronhoconstricţie semnifi-

cativă după inhalarea de Metacolină şi de 2,6 ori a şanselor de bronhodilatație semnificativă după administrarea de Albuterol. Similar, sensibilizarea la *A. lumbricoides* s-a asociat semnificativ cu o reducere a FEV_1 şi nesemnificativ cu reducerea raportului FEV_1/FVC (tab. II).

Exacerbarea astmului. S-a dovedit că sensibilizarea la *A. lumbricoides* antrenează exacerbarea astmului cu o creştere de 1,4 ori a şanselor de prezentare în urgență pentru crize de astm şi de 2,4 ori a riscului de spitalizare pentru astm, rezultatul nefiind influențat semnificativ prin eliminarea factorilor vârstă, apartenența la sex şi nivelul educațional parental.

TABELUL II

Relația dintre sensibilizarea la *A. lumbricoides* şi severitatea astmului

Teste	Neajustat	p	Ajustare pentru vârstă, sex, nivel educațional parental	p
Teste cutanate pozitive la ≥ 1 antigen	5,13 (2,38-11,09)	< 0,001	5,15 (2,36-11,21)	< 0,001
Responsivitatea căilor respiratorii la ≤ 1,98 μmol de Metacolină	1,70 (1,11-2,62)	0,016	1,61 (1,02-2,54)	0,040
Responsivitate la bronhodilatatoare	2,60 (1,44-4,69)	0,002	2,60 (1,34-5,05)	0,005
Prezentare în urgență pentru crize de astm în ultimul an	1,41 (0,85-2,34)	0,184	1,53 (0,91-2,58)	0,110
Spitalizare pentru astm în ultimul an	2,37 (0,99-5,67)	0,053	3,08 (1,23-7,68)	0,016

DISCUȚII

Cercetările au stabilit că sensibilizarea la *A. lumbricoides* s-a asociat cu inducerea reacției alergice, hipereozinofilie, niveluri crescute de IgE total şi specific, responsivitate crescută la bronhodilatatoare, reducerea funcției pulmonare şi spitalizarea pentru astm. Asocierea dintre sensibilizarea la *A. lumbricoides* şi indicatorii severității astmului este mediată de o creştere a gradului de atopie în rândul copiilor sensibilizați la *A. lumbricoides*. Exacerbarea răspunsului imun la antigenele *A. lumbricoides* în cazul copiilor cu astm şi atopie severă poate constitui o explicație plauzibilă şi asigură protecția împotriva infestației cu acest parazit.

Reactivitatea încrucişată, evaluată individual, nu poate explica în totalitate rezultatele prezentului studiu deoarece 56,8% dintre subiecții care au avut cel puțin un test cutanat pozitiv şi o parte dintre pacienții cu IgE specifice prezente, nu au fost sensibilizați la *A. lumbricoides*. Mai mult decât atât, 12,9% dintre copii cu teste cutanate negative la alergenii aerieni au fost sensibilizați la *A. lumbricoides* ceea ce sugerează că deşi majoritatea copiilor sensibilizați la *A. lumbricoides* sunt atopici, sensibilizarea la *A. lumbricoides* poate apare şi în absența acesteia la alergenii aerieni comuni. O explicație alternativă a rezultatelor prezentului studiu o constituie pierderea influenței imunoreglatorii a infestației helmintice (stimularea celulelor T reglatorii) în cazul copiilor

susceptibili ceea ce ar putea determina creşterea severității atopiei şi astmului. Sensibilizarea la *A. lumbricoides* poate fi rezultatul expunerii actuale sau un răspuns anamnestic la acest parazit sau alți helminți.

CONCLUZII

Prezenta cercetare demonstrează o asociere puternică între sensibilizarea la *A. lumbricoides*, severitatea crescută şi morbiditatea cauzată de astm. Această asociere este cel mai probabil mediată de un grad crescut de atopie în rândul copiilor cu astm sensibilizați la *A. lumbricoides*. Studii longitudinale extensive sunt necesare pentru a demonstra dacă această asociere este rezultatul stopării supresiei atopiei în cazul copiilor cu astm care primesc tratament antihelmintic şi/sau protecției împotriva helmintiazei în cazul copiilor cu astm şi atopie severă. Indiferent de mecanismul sensibilizării la *A. lumbricoides*, aceasta reprezintă un marcher important al severității atopiei, morbidității crescute şi severității bolii la copii cu astm.

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SUPINE HYPERTENSION AND ORTHOSTATIC HYPOTENSION – A MANAGEMENT CHALLENGE

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SUPINE HYPERTENSION AND ORTHOSTATIC HYPOTENSION-A MANAGEMENT CHALLENGE (Abstract): We report the case of an 80-year-old patient, treated for hypertension (HT), with history of stroke, mesenteric artery thrombosis, ischemic coronary artery disease and lower limb peripheral artery disease, who was admitted to our department for dizziness and recurrent attacks of syncope. The patient also has a recent diagnosis of Parkinson's disease. Cardiac and reflex syncope were excluded by specific tests. Syncope due to orthostatic hypotension (OH) was confirmed by active standing test. The major issue for clinician was the therapeutic approach for supine HT associated with OH. The optimal schedule for HT was established. Midodrine, the only available medication, was added with satisfactory effects on OH. Lifestyle modification was considered an essential step for achieving the therapeutic success. **Keywords**: SUPINE HYPERTENSION, ORTHOSTATIC HYPOTENSION, SYNCOPE, ACTIVE STANDING TEST.

Of the multiple aspects of blood pressure (BP) control, the coexistence of supine hypertension (HT) and orthostatic hypotension (OH) entail therapeutic challenges, given that the treatment for one condition can aggravate the other. Supine HT associated with OH is a form of autonomous dysfunction. It involves high BP levels during clinostatism and a clinically significant drop of BP when changing position to standing, which may cause dizziness, presyncope or syncope. OH can be easily diagnosed using active standing test or a tilt-test. Selecting the most appropriate therapy for each patient remains the key issue in this situation.

CASE REPORT

We present the case of an 80-year-old patient, from rural area, former heavy smoker (60 packs/year, until 6 years ago), who was admitted for dizziness, presyncope and recurrent syncope, systolic blood pressure (SBP) levels between 90-160 mmHg and intermittent claudication with walking distance of approximately 20 meters. The patient has a long history of hypertension and is treated since 2012 with Candesartan 16 mg daily and Lercanidipine 10 mg daily. The evolution was marked by numerous cardiovascular events. In 2014 the patient suffered a stroke, caused by an internal carotid artery stenosis, documented

by Doppler ultrasound scan and treated via endarterectomy. One year later, the patient has two episodes of paroxysmal atrial fibrillation, converted to normal sinus rhythm after Amiodarone treatment, but without antiarrhythmic and anticoagulant prophylaxis. In 2016, the patient is admitted for acute coronary syndrome without ST segment elevation. Coronary angiography revealed tricoronary lesions with indication for medical therapy (Rosuvastatin 20 mg daily, Bisoprolol 5 mg daily, Ramipril 5 mg daily, Clopidogrel 75 mg daily). The same year, the patient developed digestive symptoms, consisting of intense and recurrent abdominal pain, with no apparent cause following gastrointestinal endoscopy and abdominal-pelvic ultrasound scan. An abdominal CT scan was performed and revealed an incomplete thrombosis of the superior mesenteric artery, for which anticoagulant therapy was provided using Rivaroxaban 20 mg daily. In 2017, the patient presents intermittent claudication, as a clinical manifestation of lower limbs peripheral artery disease, which justified the admission into the clinic. Digital substraction peripheral angiography documents multiple bilateral superficial femoral artery stenoses and tibial-peroneal occlusion, which limits the recommendations on revascularization. A major aspect for the therapeutic approach was the association with rigid type Parkinson's disease, which was recently diagnosed and treated with Levodopa/Carbidopa 250/25 mg per day and Rasagiline 1 mg per day. Physical examination shows asymmetric facies, hypotonia and mild right upper limb tremor, right carotid and bilateral femoral murmur, absence of bilateral popliteal pulse, supine BP 160/80 mmHg, and orthostatic blood pressure 150/70 mmHg. The biochemistry report revealed the following data: fasting plasma glucose 89 mg/dL, total cholesterol 153 mg/dL, triglycerides 86 mg/dL, uric acid 6.7 mg/dL, creatinine 0.92 mg/dL, creatinine clearance 80 mL/min/1.73m^2, AST 25 U/L, ALT 18 U/L. The main issue in this case was elucidating the etiology of the episodes of dizziness, presyncope and recurrent syncope. According to the diagnostic algorithm for syncope (1), we considered the cardiac causes, reflex-related causes, and the cause that was secondary to orthostatic hypotension (OH). Echocardiography examination ruled out cardiac syncope caused by structural disorders; ECG only documented the following: concentric left ventricular hypertrophy (interventricular septum 14 mm, posterior wall 12 mm), lack of segmental abnormal wall motion and preserved ejection fraction (EF 60%), aortic valve without any hemodynamically significant lesions (peak aortic velocity 2 m/s, peak gradient 15 mmHg), normal mitral valve. The arrhythmic cause cannot be taken into consideration given the ECG maintenance of sinus rhythm at 60/min after stopping the betablocker and Holter ECG monitoring/24 hours without cardiac arrhythmias and conduction disturbances. The matter of reflex syncope was analyzed owing to carotid sinus hypersensitivity, considering the left carotid endarterectomy followed by postoperative alteration of baroreceptor sensitivity (2). Carotid sinus massage would have been necessary for confirmation, which is not recommended due to the presence of calcified atheromatous plaques on the two common carotid arteries and on the right internal carotid artery, as evidenced by Doppler ultrasound scan. Of the causes for syncope due to OH in the presented patient,

we continue to consider the primary failure of the autonomic nervous system caused by Parkinson's disease and the drug-related cause (i.e., medication used for Parkinson's disease and hypertension). As tilt-test was not available, active standing test, also known as Schellong test, was conducted and it confirmed OH without tachycardia (fig. 1). Given that Parkinson's disease is associated with OH and supine HT, we conducted a 24-hour Ambulatory Blood Pressure Monitoring (ABPM), which revealed a reverse-dipper profile (fig. 2). Regarding the therapeutic approach, the problem referred to the fact that hypertension medication can aggravate OH. Non-pharmacological measures are important for controlling both OH and HT. The patient was recommended the following (1): ingesting 500 ml fluids quickly when standing up, snacking before going to sleep, sleeping position with the head raised, compression socks, avoiding extended periods of orthostatism, as well as avoiding excessive consumption of salt. Also, it is necessary to avoid medications that trigger/maintain/aggravate OH (2). From this point of view, although it may cause OH, Parkinson's disease medication is necessary. Hypertension medication can also lead to OH. It is for this reason that the therapeutic plan included Amlodipine 10 mg daily and Losartan 50 mg daily, with adequate control of BP, along with Midodrine 2.5 mg per day for a period of 7 days, and subsequently 5.0 mg per day, well tolerated by the patient and with a satisfactory correction of OH (supine BP 140/80 mmHg, orthostatic BP 120/80 mmHg). We continued the treatment with Rosuvastatin 10 mg/day, Clopidogrel 75 mg/day and Rivaroxaban 20 mg/day as mandated by comorbidities, Omeprazole 40 mg/day.

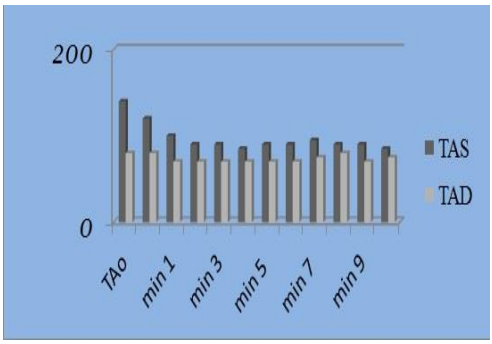

Fig. 1. Active standing test revealed a drop in SBP from 148 mmHg to 80 mmHg within 2 minutes, without stabilizing within up to 10 minutes.

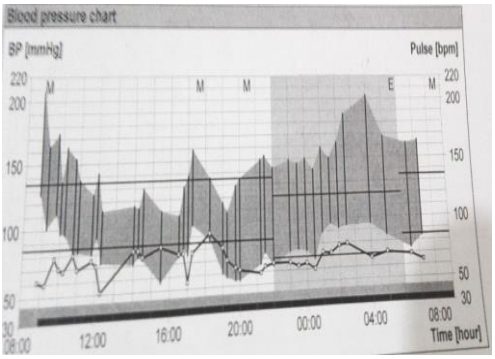

Fig. 2. Reverse-dipper BP pattern in 24-hour ABPM

DISCUSSION

The presented case highlights the importance of accurately determining the etiology for syncope and the treatment difficulties in elderly patients with comorbidities. OH is a common disorder, with a 7-30% incidence in elderly patients aged 75 years and over with normal BP levels and systemic disorders (2). OH can be caused by acute and chronic illnesses. In our patient, it was mandatory to determine chronic causes as per the ESC guidelines for the management of syncope (1). The relation with the primary autonomic dysfunction manifested in Parkinson's disease

and the age-related physiological changes is evident by means of declining baroreflex sensitivity and parasympathetic function (2, 3, 4). An additional argument is the non-tachycardic response to Schellong test, which is indicative of neurogenic OH (4). In physiological conditions, when changing position from sitting to standing there is a mild drop in BP, with activation of arterial baroreceptors and reflex increase in heart rate and myocardial contractility (5) (tab. I).

TABLE I

Physiological and pathological changes in the active standing test

Physiological changes	Pathological changes
Dropping systolic BP at least 6.5 mmHg	Dropping systolic BP > 20 mmHg
Increasing diastolic BP at least 5.6 mmHg	Dropping diastolic BP > 10 mmHg
Increasing heart rate at least 12 bpm	Increasing heart rate by >30 bpm or HR>108 bpm

In the case of autonomic failure an association of OH with supine HT would seem paradoxical, but it has a physio pathological explanation. Autonomic failure is a noradrenergic neurotransmission disorder whereby norepinephrine is not adequately released. Consequently, this impairs vasoconstriction, without a reflex increase in heart rate and reduction of intravascular volume with secondary OH (2, 6). The potential mechanisms of supine HT are the impairment of the baroreflex function, inadequate natriuresis, increased blood flow and residual sympathetic tone acting upon hypersensitive postsynaptic adrenoreceptors (5, 7, 8, 9, 10). According to other studies, in many patients both supine HT and OH were detected relatively late due to measuring BP while seating and usually resulting in normal BP levels (5, 11). According to literature data, only 2% of elderly patients with OH and HT are symptomatic (dizziness, presyncope, syncope) (10). An important practical aspect is the statement that antihypertensive medication determines OH. Antihypertensive medication is just an aggravating factor and lowering the doses alleviates symptoms (5). Clinical studies prove that the tendency for supine HT and OH in the elderly is usually plurifactorial (12, 13). Antihypertensive medication is not enough to determine the occurrence of OH, and most often there is an overlapping primary or secondary autonomic dysfunction (5, 11). Active standing test and tilt-test can confirm OH, while certain non-invasive tests such as heart rate measurement 30 minutes after the last meal, heart rate modification during the Valsalva maneuver, assessing respiratory arrhythmia during controlled breathing characterize the autonomic function (1, 2, 6). In the presented patient, the primary autonomic dysfunction within Parkinson's disease, the associated antihypertensive medication and presence of OH in Schellong test allowed for asserting the syncope etiology. What is the optimal treatment in this case? According to the 2017 Neurology Guidelines (3), the first step is to stop the medication causing OH, to the extent such a step is possible. In our patient, the medication for Parkinson's disease – which aggravated OH – could not be modified. A very important factor is the patient's education regarding the factors that can influence BP variations: avoiding rapid posture changes, extended periods of orthostatism, extensive straining during defecation or micturition, avoiding heavy meals, particu-

larly those rich in refined carbohydrates and alcohol. We would like to note the utility of swimming, as well as the minimum input of 500 ml of bolus fluids in preventing OH. Ingesting a snack before going to sleep can help reduce supine HT by inducing postprandial OH (2, 3, 4, 10, 14). As far as the antihypertensive medication is concerned, Losartan and Nifedipine R have the lowest impact on OH (4). In this case, we chose for Amlodipine instead of betablocker, considering the need to control angina and the tendency of heart rate to decrease in elderly patients as well as in patients treated for Parkinson's disease. Another useful measure could be to reduce doses in case of association with other types of drugs that have similar effects on the BP, as well as to split them in two doses – early in the morning and after lunch. This breakdown allows for 2-3 hours of activity after each dose. Also, patients must be instructed to avoid clinostatism after taking their medication. As far as OH specific medication is concerned, fludrocortisone (synthetic analogue mineralocorticoid) is contraindicated in association with supine HT (1, 11, 12). Midodrine (alpha adrenergic agonist) appears to be of an interest, as per the class II recommendations in the ESC Guidelines for syncope management (1). The important factors are up-titration to the target dose of 10 mg daily and control of the renal function (4, 11). There can also be rare occurrences of supine HT in this case (2). Pyridostigmine, a cholinesterase inhibitor, has a less marked effect on OH, but does not determine supine HT. (2) Ultimately, it can be used the parenteral administration of Octreotide, owing to its ability to generate splanchnic vasoconstriction. Droxidopa, having a similar structure to norepinephrine, is pending research for OH (2). In the presented case, Midodrine was the only available medication with satisfactory effects on OH.

CONCLUSIONS

Our case report shows the difficulty of treating HT accompanied by symptomatic OH in an elderly patient with multiple comorbidities, which requires a rigorous evaluation of the underlying etiology. In the clinical practice, patients are unique, and treatment must be customized, which reinforces the belief that "there are no illnesses, but only ill people". When pharmacological therapy is limited, it is mandatory to carefully educate the patient to improve their quality of life and ensure the therapeutic success.

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NEWS

NANOFIBERS FOR WOUND HEALING

Nanofibers resemble the natural extracellular matrix of the human body. Furthermore, polymer nanofibers provide an ideal environment for wound healing. A recent study conducted at Lund University in Sweden aimed to evaluate the pure topographical effects that an underlying scaffold material may have on fibroblast behavior in a novel *in vitro* wound model, which included poly-ε-caprolactone scaffolds with different nanofiber alignment. A stencil based wound assay with L929 murine fibroblasts was used to study the closure of circular wounds over time. Triggering fibroblast migration into the wound accelerates the remodeling of the dermis. Cell viability, morphology and mobility – in fact a combination of proliferation and migration, since both occur during the natural wound healing process - were assessed. The study emphasizes the important effect of orientation on increasing cell mobility, polarization and elongating cell morphologies compared to random fiber scaffolds. (Ottosson M, Jakobsson A, Johansson F. Accelerated Wound Closure - Differently Organized Nanofibers Affect Cell Migration and Hence the Closure of Artificial Wounds in a Cell Based *In Vitro* Model. *PLoS ONE* 2017; 12(1): e0169419)

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INFLUENŢA ŞI PREVALENŢA STEATOZEI HEPATICE NON-ALCOOLICE ÎN HEPATITA CRONICĂ VHB

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THE INFLUENCE AND PREVALENCE OF NONALCOHOLIC STEATOHEPATITIS IN CHRONIC HEPATITIS B (Abstract): The prevalence and the impact of nonalcoholic fatty liver disease (NAFLD) on chronic hepatitis B (CHB) are not well known. NAFLD as the hepatic manifestation of the metabolic syndrome (MS) is associated with obesity, diabetes mellitus and dislipidemia. **Aim**: To evaluate the prevalence and the impact of NAFLD on CHB. **Methods**: 120 patients diagnosed serologically and histological with CHB in 2006 in our center were retrospectively analyzed. Patients with self declared excessive alcohol consumption (above 20g/day for males and 10g/day for females) have been excluded. NAFLD was evaluated according to Brunt's score, while histology activity index (HAI) and fibrosis stage for CHB were estimated using Knodell score. The patients were divided in 2 groups: patients with CHB without steatosis (n=61) and with steatosis (n=59). **Results:** Out of 120 patients with CHB (male/female=76/44, median age=40.41), 49.15% had steatosis. No statistically significant differences among fibrosis stages, HAI and the aminotransferase levels was found between the two groups (p=0.320, p=0.228, and p=0.128). The presence of NAFLD associated with CHB was significantly correlated with age (p=0.001) and MS (p=0.051). In patients with CHB and steatosis, significant correlations between female sex and the presence of MS (p=0.007) as between HAI and age (p=0.003) were found. **Conclusions:** NAFLD was detected at 49.16% of patients with CHB. A positive correlation with age and MS (obesity, diabetes mellitus and/or dislipidemia) was observed. No significant correlations between NAFLD and the aminotransferase levels or histological features have been found. **Key words**: NONALCOHOLIC STEATOHEPATITIS, CHRONIC HEPATITIS B, METABOLIC SYNDROME

Infecţia cu virusul hepatitic B reprezintă o problemă de sănătate publică în lume, fiind responsabilă pentru una dintre cele mai frecvente boli la nivel mondial. Se estimează că peste două miliarde de persoane sunt infectate cu virusul hepatitic B (VHB), iar 350 milioane au infecţie cronică cu VHB (1).

Conform rezultatelor unui studiu epidemiologic recent, prevalenţa hepatitei cronice B în România este de 4,32 % (2), ceea ce înseamnă că, la o populaţie de 21 milioane locuitori, aproape 1 milion de persoane sunt infectate cu VHB.

În ultimul deceniu, boala ficatului gras nonalcoolic (NAFLD) reprezintă o altă problemă reală socială şi medicală în lume, reprezentând un spectru de condiţii clinico-patologice cu potenţial evolutiv sever ce cuprinde steatoza hepatică, steatohepatita (NASH) şi ciroza hepatică, în absenţa consumului de alcool în cantităţi hepatotoxice şi a altor etiologii de boală hepatică.

Prevalenţa NAFLD şi NASH în populaţie nu se cunoaşte cu exactitate, studiile efectuate până în prezent relevând, în funcţie de gradul de industrializare, tipul de dietă şi populaţia inclusă, valori diferite de 17-33%, respectiv 5-17% (3).

TABELUL I
Criteriile ATP de diagnostic a sindromului metabolic

3 sau mai multe din următoarele:
• Obezitate centrală abdominală (Talie>102 cm la bărbaţi, >88 cm la femei)
• Trigliceride serice >150mg/dl
• HDL-Colesterol <40mg/dl
• Tensiunea arterială >130/85mmHg
• Glicemia a jeun >110mg/dl

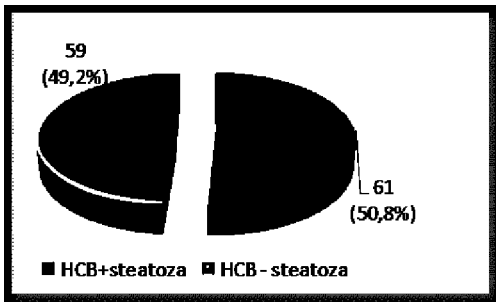

Fig. 1. Distribuţia pacienţilor în loturi de studiu şi prevalenţa steatozei hepatice.

Într-un studiu recent publicat de Poynard în Franţa, prevalenţa steatozei şi steatohepatitei (evaluate prin teste noninvazive - SteatoTest şi NashTest) în populaţia generală a fost de 18,1%, respectiv 1,3%. (4)

Prevalenţa NAFLD se estimează că va creşte în paralel cu creşterea incidenţei factorilor de risc pentru apariţia steatozei hepatice (obezitatea, insulinorezistenţa, diabetul zaharat şi alte componente ale sindromului metabolic). (5,6,7)

Având în vedere prevalenţa înaltă atât a infecţiei cronice VHB cât şi a NAFLD în populaţia generală, se estimează o incidenţă crescută a steatozei hepatice suprapuse hepatitei cronice VHB. Este bine cunoscută prevalenţa înaltă şi semnificaţia steatozei hepatice în hepatita cronică VHC. La pacienţii cu genotip 3 steatoza hepatică este direct atribuită virusului C, pe când în genotipul 1 şi 4 steatoza hepatică s-a demonstrat a fi secundară insulinorezistenţei şi componentelor sindromului metabolic. Este demonstrat faptul că atât insulinorezistenţa cât şi steatoza hepatică suprapusă hepatitei cu virus C accelerează progresia bolii, crescând gradul fibrozei hepatice. (8-11)

Comparativ cu infecţia cronică VHC, prevalenţa şi semnificaţia clinică şi prognostică a steatozei în hepatita cronică VHB este insuficient studiată.

Acest studiu analizează prevalenţa, impactul şi factorii de risc pentru steatoza hepatică suprapusă infecţiei cronice VHB la pacienţii diagnosticaţi histologic cu hepatită cronică VHB în Clinica de Gastroenterologie Fundeni, în anul 2006.

MATERIAL ŞI METODĂ

Date clinice

Au fost incluşi în studiu 120 de pacienţi diagnosticaţi serologic şi histologic cu hepatită cronică virală B (HCB) în anul 2006, în Clinica de Gastroenterologie Fundeni.

Toţi pacienţii cu AgHBs pozitiv de cel puţin 6 luni, ADN-VHB detectabil în ser şi biopsie hepatică efectuată sub ghidaj ecografic, au fost incluşi retrospectiv în studiu.

Pacienţii cu neoplazii concomitente, cu tratamente imunosupresive în ultimul an, cu anticorpi pozitivi pentru infecţia cu virusul hepatitic C sau D au fost excluşi. Alte criterii de excludere au fost : consumul excesiv declarat de alcool (> 20 g/zi pentru bărbaţi şi > 10 g/zi pentru femei), administrarea altor medicamente care pot cauza steatoză hepatică (amiodarona, corticosteroizi, tamoxifen, methotrexat), prezenţa altor etiologii de boală hepatică cronică (hepatită autoimună, ciroză biliară primitivă, boala Wilson, hemocromatoză).

TABELUL II
Distribuţia pacienţilor în funcţie de valorile transaminazelor

Steatoză da/nu			Frecvenţa	Procent	Valid Percent	Procent cumulativ
fără steatoză	Valid	Transaminaze normale	11	18.0	18.0	18.0
		Transaminaze crescute	50	82.0	82.0	100.0
		Total	61	100.0	100.0	
cu steatoză	Valid	Transaminaze normale	14	23.7	23.7	23.7
		Transaminaze crescute	45	76.3	76.3	100.0
		Total	59	100.0	100.0	

Date de laborator şi histologice

Toţi pacienţii au fost evaluaţi *clinic, antropometric* (vârstă, sex, greutate, înălţime, indice de masă corporală - BMI), *serologic* (transaminaze ALT, AST, glicemie, colesterol, trigliceride, AgHBs, încărcătură virală : ADN-VHB cantitativ) şi *histologic* (coloraţie hematoxilin-eozină, Masson trichrome, scor Knodell, steatoză hepatică : absentă $<5\%$ din hepatocite afectate şi prezentă $>5\%$ din hepatocite afectate).

Prezenţa sau absenţa sindromului metabolic a fost stabilită pe baza criteriilor ATP III (Adult Treatment Panel III) – tabelul I. Obezitatea a fost definită printr-un BMI$>$ 25 kg/m^2.

Analiza statistică

Toate datele au fost analizate utilizând programul SPSS (versiune 15.0, SPSS Inc., Chicago, IL, USA). Analiza statistică s-a făcut utilizând testul t-test pentru compararea variabilelor continue între grupuri sau în cadrul grupului, metoda chi-pătrat pentru compararea datelor calitative. P-value $<0,05$ a fost considerată statistic semnificativă.

REZULTATE

Cei 120 de pacienţi incluşi în studiu au avut vârste între 17 şi 62 ani, cu o vârstă medie de 40.41 $\pm$ 12,12 ani şi cu un raport femei/bărbaţi de 44/76 (36.7% femei, 63.3% bărbaţi).

Pacienţii diagnosticaţi cu hepatită cronică VHB au fost împărţiţi în două grupuri de studiu : pacienţi fără steatoză hepatică (61 de pacienţi) şi cu steatoză hepatică (59 de pacienţi). Prevalenţa steatozei hepatice în lotul studiat a fost de 49.16%. (Fig. 1)

Distribuţia pacienţilor cu valori normale/ crescute ale transaminazelor în cele două grupuri de studiu este descrisă în tabelul II.

Nu s-au înregistrat diferenţe semnificative statistic în ceea ce priveşte valorile transaminazelor (ALT cu valori crescute $>$N, ALT cu valori normale) la pacienţii cu steatoză hepatică suprapusă hepatitei VHB comparativ cu cei fără steatoză hepatică (p-value $=0,128$) (Fig.2).

Prezenţa steatozei hepatice la pacienţii cu infecţie cronică VHB se corelează semnificativ statistic (p-value$=0.003$) cu vârsta înaintată (mai mare decât vârsta medie >41 ani) (Fig. 3).

Din grupul de pacienţi cu steatoză hepatică, 64.4% prezintă cel puţin o componentă a sindromului metabolic (MS), comparativ cu doar 18% din pacienţii lotului fără steatoză hepatică asociată.

Prezenţa a cel puţin unei componente a

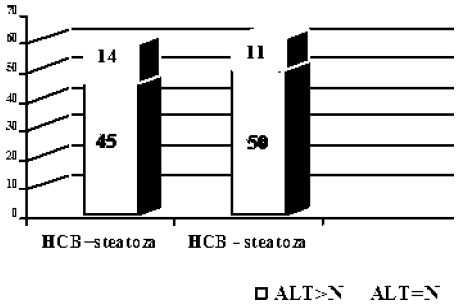

Fig. 2. Repartiţia pacienţilor în funcţie de nivelul transaminazelor.

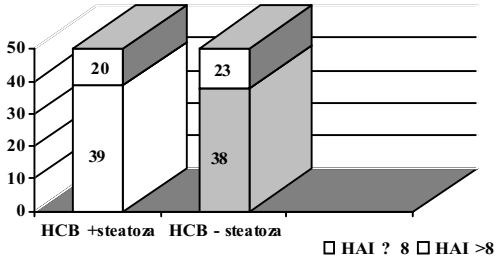

Fig. 4. Repartiţia în funcţie de activitatea necroinflamatoare.

sindromului metabolic se asociază semnificativ statistic (p-value=0.051) cu prezenţa steatozei hepatice.

În ceea ce priveşte necroinflamaţia hepatică evaluată prin scorul Knodell (considerând inflamaţie uşoară HAI ≤ 8 şi semnificativă HAI >8), nu există diferenţe statistic semnificative (p-value=0,320) între cele două grupuri analizate (Fig.4).

Dintre pacienţii care asociază steatoză hepatică 50.8% prezintă fibroză semnificativă (stadiul fibrozei ≥ 2), comparativ cu 6.1% din pacienţii fără steatoză hepatică.

Prezenţa steatozei hepatice la pacienţii cu hepatită cronică VHB se corelează semnificativ statistic (p-value=0.001) cu stadiul fibrozei hepatice (Fig.5).

În grupul de pacienţi cu steatoză hepatică supraimpusă hepatitei cronice VHB, s-au decelat corelaţii semnificative statistic între genul feminin şi prezenţa a cel puţin unei componente a sindromului metabolic (p-value =0.007).

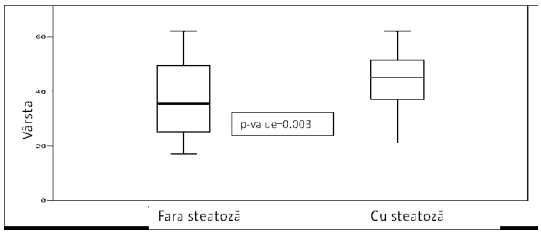

Fig. 3. Asocierea steatozei hepatice la pacienţii cu HCB.

În grupul de pacienţi cu steatoză hepatică au fost evidenţiate corelaţii semnificativ statistic (p-value=0.003) între scorul necroinflamator (HAI) şi vârsta avansată (Fig.6).

DISCUŢII

Acest studiu analizează prezenţa, impactul şi factorii de risc pentru steatoza hepatică asociată hepatitei VHB la un grup de pacienţi evaluaţi în anul 2006 în Clinica de Gastroenterologie Fundeni, Bucureşti.

În studiul nostru, prevalenţa steatozei hepatice a fost de 41.16% la pacienţii cu hepatită cronică VHB dovedită prin puncţie biopsie hepatică.

Prevalenţa a fost similară cu cea din alte studii (32-77%) (12-15), dar mult mai înaltă comparativ cu studiile din Grecia şi America de Nord, cum ar fi cel al lui Thomopoulos et al. (18%), Sanyal et al. (4,5%) şi Czaja et al. (24%). (16-18)

Este posibil ca variaţia mare a prevalenţei dintre studiile menţionate să se datoreze numărului relativ mic de pacienţi incluşi, diferenţelor etnice, dar şi a modului/corectitudinii de apreciere a consumului declarat de alcool.

În studiul nostru nu s-a evidenţiat o corelaţie pozitivă între prezenţa steatozei hepatice suprapuse infecţiei VHB şi valorilor crescute ale transaminazelor serice.

Acest studiu a plecat de la ipoteza că steatoza hepatică agravează atât necroinflamaţia ca şi fibroza hepatică în hepatita cronică VHB, similar cu hepatita cronică VHC.

Nu s-au găsit corelaţii semnificative statistic între prezenţa steatozei hepatice şi sco-

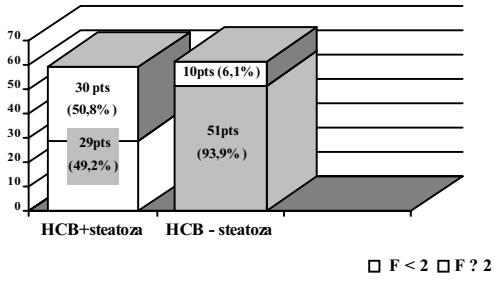

Fig. 5. Asocierea fibrozei hepatice.

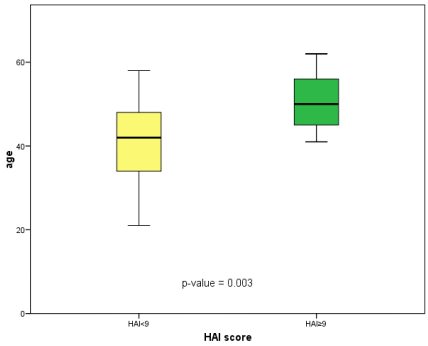

Fig. 6. Corelaţia scorului necroinflamator cu vârsta înaintată.

rul necroinflamator, nesusţinându-se astfel premisa agravării inflamaţiei hepatice de către steatoza hepatică.

Diferenţe semnificative statistic au fost demonstrate în ceea ce priveşte stadiul fibrozei hepatice între cele două grupuri de pacienţi analizate. Steatoza hepatică suprapusă infecţiei cronice VHB s-a asociat cu prezenţa fibrozei semnificative (F=2), demonstrând astfel, similar cu datele din studiile efectuate la pacienţi cu hepatită cronică VHC, rolul steatozei în progresia fibrozei la pacienţii cu hepatită VHB.

Studiul nostru demonstrează că prezenţa a cel puţin unei componente a sindromului metabolic (dislipidemie, hiperglicemie, obezitate) se asociază cu prezenţa steatozei hepatice la pacienţii cu hepatită cronică VHB, sugerând astfel că NAFLD în infecţia cronică VHB este mai probabil rezultatul factorilor metabolici ai gazdei, decât efectul direct al virusului VHB.

Adiţional, în grupul de pacienţi cu steatoză hepatică s-au evidenţiat corelaţii semnificative statistic între vârsta avansată şi scorul inflamator, precum şi între sexul feminin şi prezenţa a cel puţin a unei componente a sindromului metabolic.

În concluzie, steatoza hepatică se întâlneşte relativ frecvent la pacienţii cu hepatită cronică VHB, fiind puternic asociată cu factorii metabolici. Comparativ cu hepatita cronică VHC, steatoza în hepatita cronică VHB se asociază cu vârsta şi cu fibroza hepatică avansată, dar nu se corelează cu necroinflamaţia hepatică şi valoarea transaminazelor (19-21).

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HIGH MYOPIA AND GLAUCOMA - A CHALLENGE IN DIAGNOSIS. REVIEW

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HIGH MYOPIA AND GLAUCOMA - A CHALLENGE IN DIAGNOSIS. REVIEW (Abstract). The increasing global prevalence of myopia has significant consequences, as it predisposes to severe ocular pathologies. Differentiating early-stage glaucoma from myopia is challenging due to common clinical features. Diagnosing glaucoma in a highly myopic patient who has an abnormal optic nerve and an abnormal visual field, but normal intraocular pressure is a problem. Although the mechanisms responsible for glaucoma in myopic patients are poorly understood, it has been suggested that the optic nerve head in myopic eyes is more susceptible to glaucomatous damage. Mechanical stress induced by increased axial length of the globe and oxidative stress play a key role in the development of glaucoma in myopic eyes. Optic coherence tomography assessment of macular ganglion cells layers rather than retinal nerve fiber layer is valuable. Visual field testing should be used routinely in myopic patients, in order to facilitate the detection of early glaucoma. This review summarizes the difficulties in diagnosis of glaucoma in high myopic patients. **Keywords:** HIGH MYOPIA, GLAUCOMA, OPTIC COHERENCE TOMOGRAPHY, VISUAL FIELD.

Glaucoma is the leading cause of irreversible blindness in the adult population worldwide (1). It is a progressive optic neuropathy characterized by progressive structural loss of retinal ganglion cells and optic nerve damage that results in characteristic visual field loss. Early identification of the disease is very important, because appropriate treatment can prevent the progression of the disease.

Because primary open-angle glaucoma (POAG) is usually asymptomatic until the advanced stage, it is important to establish an effective screening tool for early detection of the disease. Many studies have investigated the risk factors associated with glaucoma (2). Elevated intraocular pressure (IOP) is an important risk factor for POAG (3). Evidence shows that lowering IOP reduces the risk of development of glaucoma (2). In addition, there is growing evidence that other risk factors like age, gender, race, refractive errors, heredity and systemic factors may play a role in glaucoma pathogenesis (4). Epidemiologic studies support the idea that high myopia is a risk factor for the progression of glaucomatous optic neuropathy (5).

The increasing global prevalence of myopia has significant consequences as it predisposes individuals to severe ocular pathologies. Differentiating early-stage glaucoma from myopia is challenging due to common clinical features. Diagnosing glaucoma in a patient who is highly myopic and has abnormal optic discs and an abnormal visual field, but normal pressures is a challenge. There are three main ways to assess glaucoma: changes in the optic nerve, which can be measured by fundus photos and optic coherence tomography imaging (OCT), changes in the visual fields (VF) indicating a progressive loss of retinal nerve fiber layer (RNFL) and elevated intraocular pressure (IOP) (6). Although the mechanisms responsible for glaucoma in myopic patients are poorly understood, it has been suggested that the optic nerve head in myopic eyes is more susceptible to glaucomatous damage because of the changes in connective tissue structure (2).

Myopia is the most frequent refractive error, which typically accelerates during the teenage years (7). Near-work activities were thought to contribute to the development of myopia by leading to constant ciliary muscle contraction and more accommodation, to which the eye responds by axial elongating. More time spent indoors has also been implicated, since exposure to light stimulates dopamine release, which is thought to reduce the axial length (8). Teenage and adult-onset myopia is characterized by an axial length of < 26 mm and refractive errors of up to -5 diopters (D). In high myopia, the extreme refractive errors of more than -6.00 D and an axial length of > 26 mm are associated with progressive degenerative changes of the fundus, optic disc deformation, retinal thinning, and choroidal atrophy (9).

The link between myopia and glaucoma

High myopia is associated with an increased risk of ocular complications such as premature cataracts, glaucoma, retinal detachment, and macular degeneration (10, 11). The prevalence of myopia varies across populations of different regions and ethnicities. High myopia is more common in Asia (12, 13). Scientists have not yet establish an effective treatment to prevent the progression of high myopia. Population-based studies indicate that the risk of glaucoma increases with the increasing degree of myopia. Most studies have shown that high myopia is associated with an increased risk of POAG. The Blue Mountains Eye Study found a strong relationship between POAG and myopia (14). In the Barbados Eye Study, a myopic refraction was one of risk factors for POAG in adult black people (15). The Beaver Dam Eye Study suggested that, after considering the effects of age and sex, persons with myopia were 60% more likely to have glaucoma than those with emmetropia (16). Patients with moderate or high myopia had a 3 times higher risk of POAG compared with those with emmetropia (17).

Pathogenesis of myopic glaucoma

Given a strong epidemiologic relationship between myopia and glaucoma, there is great interest in understanding the mechanisms between the two diseases. Abnormalities of the lamina cribrosa associated with high myopia may explain the higher susceptibility of myopic eyes to glaucoma at any IOP level (18). The mechanisms by which myopia predisposes to glaucoma development are still unknown. Though, two principal theories try to explain the progressive damage of retinal ganglion cell layer (GCL). The mechanical theory sug-

gests that the damage is to the lamina cribrosa, where axons exit the eye. This can be induced by increased IOP and a fragile sclera or by exacerbated shearing forces due to elongation of the eye. In high myopia there is a loss of collagen and proteoglycans at the scleral level. This changes lead to loss of sphericity, the scleral canal is widened, the lamina cribrosa is thinner and the choroid atrophy occurs.

The vascular theory supports that insufficient ocular perfusion secondary to increased IOP is a risk factor for glaucomatous optic neuropathy (18). One important factor implicated in the vascular theory is the oxidative stress, as a result of myopia-associated retinal pigment epithelium and choroid atrophy. The increased level of reactive oxygen species and the reduced antioxidant protection mechanisms play a pathogenic role in POAG (19). Retinal tissue is highly susceptible to oxidative stress because of the high oxygen demands coupled with its high polyunsaturated fatty acid content (20).

Differentiating early-stage glaucoma from high myopia

Glaucoma is characterized by increased IOP, altered VF and damage to the optic nerve head on fundus examination and OCT measurements. Assessment of IOP alone can prove insufficient for identifying glaucoma in high myopic patients due to the fact that IOP dependents on corneal thickness. Corneal thickness is characteristically thinner in myopic eyes (21). Differences in biomechanical properties of the cornea may modify IOP measurements. Moreover, we need to keep in mind that there is also normal tension glaucoma. It is important to remember that the IOP is often more elevated in patients with high myopia, being greater during sleep. A cross-sectional study demonstrated that myopia was correlated with increasing IOP at baseline (16). Studies showed that, for a given IOP in eyes with POAG, optic nerve damage appears to be more important in highly myopic eyes with large optic discs than in non-highly myopic eyes (22). It is necessary to check the diurnal pressure, several times a day. The appearance of normal IOP is one of the factors that makes these patients challenging to diagnose. It is best to obtain multiple readings at different times of day, since one eye pressure reading is only a single measurement out of a continuum IOP fluctuation.

The structural changes of glaucomatous optic neuropathy include generalized enlargement of the optic disc cup, localized notching or narrowing of the optic disc rim, and violation of the Inferior-Superior-Nasal-Temporal rule (the thickness of disc rims beginning with the inferior rim, followed by the superior rim, the nasal rim, and the temporal rim) (23). Early detection of glaucomatous optic disc changes is difficult in patients with high myopia because myopic discs have large diameters, a larger cup disc ratio, shallow and concentric disc cupping, and a high frequency of disc tilting (24, 25). The optic disc of many high myopia patients are accompanied by tilt, torsional or pale appearance, or peripapillary atrophy, which makes the evaluation of the retinal rim difficult (26). The fundus examination is made on dilated pupil (fig. 1, 2).

There is no consensus about the best method to detect progression of VF loss. Yi-An *et al*. (27) found that IOP and high myopia were risk factors for progression of VF loss in POAG patients. Ding *et al*. (28) studied 1,434 visual fields of high myopic

patients. They found that 16.1% of defects in the myopic subjects were the same as those found in glaucoma patients, while 3 % of the eyes had defects that looked like dense arcuate scotomas. The study group did include a few glaucoma suspects, but most were healthy high myopes. The VF in high myopia reveals the enlargement of the blind spot, the reduction of retinal sensitivity, arcuate temporal scotoma and cecocentral scotoma. It is important to remember that the scotomas do not respect the horizontal meridian (fig. 3). In glaucoma, VF examination has an important role in evaluating the disease progression. The defects respect the horizontal meridian. The most frequent defects found in glaucoma patients are: paracentral scotoma, Bjerrum arcuate scotoma, nasal step, ring scotoma (29) (fig. 4).

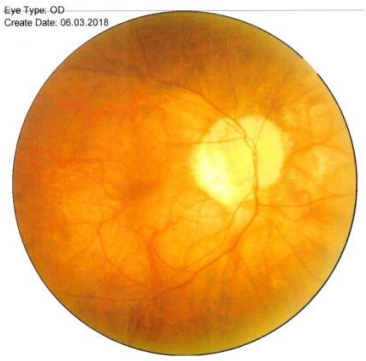

Fig. 1. Fundus examination in a high myope

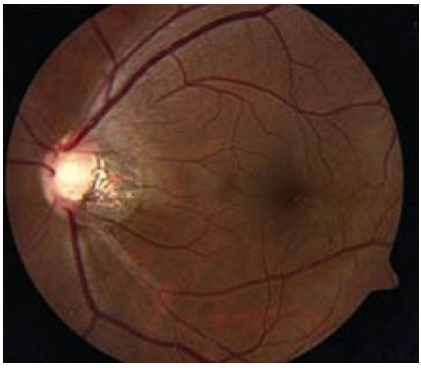

Fig. 2. Fundus examination in a patient with high myopia and glaucoma

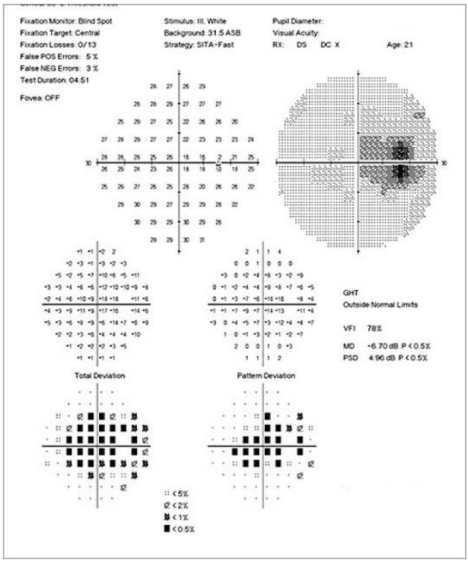

Fig. 3. Visual field - enlargement of blind spot in high myopia

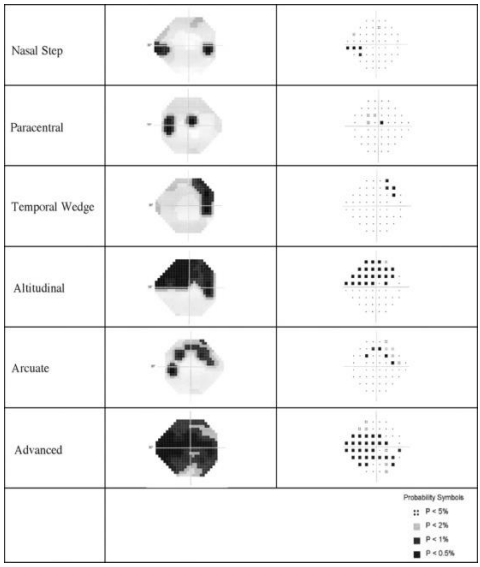

Fig. 4. Visual field defects in glaucoma (30)

Recently, computerized imaging devices such as OCT and confocal scanning laser ophthalmoscopy, have been used for the diagnosis of glaucoma. OCT is a valuable aid for detection of early glaucomatous changes. OCT images provide visualization of a thinning circumpapillary retinal nerve fiber layer (cp-RNFL), which is a classic indicator of glaucoma. However, a thin cp-RNFL is also a clinical feature of the enlarged eyes in high myopia. It is also important to remember that myopic tilt can severely affect RNFL thickness and optic nerve head parameters on OCT. Since retinal thinning typically occurs in the peripheral but not central areas, macular measurements of GCL, which are not influenced by myopic thinning of the cp-RNFL, are very important. The assessment of GCL parameters is a useful technique in association with cp-RNFL thickness, for evaluating patients with glaucoma and high myopia (31).

The OCT in high myopia shows more RNFL abnormalities in the nasal, superior and inferior quadrants, while in glaucoma in the temporal and inferior quadrants (fig.5, 6). OCT RNFL thickness map in myopic eyes requires careful consideration of the distribution pattern of the RNFL bundles. RNFL measurement is sensitive for detection of glaucoma, and the extent of RNFL damage correlates with the functional deficit in the visual field. Shoji (32) studied 93 glaucoma and 86 non-glaucoma patients with high myopia and compared the macular GCL and cp-RNFL measurements. They found that macular GCL parameters were able to detect glaucoma, and they also showed that cp-RNFL measurements were significantly related to both high myopia and glaucoma.

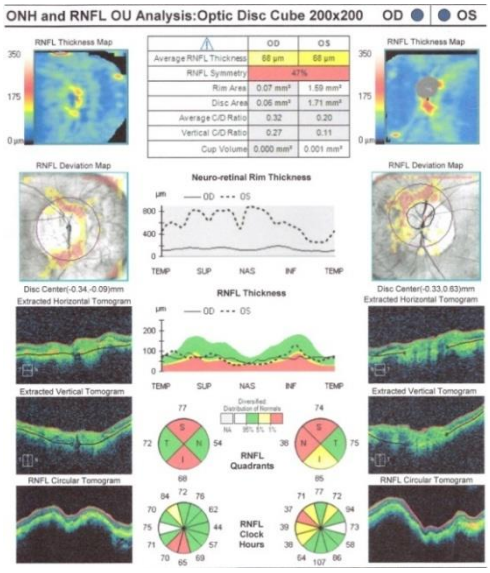

Fig. 5. OCT - RNFL in high myopia

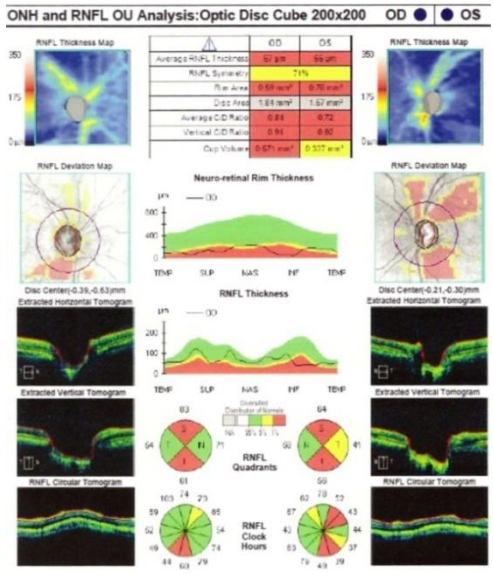

Fig. 6. OCT - RNFL in glaucoma

It remains unclear if the optic nerve damage is due to torsional changes at the level of the lamina cribrosa during axial elongation. Multiple studies have reported

cross-sectional changes in the lamina cribrosa and peripapillary atrophy using OCT imaging techniques (33-36).

New approaches

Early detection of any pathology provides potential for preventing the disease. Scientists investigate new interventions to control excessive ocular growth and limit myopic progression. One approach involves ocular strategies such as bifocals, and a new contact lens design. Another approach is based on behavioral strategies such as modified ergonomics in the classroom. The use of atropine has been shown to reduce the rate of axial growth (37). One of the most important approaches for monitoring early glaucoma changes in high myopes is to perform routine visual field testing. While analyzing the OCT, it's important to make sure that any significant defects seen on OCT correspond to visual field defects.

If it's not obvious whether the patient has glaucoma or just high myopia, one can choose to monitor the patient, looking for the earliest confirmed deterioration in test results over time. This may be an acceptable choice, depending on the age of the patient, the patient's lifestyle and the amount of reserve vision that remains unaffected (6). If one chooses to monitor the patient, it is important to make serial testing using the same machine. At first, this must be done more frequently, and then slowly spaced out if no change is detected. Furthermore, it is advisable to monitor the IOP and the optic nerve in comparison to baseline stereo disc photos.

CONCLUSIONS

From our review and summing up the studies above mentioned, we can conclude that high myopia often makes detecting glaucoma difficult, because optic nerve features may confound glaucomatous changes. Although the mechanisms responsible for glaucoma in myopic patients are poorly understood, it has been suggested that the optic nerve head in myopic eyes is more susceptible to glaucomatous damage. In addition, pathologic myopia may cause visual field defects and retinal nerve fiber layer thinning that are indistinguishable from those found in glaucoma patients. Macular ganglion cell layer parameters are able to detect early glaucoma, while cp-RNFL measurements are related to both high myopia and glaucoma. Myopic patients presenting with visual field and OCT defects should be observed conservatively unless they demonstrate progression. It is important to investigate if there is a decrease in the rate of visual field loss and RNFL thinning after cessation of progressive myopia.

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NEWS

HISTOPATHOLOGIC DIFFERENCES PARTIALLY DISTINGUISH SYNDROMIC AORTIC DISEASES

A variety of syndromic diseases such as Marfan syndrome, Loeys–Dietz syndrome, and bicuspid aortic valve with aneurysm along with risk factors of smoking and hypertension result in ascending aortic aneurysms and dissections. Historically, a complicated variety of terms have been used to describe a range of histopathology that are present in resected specimens. As a result, no consistent patterns of histopathology have been reported. We used the recent Society for Cardiovascular Pathology/Association for European Cardiovascular Pathology consensus statement on nomenclature and diagnostic criteria for noninflammatory aortic disease to blindly evaluate 148 surgically resected specimens. We found that overall patterns of histopathologic changes could separately cluster bicuspid aortic valve and no syndromic subjects from Marfan and Loeys–Dietz subjects. Marfan syndrome cases significantly had more overall medial degeneration and mucoid extracellular matrix accumulation than other syndromes. Smooth muscle cell nuclei loss was a feature of aging and not a feature of Marfan or Loeys–Dietz syndrome subjects. The authors conclude that a consistent use of histologic and histopathologic descriptors can help discriminate different etiologies of ascending aortic aneurysms (Waters KM, Rooper LM, Guajardo A and Halushka MK. Histopathologic differences partially distinguish syndromic aortic diseases. *Cardiovascular Pathology* 2017; 30: 6-11).

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HEPATITA C LA COPIL: MANIFESTĂRILE EXTRAHEPATICE ARGUMENTEAZĂ TERAPIA STANDARD ŞI LA COPIL?

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CHILDREN CHRONIC HEPATITIS C: ARE EXTRAHEPATIC MANIFESTATIONS AN ARGUMENT FOR STANDARD THERAPY? (Abstract): Chronic hepatitis C is still an important health problem in children. Adequate management of those pacients should be focused on two directions: dissapearance or reduction of the viral load (conventional antiviral therapy) and treatment of autoimmune manifestations (corticoids, plasmapheresis, cytotoxic agents). **Aim:** To associate extrahepatic manifestations with other disorders and to evaluate the evolution under specific (antiviral) and non-specific therapy. **Materials and methods:** 43 children diagnosed with chronic hepatitis C and followed in the 2nd Pediatric Clinic Iaşi between January 2005 and June 2010. **Results and discussions:** most children were diagnosed accidentally or for etiological investigations for elevated liver enzymes. Extrahepatic manifestations were found in 41.86% of pacients, most of them with articular, skin and hematologic disorders, but also depression (2 cases) in those receiving interferon. **Conclusions:** In children, extrahepatic manifestations in chronic hepatitis C infection are more rare than in adults, especially with articular involvement. In certain cases, interferon therapy can trigger those manifestations with an immune mechanism. It is essential to evaluate the autoimmunity markers before proceeding with antiviral therapy. **Key words:** CHRONIC HEPATITIS C, CHILDREN, EXTRAHEPATIC, INTERFERON

De la descoperirea VHC în 1989, cunoştinţele de epidemiologie, istorie naturală şi eficientizarea răspunsului VHC la terapia antivirală s-au acumulat, scriind o istorie de succes la adulţi. Progresul a fost mult mai lent pentru copiii infectaţi, existând lacune majore în privinţa datelor care sprijină deciziile clinice, prevalenţa mai mică în cadrul acestui grup special de pacienţi şi având în vedere consideraţiile de ordin social, material şi etic în derularea studiilor clinice la copii (1). În România, prevalenţa VHC este de 4,9%, plasând ţara noastră pe locul 1 în Europa ca număr de hepatite C înregistrate şi pe locul 4 ca rată a mortalităţii cauzate de bolile hepatice (Institutul de Sănătate Publica Cluj-Napoca). Copiii infectaţi cronic cu VHC sunt deseori subdiagnosticaţi şi, ca atare, netrataţi multă vreme, vor ajunge la o afectare hepatică severă pe termen lung. De asemenea, majoritatea sunt asimptomatici, uneori prezentând doar astenie sau durere abdominală (semne necaracteristice), fiind astfel dificil de identificat. La acestea se adaugă transmiterea verticală, cauza primară în epidemiologie (în 60% din cazuri), deşi riscul mediu al transmiterii de la mamă la copil este doar de 5 %, mult mai mic decât în cazul hepatitei B, şi faptul că vârsta adolescenţei îi aduce la un grad de risc crescut pe cei consumatori de droguri, pe amatorii de tatuaje şi piercing, pe lângă riscul legat de transfuziile de sânge şi produse derivate.

Într-o meta-analiză care evaluează riscul transmisiei verticale a VHC, s-a arătat că şansa de transmitere este dublă la mamele coinfectate VHC/HIV, cu o rată a transmisiei verticale a VHC fără HIV care variază între 4-10%, şi la mamele coinfectate VHC/HIV cu viremie VHC detectabilă - un risc de 2,82 ori mai mare (3). Iată de ce conştientizarea problemei şi identificarea precoce a copiilor infectaţi sunt prioritare, asigurând şanse mai mari de intervenţie terapeutică eficientă.

Caracteristica principală a hepatitei cronice cu virus C este persistenţa infecţiei virale în timp, cu progresia lentă a fibrozei parenchimului hepatic şi, de asemenea, tropismul limfatic demonstrat. În general, copiii au mai puţină inflamaţie, fibroză şi steatoză decât adulţii, probabil datorită duratei mai scurte a infecţiei. Fibroza semnificativă şi chiar ciroza pot apărea însă şi în perioada copilăriei, aceşti pacienţi având risc crescut de boală hepatică terminală când vor deveni adulţi. Rămân necunoscute rata progresiei bolii, factorii de risc ai progresiei, precum şi dacă această progresie se face sau nu după un model liniar. În ceea ce priveşte clearance-ul spontan, acesta este mai frecvent la copiii care au dobândit infecţia la vârstă mică şi la cei cu transaminaze normale (2,4). Genotipurile 1a şi 1b sunt prevalente la adolescenţi şi adulţi tineri, iar la copil sunt mai frecvente infectările cu genotipurile 3 şi 4.

Totuşi, în ţara noastră, majoritatea cazurilor sunt cu genotip 1. Copiii infectaţi cu genotipul 3 au cea mai mare probabilitate de apariţie a clearance-ului spontan şi răspund în mare măsură la tratamentul cu IFN (4,5). Interacţiunea complexă între anumite caracteristici virale şi răspunsul imun al gazdei determină o mare variabilitate genetică a VHC, ca mecanism prin care VHC evită supravegherea imună. Prezenţa simultană la acelasi individ a variantelor virale diferite, dar strâns înrudite au primit denumirea de quasispecii. Acestea apar în special la genotipul 3 şi pot compromite răspunsul mult mai bun la tratament al acestui genotip. La cei cu TGP normal sau uşor crescut s-a demonstrat existenţa quasispeciilor complexe, cu creşterea numărului şi a diversităţii genetice ale clonelor virale. O heterogenitate mai mare şi o complexitate a quasispeciilor la copiii cu absenţa dovezilor biochimice a leziunii hepatice poate sugera că sistemul imun al gazdei la aceşti copii exercită o presiune imună mai mare asupra VHC, ceea ce determina implicaţii în viitor pentru terapie.

În urmă cu aproximativ 10 ani, mulţi autori au descris asocierea HVC cronică cu un grup de afecţiuni non-hepatice, heterogene, cum ar fi fibroza pulmonară, vasculitele cutanate, glomerulonefrita, ulcerul Mooren, porfiria cutanea tarda, lichenul plan (2), care au fost considerate drept manifestări extrahepatice ale infecţiei cronice HVC. Cercetătorii şi-au menţinut interesul stabilirii unor conexiuni între hepatita cronică cu virus C şi unele afecţiuni autoimune, cum sunt crioglobulinemia, sindromul Sjogren, artrita reumatoidă, poliarterita nodoasă, sindromul antifosfolipidic şi lupusul eritematos sistemic. Diagnosticul şi tratamentul bolilor autoimune legate de existenţa unei HVC cronice au devenit de primă importanţă în preocupările specialiştilor, având în vedere prognosticul sumbru al acestor pacienţi. Deşi scopul tratamentului antiviral este ţintit pe afectarea hepatică primară, există suficiente date asupra eficienţei şi siguranţei utilizării acestor terapii la pacienţii care prezintă manifestări extrahepatice asociate. Managementul adecvat al acestor pacienţi trebuie axat pe două direcţii : eradicarea sau reducerea încărcăturii virale (terapia convenţională antivirală) şi tratamentul manifestărilor autoimune (corticosteroizi, plasmafereză, agenţi citotoxici) (2,5,6). Pe lângă manifestările extrahepatice enunţate în literatură, anumite comorbidităţi (care nu pot fi încadrate în acest grup) trebuie avute în vedere atunci când se instituie tratamentul specific, anti-

viral sau corticosteroid. Patologia asociată este un element important în evaluarea bolii, atât în ceea ce priveşte evoluţia pacientului care urmează sau a urmat un anumit tratament, cât şi în estimarea necesităţii instituirii unei anumite terapii sau în ceea ce priveşte oportunitatea modificării liniei terapeutice.

Tratamentul infecţiei cronice HVC cuprinde monoterapia cu IFN alfa sau asocierea acestuia cu Ribavirinul. Utilizarea acestora la bolnavii cu manifestări extrahepatice este controversată, pe de o parte, datorită excluderii acestor pacienţi din trialurile multicentrice (4), iar pe de altă parte, lipsei răspunsului la tratament sau apariţiei unor efecte secundare grave care au impus, în final, abandonarea terapiei. Există, totuşi, date care să ateste o oarecare eficienţă a tratamentului antiviral asupra HVC cronică cu determinări extrahepatice. Interferonul alfa s-a dovedit a fi eficace la unii pacienţi cu crioglobulinemie, mai ales la cei la care viremia ARN-VHC devine nedetectabilă în ser. Totodată, trebuie avută în vedere o monitorizare strictă a terapiei, din punctul de vedere al efectelor secundare imediate sau la distanţă. Noul IFN pegylat este superior ca şi eficacitate, dar şi prin incidenţa mai mică a efectelor secundare. Ribavirina, utilizată în monoterapie sau asociată cu IFN alfa, ar putea fi eficace şi în tratamentul crioglobulinemiei asociate HVC, însă utilizarea acesteia la pacienţii cu manifestări renale trebuie atent monitorizată. Missiani et al a dovedit că ribavirina poate avea efecte secundare severe, cum sunt anemia şi rashul pruriginos, care pot determina întreruperea terapiei.

În ceea ce priveşte utilizarea corticosteroizilor, există puţine date care să ateste eficacitatea la pacienţii cu HCV cronică. Unii autori au observat creşterea viremiei în timpul tratamentului, reversibilă la întreruperea terapiei. Thyele et al. a găsit un răspuns favorabil la corticosteroizi, mai ales în cazul asocierii cu azathioprina. De asemenea, tratarea pacienţilor cu VHC şi manifestări extrahepatice cu agenţi imunosupresivi este controversată. S-au observat rate crescute de morbiditate şi mortalitate la cei care au primit tratament imunosupresiv, probabil şi datorită severităţii bolii hepatice sau extrahepatice (5).

Liniile directoare de tratament sunt bine stabilite la adulţi. La aceşti pacienţi, studiile actuale au scopul de a se putea scurta durata tratamentului (la 16 săptămâni, în cazul unui genotip sensibil, şi la 24 de săptămâni, în cazul genotipului 1) pentru subiecţii cu răspuns virusologic rapid, şi de a prelungi terapia (48 săptămâni pentru genotipul 3 şi 72 săptămâni pentru genotipul 1) pentru subiecţii care înregistrează o scădere a viremiei, dar cu persistenţa ARN VHC pozitiv în săptămâna 12. Alte investigaţii în curs de desfăşurare au ca scop creşterea eficacităţii tratamentului prin adăugarea de agenţi cum ar fi telaprevir sau boceprevir, la tratamentul convenţional PEG-IFN plus ribavirină.

La copil, formele severe de boală sunt rare, însă este necesară urmărirea periodică. Terapia antivirală nu atinge ratele de creştere a eficienţei răspunsului în comparaţie cu pacienţii adulţi, însă efectele adverse sunt mai frecvente la copil şi, în unele cazuri, pot fi severe. Ţintele tratamentului HVC cronică la copil sunt reprezentate de normalizarea pe termen lung a transaminazelor, obţinerea unui răspuns virusologic susţinut, oprirea sau întârzierea degradării histologice hepatice, scăderea riscului de apariţie a cirozei şi a carcinomului hepatocelular, astfel că decizia de a trata ar trebui să ia în considerare mai multe aspecte, individualizate pentru fiecare pacient în parte (vârsta, gravitatea bolii, eficacitatea tratamentului ales, efectele sale negative, estimarea complianţei la tratament).

În prezent, utilizarea de PEG-IFN cu ribavirină, care a fost aprobat de FDA din SUA (în decembrie 2008) şi EMEA (în de-

cembrie 2009) pentru copiii mai mari de 3 ani reprezintă gold-standardul terapiei HCV cronice. Criteriile pentru eligibilitate sunt : vârsta 3-18 ani la momentul înregistrării, viremia VHC (orice metodă) detectabilă în 2 momente diferite, la distanţă de cel puţin 6 luni, boala hepatică cronică, indicată de inflamaţie şi/sau fibroză, boala hepatică compensată (Child-Pugh Grade A clinical classification), consimţământ informat semnat de părinte / susţinător legal, hemoglobina > 11 g/dL pentru fete ; > 12 g/dL pentru băieţi , TSH normal.

Rămâne de stabilit prin studii ulterioare profilul de siguranţă şi eficacitate al acestei terapii, precum şi influenţa asupra prezenţei şi a dezvoltării manifestărilor extrahepatice.

STUDIUL PERSONAL

Ideea acestui studiu a plecat de la necesitatea evaluării corecte a patologiei asociate manifestărilor extrahepatice în hepatitele cronice ale copilului, atât din necesităţi de certitudine a etiologiei, cât şi terapeutice. Unele dintre aceste afecţiuni apar independent de existenţa suferinţei hepatice de bază, însă pot influenţa starea clinică a pacientului, unele date biologice, precum şi calitatea vieţii, astfel încât se impune investigarea, tratarea şi urmărirea lor pe termen lung. Lipsesc studiile la copil legate de incidenţa, gravitatea şi semnificaţia acestor manifestări la copil.

În acest sens, am stabilit următoarele obiective :

- evaluarea incidenţei manifestărilor extrahepatice în hepatita cronică VHC la copil ;
- evidenţierea tipurilor şi subtipurilor de manifestări extrahepatice întâlnite în practică, în comparaţie cu datele furnizate de literatura de specialitate ;
- urmărirea influenţei pe care aceste manifestări extrahepatice o au în evoluţia bolii şi asupra calităţii vieţii acestor pacienţi ;
- asocierea manifestărilor extrahepatice cu alte stări patologice şi evaluarea evoluţiei naturale şi/sau sub impactul terapiei specifice (antivirale) şi nespecifice (hepatoprotectoare, hipocolesterolemiante, antibiotice etc.) asupra acestor afecţiuni şi asupra patologiei de bază.

MATERIAL ŞI METODĂ

Lotul luat în studiu a cuprins 43 de pacienţi diagnosticaţi şi urmăriţi în Clinica Pediatrie II Iaşi cu diagnosticul de hepatită cronică VHC în perioada ianuarie 2005 - iunie 2010. Distribuţia zonală a acestora a fost de 60,5% mediul urban şi 39,5% mediul rural, iar pe sexe 67,44% masculin şi 32,55% feminin. Majoritatea copiilor au provenit din mediul familial, doar unul dintre ei fiind instituţionalizat. Ca modalităţi de infectare, 3 cazuri (6,97%) au fost certificate ca fiind urmarea transmiterii verticale de la mamă, intervenţiile chirurgicale fiind incriminate în alte 3 cazuri, în restul cazurilor neputându-se preciza cauza infecţiei. Diagnosticul de hepatită cronică s-a realizat pe baza criteriilor clasice, iar anamneza a adus informaţii privind etiologia şi debutul infecţiei hepatitice. Examenul clinic efectuat la fiecare control în clinică şi examinările paraclinice şi histologice (puncţia hepatică, Fibroscan) efectuate în timpul urmăririi cazului, au ilustrat, deasemeni, prezenţa suferinţei hepatice cronice şi, la unii pacienţi, a manifestărilor extrahepatice şi/sau a patologiei asociate.

S-au efectuat analize paraclinice la momentul diagnosticului şi/sau intrării în studiu, pe parcursul evoluţiei, înainte şi după administrarea terapiei : hematologice (leucocite, hemoglobina, trombocite), teste hepatice (transaminazele, proteinele totale şi fracţiunile proteice, timpul de protrombină, bilirubina totală şi directă), autoanticorpii (antinucleari, antimitocondriali, anti muşchi neted), factorul reumatoid, crioglobulinele, celulele lupice. Pentru fiecare pacient valo-

rile obţinute au fost încadrate în funcţie de vârsta pacientului în valori crescute, valori normale sau valori scăzute. Analiza acestor rezultate s-a făcut în funcţie de valoarea medie şi de încadrarea în grupele respective. Markerii virali analizaţi au fost anticorpii anti-HCV şi viremia ARN-VHC. Ca investigaţii histologice, puncţia biopsie hepatică a fost efectuată la pacienţii diagnosticaţi şi trataţi înainte de 2008, ceilalţi au beneficiat de metodele Actitest şi Fibroscan.

În funcţie de tratamentul administrat, cei 43 de pacienţi luaţi în studiu au fost grupaţi în următoarele loturi : tratament cu interferon (12 pacienţi, 27,9%), tratament cu interferon şi Ribavirin (1 pacient, 2,32%), tratament cu Pegasys şi Ribavirin (2 pacienţi, 4,64%), tratament hepatoprotector (29 de pacienţi, 67,44%).

REZULTATE

La pacienţii infectaţi, viremia a fost peste 2 milioane de copii virale la 30% cazuri, între 1-2 milioane de copii virale în 60% cazuri, iar la 10% dintre copii a însumat între 600.000-1.000.000 de copii virale.

Diagnosticul de hepatită cronică VHC la pacienţii din lotul studiat s-a făcut, în majoritatea cazurilor, întâmplător sau cu ocazia unor determinări biologice cu valori anormale (transaminaze crescute, markeri virusologici pozitivi pentru hepatita virală tip C).

Frecvenţa manifestărilor extrahepatice a fost de 41,86%, acestea fiind raportate la 18 pacienţi, cele mai frecvente fiind de ordin articular (artralgii în 4 cazuri) şi dermatologic (urticarie cronică şi pitiriazis versicolor, 3 pacienţi). Manifestările hematologice (6 pacienţi cu anemie şi 3 cu trombocitopenie), precum şi depresia severă (2 cazuri) s-au raportat în cadrul tratamentului cu IFN alfa. Aceste estimări se încadrează în datele furnizate de literatura de specialitate, având în vedere că manifestările de ordin articular, cutanat şi hematologic sunt cele mai frecvente, în special la pacienţii cu hepatită cronică de tip viral.

Explorările hematologice, biochimice şi imunologice au arătat diferenţe statistice semificative în ceea ce priveşte nivelul mediu al hemoglobinei (12,2 mg/dL faţă de 14,2 mg/dL la cei fără manifestări extrahepatice, $p=0.000053$) şi al trombocitelor ($204.000/mm^3$ faţă de $232.000/mm^3$, $p=0.010021$). Diferenţele observate în nivelul transaminazelor la cei cu manifestări extrahepatice, cu valori de aproximativ 2 x valoarea normală sunt nesemnificative statistic.

Ca şi patologie asociată, remarcăm prezenţa steatozei cu obezitate şi hipercolesterolemie la 8 pacienţi (18,6%), a gastritei cu sau fără esofagită la 3 pacienţi (6,97%), a colemiei Gilbert la 4 cazuri (9,3%), a osteopeniei la 5 pacienţi (11,62%), a spasmofiliei la 11 pacienţi (25,58%) şi a urticariei la un pacient (2,32%). Pacienţii cu steatoză au răspuns mai puţin la tratamentul antiviral, fără a arăta îmbunătăţiri semnificative.

TRATAMENTUL CU INTERFERON, MANIFESTĂRILE EXTRAHEPATICE ŞI PATOLOGIA ASOCIATĂ

Manifestările articulare şi cutanate au evoluat independent de prezenţa tratamentului specific antiviral, nefiind observate ameliorări semnificative ale simptomatologiei, din datele obţinute de la pacienţi. Manifestările de ordin hematologic, în principal trombocitopenia (cu valori minime de 44000 tr/mm^3), au apărut după tratamentul cu Interferon la 3 (6,97%) din pacienţii trataţi, faţă de doar un caz cu valori plachetare la limita inferioară a normalului înainte de iniţierea tratamentului. Anemia s-a înregistrat la 6 dintre pacienţii trataţi (13,95%), fiind asociată sau nu cu leucopenia şi tombocitopenia, cu valori între 9,1 şi 11 mg/dL. De asemenea, s-a înregistrat un caz care a evoluat spre anemie aplastică, după finalizarea terapiei antivirale. Trebuie menţionată şi depresia, care a apărut la 2

(4,65%) din pacienţii incluşi în lotul de studiu.

S-a observat că tratamentul gastritei (cu sau fără prezenţa *H. pylori*) a ameliorat citoliza hepatică înregistrată concomitent la cei mai mulţi din pacienţii incluşi în studiu, această patologie fiind decelată cel mai frecvent. Numai la unul dintre cei 3 pacienţi diagnosticaţi s-a putut corela prezenţa gastritei şi a sindromului dispeptic cu tratamentul antiviral efectuat anterior cu Interferon alfa.

CONCLUZII

1. La copil manifestarile extrahepatice în hepatita C sunt mai rare decât raportările din literatură la adult, cu preponderenţa celor de ordin articular.
2. În unele cazuri, Interferonul declanşează sau poate agrava unele manifestări extrahepatice cu mecanism imun (în principal manifestările de tip cutanat), ceea ce face ca atenţia îndreptată acestor situaţii să fie crescută, sau chiar să impună reconsiderarea opţiunilor terapeutice. Aşadar, este necesară o evaluare cât mai corectă a pacienţilor înaintea administrării tratamentului cu Interferon pentru a evita apariţia sau agravarea unor manifestări preexistente. Este esenţial să se cerceteze toţi markerii de autoimunitate înaintea terapiei antivirale.
3. Putem aprecia faptul că manifestările hematologice sunt cele mai frecvente din manifestările extrahepatice apărute ca urmare a tratamentului antiviral cu Interferon, dar şi că întreruperea terapiei nu influenţează semnificativ evoluţia manifestărilor extrahepatice, reversibilitatea acestora fiind evidentă doar în cazul depresiei.
4. De remarcat este faptul că existenţa gastritei sau esofagitei nu s-a corelat cu durata bolii sau cu tipul de tratament antiviral urmat, fibroscopia digestivă superioară fiind efectuată la aceşti pacienţi datorită acuzelor subiective de tip anorexie, dureri epigastrice, pirozis.
5. În ceea ce priveşte tratamentul specific al hepatitei cronice, cu antivirale de tipul Interferonului, pot apărea situaţii în care curba ponderală se modifică, în sensul crescător sau descrescător. În literatura de specialitate nu s-a citat o legătură evidentă între excesul ponderal sau obezitate şi hepatita cronică la copil, însă uneori se pot stabili corelaţii ale creşterii curbei ponderale prin manifestări de tip bulimic (acestea putând apărea în urma tratamentului specific antiviral). În studiul nostru s-a remarcat o evoluţie favorabilă a obezităţii, cu scăderea curbei ponderale şi a indicelui de masă corporală la unii pacienţi care au primit tratament antiviral cu Interferon alfa, în unele cazuri scăderea în greutate corelându-se şi cu scăderea gradului de steatoză hepatică decelată ecografic.
6. Prezenţa steatozei hepatice la copiii cu hepatită cronică cu VHC poate fi explicată de dislipidemie în majoritatea cazurilor. În aproape o treime din cazuri însă, steatoza pare cauzată de prezenţa infecţiei cronice VHC, ca factor limitant în ceea ce priveşte tratamentul şi cu un posibil rol favorizant asupra apariţiei fibrozei hepatice. Deşi steatoza nu influenţează semnificativ răspunsul virusologic susţinut, prevenirea şi corecţia obezităţii la copil pot avea un rol important în ameliorarea prognosticului acestor pacienţi.
7. Tratamentul optim al manifestărilor extrahepatice şi al patologiei asociate hepatitelor cronice rămâne în discuţie, datorită datelor insuficiente care să ateste eficacitatea terapiilor utilizate. Se impune, astfel, necesitatea elaborării unor ghiduri terapeutice care ar trebui bazate în principal pe partea simptomatică, de vreme ce patogenia acestor manifestări rămâne incertă. Oricum, atât terapia

antivirală, cât şi cea imunosupresivă şi plasmafereza şi-au dovedit utilitatea, cu toate că administrarea lor în monoterapie sau în combinaţie comportă riscuri. Exacerbarea manifestărilor autoimune trebuie monitorizată atent, atât înainte, în timpul, cât şi la terminarea tratamentului. Se impun, aşadar, cuantificarea funcţiei renale şi a viremiei pe toată perioada administrării terapiei. De asemeni, tratamentul trebuie individualizat, în funcţie de eficacitate, eligibilitate, cost şi riscuri, putând fi recomandat şi susţinut în cazurile când beneficiul depăşeşte aceste piedici.

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CARDIOPULMONARY STRESS TEST PERFORMANCE IN PATIENTS WITH MODERATE-SEVERE OBSTRUCTIVE SLEEP APNEA

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CARDIOPULMONARY STRESS TEST PERFORMANCE IN PATIENTS WITH MODERATE-SEVERE OBSTRUCTIVE SLEEP APNEA (Abstract): **Introduction**: Cardiopulmonary stress test (CPET) is an important part of risk stratification and prognostic assessment of a cardiovascular patient. Since obstructive sleep apnea (OSA) is a frequent finding in patients with cardiovascular and metabolic comorbidities, the role of CPET in the evaluation of patients with sleep disordered breathing is a subject of great interest. **Aim:** To illustrate the role of CPET evaluation in OSA patients, before and after noninvasive ventilation. **Material and methods**: We performed a prospective study that included 27 patients with newly diagnosed moderate-severe OSA (apnea hypopnea index (AHI) ≥ 15) who underwent a cardiopulmonary stress test before and after 2 months of continuous positive airway pressure (CPAP therapy). We compared baseline CPET performance between OSA patients and 27 age and BMI matched controls. **Results**: OSA severity was not significantly correlated with any of the analyzed baseline CPET parameters. CPET performance did not significantly differ between controls and patients with moderate-severe, moderate or severe OSA. Eight weeks of CPAP therapy lead to a significant improvement in weight status and several CPET parameters, including effort capacity (maximal and percent predicted load), oxygen pulse and percent-predicted oxygen uptake. **Conclusions**: Age, but not AHI, influence baseline CPET results. Although we did not find significant differences between CPET performance in OSA patients and controls, 2 months of CPAP therapy was associated with a significant improvement in exercise tolerance and CPET parameters, suggesting that OSA negatively impacts exercise tolerance. **Keywords**: OBSTRUCTIVE SLEEP APNEA, CARDIOPULMONARY EFFORT TEST, CONTINUOUS POSITIVE AIRWAY THERAPY.

Repetitive total or partial collapse of upper respiratory tract occurring in obstructive sleep apnea (OSA) causes oxygen desaturation, micro-awakenings and daytime sleepiness (1). OSA induces sympathetic activation, oxidative stress, endothelial dysfunction and activation of the angiotensin-aldosterone system, explaining the frequent association of cardiovascular and metabolic comorbidities in OSA patients (1-3).

Cardiopulmonary exercise testing (CPET) is a useful instrument in cardiovascular rehabilitation, as it is able to discriminate between the three potential causes of

exercise limitation: pulmonary, cardiac or muscular dysfunction (3). CPET evaluation is indicated for risk stratification and prognostic assessment in patients with heart failure, ischemic heart disease, pulmonary hypertension (4), but recent reports have emphasized the potential benefit of cardiopulmonary exercise evaluation in OSA subjects. Impaired muscle energy metabolism caused by nocturnal hypoxemia (5), the frequent association of obesity, as well as impaired stroke volume (6) and diminished effort ventilation (7) contribute to exercise limitation in OSA patients. Previous reports regarding CPET performance in obstructive sleep apnea are inconsistent: some studies report a reduced exercise tolerance (7-11), while others state that exercise capacity is not affected by OSA (12-15). However, various studies have shown that even short-term continuous or bi-level positive airway pressure (CPAP or BiPAP), the current gold standard therapy option for moderate-severe OSA (16), is able to improve both symptoms and exercise performance in OSA patients (3, 17-19). The purpose of this study is to illustrate the role of CPET testing in OSA patients admitted in a cardiovascular rehabilitation setting, before and after CPAP therapy.

MATERIAL AND METHODS

We performed a prospective study including patients with newly diagnosed moderate-severe OSA (apnea hypopnea index (AHI) $\geq$ 15), diagnosed using cardiorespiratory polygraph. Recruited were 27 subjects who underwent peak or symptom limited cardiopulmonary stress test, performed before and after 2 months of CPAP therapy. Three patients did not tolerate CPAP therapy and another subject did not return for the second clinical evaluation. Average CPAP use was 4 hours. We compared the baseline CPET results of our OSA patients with that of 27 controls (matched for age, BMI and sex) and also with the results of CPET reevaluation, performed after 8 weeks of CPAP in our OSA group.

Statistical analysis was performed in *SPSS 20.0.* Chi square and student's t test were used for comparisons between groups. The existence of a relationship between variables was evaluated using Pearson correlation coefficient. Descriptive data is presented as mean ± standard deviation, as appropriate. A p value <0.05 was considered statistically significant.

RESULTS

Our sleep apnea group included 11 subjects with moderate and 16 patients with severe OSA (average apnea hypopnea index-AHI 23.57 and 56.03, respectively) (tab. I).

No serious complications were observed during CPET testing in our OSA and control groups. Four OSA patients and four controls stopped the test at submaximal ventilation due to dyspnea, muscle tiredness or elevated BP (>220/120 mmHg). CPET parameters did not significantly differ between our control and OSA groups (Table II) or between controls and patients with severe or moderate OSA, respectively ($p>0.05$).

Although AHI was not significantly correlated with any of the analyzed baseline CPET parameters, we found significant negative correlations between age and basal metabolic rate, maximal effort, peak oxygen uptake (VO_2), anaerobic threshold (AT), minute ventilation (VE) and peak heart rate (HR) (tab. III).

TABLE I

Descriptive statistics – OSA group

	OSA	Moderate OSA	Severe OSA
N	27	11	16
M:F ratio	20:7	7:4	13±3
Age	55.88±8.63	55.54±7.1	56.12±9.76
AHI	42.80±19.17	23.57±3.00	56.03±13.17
DI	40.40±20.26	23.27±4.51	52.18±18.31
Average nocturnal SaO_2	91.28±3.89	92.09±3.47	90.72±4.18
Minimum nocturnal SaO_2	72.37±12.37	79.27±7.97	67.62±12.80
CPAP pressure	11.48±2.68	9.84±2.50	12.47±2.34
BMI	34.95±5.59	34.96±6.77	34.95±4.85
WC	115.46±11.89	116.65±13.47	114.66±11.14

OSA – obstructive sleep apnea; N – number; M – male; F – female; AHI – apnea hypopnea index: DI – desaturation index: SaO_2 – oxygen saturation; CPAP – continuous positive airway pressure; BMI – body mass index; WC – waist circumference.

TABLE II

CPET parameters in our control vs study group

	Control group	OSA group	p
N	27	27	
M:F ratio	20:7	20:7	
Age	57.85±10.94	55.88±8.63	0.46
BMI	33.96±3.85	34.95±5.59	0.45
Peak VO_2	1341.18±282.32	1356.62±465.37	0.88
Percent-predicted peak VO_2	63.70±15.23	56.88±14.26	0.09
Weight indexed peak VO_2	13.57±2.51	13.08±4.03	0.58
AT	1167.13±320.64	1050.04±346.42	0.23
Weight indexed AT	11.86±2.65	10.37±3.02	0.08
Max Watt	94.18±25.03	95.40±34.30	0.88
Percent-predicted Watt	55.67±11	51.44±14	0.23
VE	44.80±8.58	43.20±14.67	0.63
Max HR	114.22±19.51	114.76±17.81	0.91
Percent-predicted HR	71.30±9.26	69.88±10.00	0.60
O_2 pulse	12.62±2.77	12.34±3.37	0.74
Max SBP	186.44±24.06	186.08±30.33	0.96
Max DBP	102.96±14.89	102.28±14.11	0.86
RER	1.03±0.09	1.03±0.05	0.91

CPET – cardiopulmonary effort test; N – number; M – male; F – female; BMI – body mass index; VO_2 – oxygen uptake; max – maximum; AT – anaerobic threshold; VE – minute ventilation; HR – heart rate; O_2 – oxygen; SBP – systolic blood pressure; DBP – diastolic blood pressure; RER – respiratory exchange ratio.

TABLE III

Significant correlations in our OSA group

	R	p
Age and Basal metabolic rate	-0.61	0.002
Max Watt	-0.48	0.01
Max VO_2	-0.38	0.04
AT	-0.55	0.005
VE	-0.42	0.04
Max HR	-0.42	0.02

R – Pearson correlation coefficient; SaO_2 – oxygen saturation; max – maximum; VO_2 – oxygen uptake; AT – anaerobic threshold; VE – minute ventilation; HR – heart rate

Eight weeks of CPAP therapy lead to significant improvement in weight status and several CPET parameters, including effort capacity (maximal and percent predicted load), oxygen pulse and percent-predicted VO_2 (tab. IV).

TABLE IV
CPAP effect on CPET performance*

	Baseline	After CPAP	p
Weight	106.86±19.15	105.43±18.77	**0.01**
BMI	35.25±5.68	34.72±5.69	**0.04**
Peak VO_2	1405.17±483.20	1577.21±489.43	0.11
Percent-predicted peak VO_2	56.14±13.35	67.38±18.24	**0.01**
AT	1069.94±396.71	1272.76±456.04	0.07
Weight indexed AT	9.95±3.32	11.97±4.39	0.06
Max Watt	98±35.97	115.09±39.77	**0.03**
Percent-predicted Watt	50.71±14.32	63.14±17.43	**0.0003**
VE	44.73±15.57	49.37±15.66	0.19
Resting HR	81.27±14.98	76.18±11.71	**0.02**
Max HR	117.22±19.89	114.31±19.14	0.39
Percent-predicted HR	71.13±11.17	69.36±10.11	0.39
O_2 pulse	12.38±3.75	16.10±8.16	**0.03**
Max SBP	188.66±33.11	188.42±21.13	0.96
Max DBP	103±15	102.38±9.99	0.81
RER	1.02±0.05	1.03±0.07	0.47

*analysis performed on the 23 subjects included in our second evaluation

CPAP – continuous positive airway pressure; CPET – cardiopulmonary effort test; BMI – body mass index; VO_2 – oxygen uptake; AT – anaerobic threshold; max – maximum; VE – minute ventilation; HR – heart rate; O_2 – oxygen; SBP – systolic blood pressure; DBP – diastolic blood pressure; RER – respiratory exchange ratio.

DISCUSSION

Previous studies on CPET performance in OSA patients have yielded contradictory results. Lin *et al*. (9) found that overweight patients with moderate-severe OSA present significantly impaired workload, VO_2, AT, maximum VE and oxygen pulse when compared to matched controls. Another report conducted on a larger number of subjects (42 patients and 50 controls) with morbid obesity found a significantly lower weight-indexed VO_2 max in OSA patients compared to controls, as well as a significant negative correlation between AHI and VO_2 (11). The authors also report higher DBP values and decreased HR rise and recovery during and immediately after exercise in OSA patients (11). A correlation between OSA severity and BP increase during exercise, but no significant difference regarding peak VO_2 and VE between patients with mild-moderate and severe OSA have been reported by Przybylowski *et al*. (7), who performed CPET in 111 obese OSA patients. A more recent study found a significant correlation between OSA severity and percent predicted peak VO_2 (8), and Stavrou *et al*. (10) not only show that moderate-severe OSA is associated with reduced peak VO_2 and exercise duration, but also propose a predictive model that differentiates controls from OSA patients with 78% accuracy.

Most studies demonstrating that OSA is

associated with impaired exercise capacity have analyzed obese or overweight individuals. Rizzi *et al*. (12) compared CPET results between norm ponderal OSA patients and controls and found no statistically significant difference concerning peak VO_2, AT and RER. These results can be influenced by a relatively low AHI in the studied OSA population (15.4±9.2) and by the fact that males are greatly underrepresented in the analysis (37%) (12). Another study (13) compared CPET performance in lean and obese patients, with and without OSA, respectively, and found obesity to be the most important factor leading to impaired exercise and ventilatory capacity (illustrated by VO_2 and VCO_2). Despite an altered HR and ventilatory response to exercise, Hargens *et al*. did not find a significant difference regarding VO_2 between young males with OSA and controls (14, 15).

Previous studies of CPAP therapy effect on CPET performance have been conducted on a limited number of subjects. The study of 31 subjects with severe OSA (average AHI 54.25) but with anthropometric characteristics comparable to those of our group (M:F ratio 27:4, average age 53.41, BMI 31.43) reported improved work peak (Δ=16.9W) and percent-predicted-oxygen uptake (Δ=7.6%), but also positive changes in HR reserve (Δ=-6.8%), maximal HR (Δ=6.7 bpm) and VE (Δ=10.3 l/min) after 8 weeks of CPAP therapy (3). Similarly, after 2 months of CPAP therapy, our OSA group presented a significant increase in maximum and percent-predicted-maximum load. Although in our analysis peak oxygen uptake and anaerobic threshold did not significantly improve after CPAP ($p>0.05$), oxygen pulse and percent-predicted-oxygen-consumption exhibited a statistically significant improvement.

Other authors (17) found that 2 month-CPAP therapy improves not only CPET parameters (VO_2, oxygen pulse, work peak, AT and VE) but also BP values and right ventricular ejection fraction, suggesting that the benefits are due to an improvement of overall fitness and cardiac function (17). Goel *et al*. (18) also reported that exercise duration, maximum load, VO_2, oxygen pulse, AT and VE show a statistically significant improvement after 4 weeks of CPAP. However, the analysis included only a small number of subjects (15 patients and 5 controls) and the authors did not compare baseline CPET results between OSA subjects and controls (18). Our patients also presented a small but statistically significant weight loss and a decrease in resting heart rate, but no significant changes in resting and peak blood pressure values.

An interesting study showed that noninvasive ventilation prevents impairment of exercise capacity (decrease in VO_2, workload and AT) in compliant versus non-compliant OSA patients (19). Surprisingly, the analysis showed a decrease in maximal VE among compliant patients (Δ=-6,4 l/min). The authors consider that the short duration of CPAP therapy (42 days) could explain the lack of significant improvement in the analyzed CPAP-compliant group (19).

In another report, 15 obese patients presented no significant improvement in peak VO_2, workload, HR, oxygen pulse or ventilation after 3 months of CPAP (20).

Although our study failed to illustrate a significant difference in baseline CPET results between OSA patients and controls, we found that 2-month CPAP therapy improves several exercise-related parameters. The lack of association between AHI and CPET results can be explained by the fre-

quent association of cardiovascular comorbidities (impaired glucose metabolism – 59.25%, grade 3 hypertension 66.66%, obesity-77.77%) and the high prevalence of former or current smokers (62.96%) in our OSA group. Other limitations of our study are the diagnosis of OSA using cardiorespiratory polygraphy instead of polysomnography and the lack of screening for OSA in our control group. Polygraphy is an accepted alternative to standard polysomnography in the diagnosis of OSA, its major disadvantage being a potential underestimation of OSA severity, which does not interfere with the basic methodology of this study (21, 22).

CONCLUSIONS

Our study showed that some baseline CPET results are influenced by age, but not by OSA severity. The lack of statistically significant correlation between AHI and CPET performance can be explained by the small number of patients included in our analysis as well by the presence of various cardiovascular comorbidities in our OSA group. However, the fact that CPAP therapy was able to effectively improve several CPET variables in our patients shows that sleep apnea negatively impacts exercise tolerance. Further studies are required in order to determine the usefulness of CPET in the monitoring of OSA patients.

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NEWS

PRENYLATED COMPOUNDS FROM LEGUMES INDUCE ANTIBACTERIAL AND MEMBRANE PERMEABILIZATION OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI*

Under (a)biotic stress, plants from Fabaceae family, produce prenylated phenolic compounds as a defense mechanism, which have shown very good antibacterial activity.Aim of the study: To study the antibacterial proprieties and the effects on the permeability of the bacterial membrane. Materials and methods: *L. angustifolius, A. hypogaea* and *G. max* under-went germination, CC extraction, RP-UHPLC-UV-MS analysis, antibacterial susceptibility and cell membrane permeability tests using fluorescent propidium iodine. Results: Pools contained 165-350 mg prenylated phenolics/g. Chain prenylated isoflavones (cIsf, *L. angustifolius*) were the most active against *L. monocytogenes* (10 μ/ml) compared to controls. None of the pools showed activity against *E. coli* alone. However, cIsf realized the most efficient permeabilization in combination with efflux pump inhibitor (EPI) PaβN, in a dose-dependent manner. Conclusion: Co-administration of prenylated isoflavones with EPI in-creased bacterial membrane permeabilization and antimicrobial effect has been shown. Further experiments with different phenolic classes are necessary to confirm the antibacterial activity (Carla Araya-Cloutier, Jean-Paul Vincken, Roan van Ederen et al. Rapid membrane permeabilization of Listeria monocytogenes and Escherichia coli induced by antibacterial prenylated phenolic compounds from legumes. *Food Chemistry* 2018; 240: 147-155).

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SINDROMUL DE APNEE OBSTRUCTIVĂ ÎN SOMN (SAOS) ŞI ACCIDENTELE RUTIERE

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OBSTRUCTIVE SLEEP APNEA (OSA) SYNDROME AND TRAFFIC ACCIDENTS (Abstract): For many years traffic accidents represent an important concern in the world and was proven that excesive sleepiness, decreased vigilance of falling asleep in traffic are involved in many car accidents. **Aim**: To asses the correlation between obstructive sleep apnea (OSA) syndrome severity, the lenght of nocturnal arterial oxygen desaturation period and the patients' answers to the Epworth Sleepiness Scale Questionnaire (ESS) and Berlin Questionnaire (BQ). **Material and method**: We used the records of patients admited in Iaşi Pneumology Clinical Hospital with obstructive sleep apnea syndrome between 2007-2008, including only those patients which drive the car daily and excluding those patiernts with comorbidities which could increase the nocturnal arterial oxygen desaturation. The study group (33 patients) have been assessed through polysomnography and some specific questions from ESS and BQ. We identified those patients involved in traffic accidents due to sleepiness. The correlation between obstructive sleep apnea syndrome severity (reflectd by Apnea-Hipopnea Index - AHI), the lenght of the nocturnal arterial oxygen desaturation period ($<90\%$) and the answers the answers to ESS and BQ were assessed. **Results**: Two patients presented mild severity sleep apnea syndrome, 15 presented moderate severity and 16 high severity sleep apnea syndrome. The correlation of AHI with ESS ($r=0,59$, $p<0.001$) and BQ ($r=0.47$, $p<0.05$)were significant while the correlation with nocturnal arterial oxygen desaturation period had no significance ($r=0.29$, $p<0.05$). **Conclusion**: Patients with moderate or severe OSA present a high degree of diurnal sleepiness which could affect the driving capacity. **Key words**: SLEEP APNEA SYNDROME, POLYSOMNOGRAPHY, TRAFFIC ACCIDENT

În ultimii ani problema accidentelor de trafic a constituit o preocupare frecventă la nivel internaţional, siguranţa circulaţiei atrăgând atenţia că cele mai multe astfel de accidente se datorează somnolenţei excesive, respectiv adormirii la volan sau reducerii vigilenţei.

În Franţa, Agenţia Naţională pentru Siguranţa Circulaţiei estima că un sfert din accidentele rutiere fatale care au avut loc în anul 2003 au fost direct corelate cu somnolenţa excesivă (SE). Responsabili de SE sunt numeroşi factori: perioadele lungi de nesomn, consumul de alcool, consumul de droguri, anumite medicamente, scăderea cronica a duratei somnului şi tulburările de somn. Cele mai multe tulburări de somn au drept consecinţă SE, iar sindromul de apnee obstructivă în somn (SAOS) este una dintre ele.

SAOS este o afecţiune frecvent subestimată şi subdiagnosticată, care afectează aproximativ 4% din populaţia generală de sex masculin şi aproximativ 2% dintre femei. Aceste procente cresc semnificativ odată cu vârsta, indicele de masă corporală sau conformaţia anatomică a căilor aeriene supe-

rioare. SAOS se caracterizează prin prezenţa în timpul nopţii a pauzelor de respiraţiei, cui o durată de cel puţin 10 secunde, însoţite de scăderea saturaţiei în oxigen cu cel puţin 4% în prezenţa efortului respirator toracic şi abdominal (1). Pe lângă desaturările nocturne, acre au importante consecinte hemodinamice, episoadele apneice sunt însoţite de microtreziri care fragmentează sistematic somnul pacientului, fiind unul din factorii responsabili de instalarea si menţinerea SE. Studii retrospective ce au la baza date subiective şi obiective, au arătat că frecvenţa accidentelor de circulaţie printre şoferii cu SAOS este mai crescută (2,3,4), însă, datorită numărului scăzut de subiecţi incluşi în aceste studii, acestea au fost ţinta numeroaselor critici.

Teran-Santos et al. au raportat într-un studiu (5) existenţa unei asociaţii puternice între SAOS şi riscul de accidente rutiere. Autorii au observat că şoferii care s-au prezentat la camera de gardă după implicarea într-un accident rutier aveau un risc mult mai crescut decât populaţia generală de a fi diagnosticaţi cu SAOS. Relaţia dintre SAOS şi accidentele rutiere rămâne semnificativă chiar după ajustarea potenţialilor cofactori : consumul de alcool, vârsta, indicele de masă corporală, experienţa ca şofer, utilizarea medicamentelor care au ca efect secundar somnolenţa sau antecedentele de accidente de circulaţie. Rezultatele acestor studii confirmă că rata accidentelor de trafic la persoanele cu SAOS este de 3-4 ori mai mare decat la cele care nu au această boală (6,7, 8). Tratamentul corect al acestei afecţiuni reduce riscul de a adormi, creşte rezulatele obţinute la testele de performanţă şi vigilenţă şi scade numărul accidentelor raportate de persoanele cu apnee de somn (8,9,10,11, 12,13).

În laboratorul de polisomnografie al Spitalului Clinic de Pneumologie din Iaşi, posibilitatea testării vigilenţei sau performanţei la pacienţii adresaţi cu suspiciune de SAOS este limitată. Astfel, am corelat datele obţinute de la pacienţii care au fost înregistraţi polisomnografic pentru a estima riscul de scădere a vigilenţei în timpul şofatului. Există date în literatură care corelează hipoxemia nocturnă intermitentă întâlnită la pacienţii cu SAOS cu scăderea vigilenţei. Pentru estimarea subiectivă a somnolenţei diurne excesive aplicăm fiecărui pacient scala de somnolenţa Epworth (Epworth Sleepiness Score--ESS). Akiko N. şi colaboratorii raportau în articolul "*Daytime sleepiness and automobile accidents in patients with obstructive sleep apnea syndrome"* publicat în 1998 existenţa unei corelaţii semnificative pozitive între rezultatele obţinute la evaluarea somnolentei diurne şi riscul de accidente rutiere ($r=0.56$, $P<0.01$). Pacienţii cu afecţiuni repiratorii nonapneice, care se însoţesc de hipoxie cronică prezintă, de asemenea, alterarea funcţiilor cognitive şi scăderea performanţei şi a vigilenţei (14,15).

Scopul acestei lucrări este evaluarea corelaţiilor dintre severitatea SAOS, timpul petrecut la o saturaţie inferioară celei de 90% şi răspunsurile pacienţilor la intrebările caracteristice din chestionarele Epworth şi Berlin (Berlin Questionaire-BQ).

MATERIAL ŞI METODĂ

Am reanalizat fişele pacienţilor care s-au adresat Laboratorului de Polisomnografie din cadrul Spitalului Clinic de Pneumologie din Iaşi pentru suspiciunea de sindrom de apnee în somn în perioada 2007-2008, şi care în urma investigaţiilor au fost diagnosticaţi cu SAOS. Am selectat doar pacienţii care aveau carnet de conducere şi conduceau efectiv maşina ca activitate zilnică. De asemenea, am evaluat numărul pacienţilor care au declarat implicarea într-un eveniment rutier datorat somnolenţei, în absenţa consumului de alcool sau a medicaţiei care să determine SE. Am exclus 1 pacient datorită diagnosticului de SAOS asociat cu narcolepsie şi 7 pacienţi care au

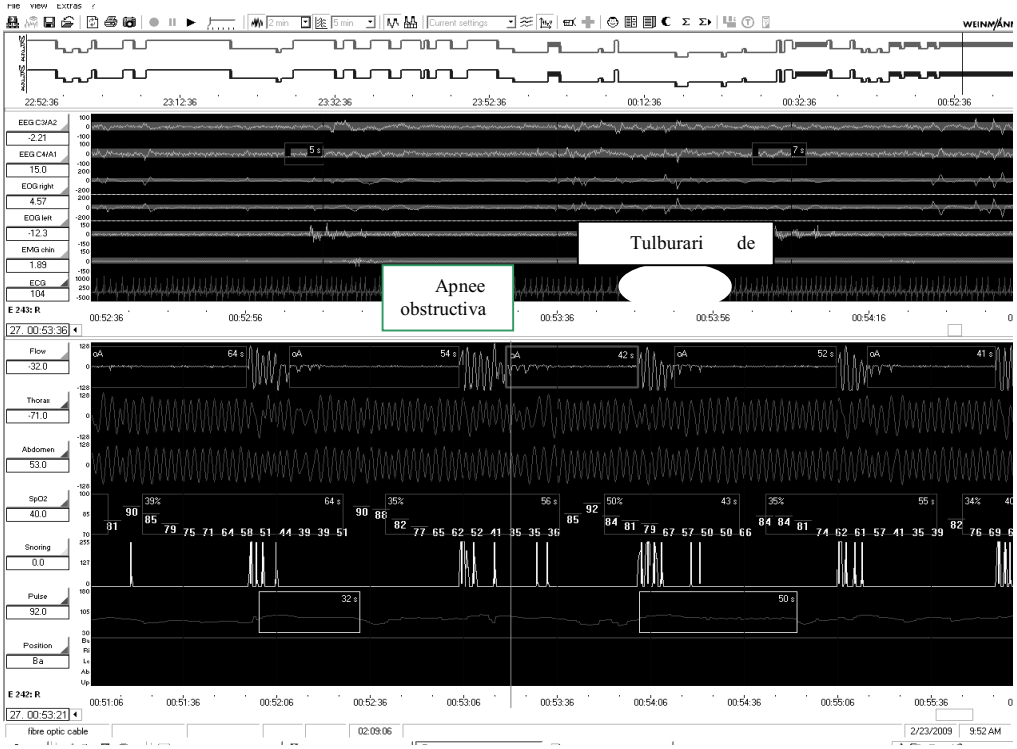

Fig. 1. Imagine dintr-o înregistrare polisomnografică a unui pacient cu SAOS sever

refuzat să completeze întrebările corespunzătoare din ESS si BQ. Au fost excluşi şi pacientii cu comorbidităţi care accentuau desaturările nocturne : 5 pacienţi cu BPOC si 3 pacienţi cu astm bronşic. În final am analizat rezultatele a 33 de pacienţi (6 femei) cărora li s-a indicat polisomnografie completă şi care au fost diagnosticaţi cu SAOS, în toate formele. Toţi pacienţii au completat la internare formularul de consimţământ informat privind utilizarea datelor personale. Pacienţii au fost înregistraţi cu polisomnograf Weinmann Somnolab 1.29, soft SomnoManager 1.3. Polisomnografia completă a constat din inregistrarea următorilor parametri :

a). Parametri neurofiziologici

1. EEG (electroencefalograma) : s-au utilizat două canale, electrozii fiind plasati conform standardelor actuale ale AASM (American Academy of Sleep Medicine, 2007) ;
2. EOG (electrooculograma)
3. Electromiograma muşchilor mentonieri
4. Electromiograma muşchilor pretibiali, pentru un singur membru inferior.

Pentru parametri descrişi la punctele 1-3 s-au utilizat electrozi Grass EEG, iar pentru fixarea acestora s-a utilizat crema EC Grass 2.

b) Parametri cardiorespiratori

1. Electrocardiograma
2. Flux respirator : s-au utilizat fie canula nazală, fie termistorul nazal, care au înregistrat de asemenea şi sforăitul.
3. Saturaţia în oxigen, pulsul periferic şi pletismografia înregistrate prin intermediul pulsoximetrului
4. Efortul respirator toracic şi abdominal : centura toracică şi, respectiv cea abdominală, prevazute cu sisteme piezoelectrice pentru detectarea efortului de întindere.
5. Detector al poziţiei corpului în timpul

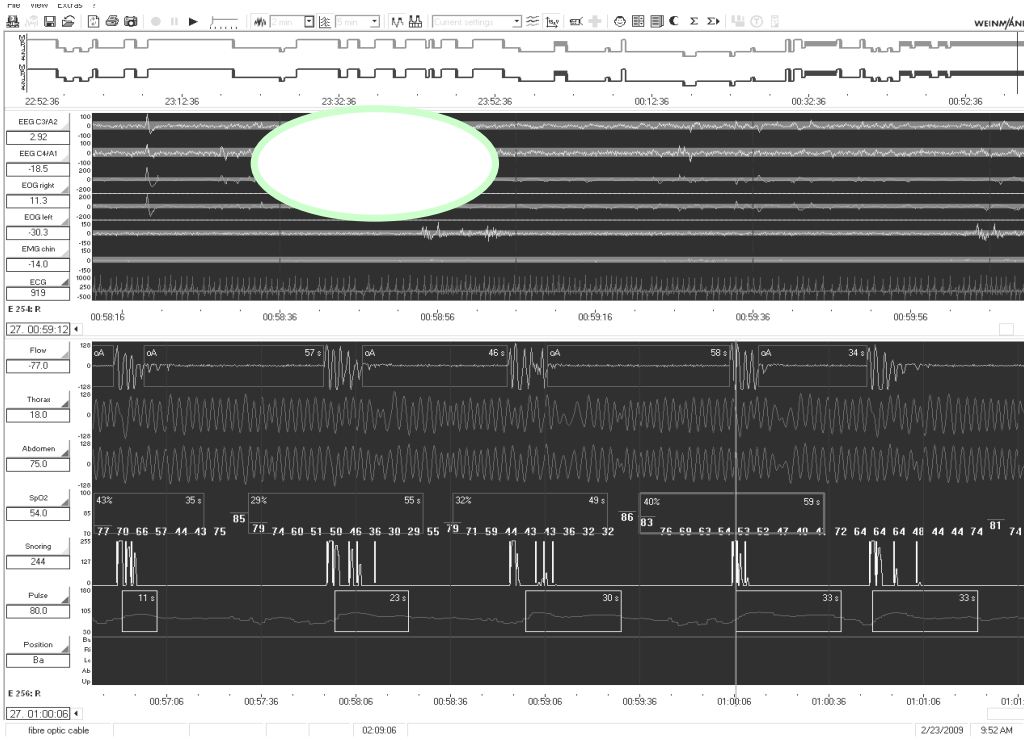

Fig. 2. Microtrezire, stadiul REM. Se observă mişcările în oglindă ale globilor oculari pe electrooculogramă. Severitatea evenimentelor respiratorii este agravată de somnul REM. În imaginea de mai sus, pacientul desaturează până la o valoare a SaO2 de 36%, iar durata apneilor obstructive este în creştere. Evenimentele respiratorii obstructive repetate sunt însoţite de variabilitate cardiacă şi tulburări de ritm

somnului

6. Microfon pentru înregistrarea zgomotelor din camera
7. Camera video, prevazută cu infraroşu, pentru înregistrarea imaginilor în timpul somnului pacientului.

Ora de pornire a înregistrării a fost cuprinsă între 20 : 50 şi 22 : 45, iar ora de întrerupere a înregistrarii a fost între 5 : 30 si 7 : 00.

Fiecare înregistrare a fost analizată pentru determinarea stadiilor somnului, microtrezirilor, mişcărilor periodice ale membrelor, tulburărilor de ventilaţie de tip obstructiv/central/mixt şi desaturărilor conform recomandărilor AASM. Validarea a fost efectuată manual. Astfel, pentru încadrarea nozologică a tulburarilor respiratorii în timpul somnului s-au utilizat următoarele caracteristici :

- Apnee obstructivă : sistarea fluxului respirator (semnal Flow) pentru cel puţin 10 secunde, însoţită de o desaturare de minim 4% şi de menţinerea efortului respirator.

- Hipopnee : reducerea amplitudinii fluxului respirator cu cel puţin 50%, cu o durată de cel puţin 10 secunde şi însoţită de desaturare cu cel puţin 4% faţă de valoarea anterioară. Pe înregistrările polisomnografice, desaturările preced evenimentele obstructive şi se desfăşoară concomitent cu apneile de tip central.

Fişa polisomnografică a pacientului cuprinde datele completate în prezenţa asistentei medicale sau a medicului, ESS şi BQ, datele privind acuzele, tratamentul medicamentos urmat, relaţia cu fumatul, consumul declarativ de alcool, comorbidităţi. S-a măsu-

TABELUL I
Principalele date ale pacienţilor

	Media	DS	Min	Max
Vârsta	46,66	11,86	23	66
AHI	36,15	19,6	13	98
ESS	10,33	2,02	6	14
CT90	47,81	36,07	4	162

rat perimetrul gâtului şi cel abdominal ; s-a calculat indicele de masă corporală (IMC). Datele celor 33 de pacienţi pe care le-am utilizat în aceasta analiză sunt prezentate în tabelul 1 : valorile medii, deviaţia standard, valoarea minimă şi maximă pentru fiecare parametru. Răspunsul la întrebarea „Aţipiţi la volan ? ” din BQ a fost un înregistrat astfel : răspuns pozitiv – 1, răspuns negativ- 0.

REZULTATE

Severitatea SAOS este cuantificată prin indicele apnee-hipopnee pe ora de somn (AHI). Un indice cuprins între 0 şi 5 indică absenţa SAOS, 5 > AHI > 15 indică SAOS uşor, AHI cuprins între 15 şi 30 indică forma moderată a bolii, iar un indice mai mare de 30 indică forma severa de boală. Toate datele pacienţilor au fost introduse în tabele (Microsoft Office Excell, 2003). Pentru analiza statistică s-a utilizat programul de statistică al Office Excell. Corelaţia s-a efectuat cu ajutorul funcţiilor correl, covariance şi coeficientul Pearson.

Din cei 33 de pacienţi 2 au avut SAOS uşor, 15 SAOS moderat şi 16 au avut forma severă de boală.

Astfel, analizand în paralel AHI şi ESS observăm existenţa unei corelaţii pozitive

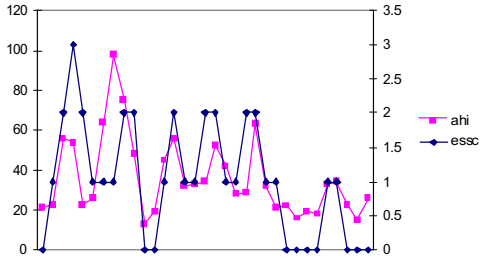

Fig. 4. Relaţia AHI –ESS spec.

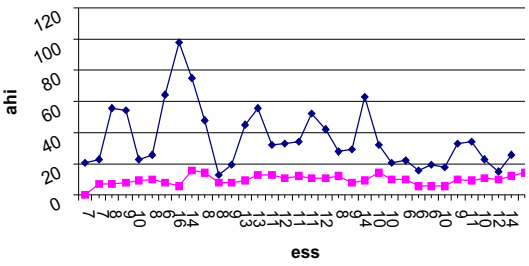

Fig. 3. Relaţia AHI-ESS

semnificative între cei doi parametri (r = 0.59, p < 0.001) (fig. 3).

Aplicând acelaşi procedeu am dorit să vedem dacă se menţine corelaţia şi în cazul răspunsului la întrebarea selectată din ESS (ESSspec). Astfel, indicele de corelaţie dintre AHI şi ESS spec este de 0.59, arătând acelaşi grad de corelaţie puternică pozitivă (fig. 4).

Observăm însă o slabă corelaţie între saturaţia în oxigen din timpul nopţii, în special între timpul petrecut sub valoarea de 90% a SaO2 şi SE (r = 0.29, p < 0.05), lipsită de valoare statistică.

Deşi de tip dihotomic, răspunsul la întrebarea selectată din BQ este strâns legată de severitatea bolii. Observăm pe grafic (fig. 5) că doar o mică parte din pacienţii cu forma moderată şi toţi cei cu forma uşoară de boală răspund „nu” la aceasta întrebare, iar cei cu forma severă de boală şi cei mai multi cu forma moderată răspund „da”.

DISCUŢII

Având în vedere că în lotul ales distribuţia în funcţie de severitatea bolii nu a

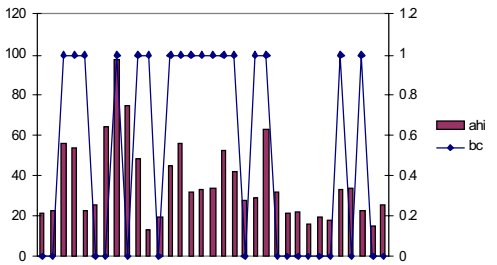

Fig. 5. Relaţia AHI- BQ ; r = 0.47, P < 0.05

fost echitabilă, datorită prezenţei în număr redus al pacienţilor cu forma uşoară a bolii, este necesară verificarea acestor date pe un lot mai mare numeric şi mai bine reprezentat. Numeroasele condiţii pentru ca un pacient să fie inclus în această analiză a limitat sever numărul pacienţilor admişi. Prezenţa copleşitoare a pacientilor cu forma severă de boală la diagnostic este, pe de o parte, meritul unei selecţii atente a pacienţilor, utilizarea diagnosticului în ambulator prin poligrafie cardiorespiratorie ambulatorie, iar pe de altă parte un marker al subdiagnosticării formelor uşoare şi moderate în serviciile de medicină primară.

Modalitatea cea mai simplă de a scădea riscul de accidente datorat SAOS este creşterea vigilenţei asupra acestei boli, ceea ce va duce la creşterea şanselor de identificare şi diagnostic. Acest aspect poate fi realizat prin educaţie, în special privind semnele şi simptomele acestei afectiuni, mai ales că ele sunt usor de recunoscut de anturaj. Deşi diagnosticul SAOS este o problemă rezolvabilă, existând mijloace şi metode bine structurate, nu există posibilitatea ca în urma analizarii polisomnografice, a datelor de laborator cu care se coroborează datele obţinute la polisomnografie, testelor de evidenţiere a SE, testarilor diurne (MSLT, MWT) de a prezice care şofer diagnosticat cu apnee de somn va avea un accident rutier. În acelaşi timp există mulţi pacienţi care fie au evitat în ultimul moment un accident, fie nu au avut unul, astfel că nu se poate pune problema limitării accesului lor la volan. In plus, efectuarea tratamentului aduce rezultate spectaculoase, atât în planul SE şi al vigilenţei cât şi în privinţa comorbidităţilor asociate.

Datele obţinute din analiza prezentată doar deschid drumul către necesitatea administrării altor teste tuturor soferilor care se adresează laboratorului de polisomnografie şi sunt diagnosticaţi cu SAOS. Astfel, se impune evaluarea vigilenţei, fie prin teste de tipul cititului, fie prin testul Osler, fie prin MSLT, însă acesta din urma este foarte consumator de timp şi de resurse. Teste de tipul simulării condusului nu sunt accesibile în laboratorul nostru. Aceste testări trebuie efectuate atat la diagnostic cât şi ca marker al eficienţei tratamentului recomandat.

Deşi hipoxemia nocturnă din afecţiunile respiratorii cronice (BPOC) a fost incriminată în determinarea unor tulburări cognitive şi deficit de atenţie, datele noastre, efectuate pe un lot redus de pacienţi, nu arată acelaşi lucru ca cele din bibliografie.

Începând din 1997 anumite state care au încercat să legifereze necesitatea efectuării polisomnografiei pentru şoferi (16), s-au lovit de reticenţa medicilor în a divulga date care le-ar vătăma relaţia de confidenţialitate pe care o au cu pacienţii.

În 1991, un grup de experţi din Federaţia Transportatorilor Americani recomandau ca şoferii cu suspiciune de SAOS sau cu SAOS netratat să fie consideraţi necalificaţi medical pentru a conduce vehicule comerciale până când fie diagnosticul elimină suspiciunea de SAOS, fie până când sunt trataţi adecvat. Acest grup recomandă ca şoferii trataţi pentru SAOS să-şi continue terapia pe toata durata menţinerii carnetului de şofer profesionist şi să efectueze anual o testare MSLT (16). Aceste reguli însă nu au fost încadrate într-o formă legală, iar recomandările nu au fost incluse în ghidurile medicilor care examinează şoferii profesionişti. În contrast însă, Federaţia Piloţilor are reguli clare în privinţa diagnosticării şi tratării SAOS, fiecare pilot fiind nevoit să efectueze anual o polisomnografie şi un test de menţinere a vigilenţei (MWT). Controale regulate sunt clar specificate pentru a se evalua eficacitatea terapeutică.

CONCLUZII

Pacienţii cu SAOS moderat sau sever prezintă un grad înalt de somnolenţă diurnă excesivă, somnolenţă care se repercutează

şi asupra statutului de şofer. Corelând date subiective privind somnolenţa la volan din chestionarele completate de pacient, cu date obiective, obţinute din înregistrările polisomnografice, putem afirma că SAOS este o afecţiune care alterează gradul de vigilenţă la volan, iar gradul de alterare este corelat puternic cu severitatea bolii. Acest efect nu se datorează exclusiv hipoxiei nocturne ; biciuirea sistematică simpatică a sistemului nervos central şi afectarea calităţii somnului contribuie la somnolenţa din timpul zilei. Rămâne ca pe viitor să verificăm aceste date pe un număr mare de pacienţi, iar datele subiective sa fie corelate cu date obiective, obţinute în urma unor teste standardizate.

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WHY 3D ULTRASOUND AND HYSTEROSCOPY SHOULD REPRESENT THE GOLD STANDARD FOR DIAGNOSIS AND MANAGEMENT OF UTERINE OSSEOUS METAPLASIA? CASE REPORT

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WHY 3D ULTRASOUND AND HYSTEROSCOPY SHOULD REPRESENT THE GOLD STANDARD FOR DIAGNOSIS AND MANAGEMENT OF UTERINE OSSEOUS METAPLASIA? CASE REPORT (Abstract): Osseous metaplasia of the endometrium represents a disorder of the uterus characterized by the presence of mature or immature bone in the uterine cavity. In most of the cases infertility or menorrhagia can be present, although sometimes this condition can be asymptomatic. Endovaginal ultrasound and particularly three-dimensional ultrasound are very important diagnostic tools. Hysteroscopic removal of the bony fragments helps in treating both infertility and menorrhagia. We present a case of 28 years-old women with osseous metaplasia and uterine synechia. Three-dimensional ultrasound was useful for the diagnosis and both affections were solved by hysteroscopic approach. **Keywords**: OSSEOUS METAPLASIA, 3D ULTRASOUND, HYSTEROSCOPY.

Osseous metaplasia of the endometrium, represents a rare disorder of the uterus, characterized by the presence of mature or immature bone in the uterine cavity. Although a rare condition, with the increasing use of ultrasound, the condition is being diagnosed more frequently. Osseous metaplasia can induce infertility or menorrhagia. Removal of the bony fragments helps in treating both, infertility and menorrhagia. We present, a clinical case of young women 28-years-old with osseous metaplasia associated with uterine synechia.

CASE REPORT

A G1 P0 28-year-old woman presented, with a history of menorrhagia for two months. She had been married for the last 4 years and had a missed abortion two months ago, after which she underwent dilatation and curettage at 14 weeks gestation. After the abortion, the patient had menorrhagia and the curettage was repeated. Following the second curettage, the patient still had bleeding and another gynecological examination, combined with three-dimensional (3D) acquisition was performed (Voluson E8, endovaginally probe 4-8 Hz, GE Healthcare). Longitudinal and transverse plane sonograms of the uterus revealed multiple hyperechogenic areas, of various sizes within the endometrium, with posterior

acoustic shadowing (fig. 1). The coronal plane of the uterus obtained with 3D ultrasound probe, was useful for the diagnosis, showing the irregular shape of the hyperechoic areas (fig. 2).

Based on these imaging findings and clinical history, a presumptive diagnosis of osseous metaplasia of endometrium was made and a hysteroscopy was performed. The diagnosis was confirmed, many bony fragments were observed and extracted under direct visualization, one of them resembling a long bone of the fetus (fig. 3, 4). Moreover, during hysteroscopy, a uterine synechia was identified and managed by hysteroscopic approach (fig. 5).

After the procedure the metrorrhagia resolved and the menstrual resumed as normal.

Fig. 1. Two-dimensional ultrasound- longitudinal plane of the uterus- hyperechogenic linear structure centrally positioned in the uterine cavity and irregular hyperechogenic structure at the isthmus; both images have posterior acoustic shadowing

Fig. 2. Three-dimensional ultrasound- coronal plane of the uterus- hyperechoic structure in the uterine cavity and at the level of the isthmus

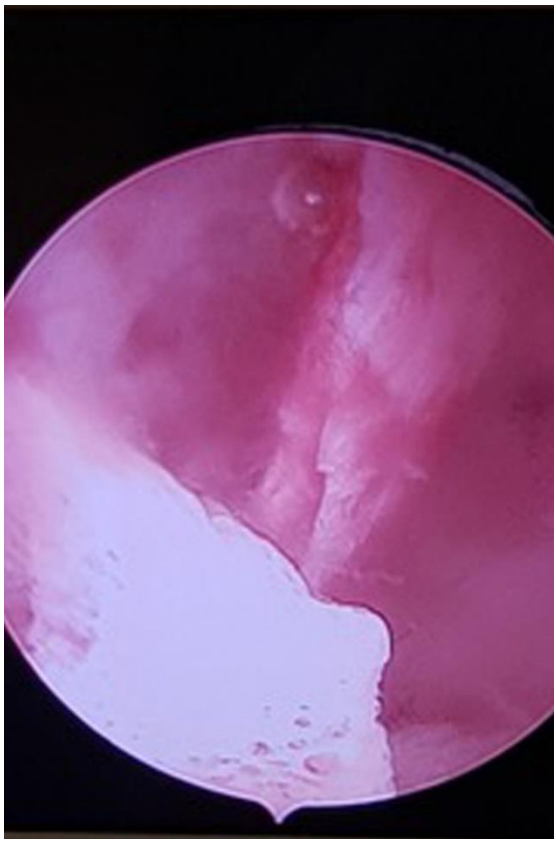

Fig. 3. Hysteroscopic image - bony fragments in the uterine cavity

Fig. 4. Hysteroscopic image- bony fragments in the uterine cavity

Fig. 5. Image of the extracted bony fragments from the uterine cavity.

DISCUSSION

Osseous metaplasia is a rare condition and in literature, between 1928 and 2013 there have been published, a total of 293 patients in 155 case reports and case series (1). In infertile women, the prevalence of osseous metaplasia is estimated to be approximately 0.002% (2). In the general population, the prevalence is unknown but is lower than in infertile women. The number of cases reported increased with the routine use of ultrasound exploration, in gynecology. The condition is also known as ectopic intrauterine bone, heterotopic intrauterine bone and endometrial ossification.

There have been several theories proposed for this condition. The origin of the bone fragments could be either retained fetal bone from prior termination of pregnancy, or osseous endometrial metaplasia. The theory of ossification of fetal residual tissue is sustained by the observation that in almost all cases, patients with endometrial ossification have a past medical history of abortion, ranging from 8 weeks to 37 years prior to the diagnosis of osseous metaplasia (3, 4). In the case of stromal metaplasia, hormonal, chronic endometritis or exogenous stimuli (like intrauterine device) are considered the main triggering factors. In these cases, the process of metaplasia could represent an adaptive change of one cell for another, due to the local adverse environment (5). This theory may explain the few published cases of osseous metaplasia in women with no obstetrical history (6).

Usually, the main complication associated with osseous metaplasia, is secondary infertility. The infertility occurs, because of the osseous fragments, which act like a foreign body and mimics the action of an intrauterine device (IUD). In other cases, osseous metaplasia can cause pelvic pain, dysmenorrhea, and menstrual disorders (7). Osseous metaplasia can sometimes be an incidental finding and may act as an involuntary contraception (8).

The ultrasound examination shows a characteristic pattern in the uterine cavity with linear or irregular hyperechoic images with posterior acoustic shadowing (9, 10,

11). Sometimes these patterns may by misdiagnosed with an IUD. In these cases, 3D ultrasound can be used, because it is a powerful tool that enables us to examine coronal plane of the uterus (12). In osseous metaplasia, in the coronal plane we can clearly view the number, the contour and the position of the osseous material in the cavity.

The differential diagnosis includes the presence of IUCD, foreign bodies, Asherman's syndrome, calcified submucosal fibrosis, and Mullerian tumor (4).

The management should be complete removal of the bony fragments from the endometrial cavity, by hysteroscopy. An alternative is uterine curettage. However, vigorous curettage should be avoided, because sometimes this method cannot ensure the extraction of all the fragments and moreover has the risk of synechiae formation as described in our case.

CONCLUSIONS

Osseous metaplasia is a rare uterine condition that can induce infertility or menorrhagia. Sonographic examination, and in particular 3D ultrasound plays a primary role in the diagnosis. The characteristic ultrasound pattern, a hyperechoic image in the uterine cavity, is strongly suggestive of osseous tissue within the uterus. The ultrasound examination should be confirmed by hysteroscopy, the gold standard for the management of this entity.

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MYTH OR REALITY IN *"STARRY NIGHT"* (1889). DID VINCENT VAN GOGH PAINT HIS *MORBUS LOCI* ? WHIRLPOOL GALAXY, HIPPOCAMPAL FORMATION OR JUST A PAINTBRUSH MOVE ?

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MYTH OR REALITY IN "*STARRY NIGHT"* (1889). DID VINCENT VAN GOGH PAINT HIS *MORBUS LOCI* ? WHIRLPOOL GALAXY, HIPPOCAMPAL FORMATION OR JUST A PAINTBRUSH MOVE ? (Abstract): Vincent Van Gogh's entire career has been marked by a variety of colors, which have undergone numerous changes: from gray to vivid colors. This is due to the multiple affections that the author acquires over the course of his life and the treatments adjacent to his affections. A special feature of his artistic life is *tripartite nocturnal series* during the time the artist was admitted to the Saint Paul Asylum. Nowadays this series of paintings are known around the world and listed at exorbitant prices. Much of the paintings made by the artist in the second half of his life have a common element about a representative geometric model, a brush motion. Long after the artist's death, art critics, doctors and ordinary viewers have associated this geometric model with the anatomy of the hippocampus. The previous idea was recently exposed by a group of authors in the article: Neuroanatomic Interpretation of the Painting *Starry Night* by Vincent van Gogh. Our opinion after lengthy analyzes of the artist's biography is that he did not have neuroanatomic knowledge and therefore the theory that Vincent Van Gogh painted his *morbus loci* cannot be sustained. A more likely explanation is that this brush movement (amplified by the pathology suffered by the painter) perfectly corresponds to astronomical elements discovered at that time, *Whirlpool Galaxy* described and drawn first by Sir William Parsons, 3rd Earl of Rosse (1800-1867) in 1845. **Conclusions:** The details given by Van Gogh in the sky of Saint Remy are his visions as a painter and are unrelated to the anatomical structure of the hippocampus. **Keywords:** VAN GOGH, TRIPARTITE NOCTURNE SERIES, STARRY NIGHT, HIPPOCAMPUS, TEMPORAL LOBE, EPILEPSY, WHIRPOOL GALAXY.

Vincent Van Gogh was a Dutch painter of post-Impressionist style. His works have deeply influenced the art of the nineteenth century. A mark of his works is the vivid colors, the wrinkling character of the traces left by the brush and the emotional effect that catches the viewer.

The artist was born March 30, 1853 in Groot-Zundert, in the south of Holland.

A key moment in his artistic career was

the encounter with the French Impressionists in 1886 when he moved to Paris and then to the town of Arles. During this period, he met his friend and collaborator painter, Paul Gauguin (1848-1903), who was to be involved in the enigmatic incident in which Vincent Van Gogh cut off his ear because of mental illness. After this incident, Vincent Van Gogh was admitted to Saint-Remy-de-Provence Hospital for one year, until 1889.

Van Gogh has made over 2,000 works of art, around 900 paintings and 1,100 drawings and sketches. He was little appreciated during his life, his fame grew in the years after his death. Today the artist is considered to be one of the most important painters in history.

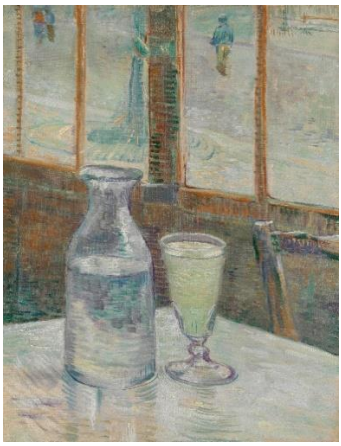

Fig 1. *Café Table with Absinthe*. Vincent van Gogh, Paris, February-March 1887. Oil on canvas, 46.3 cm x 33.2 cm. Image © Van Gogh Museum, Amsterdam (Vincent van Gogh Foundation)

Career and artistic masterpiece

His career can be divided into 4 stages: Paris (1886-1888), Arles (1888-1889), Hospital in Arles (December 1888) and the most interesting: Saint Paul Asylum (1888-1889).

In the first period of the artist's life he was focused on portraits and surrounding landscapes, the canvas created by him having a darker tone than the rest of his paintings.

Paris (1886-1888)

In 1886, he went to Paris to join his brother Théo, the manager of Goupil's gallery. In Paris, van Gogh studied with Cormon, inevitably met Pissarro, Monet, and Gauguin. Having met the new Impressionist painters, he tried to imitate their techniques; he began to lighten his very dark palette and to paint in the short brush strokes of the Impressionists' style. Unable to successfully copy the style, he developed his own more bold and unconventional style (fig. 1).

Arles (1888-1889)

At the Yellow House in Arles, van Gogh hoped like-minded artists could create together. Gauguin did join him but with disastrous results. Van Gogh's nervous temperament made him a difficult companion and night-long discussions combined with painting all day undermined his health (fig. 2).

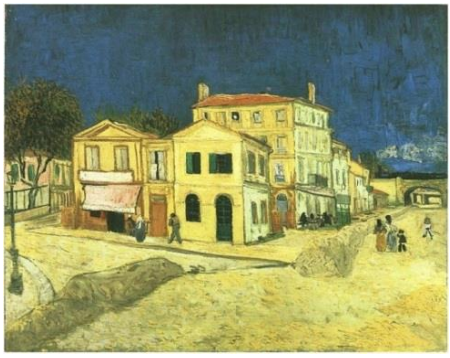

Fig. 2. *The Yellow House*. Vincent van Gogh. Oil on canvas, Arles: September 1888. Van Gogh Museum Amsterdam, The Netherlands, Europe

After the key point of his move to Paris, Van Gogh had a prolific artistic boom: he completed 200 paintings and more than 100 drawings and watercolors. He was enchanted by the local landscape and light; his works from this period are rich in yellow, ultramarine and mauve. His paintings include harvests, wheat fields and general rural landmarks from the area, including *The Old Mill* (1888), a picturesque structure bordering the wheat fields.

Hospital in Arles (December 1888)

The exact sequence of events which led to Van Gogh's mutilation of his ear is not known.

After the altercation with Paul Gauguin, Van Gogh returned to his room, where he was assaulted by voices and severed his left ear with a razor, causing severe bleeding.

Van Gogh was found unconscious the next morning by a policeman and taken to hospital. Van Gogh had no recollection of the event, suggesting that he may have suffered an acute mental breakdown.

Saint Paul Asylum (1888-1889)

In the Saint Paul Asylum, the artist made a *tripartite nocturne series* of paintings consisting of *Café Terrace at Night* (September 1888), then *Starry Night Over the Rhône* later that same month and last *The Starry Night.*

The artist considered *The Starry Night* which one day would rank among his most famous works, to be a failure, according to what he wrote to his brother. *Starry Night* depicts a dreamy interpretation of the artist's asylum room's sweeping view of Saint-Rémy-de-Provence. It offers a rare nighttime glimpse into what the artist saw while in isolation (1).

Disease and affliction

Vincent Van Gogh suffered from several diseases, especially psychiatric conditions that influenced both his life and his works. He suffered from syphilis (1882) and during periods of acute mental pressure he had epileptic seizures (the artist was suffering from a disease unknown at that time, temporal lobe epilepsy) (2).

While in Paris, he began to suffer from minor paroxysms consisting of episodes of sudden terror, peculiar epigastric sensations, and lapses of consciousness. Observers reported occasions of an initial tonic spasm of the hand and a peculiar stare, followed by a confusional-amnestic phase. His use of absinthe, an alcoholic beverage with convulsant properties favored by French artists, appears to have played a crucial role in the precipitation of van Gogh's illness (3).

Shortly after leaving Paris, the painter wrote to his brother:

"I was surely about to suffer a stroke when I left Paris. It affected me quite a bit when I had stopped drinking and smoking so much, and as I began to think instead of knocking the thoughts from my head. Good heavens, what despair and how much fatigue I felt at that time"

He explains to his brother how he can resist periods of acute emotional stress, throwing himself in work altogether and consuming large amounts of alcohol: *"If the storm within gets too loud, I take a glass more to stun myself"* (4).

Because Van Gogh used lead-based paints there are some who believe he suffered from lead poisoning from nibbling at paint chips. It was also noted by Dr. Peyron that during his attacks Van Gogh tried to poison himself by swallowing paint or drinking kerosene. One of the symptoms of lead poisoning is swelling of the retinas which can cause one to see light in circles like halos around objects. This can be seen in paintings like *The Starry Night.*

Hypergraphia is a condition causing one to need to write continuously; this disorder is commonly linked to mania and epilepsy. Some believe that the massive collection of over 800 letters Van Gogh wrote during his lifetime could be attributed to this condition (5, 6)

The tumultuous life of the genius ended abruptly and too early. Vincent Van Gogh shot himself in a wheat field in Auvers, France but did not die until 2 days later at the age of 37 years old.

An analysis of van Gogh's illness and emotionality must not obscure the fact that the great artist also had great strengths. Apart from distinct episodes of madness when he used absinthe and had seizures, he maintained a remarkable degree of lucidity during his stormy life, as is well documented in his letters (7).

Dilemma

In his painting *Starry Night* (1889), Vincent van Gogh realizes a bizarre, central and specific structure. In the literature it is assumed that this structure is very similar to the neuroanatomic structure of the hippocampus (1, 8). But in his room, there is no sketch of the human hippocampus to inspire a possible connection with *Starry Night* painting.

In this paper, the authors try to show that this structure is just a brush motion, as there are no defining elements to prove that Vincent van Gogh had knowledge of neuroanatomy (figs. 3-5). The greater likelihood is that this element of the artist's painting resemblance more to an abstract graphical representation of a *Whirlpool Galaxy*, described and drawn first by Sir William Parsons, 3rd Earl of Rosse (1800-1867) in 1845 (fig. 6). This idea was, for the first time, brought to light by writer Michael Benson, in his 2014 book *Cosmigraphics* (9).

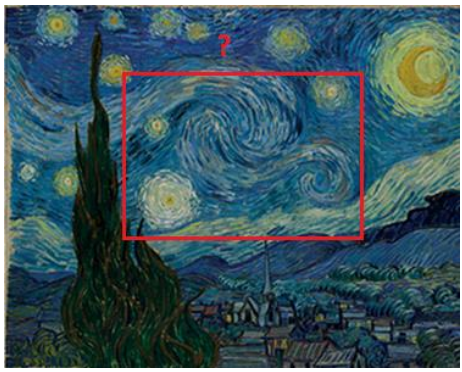

Fig. 3. *The Starry Night* Vincent van Gogh. Saint Rémy, June 1889. Oil on canvas. The Museum of Modern Art. Digital Image © The Museum of Modern Art.

Fig. 4. Structure from *The Starry Night*. Saint Rémy, June 1889. Oil on canvas. The Museum of Modern Art. Digital Image © The Museum of Modern Art

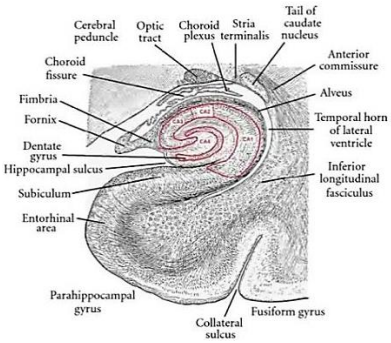

Fig. 5. J.A. Kiernan modified from Edinger, 1899 (L. Edinger, The Anatomy of the Central Nervous System of Man and of Vertebrates in General, F.A. Davis, Philadelphia, Pa, USA, 5th edition, 1899, Edited by W. S. Hall assisted by P. L. Holland and E. P. Carlton) Epilepsy Research and Treatment Vol 2012, Article ID 176157

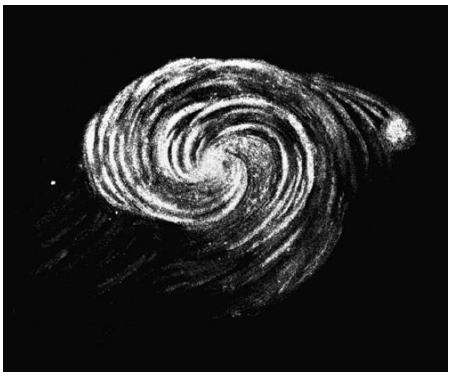

Fig. 6. Drawing of the *Whirlpool Galaxy* by William Parsons, 3rd Earl of Rosse in 1845, Cosmigraphics (2014) by Michael Benson

Starry Night is one of the most recognized pieces of art in the world. Honestly, it sometimes feels as if the painting's fame has exceeded that of its creator. It is a magnificent piece of art. That *Starry Night* resonates with so many people is a testament to how its beauty is timeless and universal.

CONCLUSIONS

The authors consider that the visual approach between the anatomical/radiological structure of the hippocampus and the formation painted by artist Vincent Van Gogh is just a coincidence. The artist did not have neuroanatomic knowledge and therefore could not paint his *morbus loci*, the structure is a simple brush motion.

An interesting approach to the interpretation of this structure is that it could represent a galaxy as it was designed a few years before *Starry Night* by Sir William Parsons.

Artistic works, either paintings, sculptures or literature, depend on a subjective view of the viewer/reader, the lover of "beauty", so it is difficult to formulate a clear opinion on the various elements of a creation.

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ETIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS CAUSING URINARY TRACT INFECTION AT "SF PARASCHEVA" INFECTIOUS DISEASES HOSPITAL

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ETIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS CAUSING URINARY TRACT INFECTION AT "SF. PARASCHEVA" INFECTIOUS DISEASES HOSPITAL (Abstract) **Aim:** Urinary tract infection (UTI) is one of the most common diagnosis in primary health care, which are treated with antibiotics. The diagnosis of UTI is suggested by clinical findings (dysuria, pollakiuria) and confirmed by microbiological examination of urine. The presence of pathogenic bacteria requires the immediate initiation of therapy in accordance with the results of the antibiogram. Management can be complicated by the increasing prevalence of antibiotic resistant strains. **Material and methods:** Microbial resistance and the etiology of bacterial pathogens was assessed among the UTI patients admitted to the "Sf. Parascheva" Infectious Diseases Hospital in Iasi, Romania. **Results:** The most frequent cause was gram-negative bacteria (GNB). Strains of *Enterococcus spp* and *Candida spp* have also been isolated. A percentage of 7.7% of the strains were beta-lactamase releasing. Regarding the antimicrobial susceptibility pattern of bacterial strains, we found increased GNB resistance to beta-lactam antibiotics. **Conclusions:** The results showed that *Escherichia coli* strains were the most frequently detected, followed by *Pseudomonas, Enterococcus, Enterobacter* species in a lower percentage. Treatment of UTI should be performed according to the results of the antibiogram, and for a sufficient period of time in order to prevent pandrug-resistant bacteria. **Keywords:** URINARY TRACT INFECTION, RESISTANCE, BACTERIAL PATHOGENS, BETA-LACTAMS.

Urinary tract infection (UTI) is an infection that affects part of the urinary tract and one of the most common bacterial infections acquired in the community and hospital settings. It is characterized by bacterial invasion and multiplication of infectious agents in the urinary tract, expressed as bacteriuria (1). Bacterial colonization in urine may be symptomatic or asymptomatic, bacteria invading the urinary tract by ascending or hematogenous (rare) routes (2).

In straightforward cases, a diagnosis may be made, and treatment given based on symptoms alone without further laboratory confirmation. In complicated or questionable cases, it may be useful to confirm the diagnosis via urinalysis or urine microscopy (3).

Urinalysis (preferably obtained from the same sample as the urine culture) may be suggestive of infection in the presence of leukocyturia (>20 PMN/mL of urine) and pathogenic organisms in urinary sediment (4). However, urinalysis can neither confirm nor exclude with certainty a diagnosis. Confirmation of UTI is obtained only by urine culture (5).

Additionally, in patients with recurrent infections other laboratory investigations, such as kidney and bladder X ray or urinary tract ultrasound are also indicated. Abdominal ultrasound can reveal anatomical anomalies of the urinary tract, urinary tract obstruction (stones, tumors) and can exclude hydronephrosis or presence of perinephric abscesses. Abdominal X ray may reveal the presence of radiopaque stones (6).

The bacteria that cause UTIs typically enter the bladder via the urethra. It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy (7).

The reports of the European Centre for Disease Prevention and Control (ECDC) on the etiological spectrum of UTIs highlight a significantly increased percentage of *E. coli* strains, both in complicated and uncomplicated infections (6). Other incriminated strains were Klebsiella sp., Proteus, Enterococcus, Pseudomonas, Serratia and others, in much smaller percentages (5, 8).

The pathogens traditionally associated with UTI are changing many of their features, particularly because of antimicrobial resistance (9). The etiology of UTI is also affected by underlying host factors that complicate UTI, such as age, diabetes, spinal cord injury, or catheterization. Consequently, complicated UTI has a more diverse etiology than uncomplicated UTI (10), and organisms that rarely cause disease in healthy patients can cause significant disease in hosts with underlying anatomic, metabolic, or immunologic disease (11).

The treatment of UTI is established after obtaining the results of laboratory investigations, being individualized in accordance with the antibiogram result (12). There are cases in which the specific treatment should be completed with a supportive treatment, respectively with drugs that increase the antibiotic efficacy and reduce the risk of UTI recurrence (e.g. phytopharmaceuticals with cranberry extract containing proanthocyanins, a substance that inhibits adhesion of bacteria to the urinary tract epithelium and blocks their multiplication (13, 14).

MATERIAL AND METHODS

For the purpose of this study, from the hospital electronic database, we extracted all urine samples collected from patients with UTI between January 1st, 2018 and September 30th, 2018 from 1,015 patients hospitalized at the "St. Parascheva" Infectious Diseases Hospital, Iasi, Romania.

The inclusion criteria in the study included: children and adults (no age limit - range 7 month-93 years) and bacterial confirmation ($\geq 10^3$ UFC / mL). Therefore, were excluded from the study, people who presented sign and symptoms of ITU, but the bacteriological examination was negative.

We collected information on demographic data (age, gender, area of residence (urban or rural), disease symptoms, the presence of underlying disease or recurrences, causes of UTI, laboratory findings (urinalysis, urine culture) and antibiogram.

The informed consent of the patient was not necessary, given that the study was a retrospective one.

RESULTS AND DISCUSSION

During the 9 months interval, urine tests were performed in 1,015 patients who presented signs and symptoms of UTI (fever, dysuria, pollakiuria) (15), either at presentation, or during their hospital admission for other medical problem.

The clinical diagnosis of UTI is essentially based on medical history (includes an evaluation of urinary tract symptoms, history of UTI or other urinary tract problems, family history and sexual history). Urine tests (urinalysis - visual exam, microscopic exam, dipstick test) are the second important element in diagnostic testing. The gold standard for a urine test is to perform a bacteriological urine culture, with identification of the pathogen, with quantification and sensitivity testing (16). In our study, of the 1,015 tested patients 467 (46%) had positive urine cultures.

Of the 467 UTI patients included in the present study 332 (71%) were women, far more numerous than the males. Regarding the age group incidence, most patients were aged 51-to 70 years old (mean age 62 years old). This agrees with literature data that show, according to international statistics reported by ECDC, that during the reproductive years the presence of bacteria in the urine is 50 times more common in women, and that 50% of women will experience an episode of urinary infection during their lifetime (17).

Also, the same study indicates that approximately 75% of UTIs acquired during hospital stay are associated with a urinary catheter (18). In our study, these cases accounted for 13.5% (63) of the cases. Another cause of UTI found in our study was represented by nosocomial infections, which accounted for 167 (35.7%) cases.

In our study group, laboratory investigations revealed plurimicrobial flora (42 urine cultures revealed simultaneous infection with 2 different bacteria, 9 cultures with 3 bacteria). As to the causative pathogen, gram negative species were identified in 337 (72.1%) patients, *Enterococcus spp* in 105 (22.4%) patients. and *Candida spp* in 25 (5.5%) patients.

Statistically significant data were obtained for gram negative bacteria. The results showed that 195 (57.8%) of the isolates were *E. coli*, followed in descending order by *Klebsiella*, *Proteus* and *Enterobacter*. Compared with previous literature, these organisms had a lower frequency. Numerous studies show that *Escherichia coli* is the most common microorganism, followed by *Klebsiella* and *Proteus spp*, in order to cause urinary tract infections. And other studies show that the presence of Gram-positive bacteria, such as *Enterococcus* and *Staphylococcus*, has increased.

A number of 28 species of Enterobacteriaceae were beta-lactamase releasing (15 of *E. coli* and 13 of *K. pneumoniae*). Extended spectrum beta-lactamases (or ESBLs for short) are a type of enzyme or chemical produced by some bacteria. ESBL enzymes cause some antibiotics not to work for treating bacterial infections. Common antibiotics, such as cephalosporin and penicillin, are often used to treat bacterial infections. With ESBL infections, these antibiotics can become useless. The most common types of bacteria that produce ESBLs include *Escherichia coli* and *Klebsiella.* When these ESBLs bacteria cause infections that can no longer be treated by normal antibiotics the doctor will find another treatment to stop the new infection that's become resistant to antibiotics.

With regard to antibiotic resistance, we had chosen to study especially the resistance of the *E. coli* and *Klebsiella spp*

strains, which were found in a high percentage in the studied group.

Currently, studies have found high rates of resistance to classes of antimicrobial agents recommended for the treatment of UTIs by the United States and European guidelines.

This is the case of our study, where the analysis of antibiotic resistance of uropathogenic *E. coli* strains isolated from patients admitted to our infectious diseases hospital revealed that the highest resistance was found to Ampicillin (72.9%), followed by Fluoroquinolones (42.3%) and Cephalosporins (34.2%). Uropathogenic *E. coli* showed low resistance to Nitrofurantoin, Amikacin, and Gentamycin. For the *Klebsiella spp* strains isolated in this study, the highest resistance was reported to Fluoroquinolones (52.6%), followed by Ampicillin and Nitrofurantoin. In both cases no Colistin-resistance were recorded.

CONCLUSIONS

In most of our cases the etiology of UTIs was microbial, sometimes even plurimicrobial, in some cases co-infection with two or more bacteria being present. *Escherichia coli* remains the most commonly isolated uropathogenic, followed by *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Proteus* and *Enterococcus* species. Urinary infections caused by Candida species were most likely influenced by the frequent and long-term use of antibiotics.

Because of the increased risk that antibiotic-resistant strains will develop and spread, new strategies for the treatment of UTIs are needed. Diagnostic algorithms can help to make the use of antibiotics more specific.

Treatment of UTIs should be initiated according to the antibiogram and continued for an adequate period of time in order to avoid pandrug resistance. The uropathogenic E. coli strains showed high resistance to beta-lactam antibiotics like broad-spectrum penicillin, while resistance of *Klebsiella* strains to fluoroquinolones has increased.

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NEWS

LIMITED INTERNAL COMBINED WITH EXTERNAL FIXATION OR SIMPLE EXTERNAL FIXATION IN TREATMENT OF OPEN TIBIAL DIAPHYSEAL FRACTURES ?

The conclusion of a recent article published in BMC Musculoskeletak Disorders is that the limited internal fixation combined with external fixation had good results compared with simple external fixation for managing open fractures. The researchers evaluated that additional internal implants did not significantly increase the risk of infection. In contrast, the combined fixation technique was beneficial for initial reduction and subsequent fixation stability, thereby permitting early rehabilitation and rapid healing with a low incidence of postoperative complications. More prospective comparative studies with larger samples and longer follow- up periods are required to accurately evaluate the clinical outcomes of this combined technique (Hao ZC, Xia Y, Xia DM, Zhang YT, Xu SG. Treatment of open tibial diaphyseal fractures by external fixation combined with limited internal fixation versus simple external fixation: a retrospective cohort study. *BMC Musculoskelet Disord*. 2019;20(1):311. Published 2019 Jul 3. doi:10.1186/s12891-019-2679-9).

EFECTELE ADMINISTRĂRII PREOPERATORII A PROSTAGLANDINEI E_1 ASUPRA FLUXULUI SANGUIN CEREBRAL LA NOU-NĂSCUTUL CU MALFORMAŢIE CONGENITALĂ CARDIACĂ DUCTO-DEPENDENTĂ

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PROSTAGLANDIN E_1 PREOPERATOR ADMINISTRATION EFFECTS ON CEREBRAL BLOOD FLOW IN NEWBORNS WITH DUCTAL-DEPENDENT CONGENITAL HEART MALFORMATIONS (Abstract) : Although the absolute number of congenital structural heart disease has not changed over the years, more and more infants undergo surgical interventions in the neonatal period, increasing the number of children and adults with post surgical status of cardiac disorders. Prostaglandin E_1 (PGE_1) is the first line drug to maintain or reopen the ductus arteriosus, until surgical intervention can be made. Cerebral blood flow (CBF) is regulated by multiple factors, like pO_2, pCO_2, pH, cardiac output and systemic blood pressure. The aim of the study was to determine how CBF changes after PGE_1 administration in these newborns. **Materials and methods** : We studied the pO_2, pCO_2, mean systemic blood pressure, and cerebral blood flow velocities by colored and pulsed Doppler ultrasonography in 36 newborns with ductal dependent cardiac malformation before and after prostaglandin E1 administration, and correlated them to normal values for the age. **Results** : We found that cerebral blood flow velocities followed the variations of blood pressure in restricted pulmonary circulation ; improvement of oxygenation went parallel with decrease of cerebral diastolic velocities, and in aortic coarctation cerebral velocities decreased after introduction of PGE_1. **Conclusions** : PGE_1 administration improved cerebral blood flow velocities in all forms of cardiac malformations, due to reorganization of cardiac output and changes in oxemia. **Key words** : CEREBRAL BLOOD FLOW, PROSTAGLANDIN E_1, CONGENITAL HEART DEFECTS DUCTAL-DEPENDENT

RETROSPECTIVE STUDY ON THE ENDOSCOPIC THIRD VENTRICULOSTOMY

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RETROSPECTIVE STUDY ON THE ENDOSCOPIC THIRD VENTRICULOSTOMY (Abstract): Hydrocephalus is an active distension of the ventricular system of the brain resulting from inadequate circulation of CSF. Hydrocephalus is the main pathology encountered in pediatric neurosurgery, making it by far the leading cause of brain surgery. **Materials and methods:** Although for a long-time shunt therapy (VPS) was the only therapy available for hydrocephalus, nowadays endoscopic third ventriculostomy (ETV) becomes a reasonable alternative method that seeks to reduce the risk associated with shunt malfunction and its lifetime dependence. The authors conducted a retrospective analysis of 157 children with hydrocephalus who were treated either ETV or VSP at Marie Curie Emergency Children's Hospital, Bucharest between 2016 and 2018.The purpose of this paper is to analyze the success rate of ETV, by counting the number patient who didn't need shunt placement within the 1 year after ETV surgery. **Results**: Among those 157 examined children, 46 underwent ETV and 111 underwent VSP as their first treatment. Among those from the first group, 18 patients were surgery-free after ETV while the others 28 needed shunt placement. We defined our success rate (39%) basing on the number of patients who didn't need any shunt placement within 1 year after ETV. We also reported a big tendency to failure for those within the ETV group who were already associated with intraventricular hemorrhage. **Conclusions**: In conclusion, according to our study ETV seems to be a reliable treatment for hydrocephalus children. Further investigations are necessary for understanding the reoperation probability in order to distinguish which treatment is long term beneficial. **Keywords:** VENTRICULOSTOMY, SHUNT PLACEMENT, SUCCESS RATE, PEDIATRICS.

Hydrocephalus is an active distension of the ventricular system of the brain resulting from inadequate passage of CSF leading to CSF accumulation. Hydrocephalus is encountered in 1-2 of every 1000 live births (1), this fact making it one of the main pa-

thology in pediatric neurosurgery. CSF is an ultrafiltrate of plasma, predominantly secreted (2) by the choroid plexus (70-80%), brain interstitial fluid, ependyma and capillaries, with a total volume of 10-60 mL in infants and 60-100 mL in children. Understanding the way this fluid circulates is an important step towards comprehending the pathology (3). Thus, from the choroid plexus which produce it, CSF circulates via the ventricular system to its point of absorption into the systemic circulation, leaving the subarachnoid space through arachnoid villi found along the superior sagittal venous sinus, intracranial venous sinuses, and around the roots of spinal nerves. Any obstruction or malformation encountered at the passage of CSF, cause an anormal accumulation of CSF and an increase in ICP, which further will lead to one or more of the following pathologic findings: atrophy of the white matter, chronic astrogliosis, neurons are selectively spared because of a more luxurious blood supply to the gray matter, spongy periventricular edema, fibrosis of the choroid plexuses, ventricular diverticula and thinning and elongation of the interhemispheric commissures.

Nowadays, the optimal treatment for pediatric hydrocephalus patients is still discussed since there exist several surgical approaches, yet their success rate was reported different according to each particular study (4).

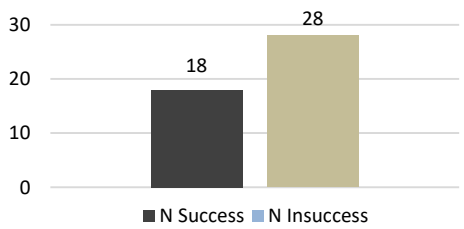

Fig. 1. Overall Success Rate

For a long time, shunt placement was the one leading approach in hydrocephalus. However, this procedure presents some disadvantages (5) such as decrease in life-quality, frequent obstructions, infections (due to an immature immune system, skin integrity and low nutrition in the first months of life). Also, even low-pressure valves could lead in time to over drainage (slit ventricles, subdural effusions and secondary craniostenosis) requiring conversion to a higher-pressure valve and anti-gravitational mechanism. Inflammation and hemorrhage (5) can complicate the shunt insertion and leads to isolated symptomatic ventricles (ataxia, cranial paresis, vital signs alteration) or multiloculated hydrocephalus.

As many significant advances have recently been made in Endoscopic Third Ventriculostomy (ETV), more and more surgeons chose this path of treatment for its benefits such as low shunt rate dependence and minimally invasive approach. According to previous literature, success rate SR of ETV depends on several important variables such as etiology, age, experience of the surgeon. For example, higher ETV SR was reported by several studies including aqueduct stenosis patients, and lower SR was observed in postinfectious and posthemoragic cases (6), facts that were also encountered and confirmed by what we present in this article.

In our study, we included our single-center experience in ETV treatment for hydrocephalus in children. Success rate was analyzed by dividing our group of patients in subgroups and a short review of literature was presented at the end.

MATERIALS AND METHODS

This is a retrospective study done be-

tween 2016 to 2018, at "Marie Curie" Emergency Children's Hospital, Bucharest, including at the beginning 157 patients who were examined for hydrocephalus. Data were collected from the medical charts and other relevant Imagistics (CT and MRI). In order to be included in the study patients had to have one of the following aspect of the pathology: triventricular hydrocephalus with lateral and third ventricles dilated, aqueduct stenosis, arachnoid cyst, history of hemorrhage or infection as a principal cause of hydrocephalus. Exclusion criteria were: multiloculated hydrocephalus or other complex cases which required multiple interventions in the past, cases with prior CSF diversion history. After establishing these criteria, those (111) who didn't meet them were excluded from the study. In the end, our study included 46 patients (28 males : 18 females) who were addressed to ETV as their first treatment for hydrocephalus. The mean age of the patients was 1 year and 8 months, ranging from neonates to 17 years old children. Out of the 46 patients, 15 were born premature, with a low birth weight and a mean birth age of 33.4 months (27 months being the minimum). From those who were born premature, 11 out of 15 were admitted for hydrocephalus due to hemorrhage or infection. The average Cranium Perimeter CP at hospitalization was 44 cm.

We analyzed several variables such as: etiology, age at the operation, birth-age, interval between operations in case of failure. Our distribution of patients according to their etiologies are showed in first table, which includes the number of cases for each pathology, the frequency of the pathology in our study and in the literature. Compared to the literature, our center reported a higher frequency of post hemorrhagic (28% to 3-13%%) and postinfectious (19% to 3-6%) cases and a lower frequency of tumors (8% to 29-47%). Based on anatomical and developmental considerations, we divided our patients in 3 groups, respectively: < 6 months Group with 30 patients, 6 months - 2 years Group with 6 patients and > 2 years Group with 6 patients to analyze the impact that age has on SR.

We defined our success rate SR basing on the number of patients who didn't need any other intervention at one-year follow-up. Diagnosis of hydrocephalus was made based on some important clinical aspects such CP growth, tight anterior fontanelle (7) and on the basis of imagistics which revealed one of the following: "Mickey Mouse" ventricles, ascending or thinning of *corpum callosum*, obstruction of the foramina Magendi and Luschka, periventricular edema.

Failure of ETV was considered when patients presented one of the following pathological aspects even after the operation: increase intracranial pressure, increase occipital-frontal circumference, bulging of the anterior fontanelle or any other symptoms of intracranial hypertension. Eventually, these patients needed to be treated by shunt placement VPS or re-ETV. About the procedure, ETV is performed with head flexed so that the burr hole site is at the highest point (8).

The optimal trajectory is achieved with a burr hole, placed just anterior to the coronal suture and slightly medial to the mid-pupillary line.

After penetrating into the lateral ventricle, foramen Monroe could be identified at the confluence of thalami-striate vein with septal vein and caudate vein. Passing through foramen Monroe, the endoscope enters into the third ventricle. ETV is per-

formed into the triangle formed between the 2 mammillary bodies and infundibulum where the floor of third ventricle is bulged, right anterior to the tip of basilar artery. Anterior to the infundibulum can be seen the optic tract and posterior to it can be seen the clivus. Penetrations into the prepontine cistern should be done carefully to avoid the injury of the basilar artery. In some cases, it would be necessary to perforate also the membrane of Liliequist, found beneath the floor of the third ventricle. The fenestrations usually around 4-5 mm, it is furtherer enlarged with a Fogarty balloon. This last step is usually done with little bleeding.

TABLE I
Distribution according to their pathologies

Types of cases	N	Frequency in our study (%)	Frequency in literature (%) (9, 10, 11, 12, 13)
Posthemoragic	13	28%	3-13%
Postmeningitis	9	19%	3-6%
Intraventricular Cyst	7	15%	5-6%
Aqueductal Stenosis	5	10%	15-31%
Tumors	4	8%	29-47%
Myelomeningocele	4	8%	3-6%
Others (Syndromes, Aneurysms)	2+2	4% + 4%	6-22%

RESULTS

In our study included a total of 46 patients diagnosed with hydrocephalus, reporting an overall SR of 39%. In the rest of the patients re-ETV or VPS was performed.

We analyzed patients according to their etiology and data set was labeled in the second table.

TABLE II
ETV SR according to each pathology

Type of Case	N	Number of Success	Success Rate
Post-hemorrhagic	13	1	7%
Post-meningitis	9	1	11%
Intraventricular Cyst	7	6	85%
Aqueduct Stenosis	5	5	100%
Tumors	4	3	75%
Myelomeningocele	4	0	0%
Genetic Syndromes	2	1	50%
Vascular Cases	2	1	50%
TOTAL	**46**	**18**	**7-100%**

The greatest SRs were 100%, 85% and 75% in the patients with aqueduct stenosis (5/5), intraventricular cyst (6/7), respectively with tectal tumors (3/4). Our lowest SRs were reported in post-hemorrhagic (13 patients) and post-infectious patients (9 patients), respectively 7% (one patient) and 11% (one patient). In patients with myelomeningocele (n=4) our SR was 0/4. We also reported syndrome-cases: Dandy Walker (1) with no SR and NF-1 (1), with SR=100%. Regarding vascular cases, we reported patients with Galen Aneurysms malformations (2 patients) with the final SR of 50%. In patients who were successfully treated, clinical symptoms were solved - the PC decreased, and anterior fontanelle lowered in tension. We reported the mean PC of 44 cm, ranging from 38 cm to 49 cm at discharge, which according to CDC Growth Charts corresponds at the 50th percentile with the age of 6 months which was the exact mean age of our successful cases. Regarding age distribution our data was collected and presented in table III.

TABLE III
Relation between age, etiology and SR

Etiology of hydrocephalus	Premature	< 6 months	6-24 months	> 24 months
Posthemoragic	5	0/12	1/1 (100%)	-
Postmeningitis	6	1/7 (14%)	0/1	0/1
Intraventricular Cyst	-	2/3 (66.7%)	2/2 (100%)	2/2 (100%)
Aqueductal Stenosis	2	2/2 (100%)	2/2 (100%)	1/1 (100%)
Tumors	-	1/1 (100%)	0/1	2/2 (100%)
Myelomeningocele	2	0/3	-	0/1
Genetic Syndromes	-	-	0/1	1/1 (100%)
Vascular Cases	-	0/1	1/1 (100%)	-
TOTAL	**15**	**6/30 (20%)**	**6/8 (75%)**	**6/8 (75%)**

We experienced 20%, 75% and 75% SR in < 6 months Group, in 6 months - 2 years Group, respectively in > 2 years Group. According to this table, SR increases with the age, being the lowest in neonates, and the highest in children above 2 years. In those who were born premature, we reported SR of 26% (4 out of 15). The mean interval between operation in case of failure was 51 days with minimum of the days for neonates and a maximum of 192 days (6 months) in 6-24 months Group. Those cases in which re-ETV was used, even though presented an initial favorable prognostic, yet they ended up with VPS. Because of the associated risks, these situations were encountered in almost all of our post-hemorrhagic and post-infectious patients (18) who were classified as unsuccess. No mortality was reported in the study.

DISCUSSION

VPS was for a long-time the standard permanent option, however this technique presents many late complications such as

infections, obstruction or over drainage with formation of slit ventricles. Nowadays, with the development of in neurosurgery, ETV seems to be the method of choice in many aspects of children hydrocephalus.

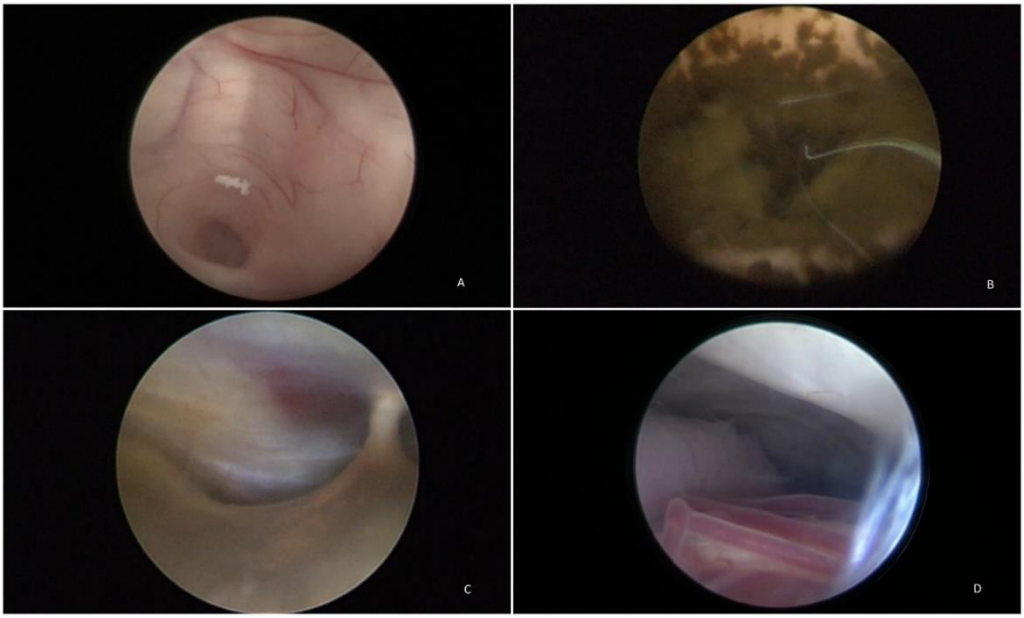

Fig. 2. Normal and pathological aspect of Sylvius Aqueduct (A, B).
Scarring of the pre-pontine Cistern (C).
Normal aspect of the pre-pontine cistern with Basilar Artery and Pre-pontine arteries (D).

In our study, we reported 46 patients with various types of hydrocephalus, from different ages, yet predominant infants < 6 months (tab. III). We defined our SR as the number of patients who didn't need within 1-year after ETV. Over time in literature there was a big controversy about whether or not it is good to use ETV in patients < 1 year (14, 16). Several authors (8, 11, 15, 16) reported very good outcomes ranging between 60-70%, while others reported poor outcomes with SR between 20-35%. In our study, in the < 6 months Group, we reported 100% SR for aqueduct stenosis (2 cases, < 6 months Group) and tumors cases (1 case, < 6 months Group), with good outcomes, SR=66.70% in intraventricular cyst cases (2 cases < 6 months Group) and poor outcomes in post-meningitis (1/7, SR of 14%) and post-hemorrhagic or other vascular cases (0), with a final overall SR of 20%. We agree that ETV is a reliable treatment that should be use for hydrocephalus in this age group, yet not only age but also etiology is an important prognostic factor for the postoperative outcome. In the other 2 groups (6 months - 2 years, and > 2 years), we reported 8 age-etiology subgroup in which SR was 100%. This again sustain the strong correlation between age, etiology and SR.

According to literature (16), age is an

important factor in the postoperative evolution of hydrocephalus children. Many studies reported a lower SR in infants < 6 months than in the rest of the patients. Bognar *et al.* (17) reported a SR of 36% in patients < 6 months, and a SR of 73% in patients > 1 year, while Kulkarni et al reported a SR of 44% and 69% in < 6 months, respectively in > 6 months Group.

The primary explanation of this ETV failure in infants might be explained by CSF poor absorption (18). After being produced by the choroid plexuses CSF circulates through the ventricular system and finally leaves the subarachnoid space through arachnoid villi found along the superior sagittal venous sinus (3 to 5 mmHg pressure gradient between the subarachnoid space and venous sinus), intracranial venous sinuses, and around the roots of spinal nerves. However, in infants these arachnoid granulations are poorly developed, increasing the risk of accumulation of CSF into the brain. Moreover, because the skull sutures are not closed very well, ICP cannot get high enough to help CSF be absorbed. Last but not the least, ETV failure in infants < 6 months could be correlated with a high tendency to develop new arachnoid membranes, usually on both sides of the basal artery between the clivus and pons, but not only. Regarding this last point, we reported at least 6 cases of multiloculated hydrocephalus, that were not included in this study due to the exclusion criteria, with severe complications which needed multiple endoscopic interventions and shunt revision till the equilibrium was achieved. In these situations, it is important to acknowledge that the surgeons usually face a distorted anatomy which is accompanied by many deposits of hemosiderin (fig. 2.B) due to blood degradation, without the normal landmarks as in figures 2.A, 2.D. From our experience one of the prognostics factors for an ETV failure is the scarring of the pre-pontine cisterns (fig. 2.C), which happened in almost all of the 6 cases mentioned above. In this situations success is barely obtained and the primary goal would be rather to lower the intracavitary pressure, reduce the number of catheters and number of revisions. From our experience, myelomeningocele surgeries (4) were unsuccessful. One possible explanation is that 3 out of 4 cases were patients < 3 months and may have suffered from infant CSF flow problems.

CONCLUSIONS

In conclusion, our study analyzed several factors such as: etiology, age at the operation, birth-age, interval between operations in case of failure, being an observational report describing whether or not this factors influence the success rate. According to our study ETV seems to be a reliable treatment for hydrocephalus children (16). Successful rate of ETV depends on age, being lower in infants under 6 months, and higher in children above 2 years, but also depends on each etiology being higher in aqueduct stenosis, tumors, intraventricular cysts patients, and lower in post-hemorrhagic and post-infectious patients. Our conclusions are still preliminary, and we believe that further investigations with a larger sample of patients will be necessary in order to report more statistically relevant data.

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No conflict of interest. Original study.

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RETROGRADE SEMIRIGID URETEROSCOPY VERSUS EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY IN THE TREATMENT OF URETERAL CALCULI BETWEEN 5-15 MM

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RETROGRADE SEMIRIGID URETEROSCOPY *VERSUS* EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY IN THE TREATMENT OF URETERAL CALCULI BETWEEN 5-15 mm (Abstract): Ureteral calculi below 15 mm are treated either by retrograde semirigid ureteroscopy or extracorporeal shock wave lithotripsy (ESWL). Controversy regarding the best method still exists, and authors favor one or another procedure. The aim of this retrospective study was to compare these two procedures. **Material and methods:** The study compared the results of 71 patients treated by retrograde ureteroscopy and 57 patients treated by extracorporeal shock wave lithotripsy. **Results:** Out of the 71 patients treated by ureteroscopy, 60 (84.5%) were stone-free, one month after the procedure. In 11 cases (15.5%), the stone could not have been reached or the fragments migrated in the pyelocaliceal system (residual fragments). Out of the 57 patients treated by ESWL, 21 (36.9%) required only one session to break the stone, 33 (57.9%) needed two sessions, one patient, three sessions and in two cases, the stone could not be focalized. The stone-free rate was evaluated three months after the last session, and it was 87.7% for ESWL. There was only one complication in each group. **Conclusions:** The difference of stone-free rate between the two procedures was not statistically significant. Ureteroscopy achieved the status stone-free earlier, in contrast with ESWL, who needed a longer time. Both procedures had lower rate of complications. **Keywords:** RETROGRADE URETEROSCOPY, ESWL, STONE-FREE RATE.

Retrograde ureteroscopy and extracorporeal shock wave lithotripsy (ESWL) are both, utilized methods in the treatment of ureteral lithiasis, for stones under 15 mm in the long diameter, according with EAU Guidelines (1). Some studies shows a better outcome for ureteroscopy (2, 3, 4). Other studies demonstrate a higher stone-free rate for ESWL (5, 6, 7), while different authors observed a similar, good rates of success (8, 9). There are also controversies regarding the preoperative stenting, as some authors demonstrated that the use of ureteral stents lower the stone-free rate after ESWL (10, 11), while others considered that there were no differences (9). Due to these con-

flicting results, some urologists tend to indicate as the first line of treatment ureteroscopy, while others indicate ESWL, according to their experience. We have conducted a study in which we compared the stone-free rates of the two procedures, the time to achieve that goal and the rate of complications.

MATERIAL AND METHODS

We have enrolled in the study 71 consecutive patients with ureteral stones with the size between 5-15 mm, which were operated by ureteroscopy between January and April 2018 in the Urological Clinic of "Dr. C.I. Parhon" Hospital Iasi with a *Olympus* Ureteroscope and 57 consecutive patients with the same diagnosis, who were treated by ESWL in the "Elytis" Hospital Iasi with a *Siemens Lithostar* unit, between August 2017 and December 2018. The diagnoses were based on echography, Plain radiological films (KUB), and in many cases, urography, when the diagnoses were in doubt. Control for the remaining fragments or stones after the two procedures were made in the same mater as for diagnosis. The status stone free was evaluated at one month for ureteroscopy group, because these patients came back for the extraction of the double JJ stents, routinely placed after the procedure, at 2-4 weeks after the procedure. The patients was considered stone free, if there were no radiopaque images on the KUB X-ray and no stones or pyelocaliceal distention on the ultrasound examination. The same control procedure was applied for searching residual fragments in the ESWL group, but the control was made at 3-4 weeks and at three month after the procedure. We waited three month even when the stone was fragmented, due to the long time needed for fragments to pass spontaneously. So, the stone free rate was compared at one month for ureteroscopy group, and at three month for ESWL group. We named residual fragments, all stones over 3 mm. The decision for the specific procedure was made by the patients on the basis of an informed consent form. We have enrolled in the study all these patients with ureteral stones, with or without previous inserted ureteral JJ stents, with a BMI under 35. The exclusions criteria were patients with lost follow up, with stones over 15 mm, or multiple ureteral lithiasis. We have compared the stone free rates between the two groups, at one and three months and the rate of complications.

Statistical analysis. Data were presented as means with standard deviation or numbers and percentages frequency, as appropriate. Between-group comparison was performed using the Chi-square test or Fisher's exact test for categorial variables and the independent t test for the remaining variables. We considered a 2-sided $p<0.05$ to be significant. All statistical analysis were performed using the *SPSS for Windows, version 19.0.1* (SPSS Inc, Chicago, Illinois).

RESULTS

There were 128 patients included in the study. The mean age of the patients was 51,5 years. A total of 61(47,7%) were males. The most stone location was the lumbar region (52.3%) and the majority of patients, 81 (63.3%) required only 1 session of ESWL or ureteroscopy. 65 patients (50.8%) had the stone under 10 mm, 74 patients (57.8%) had previous inserted JJ stents and 59 patients (46%) had the stone on the left side. Only 34 patients (26.6%) were with ureterohydronephrosis, all other were without obstruction, or with JJ stents (tab. I).

TABLE I

Characteristics of the two groups

		ESWL		Ureteroscopy		
		N	%	N	%	p value
Mean age		45.7±12.4		56.0±15.2		<0.001
Number of patients	Men	31	54.4	30	42.3	0,17
	Women	26	45.6	41	57.7	
Localization of the stone	Lumbar	31	54.4	36	50	0.81
	Iliac	3	5.3	14	20	0,03
	Pelvic	23	40.3	21	30	0.37
Size of the stone	5-10 mm	30	52.6	35	49	0,71
	11-15 mm	27	47.4	36	51	
With obstruction (ureterohydronephrosis)		20	35	14	19.7	0.13
Without obstruction		14	24.6	6	8.5	0.03
With preoperatory JJ stents		23	40.4	51	71.8	<0.001
With obstruction and JJ stents		43	75.4	65	91.5	
Right ureteral stone		34	59.6	35	49.3	0.52
Left ureteral stone		23	40.4	36	50.7	

Patients that had performed ureteroscopy were older, had a higher incidence of iliac stone location, were more likely to have a previous JJ stent insertion and required more frequently only 1 session than patients that had performed ESWL. In ESWL group, patients had more frequent obstruction. All other characteristics of the two groups were similar, without statistically significant differences. The difference characteristic between the two groups does not influence the outcome of the procedures, so they are not a bias factor (tab. II).

TABLE II

Results of the procedures

	ESWL		Ureteroscopy		
Number sessions	N	%	N	%	p value
1	21	36.9	60	84.5	<0.001
2	33	57.9	5	7	<0.001
3	1	1.7	-	-	-
Unsuccessful procedure cannot focalized the stone	2	3.5	-	-	-
	5	8.8 with residual fragments	11 cannot reach the stone or fragments migrated in pyelocaliceal system	15.5	0.65
Stone free	50	87.7	60	84.5	0.88

Out of the patients with residual fragments in the ureteroscopy group, 9 (12.7%) had the stone in lumbar position, 1 (1.4%) was iliac and 1 (1.4%) was in pelvic region. Out of the 5 patients with residual fragments in the ESWL group, 4 had JJ stent inserted.

As complications, in ureteroscopy group, 1 (1.4%) post operatory fever, over 38 degrees Celsius was recorded and was cured with intravenous antibiotics, while in the ESWL group, 1 (1.8%) subcapsular hematoma, did not require drainage and was in resolution three months after the procedure.

DISCUSSION

Our study has some major particularities. Many patients in the ESWL group (40.4%) had previously inserted JJ stents, which is known to affect the success of the procedure (10, 11), so, it was expected to see a lower proportion of stone-free patients. On the contrary, a very good success rate was noticed, higher than in other studies (3, 12, 13), probably due to the fact that we performed two or even three sessions to break the stones and we applied a relative high energy level, according to manufacturer's specifications. This good result came with a higher risk of renal injury in lumbar stones, and the reason for some studies to report a higher complications rate after ESWL (14). Because many patients had JJ stents, we did not report any case who required post ESWL stenting as in other studies (15), having the advantage of a very low risk of obstruction. Many studies (3, 16) report a low success rate after one session of ESWL as in our study, because, without JJ stent, the risk of obstruction and renal impairment is high and it is not expected to wait, to perform another session. So, preprocedural JJ stent insertion has disadvantages but also advantages, allowing multiple sessions, until reaching the goal of stone-free. We evaluated the proportion of patient cured, without residual fragments, at three month, and not at one month and this fact can also explain the higher stone-free rate after ESWL.

Another particularity is that in the ureteroscopy tents, previously placed for infected ureterohydronephrosis, or recurrent renal colic. The ureteroscopy was performed weeks after the placement of the JJ stent and it was expected to see a higher rate of success, as in other studies (2, 4, 6, 17) of over 95%. Our lower rate can be explained by the fact that after weeks of stenting, the ureteral mucosa is inflamed, and can create difficulties in advancing and reaching the stone. The ureteroscopy is performed routinely after maximum 2 weeks after the placement of the JJ stent, in centers with a smaller waiting list for the patients. Compared with other studies, our complication rate was much lower as in other studies (16-20), similar to the ESWL group ($p<0.001$) (3).The major drawback for successful ureteroscopy, was the location in the lumbar region, and even with the previously inserted JJ stents, there were 9 patients (12.7%) in whom the stone couldn't be reached or with fragments migrated in the pyelocaliceal system.

When we compare the two procedures, we see that the stone-free rate is similar ($p<0.001$), but for the ESWL group the status stone-free is achieved later. The rate of complications is very low in both groups, these methods are safe, and the results are better than those mentioned in the European guidelines (1) with a higher potential of complications for ureteroscopy. In our experience, the risk of complications

does not constitute a reason for the patients to refuse the ureteroscopy. In our study, the patients have chosen the ESWL, because of the potential risk of anesthetic complications during ureteroscopy or for personal reasons.

CONCLUSIONS

In our study, retrograde semirigid ureteroscopy and ESWL are both effective to achieve the goal of the procedures, the stone-free status, with comparable results, even when JJ stent is placed preoperatively. Ureteroscopy cure the patients quicker, with a similar rate of complications as ESWL. However, for stones in lumbar ureter, the success rate drop sharply, so ESWL is recommended as the first line of treatment. When available, flexible ureteroscopy and not semirigid ureteroscopy can be recommended if the patients accept the anesthetic risk. Finally, in our opinion, the option for a procedure or another should be the patient's choice, after a thorough counseling.

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NEWS

DEVELOPMENT OF EFFECTIVE VACCINES FOR ENTEROTOXIGENIC *ESCHERICHIA COLI*

Diarrhea in children is caused mostly by enterotoxigenic *Escherichia coli* (ETEC). Vaccination could be an effective way to prevent ETEC diarrhea. A specific vaccine was not developed yet due to ETEC strains heterogeneity. In The Lancet Infectious Diseases, Firdausi Qadri and colleagues examined the safety and immunogenicity of enterotoxigenic *E. coli* vaccine (ETVAX). ETVAX is an oral, inactivated, vaccine which contains four E. *coli* strains that overexpress adhesins CFA/I, CS3, CS5, and CS6, mixed with a recombinant protein, LCTBA. It previously shown to be safe and highly immunogenic in Swedish and Bangladeshi adults. The study was based on a randomized phase 1/2 trial in children and infants. They showed that ETVAX was safe and well tolerated by children. At a quarter of an adult dose, adverse events like mild vomiting or fever were rare. They observed that the immunogenicity of ETVAX was age-dependent: children aged 12–59 months had significant increases in IgA antibodies in lymphocyte secretions (ALS) targeting all five vaccine antigens, but children aged 6-11 months had significant ALS and fecal IgA responses to only three antigens (CFA/I, CS3, and LTB). The accomplishment of ETVAX in ETEC-endemic regions it's shaded by some concerns like the multitude of ETEC adhesins that are not targeted in the vaccine, or the immunity to the B subunits of heat-labile toxin, but not to the toxic heat-labile A subunit, nor to the heat-stable enterotoxin (Qadri F, Akhtar M, Bhuiyan T, et al. Safety and immunogenicity of the oral, inactivated, enterotoxigenic Escherichia coli vaccine ETVAX in Bangladeshi children and infants: a double-blind, randomized, placebo-controlled phase 1/2 trial. *Lancet Infect Dis* 2019; https://doi.org/10.1016/ S1473-3099(19)30571-7).

PREDICTIVE FACTORS OF THE OUTCOME OF SURGICAL PROCEDURES FOR ENTERAL FEEDING IN PATIENTS WITH MALIGNANT *VS.* NONMALIGNANT DISEASES

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PREDICTIVE FACTORS OF THE OUTCOME OF SURGICAL PROCEDURES FOR ENTERAL FEEDING IN PATIENTS WITH MALIGNANT *VS.* NONMALIGNANT DISEASES (Abstract): Surgical feeding procedures are required as a treatment for malnutrition that occurs in a wide range of malignant or benign, digestive or extradigestive diseases. Choosing the best approach in each case is important for achieving a correct nutritional support. **Aim**: To identify the predictive factors of the efficacy of the different surgical feeding procedures, comparatively in benign *vs.* malignant pathology. **Material and methods**: Retrospective, cross-sectional, observational study of a group of 276 cases with various benign and malignant diseases. Correlations were established between the initial disease, type of surgical techniques for enteral feeding and the postoperative outcomes. Statistical analysis was performed using the Chi-square test for dichotomous variables and the Mann-Whitney and Kruskal-Wallis tests for ordinal and continuous variables. **Results:** Most patients with either benign or malignant digestive diseases underwent Gavriliu's high gastrostomy with peritoneal cuff (73.6%; 91% respectively), performed under local anesthesia. The patients with benign extradigestive diseases and 35% of the patients with malignant extradigestive diseases underwent percutaneous endoscopic gastrostomy (PEG), but monitoring was short, 30 days, respectively. In early postoperative follow-up, PEG is associated with faster recovery and lower gastric stasis volume. Classic gastrostomy is still an option in cases with severe strictures due to esophageal or head and neck malignancies. The main long-term prognostic factor is the possibility of ensuring the correct number of calories and volume of food. **Conclusions:** The efficiency of feeding techniques is long-lasting, largely uninfluenced by the underlying disease. The acquisition of the nursing elements would makes it possible to maintain these surgical feeding solutions both in the hospital setting, but especially in the outpatient setting. **Keywords**: GASTROSTOMY, TUBE FEEDING, PERCUTANEOUS ENDOSCOPIC GASTROSTOMY, MALIGNANCY, PREDICTIVE FACTORS.

Surgical feeding procedures are required as a treatment for malnutrition that occurs in a wide range of malignant or benign, digestive or extradigestive diseases. Impossibility of normal feeding has extremely serious consequences on the

metabolism of carbohydrates, lipids, and proteins, as well as on the immune system. Malnutrition in cancer patients can limit their response to treatment, surgery, chemotherapy, and radiation therapy (1). A loss of over 10% in body weight at diagnosis is a poor prognostic factor for survival (2, 3). An adequate calorie intake and the restoration of a normal body mass index (BMI) are essential before a curative intervention, both for malignant and nonmalignant upper digestive stenosis.

Numerous conditions may compromise the passage of food along the digestive tract, even when permeable, such as severe head injury, brain tumors, cranioencephalic trauma, cerebral hemorrhage and cerebral infarction, because of gag reflex impairment or dysfunction. In these critically ill patients, enteral nutrition support plays an important role, many studies demonstrating the positive effect of enteral feeding vs parenteral feeding in preventing septic complications in ICU patients (4-7).

There is a wide variety of surgical feeding procedures described in the specialized literature, using the classical open, laparoscopic, or endoscopic approach, but there is relatively little data on the clinical parameters with predictive value of their maintenance and long-term efficacy.

AIM

To identify the predictive factors of the efficacy of the different surgical feeding procedures, comparatively in benign *versus* malignant diseases.

MATERIAL AND METHODS

Retrospective, cross-sectional, observational study of a group of 276 cases admitted over a 10-year interval, with various benign and malignant diseases characterized by the long-term impossibility of physiological nutrition, which is an indication for enteral nutrition. The studied parameters were gastric stasis volume at 24, 48, 72 hours, calorie intake, gastric capacity at 1, 3, 6 and 12 months, peristomal erythema, body mass index (BMI), corroborated with the sequential assessment of biological parameters over different follow-up intervals. Correlations were established between the initial disease, type of surgical feeding procedure, and postoperative outcomes. Statistical analysis was performed using the Chi-square test for dichotomous variables and the Mann-Whitney and Kruskal-Wallis tests for ordinal and continuous variables.

RESULTS

Most patients (56.52%) had a malignant digestive disease, followed by benign esophageal stenosis (21.73%), and extradigestive neoplasia (17.39%). In 12 cases (4.34%) the surgical feeding procedure was performed for benign extradigestive disorders (neurological diseases, patients hospitalized for a long time in ICU).

In the study group, men aged 41 to 60 years were most susceptible to benign digestive diseases as compared to women whose percentage was much lower and attributed to a much younger age (18-40 years). Males are at a much higher risk of developing both digestive and extradigestive diseases than females; the odds of the disease being malignant, whether digestive or extradigestive, is also higher in males (χ^2=8.508, p = 0.004) (tab. I).

Of the total study cases, 22.82% were admitted to hospital as emergencies for febrile syndrome, severe cachexia or dysphagia with odynophagia. Preoperative clinical parameters were particularly relevant

for malignant digestive disease. Thus, of the patients diagnosed with digestive malignancies 57.1% had fever, 56.5% dysphagia, 62.8% odynophagia, 55% abnormal BMI, 66.7% sialorrhea, 57.7% cough, 52.6% low hemoglobin levels, 45.1% leukocytosis, 61.9% nitrogen retention, and 58% coexistence of radiological lung opacities (tab. II).

TABLE I

Age and sex distribution of the study cases

	Age groups (years old)											
	Female (%)						Male (%)					
	18-30	31-40	41-50	51-60	61-70	71-90	18-30	31-40	41-50	51-60	61-70	71-90
Benign digestive diseases	22.2	25.9	11.1	22.2	14.8	3.7	3.0	9.1	45.5	30.3	12.1	0.0
Malignant digestive diseases	0.0	0.0	5.6	27.8	38.9	27.8	0.0	1.4	18.1	34.1	34.1	12.3
Benign extra-digestive diseases	15.2	15.2	8.7	23.9	23.9	13.0	2.7	23.6	32.4	29.7	11.0	15.2
Malignant extra- digestive diseases	14.3	28.6	0.0	42.9	14.3	0.0	0.0	0.0	17.1	43.9	29.3	9.8

TABLE II

Preoperative clinical parameters by disease type

	Benign digestive diseases (%)	Malignant digestive diseases (%)	Benign extradigestive diseases (%)	Malignant extradigestive diseases (%)
Fever	**21.4**	**57.1**	**3.6**	**17.9**
Dysphagia	**21.7**	**56.5**	**4.3**	**17.4**
Odynophagia	**24.3**	**62.8**	**2.8**	**10.1**
Abnormal / low BMI	21.8	**55.0**	4.0	19.3
Sialorrhea	33.3	**66.7**	0.0	0.0
Cough	22.1	**57.7**	3.4	16.9
Low hemoglobin	23.9	**52.6**	4.3	19.1
Leukocytosis	28.2	**45.1**	4.2	22.5
Nitrogenous retention	16.9	**61.9**	3.8	17.4
Low blood glucose level	40.8	***34.7***	4.1	20.4
Lung opacities (Rx)	23.2	**58.0**	3.6	15.2

Choosing the surgical procedure for enteral feeding was significantly influenced by the diseases type: most patients with either benign or malignant digestive diseases underwent Dan Gavriliu's high gastrostomy with peritoneal cuff (73.6% and 91%, respectively), performed under local anesthesia. The patients with benign extradigestive diseases underwent surgery by endoscopic approach, but monitoring was short, 30 days, respectively. A significant percentage (approx. 35%) of patients with malignant extradigestive diseases underwent percutaneous endoscopic gastrostomy, but the monitoring was also short, 30 days, respectively.

The patients were followed-up at 24, 48, and 72 hours, 7 days, 3, 6, 9 and 12 months postoperatively. The followed descriptive postoperative parameters were coded as 0 = absent and 1 = present and were analyzed using *IBM SPSS Statistics version 20.0* to find the most relevant parameters in terms of clinical variation and disease course (tab. III).

Statistical analysis showed that showed statistically significant variation during the first 7 days of postoperative monitoring were: fever and gastric liquid volume and appearance. The variation of these parameters was not influenced by the benign or malignant nature of the underlying disease, but by the surgical technique used.

During the first 24 hours, the most relevant parameters for monitoring the postoperative course were: gastric stasis volume (small or medium), biliary stasis (0.889) and fever (0.707). The clear appearance of gastric stasis has no significance, being within the normal postoperative variability (tab. IV).

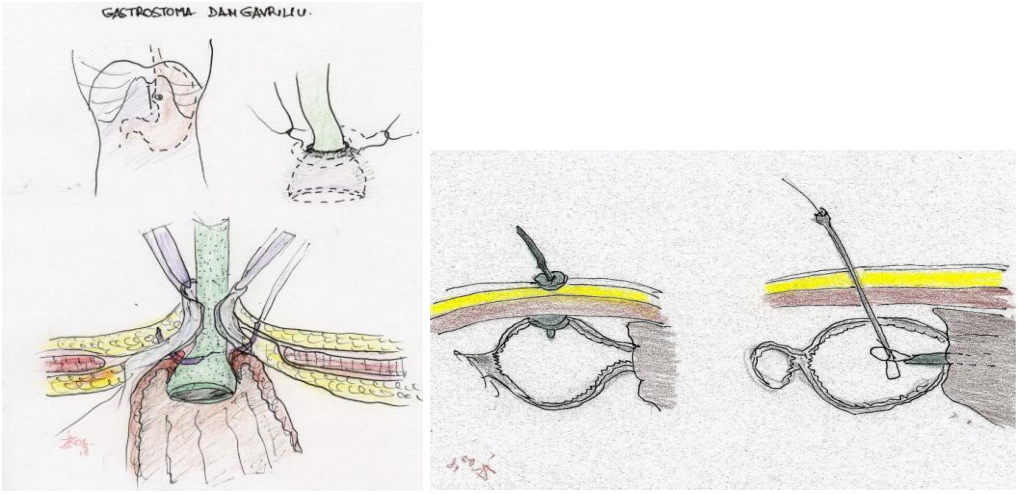

Fig. 1. a) *Dan Gavriliu's* high gastrostomy with peritoneal cuff; b) percutaneous endoscopic gastrostomy (PEG)

TABLE III

Surgical procedures for feeding tube placement by diseases in the study group

	Classic gastrostomy with peritoneal cuff (Gavriliu) (%)	Gastrostomy + / trans gastric Pezzer jejunal feeding tube (%)	Gastrostomy + jejunostomy (%)	Jejunostomy (%)	PEG (%)	PEG -j (%)	Total
Benign digestive diseases	**73.6**	6.7	1.7	18.3	0	0	60
Malignant digestive diseases	**91**	0.6	0.6	6.4	1.3	0	156
Benign extradigestive diseases	0	0	0	0	91.7	8.3	12
Malignant extradigestive diseases	64.6	0	0	0	35.4	0	48
Total	216	5	14	21	19	1	276

TABLE IV
Variance of the postoperative clinical parameters

Descriptive Statistics				
	Minimum	Maximum	Mean	Std. Deviation
Fever	0	1	.11	.312
BP	0	1	.03	.161
Surgical wound	0	1	.01	.085
Pain	0	1	.01	.085
Incontinence of gastrostomy	**0**	**0**	**.00**	**.000**
Peristomal erythema < 2 cm	0	1	.01	.118
Peristomal erythema > 5 cm	**0**	**0**	**.00**	**.000**
Gastric stasis 0-50 mL	0	1	.56	.496
Gastric stasis 50-100 mL	0	1	.36	.481
Gastric stasis 100-150 mL	0	1	.08	.266
Serosanguineous gastric stasis liquid	0	1	.03	.168
Biliary stasis liquid	0	1	.26	.441
Clear stasis liquid	0	1	.71	.455

During the postoperative 24 hours - 7 days interval, the statistically significant parameters recommended to be monitored are of gastric stasis appearance [(normal, clear gastric stasis (R2 = 0.951), biliary stasis (R2 = 0.942)] and gastric stasis volume (R2 = 0.703). The incidence of fever (R2 = 0.272) is a parameter considered unimportant during this postoperative interval. Therefore, during the postoperative 1-7 days interval, the clinical course of the patient carrying a feeding tube for enteral nutrition can be best monitored by monitoring gastric stasis volume and appearance.

Kruskal Wallis test was used to verify whether the 6 surgical procedures for enteral feeding used in the study group led to different performances of the postoperatively monitored parameters. The most obvious differences generated by the surgical feeding technique are at the level of biliary/gastric stasis. To explore these differences, the Mann-Whitney test was applied, comparing the surgical procedures two by two. The means of the ranks indicate favorable results when using the classic Dan Gavriliu's gastrostomy technique compared to gastrostomy - jejunostomy with respect to avoiding fever, low BP values, and ensuring the best possible outcome for the surgical wound (sig.=0.000).

The probability of gastric stasis with bilious appearance is higher with the classic Gavriliu's gastrostomy technique than with PEG (sig. =0.018). This can be attributed to gastric paresis associated to surgical trauma, which is more significant in the classic vs endoscopic approach. However, there are limitations of the study generated by the different number of cases analyzed for each surgical technique used.

As to long-term follow-up, between 1 and 12 months, the statistical significance of the monitoring parameters depended both on the type of feeding procedure and the disease that required the surgery. Elements such as: good stoma continence, clear, small gastric liquid volume (0-50 mL), and peristomal erythema < 2 cm are indicators of favorable outcome, associated with the possibility of managing a high calorie intake and higher volumes of food

administered per day, in both benign and malignant groups, with improvement of BMI and metabolic imbalances. On the contrary, the signs that suggest sepsis, such as fever, malignancy, pulmonary and mediastinal X-ray lesions, nitrogen retention, and low BMI are risk factors for complications such as stoma incontinence, large erythema, with the impossibility of administering the minimum number of daily calories. The statistical analysis was carried out to identify those parameters that influence the presence of feeding stoma incontinence, stasis volumes greater than 100 mL, large skin erythema > 5 cm, and tolerated feeding volume. The analysis of statistical data showed statistically significant differences between the minimum tolerated volume and the number of calories administered in benign vs malignant diseases (sig. = 0). In the study group, statistically significant correlations were recorded only for the malignant digestive disorders treated by palliative Dan Gavriliu's gastrostomy.

At 1-month follow-up, in these patients normal hemoglobin levels proved to be a significant predictor of volume of stasis < 50 mL, suggesting a favorable course (probability 81.1%, sig. < 0.05). At the 3-month follow-up, the absence of radiological pulmonary abnormalities was a significant predictor (sig. = 0.004) for the presence of peristomal erythema < 2 cm, a factor of favorable outcome (prediction value 64.7%). Statistical analysis also showed a strong correlation between continence and volume of gastric stasis < 50 mL (prediction value 95.7%).

At the 6-month follow-up, statistical analysis showed that the presence of peristomal erythema < 2 cm in the group of patients with malignant digestive diseases who underwent Gavriliu's gastrostomy was correlated with: tolerated volume per administration, number of meals, volume administered per day (sig. = 0.001), and overall the possibility of administering the necessary daily calories (p = 0.037). The impossibility of administering the necessary calories is also a significant predictor of Rx pulmonary damage 6 months after surgery in this group (sig. = 0.004). The negative saturation coefficient B = -.032 from Step 2, Logistic Regression actually indicates that patients with malignant digestive disorders, who are given more calories are less likely to develop lung parenchymal damage (the probability of prediction is 97.3%).

DISCUSSION

The surgical procedures for enteral feeding are becoming increasingly important by expanding their use in a medical conditions whose complexity and diversity require effective long-term calorie support. In order to ensure the hydroelectrolitic necessities, it is important to consider the fluid losses through fistulas, diarrhea, perspiration, urine, based on which the qualitative and quantitative supply will be calculated (8). The amount of fluids to be administered per 24 hours is estimated, on average, at 2,000-2,500 mL, to which NaCl, KCl, calcium, magnesium and other electrolytes are added, depending on the data provided by the blood and urine ionogram. The nutritional requirement is calculated considering the basal energy requirement and the additional energy consumption related to patient's suffering and initial nutritional status. The caloric requirement is about 25 kcal/kg body weight multiplied by a correction factor called stress index. The assessment of nutritional status is taking into account clinical criteria (such as

BMI, arm circumference, subcutaneous adipose fold) and biological criteria (total protein, albuminemia, prealbumin or transthyretin (TTR) with normal values of 310 ± 35 mg/L, transferrin 2.8 ± 0.3 g/L, retinol binding protein (RBP) with normal values of 62 ± 7 mg/L. These objective parameters can be used to assess malnutrition and to identify an improvement in nutrition after surgery (9, 10).

The literature on the prospective outcome factors of enteral tube feeding surgery is relatively limited. Surgical feeding techniques are numerous, but details on the long-term effectiveness of the methods or nursing challenges are not given. This study is assessing the predictive factors of the outcome of surgical procedures for enteral feeding. It was confirmed that the identification and monitoring of these parameters are important from stage I until the abolition of feeding stoma, the major goal being to maintain the necessary caloric values administered.

Most of the study patients underwent Dan Gavriliu's gastrostomy with peritoneal cuff, which aims, as major principles of continence, at creating an efficient gastric volume and maintaining the local morpho functional integrity). This procedure remains an effective surgical technique in the modern era, being characterized by a wide addressability (it can be practiced in any surgical department with minimal instruments and even under local anesthesia), indicated in patients with severe esophageal stenosis in different stages of therapy (8). Another benefit is the possibility of passing blended real food through G-tube, providing the necessary number of daily calories at affordable cost in low income patients requiring long-term enteral feeding. Quick recovery and easy nursing principles comply with the international efforts made to produce and sustain a change in medical practice in the direction of reducing hospitalization costs, with the concomitant development home-care health assistance, recuperative and palliative medicine services (11).

The main drawback of this technique is early postoperative gastric stasis, which is significantly increased compared to the minimal invasive approach.

PEG can be an excellent minimally invasive option in cases with a permeable digestive tract (12, 13), but there are limitations that need to be considered such as the costs and availability of the PEG kit and pre-hydrolyzed nutrient solutions, as well as the coexistence of a upper GI endoscopy service. Clinical studies revealed a low risk of respiratory complications in the case of neoplastic patients wearing both tracheostomy and gastrostomy, probably due to the smaller diameter of the tube. The clinical studies demonstrated that in patients diagnosed with inoperable head and neck who need tracheostomy it is recommended to perform a PEG while the digestive tract is still permeable and place a "stand-by" G-tube before the progression to dysphagia with a progressive degradation of the nutritional balance and cachexia (14-19). On the long-term follow-up of patients who underwent PEG, there may be limitations regarding the maximum daily calorie intake due to the osmotic effect and secondary induced diarrhea associated with high volumes of feeding solutions (20), but further studies are needed due to relatively short follow-up in this group.

Surgical gastrostomy is alternative when an endoscopic approach is not feasible. Post pyloric nutrition is indicated in patients with gastric feeding intolerance

and may be achieved through PEG with jejunal extension, percutaneous endoscopic jejunostomy, and surgical jejunostomy (10, 21, 22).

CONCLUSIONS

The classic and endoscopic percutaneous surgical feeding procedures complement each other, technical support and skills being important to perform both categories of techniques, select the technique carefully for the appropriate indication. After analyzing a significant number of surgical feeding procedure, we conclude that their integrity and long-term outcome were not influenced by the type of disease that required them. In early postoperative follow-up, the main difference between classical and minimally invasive surgical techniques was the reduced volume of gastric stasis after PEG and PEG-J, possibly influenced by their minimally invasive nature, the duration and type of anesthesia with reduced postoperative paresis, and non-digestive diseases for which these techniques were used (predominantly neurological). Recovery after PEG is faster than after surgical gastrostomy, but still, there are indications for a surgical gastrostomy: severe esophageal strictures, advanced head and neck malignancies, technical failure of PEG, or unavailability of the facility or funds to perform PEG or of the specific nutrient solutions. Even though surgical gastrostomy and jejunostomy are simple procedures, they should be done meticulously to achieve good results and avoid complications as these patients are already nutritionally compromised. The main long-term prognostic factor is the possibility of ensuring the correct number of calories and amount of food. The acquisition of nursing elements would make it possible to maintain these surgical feeding solutions both in the hospital setting, but especially in the outpatient setting.

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SALVAGE OF THE LIMB WITH CRITICAL SIZE BONE DEFECT USING FREE VASCULARIZED FIBULA

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SALVAGE OF THE LIMB WITH CRITICAL SIZE BONE DEFECT USING FREE VASCULARIZED FIBULA (Abstract): The critical bone defect is not yet well defined, but it is well known that the free vascularized fibula (FLAP) transfer represents the gold standard in the reconstruction of complex bone defects associated with other soft parts defects, both post-traumatic and belonging to other etiologies. **Material and methods**: We will be presenting a lot consisting of three male patients, that in regard to the wounds caused by an accident with industrial machines involved, were diagnosed with forearm-localized type III C Gustilo-Anderson open fractures, and residually displayed radius defects across a length of 10-12 centimeters, which required us to opt for free vascularized fibula transfer with cutaneous island, in all three cases. **Results:** The results were good in all cases. Within one single case the cutaneous island displayed a discreet venous congestion which demanded the use of leeches, and eventually turning out very well. **Conclusions:** The treatment of the critical bone defects required ample, microsurgical interventions performed in multidisciplinary teams, the autologous bone remaining the best option for bone reconstruction, being the best growth factor and bone stimuli source, with no immunological modifications or risk of disease transmission. **Keywords :** LIMB, CRITICAL BONE DEFECT, FLAP.

The management of post-traumatic bone defects and vicious consolidations and post excision for tumor pathology or other etiologic factors, represent a challenge within the everyday life orthopedic practice (1). The absence of a standardized definition of critical bone defects is frequently referenced in literature and determines conflicts of opinion regarding their management (2-4). A widely accepted definition in literature describes critical bone defects as being bigger than 2 centimeters and takes more than 50 percent of the long bones' circumference, but this theory is controversial when it comes to the anatomical region and the state of the soft tissue in the vicinity of the defect (3).

The relative rarity of this type of pathology leads to the lack of an evidence and some management guides. Therapeutic attitude in regard to bone defects requires a multidisciplinary understanding of the pathology, as well as considering the fac-

tors concerning the patient age, the existence of other chronic conditions, the nutritional status, smoking, the status of the neighboring tissues, the local vascularization and the severity and extension of the wound) and the factors that have to do with the surgeon's experience (1). The election treatment for critical bone defects remains the autologous bone transfer, due to the safety that it offers when it comes to the transmission of illnesses and the immunological response, as well as the fact that it is the most available source of growth factors and bone stimuli (5). It is the technique that conglomerates osteogenetic, osteoinductive and osteoconductive effects, which is the main reason why it is still regarded as the gold-standard method in the treatment of critical bone defects treatment (6). We will be presenting a series of three post-traumatic bone defects involving radius which demanded the free vascularized fibula transfer with cutaneous island.

MATERIAL AND THE METHODS

We have studied a series of three male patients with ages between 46 and 59 years. They were all the victims of some accidents caused by industrial instruments, displaying open, forearm-localized type III C Gustillo-Anderson fracture. In two of the cases, the left thoracic limb was involved and only in one of the cases, the right thoracic limb was, all of the three patients being right-handed. All three patients underwent surgery in the plastic surgery and reconstructive microsurgery clinic in Iasi, after the informed consent on their behalf had been obtained. The three patients residually displayed, after the emergency performed surgical intervention, radius-localized bone defects of between 8-10centimetres in size, placed in the superior third of the forearm (fig. 1).

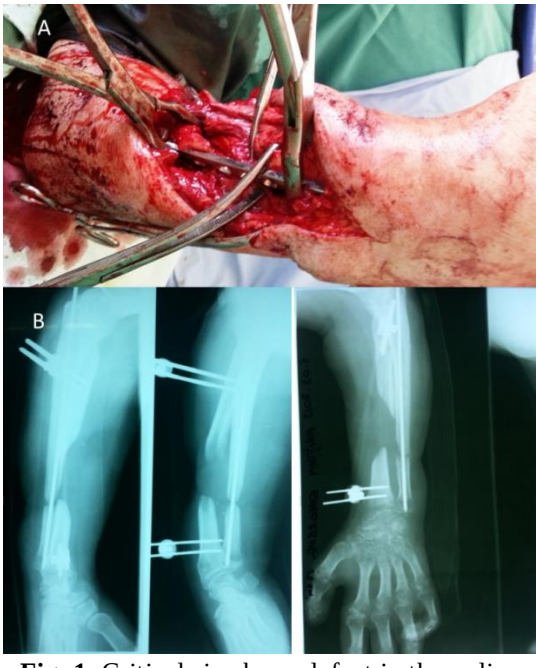

Fig. 1. Critical size bone defect in the radius
A. intraoperative image; B. radiological aspect

After the thorough analysis of the clinical parameters, associated pathologies and the vascular status of the donor, receptor areas and the radiologic examination performed in order to precisely estimate the bone defect.

In all of the cases, there were registered massive muscular losses in regard to the soft tissue parts, the initial wounds using cut by crushing mechanism. In all of the cases, a pre-surgery sketch was made after a Doppler exam, by marking the receptor vessel, the one included in the flap, the cutaneous island the bone segment that eventually required collection. In all of the cases the receptor area was around the pelvic floor, localized on the same side as the traumatized thoracic one (fig. 2).

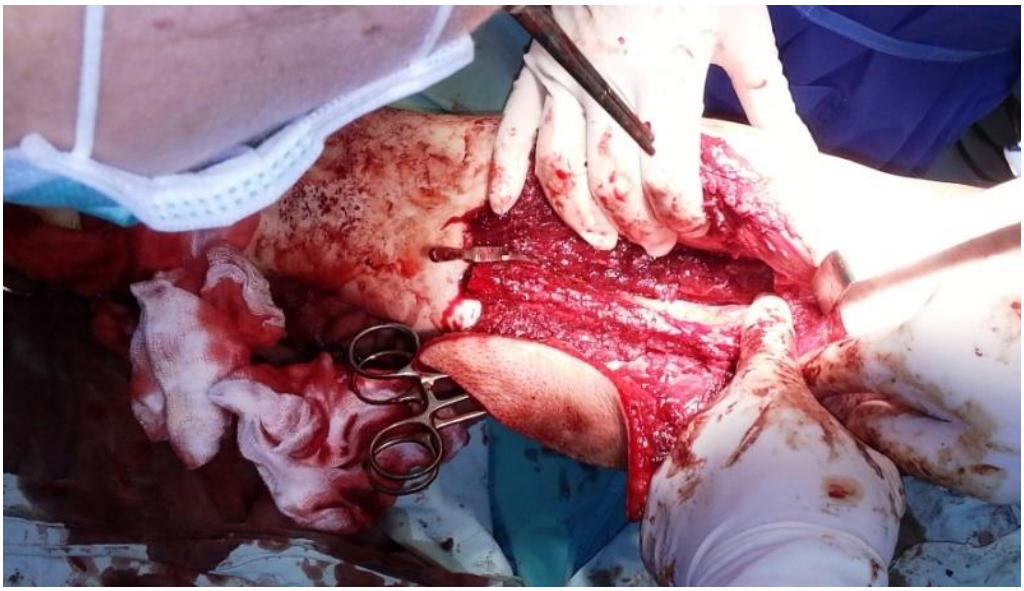

Fig. 2. Donor site

he surgical intervention took place under general anesthesia with oro-tracheal intubation. The osteosynthesis was done through the internal fixation stabilized with a plate and nails, these offering the advantage of an active mobilization with an important role in active and complete recovery (fig. 3, fig. 4).

The microsurgical intervention time went smoothly in all cases, with no incidents or accidents during the surgery. The microsurgical anastomosis was made between the peroneal artery of the flap and radial artery in all of the three cases. Two venous anastomosis were performed at each transfer. The post-surgical care was on average, 10 days. In all cases the flap included both the bone segment and the cutaneous island with sizes between 6 and 10 centimeters which was necessary for the coverage of the bone and the monitoring of the flap's viability. The closure of the donor zone was performed using a drainage. The average period of time in which the bone consolidation was obtained, radiologically objectified, was 5 months.

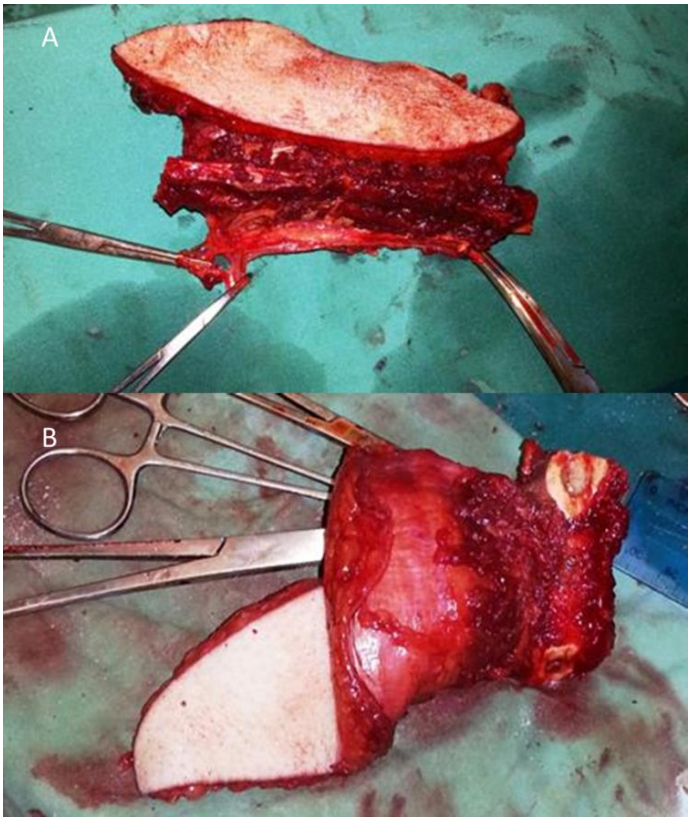

Fig. 3. A. Osteofasciocutaneous flap harvested from fibula and B. ready to be anastomosed at the recipient site

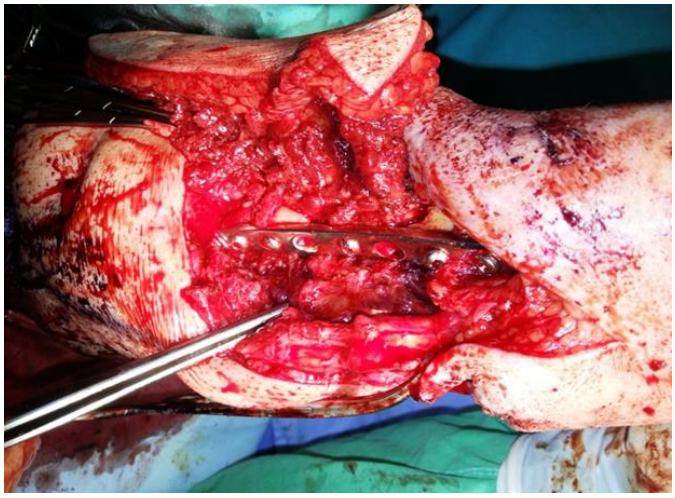

Fig. 4. Osteofasciocutaneous flap in place, after osteosynthesis with plate and screws and microsurgical arterial and venous anastomosis

RESULTS

In all three cases the post-surgical evolution was good. However, in the case of the oldest patient a slight venous congestion could be noticed, which was eventually treated by applying biological leeches for 3 days.

The cutaneous integration was qualitative 14 days after the surgical intervention.

The bone integration's assessment started 3 months after the surgery with a final evaluation expanding to 3 years (fig. 5).

Fig. 5. Radiological aspect A. Immediate postoperatively; B. At 3 years follow-up

In all cases the outcomes were very satisfactory. There were no registered immediate or distant complications and only in one case there was identified a venous congestion that was treated using biological leeches for 3 days (fig. 6).

Fig. 6. Clinical aspect at 3 years postoperatively. Morbidity on the donor area's level was minimum.

DISCUSSION

The indications concerning the reconstruction of a limb expanded throughout the last decades, which helped with avoiding amputation in a lot of cases. Vascularized bone transfer could be performed only after the 1960's, when Jacobson and Suarez perfected microvascular anastomosis techniques and the microsurgical instruments (7). In 1975, was performed the first free bone transfer for the reconstruction of a tibia defect. Since then and until now, it remains the workhorse flap for the reconstruction of limb-localized critical bone defects (8). FVFF passed the time stage and further enjoys success due to the several advantages that it incorporates (9). Because the fibula is a straight, tubular bone with a high density of the cortical and is sectionally triangle-shaped, it is regarded as ideal for the reconstruction of critical bone defects on the limbs (10, 11). Adults can have this bone harvested as a straight bone segment with a length of 26 centimeters. The fibula, having a diameter similar to the one of the radius and the ulnar bone and can easily enter the medullar channel of the tibia, femur and humerus (12, 13). Even though it benefitted from technological progress throughout the time, FVFF is still a complicated technique that requires a long surgery time that may increase the rate of possible complications and comorbidities when it comes to the donating areas, simultaneously introducing the idea of a lengthy anesthesia use, thus the indications concerning FVFF are extremely important and must be considered at all costs.

Critical post-traumatic bone defects are frequently associated with complex lesions of the soft tissues presenting reconstruction difficulties. Due to these reasons the use of the fibula as a composite osteo-fascio-cutaneous flap offers advantages to the concealing of a long and straight bone defect. At the same time, the reconstruction of a soft tissue defect of dimensions between 24/10 centimeters, through a unitary flap, anastomosed at only one vascular pedicle. The cutaneous island of the composite flap has an extremely important role in the post-surgery monitoring of the flap's viability (14). Among the FVFF indications there are included: segmentary loss of bones larger than 6-8 centimeters after tumor resection, traumatic bone loss, osteomyelitis, or the lack of the fracture's consolidation because of the infection (15, 16).

In the included cases of this study FVFF was used as a method of reconstruction of bone defects with sizes between 8 and 10 centimeters (17, 18). FVFF can be also suggested for smaller defects where the biological healing had failed, or a high risk of failure exists. The free vascularized fibular transfer can be considered as a therapeutic solution in the case of avascular bone necrosis, the loss of the bone and the soft tissues that require complex reconstructions and the young patients that require the conservation of the growing cartilage (19). There are cases in which the great defects of the soft tissues cannot be treated by using other techniques (20).

All patients included in this study were males, as well as more exposed to environments with higher risk of being traumatized (21, 22).

The estimated success rate in specialized literature varies between 76 and 100 percent (23, 24). The use of hirudotherapy for the treatment of venous congestion is frequently referenced in literature (25). The free transfer of vascularized fibula is an ample surgical intervention during which there can appear complications when it comes to the donor site. Complications when it comes to

the donor site can appear both during the free transfer of vascularized fibula and the anastomosis to the level of the receptor area and at the moment of bone fixation. The osteosynthesis mode and the consolidation time in the reported case are similar to the ones reported in literature.

CONCLUSIONS

The free transfer of vascularized fibula is commonly used for the reconstruction of critical size bone defects and leads to good results. Although technically, it is a complex intervention, it is preferred because the bone integration is qualitative and fast, the autologous bone remaining the best option for bone reconstruction, being the best source of growth factors and bone stimuli, with no immunological modifications or risk of disease transmission.

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NEWS

CANNABINDIOL LOADED MICROPARTICLE – A POTENTIAL DRUG TO IMPROVE BREAST CANCER HEMOTHERAPY

Breast cancer chemotherapy treatment toxicity is still a challenge for clinicians because, even if the cancer is treated, the quality of life is reduced due to the persistence of adverse reactions caused by anticancer drugs. A study recently published in *International Journal of Pharmaceutics* demonstrates the antiproliferative effect of Cannabidiol (CBD) as loaded microparticles on tumor cells models in vivo and *in ovo*. The study shows that CBD microparticles are efficient as monotherapy, and also in co-administration with Paclitaxel (PTX) and Doxorubin (DOX) relieving extended antiproliferative activity. The co-administration of CBD and PTX or DOX showed a synergistic effect, with the possibility to lower the doses for anticancer drugs, and so to decrease toxicity of these drugs and, in the same time, to improve the overall anticancer effect. On the other hand, it's proved that CBD is efficient in treating the nausea and pain, as side effects of chemotherapy, so the benefit is double. In conclusion, this study provides, for the first time, evidences of the potential use of CBD-loaded microparticles in combination with paclitaxel or doxorubicin in the treatment of breast cancer. (Fraguas-Sánchez AI, Fernández-Carballido A, Simancas-Herbada R, *et al*. CBD loaded microparticles as a potential formulation to improve paclitaxel and doxorubicin-based chemotherapy in breast cancer. *Int J Pharm* 2020; 574: 118916).

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WHAT GOES WRONG IN SURGICAL PATIENTS WITH CONCOMITANT *CLOSTRIDIUM DIFFICILE* INFECTION DISEASE? RISK FACTORS FOR IN-HOSPITAL MORTALITY

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WHAT GOES WRONG IN SURGICAL PATIENTS WITH CONCOMITANT *CLOSTRIDIUM DIFFICILE* INFECTION DISEASE? RISK FACTORS FOR IN-HOSPITAL MORTALITY (Abstract) **Aim:** to evaluate the possible anthropometric, clinical, biological, and pathological factors that could be associated with an increased mortality of the surgical patients with concomitant CD infection. **Material and methods:** The study was carried out on a group of 123 surgical patients who were operated in the IIIrd Surgical Unit of the "Sf. Spiridon" University Hospital Romania and were diagnosed with CDI during hospitalization. **Results:** We noted 20 deaths among the patients from our study group. Characteristic for the deceased patient's group was the presence of sepsis before or after CDI diagnosis ($p = 0.001$). The involving of a parenchymal organ ($p = 0.001$) and the presence of metastases ($p = 0.025$) were significantly higher in the deceased patient's group. Reintervention induced an estimated risk of death approximately 2-fold higher ($p = 0.002$). The estimated risk of death was slightly higher in patients with renal chronic disease ($p = 0.001$), obesity ($p = 0.009$), Diabetes Mellitus ($p = 0.005$) and chronic cardiac insufficiency ($p = 0.05$). All patients who died had surgical diseases requiring postoperative antibiotic treatment ($p = 0.001$) especially with Cephalosporin ($p = 0.003$) and Clindamycin ($p = 0.047$). The use of proton pump inhibitor and anticoagulant medication was statistically higher in patients who died. **Conclusions:** Unfavorable evolution of surgical patients with CDI is highly associated with obesity, renal chronic disease, chronic cardiac insufficiency, diabetes, chronic oral anticoagulants prior to CDI, treatment with third generation cephalosporin, clindamycin, and PPI after surgery. **Keywords:** *CLOSTRIDIUM DIFFICILE* INFECTION, SURGICAL PATIENTS, MORTALITY IN SURGICAL PATIENTS WITH CD INFECTION.

Clostridium difficile infection (CDI) is the most frequent nosocomial infection. Infection of the colon with the *Gram-positive* bacterium *Clostridium difficile* is potentially life threatening, especially in elderly people and in patients who have dysbiosis of the gut microbiota following antimicrobial drug exposure (1). There are some medical conditions, treatments and other factors that have already been demonstrated to increase the risk for CDI. Most important of those are sex, age, antibiotic treatment, and proton pump inhibitor (PPI) use, preexistent inflammatory bowel disease, healthcare facility contact and

obesity (2-7).

The aim of the study was to analyze the in-hospital mortality of the surgical patients diagnosed with CDI. We also aimed to evaluate the possible anthropometric, clinical, biological and pathological factors that could be associated with an increased mortality of the surgical patients with concomitant CD infection.

MATERIAL AND METHODS

We studied the files of all consecutive surgical patients who were operated in the IIIrd Surgical Unit of the "Sf. Spiridon" University Hospital Iasi, Romania, between 2015 and 2019 and were diagnosed with CDI during hospitalization. Data were retrospectively collected, and we included in our study emergency and elective surgical cases, with oncologic and nononcologic diseases. The inclusion criteria were surgical patients of more than 18 years old who developed postoperatively CDI. Patients with relapsed CDI were excluded from our study cohort. Our study group was We noted the general data, such as sex, age, blood type and provenience of the patient. The main surgical diagnosis, the type of the disease (oncologic, emergency), the associated diseases and postoperative complications were also noted. Regarding the surgical treatment of the patients, accordingly to the main disease, we evaluated the type of intervention in terms of the affected organ (parenchymal or hollow viscus), the type of operation (emergency or elective surgery), the need of mechanic bowel preparation before surgery, antibioprophylaxis, prophylaxis of CDI, the using of any other medication, the treatment of CDI and the length of stay before and after diagnosis of CDI. The infection with *Clostridium difficile* was documented by EIA (enzyme immunoassay) testing of toxin A and B from the stools. The studied group was divided into two subgroups, consisting in patients who survived and patients who died. *Statistical analysis*. Categorical variables were reported as percentages, and differences between groups were statistically assessed using *ANOVA* and *Fisher's exact test.* Statistical analyses were performed using *SPSS 11.0* (SPSS Inc., Chicago, IL, USA) and $p < 0.05$ was considered statistically significant.

RESULTS

143 patients with surgical interventions and postoperative developed CDI were initially included in the study. Of those, 20 patients presented one or multiple relapsing infections, so they were excluded. In the end, 123 patients were eligible, of whom we noted 20 deaths. None of the patients in our study developed toxic megacolon and surgical intervention as treatment for CDI was not indicated in any patient, as all the patients in our study had mild-moderate forms of CDI.

The group of patients who developed CDI postoperatively and died had a slightly higher average age compared to the survivors (67.55 *vs.* 65.61 y; $p = 0.526$). They were predominantly male (65% *vs.* 54.4%; $p = 0.464$) and had urban origin (60% *vs.* 58.3%; $p = 0.545$) (tab. I).

Characteristic for the deceased patient's group was the infection with both types of toxin (A and B) (100% *vs.* 76.9%; $p = 0.016$) and the presence of sepsis before or after CDI diagnosis (45% *vs.* 0%; $p = 0.001$). Referring to the main surgical disease, the involving of a parenchymal organ (45% *vs.* 9.7%; $p = 0.001$) and the presence of metastases (10% *vs.* 0%; $p = 0.025$) were significantly higher in the deceased patient's group (tab. I).

TABLE I

Clinical characteristics related to lethality

Parameter	*Clostridium difficile infection*		Statistically test	p
	Death (n=20)	Survivor (n=103)		
Age (years)	67.55±10.52	65.61±12.81	One-way ANOVA	0.526
Male	65.0%	54.4%	Fisher's Exact Test	0.464
Urban provenience	60.0%	58.3%	Fisher's Exact Test	0.545
O I	25.0%	57.3%	Fisher's Exact Test	**0.013**
Rh -	20.0%	5.9%	Fisher's Exact Test	**0.050**
Hospital days before infection	9.95±7.45	9.34±8.09	One-way ANOVA	0.755
Hospital days after infection	11.95±8.13	9.30±7.85	One-way ANOVA	0.172
Both toxin A and B	100.0%	76.9%	Fisher's Exact Test	**0.016**
Emergency	70.0%	62.1%	Fisher's Exact Test	0.616
Benign disease	55.0%	58.3%	Fisher's Exact Test	0.809
Malignant disease	45.0%	41.7%	Fisher's Exact Test	0.809
Associated diseases	90.0%	80.6%	Fisher's Exact Test	0.524
Digestive hollow organ affected	55.0%	70.9%	Fisher's Exact Test	0.192
Digestive parenchymatous organ affected	45.0%	9.7%	Fisher's Exact Test	**0.001**
Nondigestive organs affected	15.0%	20.4%	Fisher's Exact Test	0.762
Sepsis	45.0%	0.0%	Fisher's Exact Test	**0.001**
Metastasis	10.0%	0.0%	Fisher's Exact Test	**0.025**

When evaluating thy type of surgical procedure, the highest estimated risk of death was associated with the performance of an enterectomy (RR = 5.15; IC95: 0.34-78.9; p = 0.736) and necrosectomy (RR = 5.15; IC95: 0.77-34.5; p = 0.424), but the result cannot be extrapolated to the general population. Reintervention induced an estimated risk of death approximately 2-fold higher (RR = 1.93; IC95: 1.00-3.69; p = 0.002) (tab. II).

When we evaluated the risk of mortality in relation with comorbidities, the statistical analysis showed that the estimated risk of death was slightly higher in patients with renal chronic disease (RR = 1.91; IC95: 1.06-3.45; p = 0.001), obesity (RR = 1.39; IC95: 1.0-1.91; p = 0.009), Diabetes Mellitus (RR = 1.33; IC95: 1.04–1.69; p = 0.005) and chronic cardiac insufficiency (RR = 1.18; IC95: 1.05–1.37; p = 0.05) (tab. III).

TABLE II

Relation between type of surgical procedure and mortality in patients with concomitant surgical disease and *Clostridium difficile* infection

Surgical intervention	Death (n=20)		Survivor (n=103)		Fisher's Exact Test	RR	CI 95%
	n	%	n	%			
Bowel Mechanic Preparation	1	5.0	19	18.4	0.192	0.86	0.75-0.98
Amputation	3	15.0	8	7.8	0.542	1.93	0.56-6.66
Biliodigestive anastomosis	2	10.0	4	4.9	0.552	2.58	0.51-13.1
Cholecystectomy	2	10.0	7	6.8	0.973	1.47	0.33-6.57

Surgical intervention	Death (n=20)		Survivor (n=103)		Fisher's Exact Test	RR	CI 95%
	n	%	n	%			
Conservative treatment	1	5.0	2	1.9	0.984	2.58	0.25-27.1
Cure of hernia, bowel resection	2	10.0	10	9.7	0.968	1.03	0.24-4.35
Duodenopancreatectomy	1	5.0	3	2.9	0.631	1.72	0.19-15/7
Enterectomy	1	5.0	1	1.6	0.736	5.15	0.34-78.9
Hartmann procedure	2	10.0	6	5.8	0.843	1.72	0.37-7.90
Necrosectomy	2	10.0	2	1.9	0.242	5.15	0.77-34.5
Rectal resection	1	5.0	4	3.9	0.822	1.05	0.67-1.64
Reinterventions	6	30.0	5	4.9	**0.002**	1.93	1.00-3.69

TABLE III

Estimate risk of lethality related to associated morbidities.

Comorbidity	Death (n=20)		Survivor (n=103)		Fisher's Exact Test	RR	CI 95%
	n	%	n	%			
Diabetes mellitus	11	55.0	23	22.3	**0.005**	1.33	1.04-1.69
Obesity	8	40.0	14	13.6	**0.009**	1.39	1.00-1.91
Hypoalbuminemia	10	50.0	33	32.0	0.133	1.14	0.48-1.37
Cirrhosis	4	20.0	9	8.7	0.223	1.23	0.85-1.79
Renal Chronic Disease	7	35.0	6	5.8	**0.001**	1.91	1.06-3.45
BPOC	1	5.0	4	3.9	0.822	1.05	0.67-1.64
Chronic Cardiac Insufficiency	14	70.0	46	44.7	**0.050**	1.18	1.05-1.37

Biochemical parameters showing the presence of inflammatory syndrome had significantly higher mean values in deceased patients in comparison with the survivors (p = 0.001). The mean value of White blood cells, C Reactive Protein (CRP) and platelets lymphocyte ratio were higher in the deceased patient's group (tab. IV).

Among the deceased patients, 90% received preoperative antibioprophylaxis treatment (90% *vs.* 51.5%; p = 0.001). All patients who died had surgical diseases requiring postoperative antibiotic treatment, but prior to CDI diagnosis (100% *vs.* 65%; p = 0.001) especially with Cephalosporins (60% *vs.* 25.2%; p = 0.003) and Clindamycin (10% *vs.* 1%; p = 0.047). The use of proton pump inhibitor and anticoagulant medication was statistically higher in patients who died. Also, all deceased patients received CDI prophylaxis and CDI treatment (tab. V).

TABLE IV

Biomedical parameters in patients with *C. difficile* diseases related to mortality

Parameter	*Clostridium difficile*		One-way ANOVA p
	Death (n=20)	Survivor (n=103)	
White blood cells, n x 1,000/mmc	21.14±6,20	13.62±4.45	**0.001**
CRP, mg/dL	23.65±8.47	4.52±2.28	**0.001**
PLR	222.05±66.85	115.68±32.92	**0.001**

TABLE V
Treatment in patients with *C. difficile* diseases related to lethality

Parameter	*Clostridium difficile*		Fisher's Exact Test p
	Death (n=20)	Survivor (n=103)	
Antibioprophylaxis	90.0%	51.5%	**0.001**
Antibiotherapy	100.0%	65.0%	**0.001**
Prophylaxis of *C. difficile*	100.0%	24.3%	**0.001**
Quinolones	40.0%	22.3%	0.156
Penicillin	30.0%	32.0%	0.857
Third generation Cephalosporins	60.0%	25.2%	**0.003**
Clindamycin	10.0%	1.0%	**0.047**
Other	5.0%	38.8%	**0.003**
No antibiotics	0.0%	23.3%	**0.012**
H2 Rec Antagonist	60.0%	56.3%	0.810
Proton Pump Inhibitor (PPI)	65.0%	30.1%	**0.005**
Low molecular weight anticoagulant	98.2%	89.6%	0.243
Chronic oral anticoagulant treatment	65.0%	17.5%	**0.001**

DISCUSSION

Risk factors for CDI may be divided into three general categories: host factors (immune status, comorbidities), exposure to *Clostridium difficile* spores (hospitalizations, community sources, long-term care facilities), and factors that disrupt normal colonic microbiome (antibiotics, other medications, surgery) (7). As in our study all the patients had postoperatively developed CDI infection, we tried to find the differences between the characteristics of deceased patients and of the survivors by referring not only to the underlying diseases, types of surgery and treatment but also to the factors related to the CDI infections, meaning the risk factors and the treatment of it.

Regarding the use of antibiotics, although nearly all antibiotics have been associated with CDI, clindamycin, third generation cephalosporins and fluoroquinolones have traditionally been considered to pose the greatest risk (8, 9). Our study shows that in the deceased patient's group, the use of third generation cephalosporins and clindamycin was higher than in the survivor's group. We mention that all patients received specific treatment for CDI in accordance with the guidelines (10).

Exposure to gastric acid-suppressive medications, such as histamine-2 blockers and PPI, may be a potential risk factor for development of CDI, therefore there are studies that conclude that given that PPI are overprescribed in surgical settings, consideration should be given to stop PPI, when they are not necessary, especially in patients at high risk of CDI (11, 12). In our study, the proportion of use of the PPI was higher in the deceased patients. Another type of treatment we referred to in our study is the chronic anticoagulant treatment prior to CDI, whose percentage is higher (p=0.001) in the group of deceased patients. Up to date, we found no data in literature showing that chronic oral anticoagulants is somehow related to CDI. We consider that our statistical result shows a higher incidence of comorbidities requiring

chronic anticoagulant treatment in surgical patients who developed CDI for whom the evolution was unfavorable.

We also assessed the type of surgical disease, procedure and type of organ affected by the main disease for which the patient was hospitalized. In terms of type of intervention, we found no differences between the rate of emergency procedures *vs.* elective procedures in the two groups formed by the deceased patients and the survivors. More, the rate of death was not associated with a specific type of intervention or organ approached by surgery. Only the rate of reinterventions was statistically higher in the group of deceased patients (p=0.002). The presence of sepsis nonrelated to CDI, metastases and the parenchymatous organ (liver or pancreas) as the site of the primary surgical site of the surgical disease were associated with a higher mortality.

It has been already demonstrated that there are some types of surgical procedures that are more likely to increase the risk of CDI, such as colectomy, small-bowel resection, gastric resection and lower extremity amputation (13, 14), but there have never been demonstrated a relation between a specific type of surgical intervention and higher mortality in surgical patients developing postoperative CDI. Assessing the comorbidities of the patients, as expected, diabetes, obesity, renal chronic disease and chronic cardiac insufficiency were associated with unfavorable evolution of the patients. It is already known that diarrhea in CDI may be accompanied by mild abdominal pain and cramps and if prolonged, it may result in altered electrolyte balance and dehydration. When this occurs in patients with severe comorbidity, particularly after surgery, non-severe CDI may increase morbidity significantly (15).

CONCLUSIONS

Death of the patients with *Clostridium difficile* infection occurring postoperatively is associated with the presence of sepsis, metastases and reinterventions. Unfavorable evolution of surgical patients with CDI is highly associated with obesity, renal chronic disease, chronic cardiac insufficiency, and diabetes. Patients requiring chronic oral anticoagulants prior to CDI and patients requiring treatment with third generation cephalosporins, clindamycin and PPI after surgery are associated with a higher mortality rate.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

ACUTE PANCREATITIS DURING COVID-19

Abdominal pain is one of the symptoms associated with coronavirus disease 2019 (Covid-19). The authors report the presence of acute pancreatitis in two of three family members with severe Covid-19 infection, excluding other causes of acute pancreatitis. All three cases were admitted to the hospital emergency department, with a positive test for SARS-Cov-2 RNA, developing acute respiratory distress syndrome. The first patient presented elevated amylasemia 11h after admission (>1500U/L), with evidence of acute pancreatitis on the abdominal ultrasound. The second patient complained of epigastric and periumbilical pain at admission. High amylasemia (>934U/L) was recorded 6 days after admission. In both cases The Modified Glasgow Acute Pancreatitis Score was 5 points, suggesting severe acute pancreatitis. The third patient presented respiratory distress and gastrointestinal symptoms, but showed no clinical and paraclinical signs of associated acute pancreatitis. These cases highlight acute pancreatitis as an associated complication of Covid-19 and mark the importance of measuring pancreatic enzymes in patients with SARS-CoV2 infections, especially if patients experience abdominal pain. (Hadi A, Werge M, Kristiansen KT, Pedersen UG, Karstensen JG, Novovic S et al. Coronavirus Disease-19 (COVID-19) associated with severe acute pancreatitis: Case report on three family members. *Pancreatology* 2020; 20(4): 665-7).

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DOES LONG PREOPERATIVE URETERAL DOUBLE J STENTING INFLUENCE THE OUTCOMES OF RETROGRADE URETEROSCOPY FOR URETERAL STONES? A MATCHED-PAIRED CASE-CONTROL STUDY

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DOES LONG PREOPERATIVE URETERAL DOUBLE J STENTING INFLUENCE THE OUTCOMES OF RETROGRADE URETEROSCOPY FOR URETERAL STONES? A MATCHED-PAIRED CASE-CONTROL STUDY (Abstract): There is a lot of debate about whether stenting is required or not prior retrograde semirigid ureteroscopy (URS). Moreover, the effect of long-term preoperative stenting on stone-free rate (SFR) and complication rate of URS. The **aim** of this study was to evaluate the effect of long-term preoperative double J stenting compared with non-stenting and recent pre-stenting on the outcomes of retrograde URS. **Material and methods**: The data of 90 patients admitted to the Urology Clinic of the Iasi "Dr. C. I. Parhon" Hospital between January 2017 and March 2020 were collected. The patients were divided into three equal groups, matched by age, sex, stone size and location: 30 non-stented patients, 30 patients stented three months before URS, and 30 patients with stents placed for over three months before URS. SFR and the rate of main URS complications (febrile syndrome and hematuria) were in the three groups were compared. **Results**: There were no statistical differences between groups in terms of SFR (73%. 77%, 80%, p = 0.6). The complication rate was similar in all three groups, for both febrile syndrome and hematuria. **Conclusions**: Long term preoperative double J stenting does not influence the SFR of retrograde semirigid URS. In all groups, the complication rate is very low and not influenced by preoperative stenting. **Keywords:** RETROGRADE URETEROSCPY, LONG-TERM PREOPERATIVE DOUBLE J STENTING, STONE-FREE RATE.

Retrograde ureteroscopy (URS) is a well-established method for treating ureteral stones (1). The success rate ranges from 79 to 97% for distal ureteral stones (2, 3), and 68 to 78% for proximal stones (4, 5, 6), with a general success rate of 84.5 to 94.2% (4). A method to improve the outcomes of URS may be the insertion of a double J stent before the procedure in order to dilate the ureter and facilitate the passage of the ureteroscope. Many studies have reported conflicting results, most authors stating that preoperative stenting helps improve stone free rate (SFR) and

lowers the complication rate (5, 6, 7, 8) no matter of stone size (less or more than 10 mm) (9), while others concluded that pre-stenting does not improve SFR (10, 11) and rises the likelihood of complications (12). Moreover, little information exists about the effect of long-term preoperative stenting, most studies being conducted in patients with short-term preoperative stenting (13, 14). That is why we conducted a study in which we compared the stone free rates and complications of 3 groups of patients who underwent long-term preoperative stenting, short preoperative stenting, and no-stenting, respectively.

MATERIAL AND METHODS

We conducted a retrospective match-paired case-control study of the patients admitted for URS to the Clinic of Urology of the Iasi "Dr. C. I. Parhon" Hospital between January 2018 and March 2020 (26 months). Data were extracted for patients' medical records. All URSs were performed with a semirigid *Olympus* ureteroscope, 8CH at the tip.

First, enrolled in the study were 30 patients with double-J stents inserted for over three months before USR: 10 with pelvic stones, 10 with iliac stones, and 10 with lumbar stones. The largest stone sizes ranged from 6 to 15 mm. In each 10-patient subgroup 5 had stones less or equal to 10 mm and 5 had stones over 10 mm in size. Then, 60 control patients were enrolled: 30 patients in whom a 6 to 8 CH preoperative stent were placed for infected hydronephrosis or persistent pain and 30 patients without preoperative stents. In each of the two control groups 10 patients had pelvic stones, 10 iliac stones, and 10 lumbar stones, also matched for gender, decade of life, and stone size with the study group. All patients were diagnosed with radiopaque stones by plain films, associated with urography and/or echography. SFR and immediate surgery specific complications (febrile syndrome, postoperative hematuria) were compared between groups. The febrile syndrome was defined as a temperature of over 38°C detected in the first 48 postoperative hours. Also considered was postoperative macroscopic hematuria developed during the first 48 hours after URS. The patients with no ureteral opacity on the plain films performed on postoperative days 1 or 2 were declared stone-free. The patients who could not be matched in one of the three above-described groups were excluded from the study. Also excluded were the patients with stones over 15 mm, multiple ureteral stones, radiolucent stones, calcified stents, or in which the stents were not placed or ureteral stones but for other conditions, such as: ureteral stenosis, neurological bladder, and neoplasms. Patients with incomplete medical records were also excluded. Enrollment was stopped when the number of 30 cases in each group was completed.

Statistical analysis. Because all three groups consisted of 30 cases, it was considered a normal distribution of data. Means and standard deviations (SD) were used for the description of quantitative variables. The *t-Student* test was used to compare the means and chi-square test to compare the proportions. For the differences in the means between the groups we used the ANOVA test for independent measures. To compare the results between the subgroups we used the Fischer test for small numbers and chi-square for more than 2x2 groups. All the results were considered statistically significant at $p < 0.05$.

We used the online statistics package socialstatistics.com.

RESULTS

The study included 90 patients who underwent URS, mean age of 57±16 years, with a predominance of females (57/90, 63.3%). Stones were equally located on the left and right side: 45 patients had stones on the right side and 45 patients had stones on the left side. Thirty patients had lumbar stones, 30 patients had iliac stones, and 30 patients had stones located in the pelvic segment of the ureter. In 45 patients stone size was no more than 10 mm and 45 patients had stones over 10 mm. According to the study design there were 3 groups of 30 patients each, having as a criterion of classification the presence or absence of a previously inserted double-J stent, and the patients with a stent were further grouped depending on the time of stent insertion (more or less than 3 months before URS). There were no differences between groups in terms of mean age, gender, stone location and side due to the study design. The demographic characteristics of the three groups are listed in first table.

TABLE I

Demographic characteristics of the three groups

	Group 1 > 3 months stent (n_1 = 30)	Group 2 No stent (n_2 = 30)	Group 3 <3 months stent (n_3 = 30)	p
Age (yrs.) mean ± SD	56 ± 15	54 ± 17	61 ± 14	NS
Female	19	19	19	NS
Left side URS	15	15	15	NS
Lumbar stone <10 mm/ >10 mm	5/5	5/5	5/5	NS
Iliac stone <10 mm/ >10 mm	5/5	5/5	5/5	NS
Pelvic stone <10 mm/ >10 mm	5/5	5/5	5/5	NS

The stone-free radiographic status was achieved by 69 of the 90 patients (77%). When SFR was compared between groups no significant differences were found between patients who underwent URS without stenting compared to those with stents placed for less or more than three months before the procedure (22/30, 73% *vs.* 23/30, 77% *vs.* 24/30, 80%, p=0.6) (tab. II).

SFR in the 3 groups ranged from 60% to 100% (no matter of stone size) but without statistically significant differences (70% *vs.* 70% *vs.* 80%, p = 0.8 for lumbar stones SFR, 60% *vs.* 60% *vs.* 80%, p = 0.6 for iliac stones SFR, and 90% *vs.* 80% *vs.* 100%, p = 0.4 for pelvic stones SFR). Also, the general success rates of URS did not achieve statistical significance in stones less than 10 mm (4/5 patients *vs.* 4/5 patients *vs.* 4/5 patients, p = 1.0 for lumbar stones SFR, 3/5 patients *vs.* 4/5 patients *vs.* 4/5 patients, p = 0.7 for iliac stones SFR, and 5/5 patients *vs.* 5/5 patients *vs.* 5/5 patients, p = 1 for pelvic stones SFR) or in stones over 10 mm (3/5 patients *vs.* 3/5 patients *vs.* 4/5 patients, p = 0.7 for lumbar stones SFR, 3/5 patients *vs.* 4/5 patients *vs.* 4/5 patients, p = 0.4 for iliac stones SFR,

and 4/5 patients *vs.* 3/5 patients *vs.* 5/5 patients, p = 0.4 for pelvic stones SFR). Ureteroscopic failures were due to the thin ureter which did not allow acute dilation and inflammation below the stone. In both situations, the ureteroscope could not be advanced up to the stone to allow lithotripsy. There was a single case, in group 2, in which a stone fragment migrated to the pyelocaliceal system thus the patient did not achieve the stone-free status. No severe complications, such as sepsis, or avulsion, were found. One case in each group had postoperative febrile syndrome, which was treated with intravenous antibiotics. Persistent postoperative hematuria complicated the disease course of 4/30 patients (12%) in group 1, 3/30 patients (10%) in group 2, and 3/30 patients (10%) in group 3 (p = 0.9).

TABLE II

Results and complications of retrograde ureteroscopy

	Group 1 > 3 months stent (n_1 = 30)	Group 2 No stent (n_2 = 30)	Group 3 < 3 months stent (n_3 = 30)	p
Total SFR	22/30 (73%)	23/30 (77%)	24/30 (80%)	0.6
Lumbar				
<10 mm	4/5	4/5	4/5	1.0
>10 mm	3/5	3/5	4/5	0.7
Total lumbar SFR	7/10 (70%)	7/10 (70%)	8/10 (80%)	0.8
Iliac				
<10 mm	3/5	4/5	4/5	0.7
>10 mm	3/5	4/5	2/5	0.4
Total iliac SFR	6/10 (60%)	8/10 (80%)	6/10 (60%)	0.6
Pelvic				
<10 mm	5/5	5/5	5/5	1.0
>10 mm	4/5	3/5	5/5	0.4
Total pelvic SFR	9/10 (90%)	8/10 (80%)	10/10 (100%)	0.4
Complications				
Febrile syndrome	1/30	1/30	1/30	1.0
Hematuria	4/30	3/30	3/30	0.9

DISCUSSION

In our study there were no differences between groups in terms of the placement of a preoperative double-J stent and the timing of preoperative stenting. Our results agree with those reported by other researchers (10), finding no advantage in placing a double-J stent prior to URS. In practice many patients receive a double-J stent. This procedure is not justified by the facilitated ureteroscopic access before ureteral dilatation. Most patients are emergency cases and receive a double-J stent for pain relief or infected hydronephrosis. We believed that stents could help increasing the SFR, but our study failed to confirm that, as reported in the literature (15). Some studies reported more complications of long-term double-J stenting, such as calcifications or increased risk for sepsis (12, 16), while others did not (17). Our results support the harmlessness of preoperative stenting in terms of a greater risk for post-URS sepsis. Another interesting result was that there were no differences

in SFRs when stone locations (lumbar, iliac and pelvic) were compared. This finding disagrees with other studies which stated that SFR in greater in distally located stones (8). With thinner ureteroscopes, such as ours, advancement is possible without acute (18) or chronic (19) ureteral dilatation, explaining the similar results obtained in the non-stented group, compared with stented groups. Our overall SFR of 77% is lower than that reported by other authors (8, 9). The explanation for the results of over 90% SFR is that probably the patients were operated early in the course of the disease, thus avoiding inherent inflammation and edema around the calculi, which are known as factors hindering ureteroscope advancement. The postoperative complications were very rare in all three study groups, especially the febrile syndrome, probably because in all patients with urinary infections antibiotic therapy (second or third generation cephalosporins) was initiated at least 48 hours preoperatively. Another reason for this low incidence of the febrile syndrome is that we did not routinely apply high pressure fluid irrigation during surgeries, preferring to finish the procedure instead of forcing the high-pressure advancement of the ureteroscope, another reason for the low rate of iatrogenic ureteral injuries. Postoperative hematuria was a minor complication that remitted spontaneously in all cases. The stented patients had no more febrile syndrome or prolonged hematuria than non-stented patients, suggesting that stenting has no influence on URS complication rate (11), contrary to other studies in which stented patients had lower complication rates (5, 12, 8). The protective effect is probably due to other factors, including operator variability. If other urological departments reported an 8% pre-stenting rate prior to URS (20), in our department the majority of URSs are performed in pre-stented patients (21, 22).

CONCLUSSIONS

In our opinion, long-term preoperative double-J stenting before URS does not influence the SFR and incidence of complications compared with non-stenting and recent stenting, no matter of stone size and location.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE IMPACT OF TRANSCATHETER AORTIC VALVE IMPLANTATION IN PATIENTS WITH AORTIC STENOSIS

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THE IMPACT OF TRANSCATHETER AORTIC VALVE IMPLANTATION IN PATIENTS WITH AORTIC STENOSIS (Abstract): Aortic stenosis (AS) is the most common valvopathy in elderly patients, with an increasing prevalence due to aging process of population, current guidelines recommending transcatheter aortic valve implantation (TAVI) as a choice of intervention**.** Frailty is an important predictor of outcomes in AS patients who undergo TAVI, but there is no consensus in its preoperative evaluation. **Aim:** to compare the clinical outcomes of TAVI with conventionally surgically treated patients. **Material and methods:** The data of all aortic valve procedures performed in over 65 years patients, treated at Cardiovascular Institute from Iasi (January 2015-September 2019) were analyzed. The propensity score was used to compare the prognostic of patients. **Results:** The target group included 70 patients referred to TAVI compared to a second group of 98 patients who undergone surgical aortic valve replacement (SAVR). The mean age of TAVI patients was higher than SAVR group (76.47 *vs.* 64.37). The heart failure associated with the valvular pathology was more severe in the TAVI group (61.43% patients included NYHA class III and 35.71% patients NYHA class IV) compared to the SAVR group (58.16% patients were NYHA class III and 7.14% patients were NYHA class IV). Atrial fibrillation was the most frequent arrhythmia found in both groups (13.57% TAVI and 41.11% SAVR). **Conclusions:** The present study highlights the place of TAVI in current treatment of high surgically risk patients with SA. **Keywords:** FRAILTY, AORTIC STENOSIS, TRANSCATHETER AORTIC VALVE IMPLANTATION, SURGICAL AORTIC VALVE IMPLANTATION.

The prevalence of elderly patients with cardiovascular (CVD) comorbidities is constantly growing due to both the demographic trend of population ageing (81 years old) and effective options for screening, prevention and management of potentially lethal diseases in middle-aged individuals (65 years). The incidence of CVD is estimated to increase from 40% in 40-59 years old patients to more than 70% among those over 65 years old (1).

In this context, a new concept was de-

veloped, frailty, which defines that category of patients, with limited physical reserves, multiple comorbidities and increased vulnerability to stressors (2). Frailty affects 10% of adults over 65 years old, with a high prevalence, up to 60% among those with cardiovascular disease (3). The frailty phenotype is evident in patients with degenerative aortic stenosis (AS), that affects mainly patients over 60 years old, reaching about 10% among patients over 80 years old (4). Conventional risk scores, such as Society of Thoracic Surgeons (STS) score and European System for Cardiac Operative Risk Evaluation (EuroSCORE) II are relatively accurate tools in estimating the risk of mortality in isolated SAVR but limited in TAVI due to a proven tendency of overestimation (5).

The aim of this study was to perform a comparative analysis of the profile and prognosis of patients who have benefited from TAVI with a comparable group of patients with surgical aortic valve replacement (SAVR).

MATERIAL AND METHODS

Study population

An unicentric, retrospective, cohort study was conducted in Cardiovascular Institute from Iasi in order to evaluate the early prognostic of patients with severe aortic stenosis who underwent TAVI admitted between January 2015 - September 2019 compared to a group of patients with the same pathology who underwent isolated SAVR with biological prosthesis. The control group was formed by correlating the propensity score. Patients were followed up for one year after the intervention with re-evaluations at 30 days, 6 months and 12 months.

We excluded from the study patients undergoing SAVR for aortic regurgitation and those with others concomitant cardiac procedures.

Study design and Endpoint definitions

To compare the differences between the outcomes of patients undergoing TAVI or isolated SAVR, a propensity score-matched analysis was performed.

To confirm the presence of a complication, the same parameters were used both in patients who underwent TAVI or SAVR:

- angina - the presence of one or more coronary lesions treated with medications, interventional or surgical revascularization,
- perioperative mortality - mortality occurred in the first 30 days after the intervention,
- postoperative AKI - increase in serum creatinine by over 150% compared to preoperative value or need for dialysis,
- stroke or transient ischemic attack - permanent or transient motor or sensory deficit,
- minor or major vascular complications - consistent with VARC-2 recommendations (6).

Statistical methods

Pre-, intra-, and postoperative information of the patients included in the study were introduced into a database using *Microsoft Excel* 15.20 for Mac OS X and were statistically analyzed using *IBM SPSS Statistics 24.0* program for Mac OS X. The results are presented as tables, graphical representations and dedicated statistical tests. Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as total number of cases and percentages. Groups were compared using the χ^2 or Fisher exact test for categorical variables and the *ANOVA / Student's t-test* and *Wilcoxon-Mann-Whitney U / Kruskal Wallis* test for contin-

uous variables based on their distribution. Normality was checked using the *Kolmogorov-Smirnov* and the *Shapiro-Wilk W* tests. P-values <0.05 were considered significant.

The control group was selected based on propensity score matching (PSM) to remove the influence of confounding bias. PSM analysis was performed using logistic regression to create a propensity score for the TAVI and SAVR groups. Sex, BMI, age, and comorbidities were entered into the PSM, and the resulting score-matched pairs were used for further analyses.

The study was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi and the Ethics Committee of the Cardiovascular Institute from Iasi. The patients were informed about the study and signed an informed consent.

RESULTS

The study group included a total of 168 patients evaluated with AS with indications of valve prothesis, of which 70 patients underwent TAVI and 98 patients underwent isolated SAVR with biological valve. The demographic and clinical data of the patients are presented in first table.

TABLE I

Characteristics of the patient cohorts before matching

Parameter	TAVI	SAVR	p-value
Age (years)	76.47±6.83	64.37±11.76	0.002
Gender (no., % female)	37 (52.86)	41 (41.84)	N.S.
BMI (kg/m^2)	28.56±4.05	28.15±5.26	N.S.
EuroSCORE II	22.42±14.12	1.6±1.56	<0.001
AHT (no., %)	32 (45.71)	39 (39.79)	N.S.
NYHA (no., %)	NYHA II - 2 (2.86) NYHA III - 43 (61.43) NYHA IV - 25 (35.71)	NYHA II - 34 (34.69) NYHA III - 57 (58.16) NYHA IV - 7 (7.14)	< 0.001
Type 2 DM (no., %)	8 (11.43)	17 (17.35)	N.S.
AMI (no, %)	8 (11.43)	0 (0)	0.001
Stroke	5 (7.14)	5 (5.10)	N.S.
eGFR (medium) (mL/min)	78.33 ± 23.03	72.95 ± 15.83	N.S.
COPD (no, %)	12.86	12 (12.24)	N.S.
PAD (no., %)	10 (14.29)	7 (7.14)	N.S.
Hepatitis (no., %)	4 (5.88)	5 (5.10)	N.S.
LVEF (%)	53.88 ± 11.20	53.99 ± 11.12	N.S.
PPM	5 (7.14)	-	-
Prior cardiac surgery (no., %)	7 (10)	2 (2.04)	0.028
PH (no., %)	18 (25.71)	4 (4.82)	< 0.001

BMI-body mass index, AHT-arterial hypertension, NYHA-New York Heart Association, DM-diabetes mellitus, AMI-acute myocardial infarction, GFR-glomerular filtration ratio, COPD-chronic obstructive pulmonary disease, PAD-peripheral artery disease, PPM-permanent pacemaker, PH-pulmonary hypertension.

The TAVI patients were older compared to the SAVR group (76.47 ± 6.83 vs 63.37 ± 11.76 years; p = 0.002) and had a significantly increased surgical risk assessed by EuroSCORE II (22.42% ± 14.12 vs 1.6 ± 1.56; p < 0.001), these being two of the reasons why this therapeutic method was chosen. Previous cardiac surgery was a criterion for choosing TAVI, 7 (10%) of the patients having such a history, compared to 2 of patients treated with open heart surgery (2.04%). A number of 8 patients (11.43%) from the TAVI group had a history of acute myocardial infarction. The incidence of liver, kidney and chronic lung disease did not vary significantly between the two groups.

The transfemoral approach was preferred in 61 patients (87.14%), the valve implantation being performed in this way in patients without significant vascular lesions (iliac artery stenosis or occlusions, aortic aneurysm, atheromatosis with extensive parietal thrombosis and embolic risk). In 9 patients (12.85%), the valve was implanted by transapical approach due to poor ilio-femoral vessels. Overall, 48 TAVI patients had a SAPIEN 3 valve, and 22 patients had Medtronic CoreValve Evolut R. In the case of self-expandable (SE) valves, the most frequently used were those of 23 mm and 26 mm (60.41% and 29.16%) and for those balloon-expandable (BE), valves of 26 and 29 mm were used (54.54% and 45.45%). Valve under sizing was preferred in patients with extensive annular calcifications to prevent a possible rupture. Mild or moderate postprocedural valve regurgitation was identified in 17 cases (24.29%), the incidence decreasing at 6 months assessment. Relative to the total number of valves used, 9 of the SE valves (40.90%) and 8 of the 48 BE valves (16.67%) were associated with mild or moderate postprocedural regurgitation (tab. II). Although the incidence of regurgitation was almost three times higher with SE valves (p< 0.001), was not clinically significant.

The general postoperative morbidity and mortality of the two cohorts are presented in table III.

TABLE II

Risk of complications depending on valve rate

	Evolut R	SAPIEN 3
Regurgitation	9 (40.90%)	8 (16.67%)
Hemorrhagic complications	1 (4.55%)	6 (12.5%)
Vascular complications	2 (9.09%)	7 (14.58%)
PPM	1 (4.55%)	2 (4.17%)

TABLE III

Postoperative morbidity and mortality depending on the type of treatment

	TAVI	SAVR	p value
Acute kidney injury	10 (14.28%)	10 (10.20%)	N.S.
Postoperative AMI	0 (0%)	0 (0%)	N.S.
Infectious complications	7 (10%)	7 (7.14%)	N.S.
Stroke / TIA	0 (0%)	1 (1.02%)	N.S.
Hemorrhagic complications	7 (10%)	8 (8.16%)	N.S.
Postoperative arrhythmias	19 (27.14%)	45 (45.92%)	0.003
30-days mortality	5 (7.14%)	3 (3.06%)	N.S.

The most common arrhythmia found in both groups was atrial fibrillation (13 patients in TAVI group-13.57% and 37 cases in SAVR group-41.11%) (fig. 1). Four of the TAVI cohort developed atrioventricular block that required permanent pacemaker implantation (5.71%).

TAVI specific vascular complications were reported in 9 cases (12.86%) represented by: femoral artery stenosis treated with stent placement or endarterectomy with patch plasty (2), femoral artery rupture which required open surgical repair (2), pseudoaneurysm formation (2), access site bleeding and hematoma formation managed conservatively (1), vascular closure device failure which needed surgical suturing of the femoral artery (1), and iliac artery stripping resolved by femoral-femoral bypass (1). Severe vascular complications were registered in only 2 cases and were represented by an aortic dissection and annular rupture and lead to cardiac tamponade and hemorrhagic shock in both cases. Cardiac complications such as perforation of the right ventricle were recorded in only one case, the evolution being favorable under treatment.

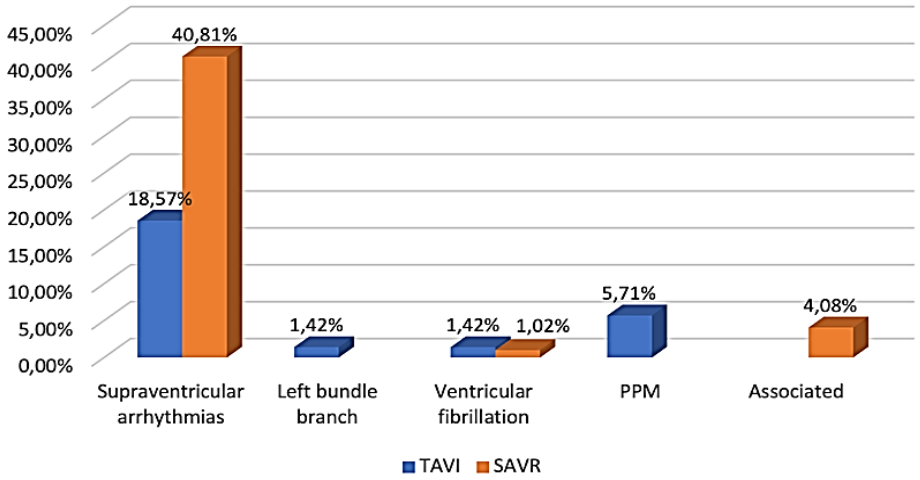

Fig. 1. Prevalence of postoperative arrhythmias

After surgery, 3 patients developed AKI, severe PH and upper gastrointestinal bleeding that led to death.

Considering the differences related to the risk profile, the SAVR group was analyzed after correlating the propensity score with the patients who underwent TAVI. From the total 98 patients with SAVR, 22 cases revealed similar preoperative profile to patients with TAVI based on PSM analysis, except EuroSCORE II. The value of EuroSCORE II could not be included because all patients treated surgically had a value below 3%, significantly lower than patients with TAVI. The obtained results are shown in table IV.

The length of post-procedural hospitalization in TAVI patients was 8.48% ± 7.28 days, but significantly shorter compared to surgically treated patients 16.10± 8.66 days.

TABLE IV

Postoperative morbidity and mortality depending on the type of treatment after correlation of the propensity score

	TAVI	SAVR	p value
Acute kidney injury	10 (14.28%)	3 (13.63%)	N.S.
Postoperative AMI	0 (0%)	0 (0%)	N.S.
Infectious complications	7 (10%)	3 (13.63%)	N.S.
Stroke / TIA	0 (0%)	0 (0%)	N.S.
Hemorrhagic complications	7 (10%)	4 (18.18%)	N.S.
Postoperative arrhythmias	19 (27.14%)	10 (45.45%)	0.003
Death < 30 days	5 (7.14%)	1 (4.54%)	N.S.

DISCUSSION

In the general analysis, the average age of patients treated with TAVI was 12 years higher compared with patients treated surgically (76.47 *vs.* 64.37 years). The surgical risk was significantly different, the average value of EuroSCORE II for patients with TAVI was 22.42% compared to 1.6% for the SAVR group. Only 4 patients with TAVI had an EuroSCORE II value < 10% compared to 96 of 98 patients with SAVR (5.71% TAVI *vs.* 97.95%). The maximum value of EuroSCORE II was 12.43% for SAVR and 54% for TAVI. Current studies show that patients who benefited from TAVI are older with multiple comorbidities and a prevalence of frailty of 63% (7, 8). TAVI was reserved for patients with a history of surgery and with multiple comorbidities, patients who could not have tolerated a conventional surgery.

The severity of heart failure was different, surgical patients being predominantly in NYHA class II and III (34.69% and 58.16%), in contrast to TAVI patients with advanced heart failure (61.43% in NYHA class III and 35.71% in NYHA class IV). Increased severity of heart failure caused the coexistence of PH in 25.71% of TAVI patients, compared to 4.82% of SAVR group.

In this study, the SE valves were associated with a higher rate of valvular regurgitation and the BE valve with several hemorrhagic complications. Vlastra *et al.*, reported a lower incidence of embolic accidents and pacemaker implantation by using transfemoral implanted BE valves (9), which was not found in our groups. The same research indicates an increased risk of bleeding complications in patients treated with BE valves, an observation that is in concordance with the results of our study. The SE valve was preferred in patients with a large aortic annuli given the availability of valves with a diameter of 34 mm compared to a maximum of 29 mm for BE valves. In the presence of extensive annular calcifications, was preferred the valvular under sizing, despite the risk of paravalvular leak or regurgitation.

Even if the rate of valvular regurgitation seems to be high (24.29%), the results are similar to those reported in the current literature, did not cause symptoms and recorded a regression on mild-term evaluation. The importance of this regurgitation is debatable given that the 2-year control performed in the PARTNER trial reveals a negative impact on survival (10). The incidence of regurgitation is lower in patients with SAVR due to the open surgical heart

procedure that allows the direct visualization of the valve, removal of cusps and annular calcifications and permits the direct measurement of the aortic ring. If the results are not satisfactory, implantation under direct visualization allows easy valvular repositioning. In patients with TAVI, the presence of extensive cusps or annular calcifications, correlate directly with the risk of paravalvular regurgitation, by quantifying the severity of calcifications on the preprocedural CT scans allowing accurate estimation of this risk.

The biological valves used in TAVI are different from those used in SAVR, resulting in lower pressure gradients which is associated with improved hemodynamic performance due to a larger orifice area (11). The technical differences between TAVI and SAVR explain the increased incidence of permanent pacemaker implantation in TAVI group, especially by using SE valves. In SAVR group, calcifications were removed, unlike TAVI group where calcifications are dislocated, leading to a potential injury of the atrioventricular bundle.

After correlating the propensity scores, although the results of comparison remained statistically insignificant due to the lower number of SAVR patients with similar TAVI comorbidities, in the TAVI group we found a lower incidence of infectious, hemorrhagic complications and postoperative arrhythmias compared to SAVR group. Indja *et al.*, showed in a recent meta-analysis a higher incidence of new-onset atrial fibrillation in the SAVR patients compared to the TAVI group. Furthermore, the study demonstrated that TAVI patients evaluated with atrial fibrillation after intervention had higher risk of stroke and mortality (12).

The value of EuroSCORE II was not used as a cofactor in the correlation of the propensity scores, the average value after correlation being 2.56% *vs.* 22.42% in the TAVI group, this situation was accepted due to the major difference in terms of risk profile would have determined the impossibility of obtaining a similar lot.

At the following reevaluation, there was a slight improvement in LVEF from 53.88% before the procedure to 57% one year after the intervention.

Acute kidney injury (AKI) is known as a common and serious complication after cardiac surgery (13). In our study, the incidence of AKI was lower (14.28%) than we expected under iodinated contrast injection. The absence of a significant difference between TAVI and SAVR could be attributed to a decreased renal perfusion during extracorporeal circulation in SAVR which contra balances the nephrotoxic effects of iodine in the TAVI group. Moreover, Kumar *et al.*, demonstrated that in patients with AS and chronic kidney disease, TAVI is a preferable approach being associated with a lower risk of AKI and dialysis requiring AKI and lower in-hospital mortality compared to SAVR (14).

Although the PARTNER study reports an incidence of stroke of 3.3% at 30 days after surgery (15), no case was observed in our study, careful selection of patients and approach, optimal progression of the implantation system through the blood vessels and good postoperative management of antiplatelet therapy have minimized this risk (0% in this case).

Wagner *et al.*, in a meta-analysis performed on 13 observational studies and 6 clinical trials, identified the association of

TAVI with low risk of major bleeding but with a high risk of vascular complications, moderate or severe paravalvular regurgitation and pacemaker implantation. In this study, paravalvular leak was reported in only 2 cases (2.86%) (16).

Even if TAVI patients revealed with multiple comorbidities, the length of post-procedural hospitalization was almost half compared to SAVR (8.48 days *vs.* 16.10 days), higher values being recorded in patients who developed complications, the obtained results being similar to the literature (17).

CONCLUSIONS

TAVI is a relevant therapeutic alternative to surgery in patients with a high operative risk. The mean age, prevalence of comorbidities and the value of EuroSCORE II are significantly increased in TAVI patients compared SAVR. TAVI performed in patients with an increased surgical risk is associated with morbidity and mortality rates similar to those recorded in patients with a reduced risk referred to SAVR. International guidelines include frailty as an indication of TAVI, but do not propose a precise tool for assessing it, further studies are needed to develop a general frailty score system applicable in current medical practice as an adjunct to prognostic scores in order to optimize preoperative status patients with subsequent improvement in prognostic and quality of life.

CONFLICT OF INTEREST AND FUNDING

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NEWS

SARS-CoV-2 FULMINANT MYOCARDITIS:
AN AUTOPSY AND HISTOPATHOLOGICAL CASE STUDY

The new SARS-CoV-2 pathogen, causing coronavirus disease 2019 (COVID-19), was identified for the first time in December 2019, in China. Cases of COVID-19 have now been reported all around the world, resulting in 2020 in more than one million of deaths, which are consecutive in most of cases to a life-threatening acute respiratory distress syndrome (ARDS). Cardiovascular involvement can also occur, such as thrombosis or myocarditis, generally associated with pulmonary involvement. To date, little is known about SARSCov-2-induced myocarditis. Several cases of fulminant myocarditis have been reported, with only few cases of histopathological assessment. In the present study, it is reported a case of isolated fulminant myocarditis, including exhaustive post-mortem histopathological, immunohistochemical, and viral analyses (Gauchotte G, Venard V, Michaël Segondy M, *et al.* SARS-CoV-2 fulminant myocarditis: an autopsy and histopathological case study. *Int J Legal Med* 2021; 135: 577-581).

OCULO-AURICULO-VERTEBRAL SPECTRUM: CLINICAL FEATURES IN A COHORT OF 37 PATIENTS

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OCULO-AURICULO-VERTEBRAL SPECTRUM: CLINICAL FEATURES IN A COHORT OF 37 PATIENTS (Abstract): Oculo-auriculo-vertebral spectrum (OAVS) is a developmental disorder involving structures derived from the first and second pharyngeal arches. The phenotype is clinically heterogeneous and is characterized by abnormal development of the ear, mandible, and defects of the vertebral spine. **Material and methods**: We performed a retrospective study on a group of 88 patients with various forms of external ear malformations and hearing loss. All patients were assessed for chromosomal abnormalities using karyotype and SALSA MLPA kits. **Results:** Of the 88 patients enrolled in the study, 37 (42.5%) were diagnosed with oculo-auriculo-vertebral spectrum based on clinical criteria. The main clinical findings were: microtia, preauricular tags and sinuses, epibulbar dermoids, mandibular hypoplasia, micrognathia, hearing loss and vertebral abnormalities. **Conclusions:** The causes, significant phenotypic heterogeneity, and variations in management make difficult creating evidence-based guidelines for diagnosis and management of this complex disorder. **Keywords:** OCULO-AURICULO-VERTEBRAL SPECTRUM, HEARING LOSS, EAR MALFORMATIONS.

Oculo - auriculo - vertebral spectrum (OAVS) is a heterogeneous disorder that affects the development of the ear, mouth, and mandible. It is characterized by facial asymmetry (due to hypoplasia of the jaw and/or mandible), abnormalities of the auricles and ear, and hearing loss; abnormalities of the inner ear, temporomandibular joint, and facial muscles may be associated. Malformations of the orbit, eye, nose, skull, neck, spine, or heart are also present, often unilaterally, but cases with bilateral involvement have been reported (1).

The spectrum ranges from mild to severe forms and includes: hemifacial microsomia, Goldenhar syndrome, oromandibular dysostosis, facial-auriculo-vertebral syndrome and lateral facial dysplasia (2). Goldenhar syndrome, which associated facial asymmetry, epibulbar dermoids, microtia, and vertebral anomalies, accounts for an estimated 10% of cases of OAVS (3).

The causes are still unclear and there are no widely agreed diagnostic criteria for OAVS. A proposed minimum criteria for the diagnosis includes asymmetric hypoplasia of

the facial structures with preauricular buds and/or microtia (4).

The prevalence of OAVS varies from 1/150,000 to 1/5,600, but an exact figure is difficult to estimate due to ethnic and geographic variability (5). Although the impairment is unilateral in most cases, approximately 10-15% are bilateral (6). Boys are more commonly affected, with a prevalence of 3:1, and unilateral impairment more often involves the right hemifacial region (4).

The American Cleft Palate-Craniofacial Association (ACPA) recommends that the management of patients with OAVS be performed by a multidisciplinary team including a pediatrician, audiologist, speech-language pathologist, oral surgeon, plastic surgeon, otolaryngologist, neurosurgeon, ophthalmologist, radiologist, psychologist, and geneticist. After cheilopalatoschisis, OAVS is the second most common congenital facial pathology that requires a multidisciplinary approach (7). Facial asymmetry caused by mandibular or maxillary hypoplasia is the main cause of craniofacial dysmorphia, dental malposition, and malocclusion (8).

MATERIAL AND METHODS

We performed a retrospective study on a group of 88 patients with various forms of hearing loss and external ear malformations. In all patients with positive family history, healthy first-degree relatives were included in the study if mutations were detected in the index case. Patients who met the minimal diagnostic criteria: unilateral microtia or various degrees of microtia, bilateral and asymmetric with facial asymmetry included. We considered skeletal involvement other than spine and ribs and symmetrical involvement of the auricles as exclusion criteria.

All patients enrolled in the study participated voluntarily and were registered under a code number to maintain anonymity. The use of the results was according to a protocol approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi (approval no. 14789) and the Ethics Committee of the Children's Emergency Hospital "Sf. Maria" Iasi (approval no. 681). The patients, parents or guardians signed an informed consent form before the start of the study. All subjects were clinically evaluated by a geneticist.

DNA was extracted from 300 µL peripheral blood samples stored with EDTA using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA).

All patients were assessed for chromosomal abnormalities using karyotype and MLPA technique (Multiplex Ligation-dependent Probe Amplification) using P036 and P070 for screening of subtelomeric region, P245-Microdeletion Syndromes which contain probes for common microdeletion/microduplication syndromes and P250-DiGeorge for 22q11.2 region (MRC-Holland, Amsterdam, The Netherlands) (9, 10).

MLPA analysis was performed according to the manufacturer's protocol. Briefly, 100 nanograms of genomic DNA were denatured and hybridized with SALSA probes at 60°C for approximately 20 h. After 15 min of ligation at 54°C, PCR was performed using Cy5-labeled primers. The fluorescent amplification products were separated based on their length by capillary electrophoresis in a CEQ 8000 GeXP Genetic Analysis System (Beckman Coulter, Brea, CA, USA), and the results were analyzed using the program Coffalyser. NET V9 (MRC-Holland, Amsterdam, The Netherlands). The probe ratio for deletion and duplication

was set to 0.7 and 1.3, respectively.

RESULTS

Of the 88 patients enrolled initially in the study, 37 (42.5%) were diagnosed with oculo-auriculo-vertebral spectrum based on clinical criteria, 17 (19.5%) with Treacher-Collins syndrome, and 10 (11%) with branchiootorenal syndrome; 24 (27%) with other syndromes or without clinical signs specific for a syndrome.

Of the 37 patients with the oculo-auriculo-vertebral spectrum, 17 of them had hearing loss and malformations of the external ear without other clinical signs. The male patients were preponderant with a sex ratio male: female of 2.7:1. The average age at diagnosis is 1 month, and approximately 80% of the included patients were diagnosed by the age of 1 year.

More than 50% of cases provided from pregnancy with parity >3 and 85% of patients from our study came from families with poor socio-economic situations. Prenatal history revealed that most patients included in the study (78% - 29 cases) had teratogen exposure (alcohol and/or antibiotics). A significant number of cases – 8 – were born prematurely; 6 of them had threatening miscarriage during pregnancy.

The main clinical signs present in our group are unilateral microtia 95% of patients, bilateral microtia in 2% cases, preauricular tags: 60% of patients, epibulbar dermoids: 30%, unilateral mandibular hypoplasia: 24%, micrognathia 15%, macrostomia 12% and preauricular sinuses 10%.

All patients included in the study have hearing loss: 33 of them have conductive hearing loss, while only 4 patients have mixed hearing loss. The main cause of addressability of these patients is hearing loss and malformations of the external ear (11).

The vertebral abnormalities were present in 15 cases, 12 of them had hemivertebrae, 2 vertebral blocks and 1 spina bifida. The occasional findings in our study include four cases with congenital heart defect, 1 case of epilepsy and 1 case of thalassemia minor.

Karyotype was normal in all cases, but MLPA showed 2 abnormal results: 1 patient had del22q11.22 between LCR D and LCR E genomic position 21.799.442-22.322.996 - hg19 and 1 patient had a deletion in exon 2 *DGCR8* gene chr22:20073612-20073613 -hg19.

DISCUSSION

The literature date indicated that phenotype in the oculo-auriculo-vertebral spectrum is variable from mild (with only hearing loss and possibly minor malformations in the external ear or mild asymmetry of the face) to severe (with complex malformations in the external ear such as vertebral, cardiac, genital, or nervous system anomalies) (12-14).

During the time were tried different attempts to classify abnormalities commonly associated with the OAVS. Thus, Vento and all. (15) elaborated OMENS classification, an acronym from **O**rbit, **M**andible, **E**ar, **NE**rve, and **S**oft tissues, while Horgan and all. transform OMENS acronym in OMENS plus by adding of extracranial damage (16). These classification systems were completed by Pruzansky and Kaban which focuses on the size and shape of the mandible and glenoid fossa. In theirs classification the grade I corresponds to mandibles with mild hypoplasia; grade IIa is associated with a functional hypoplastic and malformed condyles, grade IIb describes a severely hypoplastic and malformed condyles, with displacement from the temporomandibular joint, while grade III is represented by complete damage of

mandible, with the absence of the branch, condyle and joint (17).

External ear malformations, mandibular hypoplasia, micrognathia, hearing loss and vertebral anomalies are the most common features in our OAVS study (tab. I). A retrospective study of all patients referred for auricular reconstructive surgery during 1990-2012 (18), showed that atresia of the acoustic meatus, preauricular skin tags, hemifacial microsomia, and facial nerve palsy were present in most cases. All these clinical signs are present in the current clinical diagnosis criteria.

In our study sex ratio was male: female of 2.7:1 a little discordant reported to other studies. Bragagnolo *et al.* identified a 54% male cases from 72 patients (20), while Barisic *et al.* reported a male-to-female ratio of 1.4: 1 (19).

Although it cannot be proven concretely, recent studies propose the involvement of teratogenic factors, number of pregnancies and maternal age in the etiology of the oculo-auriculo-vertebral spectrum. Consistent with these, in our group 22% were first pregnancies but more than 50% of cases are multiparity (22). Werler suggests that twin pregnancies and those resulting from assisted reproduction are often involved in the etiology of the oculo-auriculo-vertebral spectrum. From our group, only 2 cases come from twin pregnancies and only one from assisted reproduction pregnancy, data inconsistent with the literature (23).

TABLE I

Prevalence of main clinical anomalies

Clinical findings	Our study N=37	Barisic *et al.* (19) N=259	Bragagnolo *et al.* (20) N=72	van Nunen *et al.* (18) N=155	Rooryck *et al.* (21) N=86
Microtia	95%	88%	100%	100%	70%
Preauricular tags	60%	45%	44%	12%	67%
Preauricular sinuses	10%	3%	3%	2%	7%
Epibulbar dermoids	30%	8%	15%	-	31%
Mandibular hypoplasia	24%	49%	94%	56%	76%
Micrognathia	15%	49%	76%	20%	22%
Hearing loss	90%	10%	45%	76%	68%
Vertebral abnormalities	40%	25%	8%	3%	35%

Although data from the literature suggest a higher frequency of premature births in patients with OAVS, in our group, 22% (8 cases) were born prematurely, 6 of them due to the threatening miscarriage during pregnancy (23). It was suggested that maternal diabetes may also be associated with OAVS (24) but in our study none of the mothers declared history of diabetes.

OAVS includes vertebral and spine malformations such as: hemivertebrae, vertebral blocks, scoliosis, or kyphoscoliosis, occipitalization of the atlas, and spina bifida. Identifying spinal abnormalities helps to manage neck pain, torticollis, spinal instability, or scoliosis (25-27). In our study, the main vertebral anomaly was hemivertebrae, identified in 12 cases.

Renkema *et al.* searched the nervous system abnormalities in the OAVS, and the most reported were neural tube defects, corpus callosum agenesis, intracranial lipo-

mas, Arnold-Chiari malformation, hydrocephalus, ventriculomegaly, cerebral hypoplasia and microcephaly (28). In these patients' intellectual disability is more frequent than general population, most commonly being present language development and neuro-psycho-motor delay. However, these features most likely present multifactorial causes, but frequently are found behavioral and social adaptation disorders caused by decreased self-confidence.

Ophthalmic abnormalities are common and include epibulbar or limbal dermoids, colobomas, orbital dystopia, or microphthalmia. Ophthalmological evaluation of limbal dermoids is indicated, with increased attention to astigmatism or anisometropia, management including excision and follow-up for the development of amblyopia. Epibulbar dermoids rarely require excision and are usually unilateral (25, 29-31). In our cases we found only epibulbar dermoids.

Although the group of patients with syndromic hearing loss represented by the oculo-atrial-vertebral spectrum presents clinical data consistent with the literature, in terms of genetic testing, we have only 2 abnormal results so far. The literature cites some promising arrayCGH results, but this complex test was impossible to apply to our patients (21).

First patient with abnormal MLPA result has a deletion of LCR D and LCR E segments in 22q11.22 region. Our patient had normal prenatal growth but preterm birth, with a gestational age of 37 weeks, nonspecific facial dysmorphic features (triangular face with arched eyebrows), conductive bilateral hearing loss and bilateral preauricular sinuses. Patients with this type of deletion have prenatal and postnatal growth restriction, behavior and neurological problems and cardiovascular defects (32).

The *DGCR8* gene deletion, found in another of our patients, is reported as a DiGeorge critical region, a gene whose haploinsufficiency can influence the severity of the clinical phenotype. Our patient, similar to those reported (33), had facial asymmetry with mandibular hypoplasia, multiple congenital anomalies, palatoschisis, hemivertebrae L2, malformed external ear unilateral and conductive hearing loss.

Regarding the audiological assessment, the early screening of the hearing loss helps in its treatment. Hearing is critical for language, social and cognitive development, and patients with OAVS are at risk of conductive or sensory hearing loss (34). Ear malformations, including microtia, atresia of the external auditory canal, and malformations of the eustachian tube cause conductive hearing loss. Malformations of the inner ear, vestibule, acoustic and facial nerve damage are usually associated with severe forms of OAVS and implicitly of hearing loss (35). Treatment of hearing loss is dependent on its degree, type and laterality and may include hearing aid, cochlear implantation, or surgical repair of the atresia of the ear canal (25).

In Romania, no studies have been performed on large groups of patients with syndromic hearing loss specially oculo-auriculo-vertebral spectrum. Data from the literature report a high incidence of these cases in disadvantaged families; these data coincide with our group, 85% of patients come from families with poor socio-economic situations. However, addressability of patients in rural areas is constantly increasing due to the possibility of material benefits in this pathology (36). Although attempts are being made to involve the social situation of patients in pathology, additional epidemiological and genetic studies are requested.

CONCLUSIONS

Oculo-auriculo-vertebral spectrum represents a complex craniofacial disorder that remains challenging for complete understanding. The causes, significant phenotypic heterogeneity, and variations in management make difficult creating evidence-based guidelines for diagnosis and management of this complex disorder. For the members of the craniofacial teams, this means the treatment of each individual patient is unique and different. Further research into the genetics is needed to help clarify the diagnosis.

To improve the quality of life and early treatment, it is necessary to develop diagnostic and management guidelines for cases of syndromic hearing loss.

CONFLICT OF INTEREST AND FUNDING

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PSYCHOEMOTIONAL DISTRESS AT THE MEDICAL STAFF AFTER ONE YEAR PERIOD PANDEMIC IN A FIRST-LINE COVID-19 HOSPITAL

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PSYCHOEMOTIONAL DISTRESS AT THE MEDICAL STAFF AFTER ONE YEAR PERIOD PANDEMIC IN A FIRST-LINE COVID-19 HOSPITAL (Abstract): Humankind is currently facing a pandemic of unimaginable proportions generated by a new strain of coronavirus, SARS-CoV-2, which causes significant public health problems. Material and methods: We employed a cross-sectional survey of 252 healthcare professionals from a frontline University Hospital in the "battle" with the new coronavirus after one-year pandemic. We analyzed the prevalence and associated factors with work-related psychological distress among our study group. Results: The results show that gender, marital status, the workload of treating COVID-19 patients, fear of SARS-CoV-2 infection, depression, and anxiety predicted burnout syndrome. Conclusions: The medical field is one of the most vulnerable areas where the staff is predisposed to psychological distress. This increases the probability of suffering different consequences, including burnout syndrome, depression, anxiety, post-traumatic stress disorder (PTSD). **Keywords:** PANDEMIC, BURN-OUT, PSYCHOEMOTIONAL DISTRESS, MEDICAL STAFF.

Burn-out is a syndrome defined as resulting from chronic workplace stress not successfully managed. It's a state of mental, physical, and emotional exhaustion caused by excessive and prolonged stress. It's a gradual process, first, the signs and symptoms are subtle (1).

There have been some pandemics throughout human history, infectious diseases often having devastating consequences. Plague, typhus, cholera, yellow fever, influenza, and tuberculosis are contagious diseases that have wreaked havoc over time. Nowadays, HIV infection could be considered a pandemic.

We are currently going through an exceptional period, with humanity facing a new pandemic, the SARS-CoV-2 virus infection (2). The spread of SARS-CoV-2 infection worldwide has been impressive. Today, we face more than 218 million diagnosed patients and an unknown number of people who have gone through the disease undiagnosed. Until now, there have been over 4,529,105 deaths worldwide. The first confirmed case in our country with

SARS-CoV-2 infection was on February 26th, 2020. Since then, over 1,098,765 cases have been confirmed, and over 3,570 deaths have been registered (3).

Given the developing circumstances with the new coronavirus, evidence synthesis about mental health outcomes it is necessary to produce guidance for professionals and health care facilities (4).

MATERIAL AND METHODS

According to the administrative zone health department's program report and plan, 252 healthcare professionals were working in the hospital at the time of the study that accepted to participate in our research. In addition, we obtained a list of healthcare professionals from the human resource department, and we excluded the persons on sick, annual, maternity, and family leaves.

We applied a questionnaire that contained general characteristics consisting of 11 items as follows: age, gender, marital status, education level, work experience, profession, number of rotating shifts within a month, socioeconomic status, satisfaction at work, current health condition, and behavioral factors (smoking, drugs, alcohol). Age details were collected as an ordinal variable; other questions were answered with values from 1 to 5. We also translated and adapted the *Hospital Anxiety and Depression Scale* (HADS). We used 21 items, seven about depression, seven about exhaustion, and seven about anxiety, with responses from 0 to 3.

RESULTS

Out of the entire hospital staff, 252 participated and completed the questionnaire, offering an overall response rate of 100%. The majority of participants were women (87.3% - 220). The average age was 38 years, ranging from 18 to 62 years old. The number of married participants was 1.2 higher than single participants. A high proportion of respondents had a bachelor's degree for higher education, 48.8%, high school graduates, 41.7%, and 9.5% of participants had secondary education.

As for the professional status of the participants, 35.73% are specialist doctors and resident physicians, nurses accounted for 35.31%, cares accounted for 9.52%, stretcher-bearers 4.36%, and the other workers accounted for 15.08% (economists, pharmacists, lawyers, psychologists, physiotherapists). Of all participants, 84.92% belonged to a job category that required interaction with COVID-19 patients, 84.12% perceived themselves as having a high or middle socioeconomic status. A total of 67% of participants were non-smokers, 5.15% stated that they occasionally consumed alcohol, none of them used drugs.

Regarding satisfaction at work, 32.93% declared they are satisfied and very satisfied. Of all participants, 34.54% claimed they have a good health condition, 41.66% medium, and 23.8% poor (tab. I)

As a result of the analysis of the data, aspects related to anxiety (21.03% - 53), exhaustion (17.46% - 44), and depression (11.9% - 30) are highlighted. We have noticed that overwork and burnout related to anxiety and depression are found among physicians and nurses.

There are main elements of anxiety and less psycho-emotional exhaustion among the medical staff with higher education at the beginning of their career (resident physician). In the context of this demanding period, the research focused more on psycho-emotional exhaustion and burn-out

syndrome. However, we want to emphasize that we can face challenges by activating defense mechanisms (coping / resilience).

In our study, no people were identified who could reach extreme exhaustion in the work process, on the one hand, due to good resilience and on the other hand, due to a well thought out program of work and rest and the possibility of supplementing the staff number during the pandemic by hiring nurses, carers, and stretcher-bearers for a determined period.

However, the psychological pressure at work, as well as the one felt after limiting and restricting mobility for shorter or longer periods, had an essential impact on the psycho-emotional state of health care workers, requiring further psychological reassessments and psychological support from the team sequentially at the end of pandemic and post-pandemic.

TABLE I.

General characteristics.

Variable	Value
Age (years old)	18-62 (average 38)
Gender	n=252
Female	220
Male	32
Marital status	
Married	105 (41.66%)
Divorced	30 (11.90%)
Widowed	12 (4.70%)
Single	45 (17.80%)
In a relationship	60 (23.94%)
Education level	
Higher education	123 (48.8%)
High school	105 (41.66%)
Secondary education	24 (9.54%)
Work experience	
< 1 year	65 (25.7%)
1-5 years	45 (17.8%)
5-10 years	47 (18.6%)
10-15 years	35 (13.8%)
15 years	60 (24.1%)
Profession	
Physicians	90 (35.73%)
Nurses	89 (35.31%)
Carers	24 (9.52%)
Stretcher-bearers	11 (4.36%)
Others	38 (15.08%)
Number of shifts within a month	2-12 (average 6)
Socio-economic status	
High	84 (33.33%)
Middle	128 (50.79%)
Low	40 (15.88%)
Satisfaction at work	
Very satisfied	23 (9.12%)
Satisfied	60 (23.8%)
Dissatisfied	102 (40.47%)
Very dissatisfied	67 (26.61%)
Current health condition	
Good	87 (34.54%)
Average	105 (41.66%)
Poor	60 (23.8%)
Behavioral factors	
Smoking	83 (32.9%)
Drugs	0 (0.0%)
Alcohol	13 (5.15%)

DISCUSSION

Pandemics of this magnitude have appeared in humanity about once every 100 years. Over the past decades, there has been an increasing belief that the experience of stress is associated with impairment of health (5).

The health care sector is one of the main sectors of the workforce, accounting for almost 10% of the European Union's workforce. The largest group of health workers are in hospitals. This sector has a significant imbalance between women and men; women account for almost 80% of employees. Psychosocial problems can lead to illness, accident, stigma, isolation, and death. They can also have a notable impact on the employer by lowering productivity and morale (6).

Resilience defines a person's ability to adapt quickly to problems /events with little stress. Resilient people do not reject their emotions but accept them without letting them take control or influence their decisions; for example, in the current situation, resilience can be seen as the ability to regain strength after specific changes or stressful events within the COVID-19 pandemic (7).

Coping designates any mechanism for prevention and adaptation to stress, any transaction between the subject and the environment to reduce stress intensity. Adaptation refers not only to living with anxiety. The coping mechanism is more general than the adaptation one. It aims to modify the system's functions to face the requirements of a dynamic environment and even eliminate the environmental factors that demand the new adaptation (8). There is an interconnectivity between stress, coping, and resilience so that exposure to stress brings about adaptation and triggers the adaptive mechanisms (resilience and coping) (9).

According to World Health Organization in 2009, mental health can be conceptualized as a state of well-being in which the individual is aware of his abilities, can cope with ordinary stressful situations in life, can work productively and fruitfully, can have a contribution to the community to which he belongs. In this positive way, mental health is the foundation of an individual and the community. This concept of mental health is consistent with its broad and varied interpretation of cultures (10).

Unfortunately, medical staff caring for patients with SARS-CoV-2 are often victims of this infection. Thus, 1 in 9 infected patients belongs to the medical staff. Any new disorder brings out the fear of the unknown, and the lack of information, conflicting data, or fake news can increase the perceived amount of stress by healthcare providers (11). Therefore, it is necessary to adapt and embrace policies that focus on correct dissemination of information, mutual support, and community-hospital relationships (12).

Health workers complain about accumulated fatigue and mental stress and suffer from burnout syndrome due to constant labor shortages and insufficient benefits. They can impact patients and colleagues as they have an increased risk of making wrong decisions and frequently quit their jobs because of extreme fatigue, exhaustion, fear of the unknown, fear of getting infected; many workers suffer from severe physical and mental stress, which leads to depression and trauma (1).

Psychosocial risks are related to the conception, organization, and management of work and the economic and social context of work, resulting in increased stress and severe deterioration of mental and physical health (13). Psychosocial work-related risk factors typical for the health sector include high expectations combined with time, skills, and insufficient social support; coping with pain; treating dying people, people with infectious diseases; emergencies; exposure to traumatic events; complaints and disputes (14).

Violence and harassment are also psychosocial dangers in the health sector. Violent behavior can come from patients, visitors, or colleagues. Health and social care sector reported the highest exposure to Violence at work in the European Union, 15.2%. In this sector, the incidence of

intimidation and harassment is above average (15).

The factors that determine the professional burnout of medical staff are death and suffering of patients; conflicts with colleagues and superiors; inadequate training; lack of social support; excessive workload; uncertainty about a prescribed treatment; pressure due to time; inadequate pay; conflict between career and family (16).

Health care workers are also at high risk for substance abuse due to specific risk factors such as exposure to a high volume of work, treatment of terminally ill and dying patients, inadequate training for work requirements, exhaustion, and insufficient knowledge of the dangers of alcohol and drugs, pandemic. All these psychosocial risk factors significantly influence employees' physical and mental health. Specific health problems caused by these risk factors may include stress, exhaustion, anxiety, irritability, depression (17).

We can prevent exhaustion syndrome by equitably calibrating the tasks assigned to the employee, periodic annual or biannual monitoring, communication, feedback, and setting new goals. Working in a hospital is challenging because employees usually need to handle intensive tasks involving work overload, extended working hours, high effort and energy, limited professionals, and lack of support, especially when we confront with this new virus when in the beginning, we didn't know how to protect ourselves and our family, how the virus affects us and when it will be gone (18). The global prevalence of psychological distress among medical personnel is high, providing that the threat posed by COVID-19 is a significant stressor for medical staff. Young employees should have better adaptability to the new condition represented by the pandemics than senior doctors (19).

CONCLUSIONS

Psychological stress is a natural phenomenon and can manifest in various forms. Every person is susceptible of it, being aware of this. Every person shows that he is attracted to balancing activities, even at work or home (20).

The existence of clear protocols for any possible situations, the practical training with the staff regarding the protection measures, the adequate use of protective equipment, and all actions that ensure a state of confidence and control decreases the stress level (21, 22). Understanding the challenges that doctors were facing, especially at the beginning of the pandemic, will help institutions provide psychological and organizational support. This pandemic raised an unknown social and economic problem that made the population adapt.

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ETHICAL APPROVAL

Ethical review and approval were waived for this study.

Informed consent was obtained from all subjects involved in the study.

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NEWS

SERUM FERRITIN AS A DIAGNOSTIC BIOMARKER FOR KAWASAKI DISEASE

Kawasaki disease is an acute febrile systemic vasculitis of unknown etiology. Kawasaki disease diagnosis depends on the use of adjuvant diagnostic markers, such as echocardiographic findings and laboratory biomarkers, including C-reactive protein, erythrocyte sedimentation rate, albumin, alanine aminotransferase, aspartate aminotransferase, white blood cell count, hemoglobin, platelet count, and pyuria. Although the laboratory findings observed in Kawasaki disease are not specific enough to conclusively establish a diagnosis, they can be useful, particularly in patients with a high suspicion for Kawasaki disease. Diagnosis of Kawasaki disease is occasionally delayed because it is solely based on clinical symptoms. Previous studies have attempted to identify diagnostic biomarkers for Kawasaki disease. Recently, patients with Kawasaki disease were reported to have elevated serum ferritin levels. This study aims to investigate the usefulness of the serum ferritin level as a diagnostic biomarker for distinguishing Kawasaki disease from other acute febrile illnesses. Blood samples were obtained from pediatric patients with Kawasaki disease and those with other acute febrile illnesses between December 2007 and June 2011 for measuring various laboratory parameters, including serum ferritin levels. In patients with Kawasaki disease, laboratory tests were performed at diagnosis and repeated at 2, 14, and 56 days after intravenous immunoglobulin treatment. At the time of diagnosis, serum ferritin levels in patients with Kawasaki disease (188.8 μg/L) were significantly higher than those in patients with other acute febrile illnesses (106.8 μg/L, P=0.003). The serum ferritin cut-off value of 120.8 μg/L effectively distinguished patients with Kawasaki disease from those with other acute febrile illnesses, with a sensitivity and specificity of 74.5% and 83.3%, respectively. Serum ferritin may be a useful biomarker to distinguish Kawasaki disease from other acute febrile illnesses. (Kim SH, Song ES, Yoon S, et al (2021). Serum Ferritin as a Diagnostic Biomarker for Kawasaki Disease. *Ann Med Lab* 2021; 41(3), 318322).

USING CASE-CASE STUDY DESIGN: WHICH CAN BE THE PERFORMANCE?

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USING CASE-CASE STUDY DESIGN: WHICH CAN BE THE PERFORMANCE? (Abstract): The case-case studies compare groups of patients classified by the subtype of the disease they present, to identify specific risk factors for the sub-category of patients with a characteristic of interest. **Material and methods:** This analysis brings attention to the main characteristics of case-case studies, with the aim of facilitating the understanding of methodology and the adequate way of implementing it, and the interpretation of the obtained results. **Results:** Specifically, this study design identifies the differences regarding risk factors between disease's subtypes, reflecting their etiological heterogeneity. However, the association for potential risk factors that are common to comparison sub-groups cannot be identified and the risk revealed does not represent the risk level in the general population. One conducting a case-case study must consider both the characteristics of the chronic or infectious diseases category and the advantages and the limitation of this type of study. **Conclusions:** As advantages, the case-case study design helps minimizing the selection and recall bias and can be carried out for a shorter period of time, with fewer resources than conventional studies. Selection or information bias may persist, and caution is needed for the interpretation of results. **Keywords:** CASE-CASE STUDY, CASE-ONLY STUDY, BIAS.

Case-case studies are case-only epidemiological studies, a derived category from case-control studies which compares groups of patients classified by the subtype of the disease they present, in order to identify specific risk factors for the subcategory of patients with the characteristic of interest (genetic, histological etc.).

These studies are an alternative when population epidemiological studies cannot be carried out due to the unavailability of eligible participants or when resources are limited.

In general, epidemiological studies can explore only some of a disease's dimensions, having advantages and limitations in this regard. Therefore, an appropriate study design must be used to achieve the proposed objective, taking into consideration also the available resources, convenience, feasibility, costs, and ethical issues.

When none of the known epidemiological study designs allows the exploration of the disease's characteristics of interest, the design can be adapted in terms of the method (comparative groups, subject selec-

tion, statistical analysis) and measurement instruments.

The present analysis brings attention to the latest characteristics of case-case studies, with the final objective of facilitating understanding of methodology, how to efficiently perform it and how to correctly interpret the obtained results.

Case-case studies use as comparison group one of the disease's sub-category, to reflect the level of exposure in the source population, from which the cases originate. Because the comparison group also includes cases that have more frequent exposures to risk factors than the general population, it cannot represent the latter. Consequently, the measured association will not represent the risk of the disease in the general population.

As the exposure to risk factors is high in both the case and comparison groups, the potential association will be different from the one that might be revealed through classical (conventional) case-control studies. For the exposures that are very similar between cases and controls, the potential association will be masked or underestimated. However, if the risk factors are different between the comparison groups, the association can be easily identified, which suggests the existence of etiological heterogeneity.

Studying the chronic disease category

In the case of chronic diseases, the patients' belonging to one of the comparison groups can be identified with good accuracy by using genotyping (such as the presence of certain alleles), molecular characterization (for example the presence of cellular receptors), morphological characterization (histopathological aspects) or biomarker identification (1-4). In these studies, involving molecular tests, a gene-environment interaction (comparative analysis between genetic sub-categories) or the consequences induced by the causal factor (identification of a mutation, alteration of an intermediate marker etc.) can be revealed (4).

The errors in studies involving molecular biology data may be caused by differences in laboratory testing (laboratory method's complexity, inter-laboratory validity, working protocol), difficulty in identifying the errors, confounding factors, or misinterpretation of a biological marker significance (4). The presence of a factor associated with a disease (biomarker for molecular epidemiology) may represent an intermediate stage of a physiopathological process, or the consequence of changes induced by the causal factor and not the cause (4). At the same time, these types of studies use prevalent cases that may present a better survival, and the associated factor may be in fact a prognostic factor (4).

For the *cancer epidemiology*, the case-case studies can mainly identify risk factors, but also biomarkers of aggression (the risk of an aggressive subtype) or the risk of recurrence after treatment (1-3, 5).

For example, for *breast cancer*, a recent study identified an association between alcohol consumption and a lower risk for human epidermal growth factor 2-over expressing (H2E) receptors breast cancer compared to estrogen receptor positive (ER +) breast cancer (3). Regarding the aggressiveness of hormone receptor positive breast cancer (classified by histological grade and level of proliferation), in another study, an association was identified between nulliparity and highly proliferative (presence of K167 marker) tumors, obesity and high grade (poorly differentiated) tumors, and current use of combined hor-

mone therapy and low grade (well-differentiated) tumors (1, 5).

Another recent study on *prostate cancer* identified the SNPs (single nucleotide polymorphism) genes CYP17A1 (gene related to the synthesis and metabolism of steroid hormones), AR (androgen receptor gene), LHCGR (luteinizing hormone chorionic gonadotropin hormone receptor gene) and ESR (estrogen receptor genes) as biomarkers of prostate cancer risk, and rs743572, rs6162, rs6163 (in CYP17A1 gene), rs1256049 (in ESR2 gene) and rs2293275 (in LHCGR gene) variants as aggressiveness biomarkers (2).

Studying the infectious disease category

For infectious diseases, case-case studies can identify the source of contamination (or the route of transmission) for a rapid intervention to prevent the occurrence of new cases (6-9). Thus, in an outbreak investigation, the comparison group consists of non-outbreak cases, which were previously diagnosed and identified by the in-place surveillance system. As a result, the risk factor can have different significance between comparison groups and in consequence the real contamination source may not be identified, or the exposure may be very similar (over-matching) and the association underestimated (8, 10, 11).

In the case of comparative studies of infections caused by strains differentiated through the antibiotic resistance profile, phenotypically established, there is a risk for the low-level resistance expressing strains not to be identified and to be considered sensitive, causing cases' misclassification (9, 12, 13). Also, recall bias may be an issue when the assessment of exposures is recorded through patients' self-declaration (12).

The case-case comparison of different infectious diseases surveilled through passive systems, in which only a part of cases is registered, it will use only the selected cases, which are not representative for those present in the population (6). In this comparison, a selection bias can occur, if the cases' registration in the two surveillance systems are done differently, error that can be avoided by comparing the cases with the same disease caused by different strain subtype (characterized by phage profile, antibiotic resistance, plasmid profile) (6, 8, 11, 13, 14).

At the same time, the exposures may vary over time, and if the comparison is made with a control group identified in previous periods, the difference may be due to the dynamics over time and not to a real one (6). On the other hand, etiologically different infectious diseases may have some identical risk factors, which will not be identified through these case-case studies (11, 14). Sometimes, in the surveillance systems, the reported cases can mainly originate from clusters or only the severe ones can be declared, generating differences in the disease's severity among the compared groups (14).

As an overview, in order to use cases as a comparison group in the study of infectious diseases, they must originate from a sufficiently long surveillance period or from a sufficiently large population (cumulative national data) identified by a surveillance system which is stable over time and correctly records the exposures (6, 7).

When a case-case study design is envisaged, the main advantages and inconvenient should be considered to achieve a study with valid results (tab. I).

TABLE I.

The main advantages and limitations of case-case studies (1, 4, 6, 15).

Advantages	Limits
- Minimization simultaneously of the selection and recall bias - Short duration in time and less resources needed than population studies - Selectively identify the association between a particular exposure and a disease's sub-category - Useful for biomarkers identification and for the individual disease risk assessment improvement	- The found association does not represent the risk of the disease in the population - Selection or information bias may occur - Possibility for the two selected groups to remain not comparable - It cannot measure the association for factors that are common to both cases and the control group

CONCLUSIONS

The main advantage of case-case studies is the minimization of both selection and recall bias. They are useful for the rapid identification of risk factors, consuming fewer resources than other analytical studies. The method is however restrictive to study only a part of a diseases' risk factors, being appropriate for the identification of those that cannot be highlighted by population studies. The risk factors identified by these studies allow the description of a disease's etiological heterogeneity, but do not represent the risk of the disease occurring in the reference population.

The case-case study can increase the ability to exploit data collected in routine surveillance systems and complete knowledge about causality and natural history of a disease. Nevertheless, the interpretation of the results should be done with caution, considering the compared groups (aspects related to diseases or characteristics of compared patients) and the possibility of a selection and recall bias persistence.

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NEWS

BIFIDOBACTERIUM LONGUM: PROTECTION AGAINST INFLAMMATORY BOWEL DISEASE

The prevalence of inflammatory bowel disease is generally associated with the change of the immune system and gut microbiota. Bifidobacterium longum is a symbiotic bacterium existed in the human gastrointestinal tract and has been demonstrated to be capable of relieving colitis in mice and can be employed as an alternative or auxiliary way for treating inflammatory bowel disease. Bifidobacterium longum can change the structure of the gut microbiota, induce and regulate immune responses, and reduce the expression of inflammatory cytokines and reactive oxygen species in the intestine. Besides, it can also maintain the normal intestinal barrier function by increasing the expression of the tight junction protein. Therefore, Bifidobacterium longum has great potential and can be used as a prevention, replacement, or adjuvant treatment for inflammatory bowel disease. (Yao S, Zhao Z, Wang W, Liu X. *Bifidobacterium Longum*: Protection against Inflammatory Bowel Disease. *J Immunol Res* 2021; 2021: 8030297).

EPIDEMIOLOGY PROFILE OF WEST NILE VIRUS MENINGITIS INFECTIONS IN NORTH-EAST OF ROMANIA

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EPIDEMIOLOGY PROFILE OF WEST NILE VIRUS MENINGITIS INFECTIONS IN NORTH-EAST OF ROMANIA (Abstract): Meningitis is a disease with global presence that represents a worldwide issue, with the West Nile Virus as an etiological factor. The severity of viral meningitis depends on different factors such as age, immune system and etiological agents. **Aim:** The aim of the study was to study the frequency of West Nile Virus meningitis and to bring a contribution to the existing knowledge of the WNV distribution on adult individuals in Romania. **Results**: They showed a complete etiological and symptomatic picture for WNV meningitis with the main cause being the climatic changes experienced for the sudden appearance of an increased number of WNV infections. **Conclusions**: This retrospective study may constitute a foundation for a plan to prevent a possible epidemic of meningitis caused by the West Nile virus. **Keywords**: EPIDEMIC OF MENINGITIS, WEST NILE VIRUS

Meningitis represents inflammation of the protective membranes covering the brain and spinal cord, associated with abnormal number of cells in the cerebrospinal fluid (1, 2). Aseptic meningitis represents a syndrome characterized by acute onset of meningeal symptoms and fever, with pleocytosis of the cerebrospinal fluid and no growth on routine bacterial culture (1, 3). Viral meningitis can occur at any age but is most common in children (1, 4). In a large study by Rantakallio *et al.* comprising 12,000 subjects, showed the incidence of viral meningitis to be 219 per 100,000 in children under one year of age and 27.8 per 100,000 in children under 14 years old (4). A retrospective study with a small number of subjects showed that the incidence of aseptic meningitis in individuals aged 16 years and over is lower at 7.6 per 100,000 (5). Enteroviruses are said to account for 80% of cases in adults, but a wider range of causes is increasingly implicated. Often no cause is identified; among 144 consecutive adults with aseptic meningitis, only 72 had a confirmed diagnosis (5,6).

West Nile Virus (WNV) is an arthropod-borne flavivirus which is associated with epidemics of flu-like febrile illness usually associated with occurrence of men-

ingitis, encephalitis or acute flaccid paralysis (7). However only 1% of the infected people developed a neurological infection, exhibiting a mild illness with a fever-like headache, myalgias, nausea, vomiting and chills (8, 9-13). Around 5 percent of individuals with symptomatic WNV infection develop those neurologic diseases (14). But the percentage of the manifestation of invasive diseases can vary according to many factors such as endemics or season (7).

WNV meningitis presents symptoms such as: rapid onset of headaches, photophobia, back pain, confusion, and continued fever (8). Ordinary diagnostic tests include cerebrospinal fluid (CSF) to differentiate WNV infection from other infections that could give similar symptoms (8). Furthermore, after CSF other analyzes were performed based on this test, to accurately establish that these studied cases were WNV meningitis. Analysis methods such as blood count and PCR from blood and CSF. In addition to having at least one or more of the following symptoms: fever (temperature > 38°C) or hypothermia (temperature < 35°C); cerebrospinal fluid pleocytosis (>5 leukocytes/mm^3); peripheral leukocyte count >10,000/mm^3; or neuroimaging findings consistent with acute meningeal inflammation, reinforces the diagnosis of viral meningitis (7).

In this context, in the present paper we were interested in establishing some preliminary epidemiological profile of West Nile Virus meningitis infections in North-West part of Romania.

MATERIAL AND METHODS

We performed a retrospective study, including 29 patients diagnosed with meningitis caused by West Nile Virus, hospitalized during 2017 and 2018 in the "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iași county, Romania.

RESULTS

The study group consisted of 19 males (65.51%) and 10 (34.48%) females, mostly over the age of 61 (58%) and moderate amount of cases being between age of 21 to 40 (22.48%), coming mostly from rural areas (65.51%). The incidence of the studied cases occurred almost exclusively in the months of August (48.38%) and September (40.7%) (fig. 1).

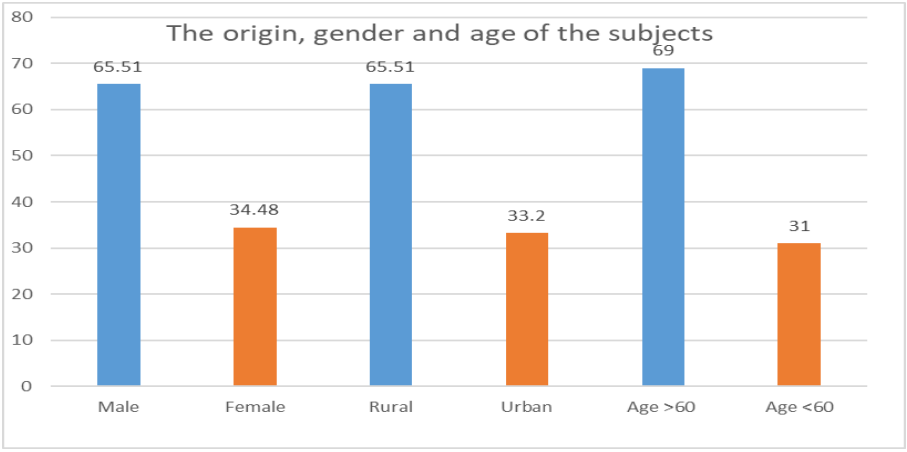


Fig. 1. Description of gender, origin and age

Of these cases, a number of 22 cases were reported with comorbidities such as: diabetes (16.12%), chronic hepatitis(16.12%), neoplasms (16.12%), CKD (9.6%) and cardiac pathology (12.9%) and for the rest there were no comorbidities (48.3%). A large proportion of patients did not use antibiotics (67.74%), but there are subjects who used a series of antibiotics before hospitalization such as: penicillins (16.1%), cephalosporins (9.6%), quinolones (6.45%).

Among the symptoms declared by the patients at admission, the most common was headache (70.96%), followed by confusion (54.83%), fever and other symptoms, with 1 to 7 days declaring the onset of the symptoms before hospitalization. Following the TGP enzyme biochemical analysis, the following values were observed: <40 (48.38%), 41-100 (25.8%), 101-200 (9.6%), 201-300 and >400 they both had the same number of cases (6.45%) and 301-400 with one case (3.2%) and the average value recorded was 99.25. The blood count analysis of the studied patients showed the following: leukocytosis (67.74%), normal number of leukocytes (32.25%) and no leukocytopenia (fig. 2).

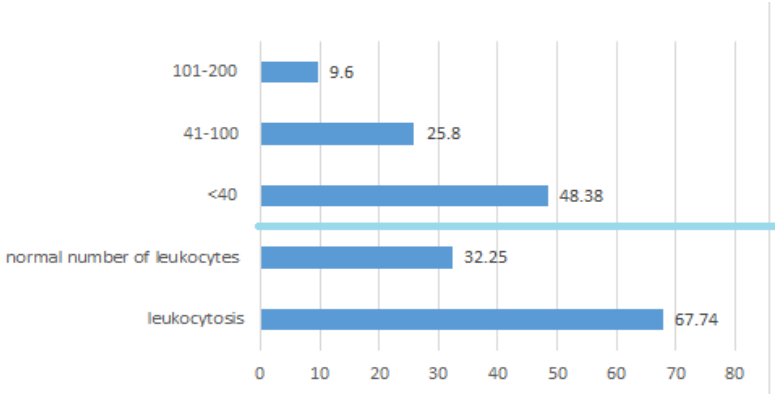


Fig. 2. Results of TGP enzyme and blood count analysis

PCR analyzes were also carried out on the cerebrospinal fluid (CSF) from the target group and the following results were obtained: age 0-10 (48.38%) followed by 21-30 (19.35%) a lower value was 11-20 (12.9%) and the values of 31-40 and >40 recorded the same percentage (9.6%). Adults over the age of 40 show the most positive results on PCR analysis, and children younger than 10 years show the fewest positive results.

The number of lumbar punctures was essential for carrying out laboratory analyses, all patients involved in the study had one puncture in order to collect cerebrospinal fluid (CSF).

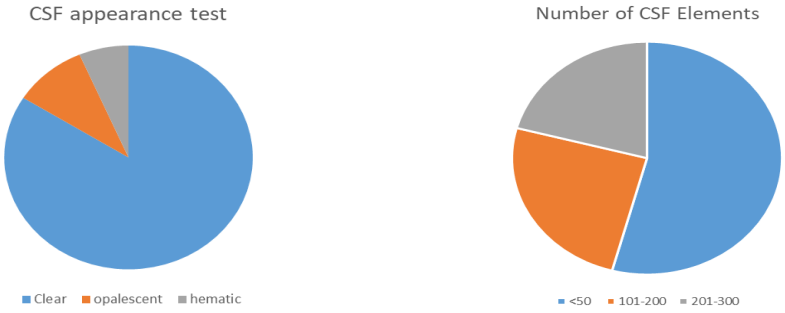
For 74.19% a second CSF collection was carried out, and for 25.8 % a third harvest was necessary, the second and third were necessary because of unfavorable clinical-biological evolution or stationary. After the CSF collection, a CSF appearance test was performed, and the majority of it was clear (83.87%), followed by opalescent (9.6%), and hematic (6.45%).

The analysis of the number of CSF elements showed the highest value for <50 (41.93%), 101-200 (19.35%), value of 201-

300 (16.12%), for the number of elements 51-100 and 301-400 recorded the same number of 9.6%.

Following the analysis of the albumin value in CSF, the highest value recorded was for 1.1-2 g (52.61%), followed by 0.5-1g (26.39%) and > 2g (21%).

The CSF glucose value analysis showed a hyperglycorrhachia (98%) in the patients involved in the study (fig. 3 a, b).



a. **b.**

Fig. 3. Result of CSF tests
a. CSF appearance test. **b.** Number of CSF elements

DISCUSSION

In the present article we traced the exponential increase for WNV meningitis, the study spanned a period of 2 years and presents major symptoms for West Nile Virus meningitis, all cases presented were specifically diagnosed with the West Nile virus. In the case of gender analysis, it can be noted that the occurrence of meningitis caused by WNV is higher in males (65.51%), compared to other viral agents WNV mainly affects adults, an aspect that is previously confirmed by other scientific sources (15). Seasonality is observable during the period of hospitalization of patients, predominantly from July to September. This season is usually characterized by a very hot dry weather, favorable for the proliferation of the viruses responsible for meningitis, which can also be observed in studies in the field (16).

Also recently, our research group become interested on the relationship between the West Nile virus and oxidative stress metabolism, as this was very rarely studied in the past. For example, it was showed that the WNV infection of rabbit PBMCs induces the transcription of HO-1 and inducible NO synthase (iNOS), suggesting that oxidative stress may be involved (17, 18). However, natural WNV strain infections do not induce stress granules (SGs) and some WNV strains could inhibit the SG formation induced by arsenite treatment (19).

The interaction of viral RNA and proteins with TIA-1/TIAR is found to interfere with the formation of SGs (20). Thus, WNV induced both ROS and the antioxidant responses. However, the infected cells do not display characteristics of oxidative stress since the antioxidant responses counteract the negative effects of ROS (19). Thus, it can speculate that the cellular redox status could be quite relevant for the life cycle of WNV.

CONCLUSIONS

In summary, the retrospective study carried out on WNV meningitis presents vital information regarding the spread of this

type of meningitis and the degree of incidence in the north-west area of the country for the period 2017-2018. These results may constitute important data for further development of prophylaxis methods against the spread of WNV meningitis.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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BREAST CANCER IN WOMEN YOUNGER THAN 35 YEARS OLD: IMMUNOHISTOCHEMICAL AND MOLECULAR CHARACTERISTICS AND IMAGING DIAGNOSIS

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BREAST CANCER IN WOMEN YOUNGER THAN 35 YEARS OLD: IMMUNOHISTOCHEMICAL AND MOLECULAR CHARACTERISTICS AND IMAGING DIAGNOSIS (Abstract): The **aim** of this study was to characterize imaging, immunohistochemical and molecular breast cancer in women younger than 35 years old. **Material and methods**: In this retrospective study, data were collected from 46 patients under the age of 35, out of a total of 1,854 patients diagnosed with breast cancer between May 2012 and February 2019 at the Regional Institute of Oncology Iasi (Department of Surgery). The following tumor characteristics were evaluated: tumor size, histological type, molecular subtype, histological grade, nodal status and the presence of systemic metastases. **Results:** Of the 46 cases, invasive ductal carcinoma was the most common histological type (n=31, 67.39%). More than half of the tumors were ER + (n=36, 78.26%) and PR + (n=33, 71.73%). In 84.78% of cases (n=39) we observed a high expression of Ki67 proliferation index (> 20%). Luminal genomic B subtype was the most common (n=27, 58.69%), and triple negative breast cancer was 13.04% (n=6). **Conclusions:** Breast tumors in young women (under the age of 35) have a much more aggressive tumor biology. Future research is required to investigate new treatment strategies especially in young breast cancer patients. **Keywords:** BREAST CANCER, YOUNG WOMEN, AGE, LUMINAL.

Female breast carcinoma is one of the most malignant diseases in the world, with approximately 1 million newly diagnosed cases annually (1). In Romania, and in other developed or developing countries, breast cancer management is a major problem not only of the medical system, but also of the social and economic system.

Although efforts have been made by the EU in recent decades to implement mammographic screening programs (2), Romania is among the last places in Europe in terms of the rate of female population that has breast-controlled at least once in life. (3). Moreover, studies have shown that the mortality rate caused by breast cancer in Romania is 36%, much higher than the European average of 29% (3). In Romania, in 2017, 21 new breast cancers were diagnosed daily, and in the same country, the main cause of female mortality is breast cancer (every 3 hours, a woman dies from this disease) (3).

Starting from these premises, the prognostic factors are important not only in understanding the course of this disease, but also in predicting the outcome for each patient, and especially for choosing the optimal therapeutic management (4).

To date, numerous prognostic factors for breast cancer have been identified, including age, tumor size, degree of axillary lymph node involvement, histopathological grade (5), hormone receptor status (4,6-8), human epidermal growth factor receptor 2 (Her2) status (9, 10), or cell proliferation marker Ki67 (5). Thus, given the urgency of an individual therapy, the outcome in these cases should be estimated more accurately (9).

The aims of this study were to evaluate the clinical, imaging, immunohistochemical and molecular manifestations of young women with breast cancer.

MATERIAL AND METHODS

Patient selection

In this retrospective study, data were collected from medical charts of patients that had undergone surgical treatment between May 2012 and February 2019 at the Regional Institute of Oncology Iaşi (Department of Surgery). Out of a total of 1854 patients, we collected data on 46 patients, aged between 18 and 35 years old. The following tumor characteristics were evaluated: tumor size, histological type, molecular subtype, histological grade, nodal status, systemic metastases. All of these features were assessed according to the 4th edition of World Health Organization (WHO) Classification of Breast Tumors (11) and the 8th edition of the American Joint Committee on Cancer / Union for International Cancer Control (AJCC/UICC) Tumor-Node-Metastasis (TNM) Classification (12).

Also, we assessed the following immunomarkers: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her2/neu) and Ki67 proliferation index, in order to establish the subtypes defined into the framework of molecular classification of breast carcinoma (13). The immunoexpression of these markers was semi-quantitatively analyzed by using suitable scores reported in the literature. ER and PR were quantified according to Allred score (14). Her2/neu status was evaluated as per the standard criteria, on a scale from 0 (negative), 1+ (negative), 2+ (equivocal) to 3+ (positive) (15), and for equivocal results the silver-enhanced in situ hybridization method was applied (16). The Ki67 index was classified as high (> 20%) or low (≤20%) (17). Based on molecular differences primarily through gene expression and presence/absence of hormonal receptors on the cell surface, four clinically relevant breast cancer subtypes have been identified, including Luminal A (ER+, PR+, Her2-, Ki67 low expression), Luminal B (ER+, Her2-, PR-, or Ki67 high expression), triple-negative (ER-, PR-, Her2-), and Her2 (Her2 overexpression) (18).

Written consent regarding the usage of clinical and pathological data of the pa-

tients included in our study was obtained and approved by the Ethics Commissions of our Hospital (Regional Institute of Oncology, Iași) and the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi Romania.

RESULTS

A total of 1854 women were diagnosed with breast cancer during this period, 46 youngers than the age of 35 years old. Of these, 4 of them had bilateral breast cancer and 6 patients had a hereditary - collateral history. Out of the very young group, 30 women presented palpable lesions and 7 axillary adenopathies on diagnosis.

Of the 46 cases, the histological type was: invasive breast carcinoma no special type (NST) (*n=31*), lobular (*n=3*), medullar (*n=3*), metaplastic (*n=1*), micropapillary + NST (*n=2*), cribriform + NST (*n=3*), tubular + NST (*n=2*) and apocrine + NST (*n=1*) (tab. I). The degree of differentiation for tumors (the degree of histological malignancy) evaluated according to the Nottingham scale allowed the tumors to be separated into: G1 invasive mammary carcinomas (*n=4*), G2 invasive mammary carcinomas (*n=13*), G3 invasive mammary carcinomas (*n=7*) and Gx invasive mammary carcinomas (*n=20*). The analyses of immunostaining for ER showed the presence of ER in 36 cases (78.26%) (fig.1.a), while the rest of 10 cases were negative for ER. Similarly, 33 of the cases (71.73%) were PR+ (fig.1.b) and 13 of the cases were PR- (28.27%). Each of the 46 immunohistochemically analyzed cases, were assigned to one of four phenotypes: classical phenotype ER+/PR+ (*n*=32), ER-/PR+phenotype- (*n*=8), ER+/PR-phenotype (*n*=3) and ER-/PR+ phenotype (*n*=3). The most commonly seen hormonal immunophenotype was the classical one with both receptors being present ER+/PR+. The interpretation of immunostaining of Her2 oncoprotein was done according ASCO-CAP recommendations, cases being classed as: score 0 (*n*=13), score 1+ (*n*=15), score 2+ (*n*=12) and score 3+ (*n*=6). The Ki67 proliferation index was significantly high in 39 cases (84.78%) (fig.2.a). The assignment of the analyzed cases according to Her 2 status was complete following in situ hybridization and showed 6 Her 3+ cases (fig.2.b) and 40 Her2- cases. The molecular subtypes revealed Luminal A (*n*=5), Luminal B (*n*=27), Her2/neu-type (*n*=8) and triple negative (*n*=6). All immunohistochemical and molecular characteristics can be seen in Table II.

All patients underwent imaging evaluations: ultrasonographic, mammographically examined and breast magnetic resonance (fig.3). On ultrasound examination, of the 40 patients examined, most patients (*n*=27) had tumors larger than 2.5 cm in diameter, and 13 patients had tumors smaller than 2.5 cm (tab. III). Multifocal masses were seen in 7 cases and microcalcifications without mass only in 5 cases. Also, 17 patients were mammographically examined, as well. Breast magnetic resonance imaging, performed in 3 cases, revealed: multicentric masses looking like invasive carcinoma and in situ (case 1), malignant expansive formation that associates the appearance of mastitis and deep axillary adenopathy (case 2) and mammary intraglandular expansive formation with indeterminate imaging characters, recommending corroboration with the anatomo-pathological examination (case3).

Regarding TNM tumor staging, the tumor size was T1 (*n*=15), T2 (*n*=20), T3 (*n*=5), T4 (*n*=4) and Tx (*n*=2). Similarly, the lymph node status was N0 (*n*=15), N1 (*n*=22), N2 (*n*=8), N3 (*n*=1) and Nx (*n*=0). The systemic metastases status was characterized as follows: M1 (*n*=1), M0 (*n*=10)

and Mx (n=4). Of the total 46 patients, 41 underwent Madden-type modified radical mastectomy, a surgical technique that involves the complete removal of the breast, including the overlying skin and nipple-areolar complex and ipsilateral level I, II axillary lymphadenectomy (19), while the breast conserving surgery with axillary lymph node dissection was preferred in 5 patients. 5 patients underwent breast reconstruction. Of the 46 patients, 30 cases underwent neoadjuvant chemotherapy.

DISCUSSION

In developed countries, breast cancer in people under the age of 40 accounts for 5-7% of all breast cancers (20). Moreover, in some countries such as China, the incidence of breast cancer in young women reaches 5-13% of all breast cancer cases (21). Various studies have shown that breast cancer in young women is different than in older women. Thus, these cancers in young women appear to have a more aggressive biological behavior, have a higher histopathological degree and a higher proliferation fraction, compared to older women (22). Also, breast cancer in young people has a worse prognosis in terms of recurrence and overall survival (23). Moreover, various authors have shown that young age is an independent factor of poor prognosis (24,25).

Of the 46 cases, invasive ductal carcinoma was the most common histological type, representing 67.39% (n=31), a result similar to Eric *et al.* (26). In his series of 79 women with breast cancer aged < 40 years, he observed that 70.9% pf patients (n=56) had invasive ductal carcinoma (26). Also, different authors have identified that invasive ductal carcinoma is one of the most common histological types of breast cancer in women under 40 years (7,27). Moreover, Copson *et al.*, in the largest prospective observational study evaluating the pathological characteristics of 2,956 women with breast cancer under 40 years, reported that the majority of tumors (86.5%) had ductal histology (28).

Currently, a more elaborate characterization of the interactions between immunohistochemistry and imaging is needed to understand not only breast cancer biology but especially the treatments adapted for each patient. Regarding breast cancer, ER and PR expression are considered the most useful predictive marker (5,29). Previous studies have shown ER expression in 50-80% of cases and PR expression in 60-70% of cases of invasive ductal carcinoma (5,30,31). Interestingly, some studies in India and Pakistan have shown a higher rate of hormone receptor-negative tumors than that found in the western population (29,32,33). In our study, ER+ status was found in 36 patients out of a total of 46 (78.26%). Also, PR+ status was observed at 71.73% (n=33). We remarked an important representation of estrogen and progesterone receptors.

Besides hormonal receptors, one of the most promising markers in breast cancer is Her2. This is a protein that is overexpressed in 18-20% of breast cancer and has been shown to have prognostic value (29-31). In this sense, Her2-expressing tumors are generally histologically high grade, tend to metastasize, and have a worse prognosis (34). The most common metastatic site for breast cancer is bone, being followed by lung, brain and liver (35).

According to ASCO-CAP recommendations, the cases in our study were classified as follows: score 0 (n=13), score 1+ (n=15), score 2+ (n=12) and score 3+ (n=6) (tab. II). In 8 (17.39%) of the patients we identified an overexpression of Her2/neu, overexpression that was reported as an aggressive immunophenotype in

breast cancer in young women (36). On the other hand, various authors have not identified a significant correlation of Her2/neu overexpression and young age in breast cancer patients (26,37).

Luminal A tumors are characterized by endocrine-responsive cancer (ER+, PR+) and low proliferative rate (low grade and low Ki67) (38) and have a better prognosis. Luminal subtype breast cancers also have the lowest recurrence rate, as opposed to triple negative subtypes that relapse the most (25,39,40). In our study, Luminal A tumors were in a small percentage of 10.86% (n=5) (tab. II). Similarly, Azim *et al.* reports that in his study, he identified Luminal A cancer in 17% of the younger and 35% of the older women (41). In another study of 46 women < 40 years and 73 patients > 40 years, Bouferraa *et al.*, observed that Luminal A subtype had a higher prevalence in patients above the age of 40, but the difference did not was statistically significant (42).

Luminal B tumors are also endocrine-responsive tumors but have a higher proliferative rate and have a worse prognosis compared to Luminal A tumors (38). In our study, we observed that compared to Luminal type A, Luminal B had a much higher prevalence rate of 58.69% (n=27), according to other studies in the literature, which reported a predominance of Luminal B tumors in young women and less often Luminal A tumors (43).

Triple-negative tumors (ER-, PR-, Her2-) usually have a very aggressive behavior, chemotherapy being the main pillar of treatment (44). We observed that in our group, triple-negative tumors were detected in 17.39 patients (n=8), according to other studies in the literature, which reported 19.9% in young women (28). Other authors have reported higher percentages, such as Carvalho *et al.* which identified a 25% rate (45) and Eric *et al.* which identified a 32% rate in the young women group (26).

The Ki67 proliferation index is a nuclear protein that is expressed in proliferating cells, being also a marker of tumor proliferative status (46). Although current guidelines from the American Society of Clinical Oncology do not include Ki67 as routine biological markers for breast cancer, however, in addition to ER, PR and Her2/neu status, Ki67 can complete the image of the histopathological pattern of the tumor. In this regard, new genetic tests have highlighted the role of proliferative genes, including the Ki67 marker, as a predictive factor (47). However, a standardization of Ki67 pathological assessment has not yet been determined (48).

In our study, of the 46 patients, 39 had Ki67 > 20%, and the remaining 7 had Ki67 < 20%. It should be noted that a significant percentage of patients (84.78%) had a high Ki67 proliferation index. In his study in which he compared the immunohistochemical characteristics of breast cancer in young women (< 40 years) and older women (> 60 years), Eric *et al.* observed that the Ki67 proliferation index was significantly higher in the young women group (26). Also, Fredholm *et al.* observed that in the group of young women (< 40 years), Ki67 was higher (> 14%) in a percentage of 79% in Luminal patients, 88.5% in Her2+ patients and 94.1% in triple-negative patients. Regarding the correlation of Ki67 value with tumor size, in our case we did not notice differences between Ki67 and tumor size. Thus, of the patients with Ki67 > 20% and which were ultrasound examined, 17 of them had tumors > 2.5 cm and 12 had tumors ≤ 2.5 cm.

Tumor size has traditionally been considered one of the most powerful prognostic factors in breast cancer (7,49,50). Moreover, this size of the breast tumor was

the basis of major staging systems for breast cancer (51-53). Some authors have reported increased tumor size as being associated with increased breast cancer-specific mortality with each joint estrogen and progesterone receptor status (54, 55). It is important to note that this information came from studies in which the authors located all stage T3 tumors (> 5 cm) in a single category (54, 55). On the other hand, the view that breast tumor size would correlate with survival in all breast cancer subtypes has been questioned (56), and several studies have found that this model is not valid for small breast tumors (57-59). In our study, we observed that there is no significant difference between tumor size and hormonal tumor expression. Thus, in the 40 patients explored ultrasonographically and ER+, 14 of them had tumors > 2.5 cm and 10 of them had tumors ≤ 2.5 cm (tab. III). Also, of the PR+ patients, 14 of them had tumors > 2.5 cm and 11 of them had tumor ≤ 2.5 cm (tab. III). In consensus, Zheng *et al.* obtained similar results, but piecewise (9). In his study of 358.870 female patients diagnosed with invasive breast cancer, Zheng *et al.* found that patients with 51-60 mm, 71-80 mm and > 80 mm tumors had similar breast-cancer specific mortality as those with 61-70 mm tumors. These results suggest in an interesting way that increasing tumor size potentially lost its prognostic value in ER+ PR+ group above the threshold of 51 mm (9). Sohail *et al.*, also observed that out of 59 ER+ patients, 22.3% had tumor sizes between 2-5 cm and 17.7% had > 5 cm. Also, of 48 PR+ patients, 18.5% had tumors between 2-5 cm and 15.4% had > 5 cm (5). In his study that included 19,541 non-Hispanic white women with node-negative breast cancer, Anderson *et al.* (54) observed that 80.3% (n=12,248) of ER+ tumors were ≤ 2 cm in size, while only 19.7% (n=2,999) were > 2 cm.

CONCLUSIONS

In our study of a group of 46 young women (under the age of 35) we identified an increased hormonal expression of estrogen and progesterone receptors, regardless of tumor size. We also identified Luminal B tumors in more than half of the patients, and a high Ki67 proliferation index. In conclusion, we believe that breast tumors in young women have a more aggressive tumor biology, and that future research is required to investigate new treatment strategies especially in this category of patients.

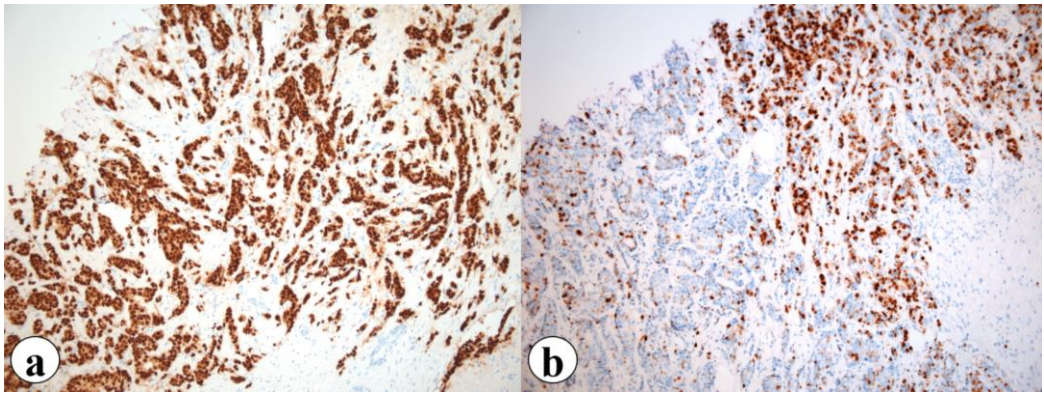

Fig. 1. Microphotograph showing invasive breast carcinoma NST:
(a) ER+ (*IHC, x10*); (b) PR (*IHC, x10*).

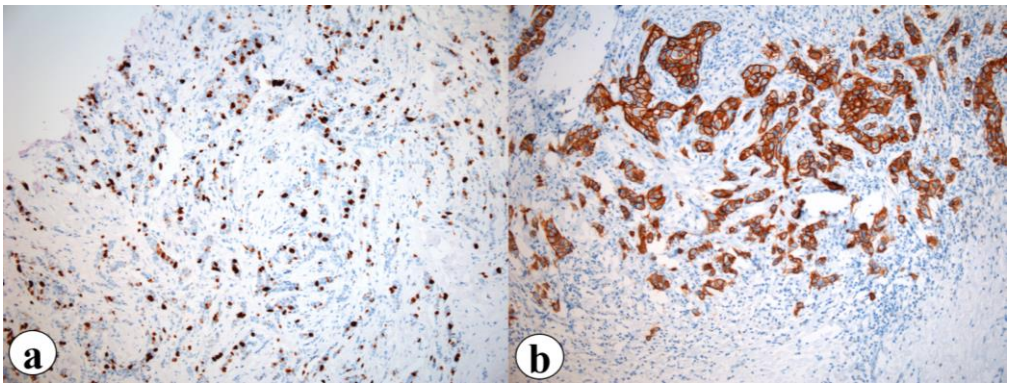

Fig. 2. Microphotograph showing invasive breast carcinoma NST:
(a) high Ki-67 proliferation index (*IHC, x10*); (b) HER-2/neu status=3+ (*IHC, x10*).

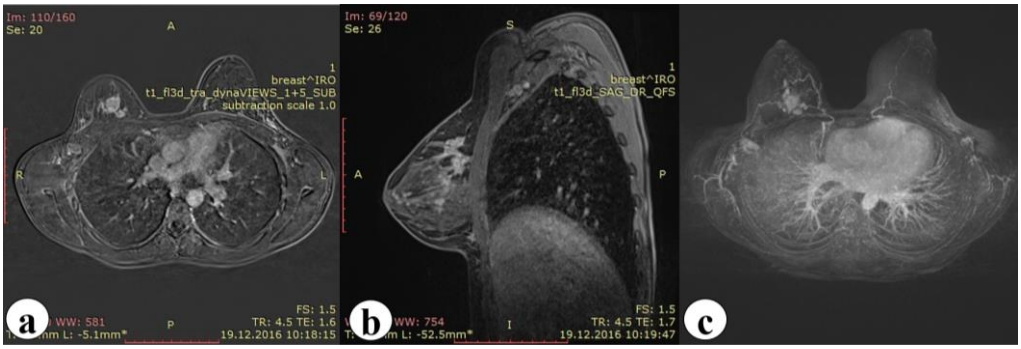

Fig. 3. Contrast MRI showing in the right breast, upper quadrants, at a distance of 8 cm from the nipple - multinodular contrast uptake that converges into a mass with a maximum size of 26/14/29 mm, intense early, with washing in late sequences, imprecisely delimited, with spicules on the contour; the posterior edge at a distance of 2 mm from the pectoral muscle. Other suspicious micronodular contrast uptakes dispersed in the central quadrant.

TABLE I.

Histological types and grades of tumors

Histological type *n* (%)			Histological grades (*n*)	
Invasive breast carcinoma NST	31 (67.39%)		0	0
Lobular	3 (6.52)		IA	3
Medullar	3 (6.52%)		IB	1
Metaplastic	1 (2.17%)		IIA	10
Micropapillary + NST	2 (4.34%)		IIB	12
Cribriform + NST	3 (6.52%)		IIIA	14
Tubular + NST	2 (4.34%)		IIIB	6
Apocrine + NST	1 (2.17%)		IIIC	0
			IV	0

TABLE II.

Immunohistochemical and molecular characteristics

	n (%)
Estrogen status	
Positive	36 (78.26%)
Negative	10 (21.74%)
Progesterone status	
Positive	33 (71.73%)
Negative	13 (28.27%)
HER-2/neu status	
0 negative	13
1+ negative	15
2+ equivocal	12
3+ positive	6
Ki67 proliferation index	
High	39 (84.78%)
Low	7 (15.22%)
Luminal A	5 (10.86%)
Luminal B	27 (58.69%)
Triple-negative	6 (13.04%)
Her2 (overexpression)	8 (17.39%)

TABLE III.

Immunohistochemical characteristics in the 40 ultrasound-examined patients

n=40/46	ER+	ER-	PR+	PR-	Ki67 high	Ki67 low
> 2.5 cm (*n*=27)	14	7	14	6	17	4
≤ 2.5 cm (*n*=13)	10	3	11	5	12	2

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DEVELOPMENT AND VALIDATION OF A LC-MS /MS METHOD FOR THE QUANTITATIVE DETERMINATION OF TRAMADOL HYDROCHLORIDE

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DEVELOPMENT AND VALIDATION OF A LC-MS/MS METHOD FOR THE QUANTITATIVE DETERMINATION OF TRAMADOL HYDROCHLORIDE (Abstract): The **aim** of this study was to develop and validated a highly sensitive, accurate, and precise liquid chromatography and tandem mass spectrometry (LC/MS/MS) method for quantitative analysis of tramadol (TMH) in human plasma using metoprolol tartrate as internal standard (IS). **Material and methods:** Quantitative analysis was done with methanol and 0.15 % formic acid in water (40:60, v/v) as the mobile phase and C_{18} as the stationary phase with a flow rate of 0.5 mL/min and MS as the detector. The mass spectrometer was operated in positive polarity mode. The method was validated for specificity, sensitivity, precision, accuracy, and other analytical parameters. The level of tramadol data was statistically analyzed using ANOVA at a 95% confidence level. **Results:** The total analytical run time was 2.5 min. The results found were satisfactory over the linear calibration range of 10-500 ng/mL for TMH. It is a simple, fast, precise and accurate liquid chromatographic method. **Conclusions:** The developed method can be used by scientific community for the quantification of tramadol in plasma samples from various clinical studies *of different dose strengths.* **Keywords:** TRAMADOL, LC/MS/MS METHOD, QUANTIFICATION.

Pain is the most common symptom of injuries, illnesses, disorders and conditions that we can experience throughout our lives. Depending on the type of pain, different medications may be recommended to relieve the discomfort. One of the most used drugs worldwide for alleviating moderate to severe pain is tramadol (TMH). It presents a very good analgesic activity by its action on central nervous system. Tramadol is considered a weak opioid due to its relatively low affinity for μ-opioid receptor, the main target for traditional opioids (1). It is available in a variety of formulations such as oral, suppository and parenteral preparations. The drug tramadol is a racemic mixture of two enantiomers in the form of hydrochlorides. The racemic

mixture of (+) dextro and (-) levo enantiomer of tramadol has synergistic analgesic effect. Some studies show that tramadol is also an effective antidepressant (2). Tramadol, in combination with adrenaline, has been used as a local anaesthetic in maxillofacial surgery. After oral administration it is rapidly absorbed from the intestine. Its oral bioavailability is 65–70 % due to first-pass metabolism (3) and it is metabolized to O-desmethyltramadol (ODT) primarily by cytochrome P450 (CYP) 2D6, and N-desmethyltramadol (NDT) by CYP2B6 and CYP3A4. ODT and NDT are further metabolized to N,O-dides-methyltramadol (NODT) by CYPs (4). Tissue distribution of tramadol is about 2.6–2.9 L/kg body weight. Tramadol does not have serious side effects, it is not addictive in therapeutic doses, as seen in other opioids such as morphine (5). Tramadol has been determined by using several chromatographic techniques such as liquid chromatography (LC) with ultraviolet (6, 7) or fluorescence detection (8, 9), LC coupled to tandem mass spectrometry (MS/MS) (10-12), gas chromatography (13, 14). Drug analysis is important in the pharmaceutical industry because it can help in dosage form selection by studying the stability of the active compound and identifying impurities in pharmaceutical formulations. Analytical determinations of medicinal substances are not only applied in the process of manufacturing medicines, but also in forensics for the quantification of prohibited substances in doping or problems related to drug abuse. This study aimed to develop a rapid, simple determination method for tramadol in human plasma using an isocratic LC-MS/MS.

MATERIALS AND METHODS

Materials

Tramadol hydrochloride (TM) and metoprolol tartrate as an internal standard (IS) were obtained from Unichem Laboratories LTD (India). HPLC-grade methanol, formic acid, and trichloroacetic acid were purchased from Merck (Germany). All other reagents were of analytical grade and commercially available.

Solutions

Stock solutions of tramadol (10 μg/mL) and IS (100 ppm) were prepared in methanol. Standard solutions of tramadol were obtained by the dilution of stock solution with methanol.

Sample preparation

0.5 mL biologic sample was treated with 0.5 mL trichloroacetic acid 5% like deproteinized. The obtained solution was ultrasonicated for 5 minutes and then centrifugated at 4000 rpm for 10 minutes. The supernatants were filtrated with a Millex-LH syringe filter (0.45 μm, 4 mm, Merck Millipore Ltd., Billerica, MA, USA) before injection into the LC. From the supernatant, a volume of 5 μL was measured and analyzed under the conditions of the method.

Chromatographic conditions

Tramadol and metoprolol tartrate in the sample were separated using a Dionex Ultimate 3000 LC instrument. Separation was performed using a Hypurity™ Elite Hypersil C18 (100 x 3 mm, 3 μm). The mobile phase consisted of methanol and 0.15 % formic acid in water (40:60, v/v). The flow rate was 0.5 mL/min, and the column temperature was set at 40 °C, and the autoinjector was set at 4 °C.

Mass spectrometric conditions

The column effluent was monitored using a triple quadrupole mass spectrometer (3200 QTRAP®, AB Sciex, Foster City, CA, USA) equipped with an electrospray probe in positive ionization mode. The ion transitions were monitored using a dwell time of 200 milliseconds for each compound: tramadol, 264.4/58.3; metoprolol 268.1/121.18. Samples were introduced to the interface through a turbo ion spray with the temperature set at 300 °C. A high positive voltage of 3.8 kV was applied to the ion spray. The pressure of the ionization gas was 20 psi, and the pressure of the auxiliary gas was 30 psi. Collision energy for tramadol and metoprolol tartrate was 1.5 mTorr. The capillary temperature was set at 320°C.

Method validation

In order to determine the *specificity* of the method, the following chromatograms were recorded under the method conditions:

- blank solution - 0.5 mL of a biological sample from each source (S1-S6);

- sample solution - 0.5 mL of a biological sample (S1-S6) was mixed with 0.025 mL standard solution of 40 ppm concentration;

- internal standard solution - 0.5 mL biological sample (S1-S6) was mixed with 0.025 mL solution 40 ppm internal standard solution.

Selectivity was studied by investigating the magnitude of any interference caused by drug metabolites. In order to establish the influence of interferences, a number of drugs such as metoclopramide, famotidine, granisetron, pantoprazole and fentanyl were co-administered with tramadol. It was considered: Metoclopramide - ESI (-) 301.0 → 190.0 (15), Famotidine - ESI (+) 338.3 → 189.2 (16), Granisetron - ESI (+) 313.1 → 138.2 (17), Pantoprazole - ESI (+) 384.1 → 200.0 (18), Fentanyl - ESI (+) 337.3 → 188.26 (19).

The study of the residual effect on the column

Analyte traces that can be found in the samples are identified by observing similar signals. These signals must have an area that does not exceed 0.2 of the lowest concentration in the studied range, and in the case of components that are internal standards must not be greater than 5%.

Solutions used:

- blank solution - a volume of 0.5 mL of biological sample;

- sample solution at the upper limit of quantification (ULOQ) - 0.5 mL of biological sample was mixed with 0.025 mL of the standard solution of 40 ppm concentration;

- sample solution at the lower limit of quantification (LLOQ) - 0.5 mL biological sample was mixed with 0.02 mL 1000 ppb concentration standard solution.

- internal standard solution - 0.5 mL of a biological sample (S1-S6) was mixed with 0.05 mL of 40 ppm internal standard solution, resulting in a solution with a concentration of 1000 ppb.

The formula used to calculate the residual effect is shown below:

$$\%\, residual\ effect = \frac{signal\ area\ (analyte\ blank\ solution)}{signal\ area\ \ (analyte\ sample\ solution)\ (LLOQ)} \times 100$$

Linearity. For the study of the linearity of the method, 3 sets of working solutions were prepared, on the concentration range 10-500 ppb for tramadol (20). The internal standard used for each solution was 1000 ppb metoprolol tartrate (tab. I).

TABLE I.
Linearity of the method

	P1	P2	P3	P4	P5	P6
TMH (ppb)	10	30	100	250	375	500
MT (IS) (ppb)	1000	1000	1000	1000	1000	1000

The precision of the method

It was established by determining repeatability and intermediate precision. A number of 3 samples were prepared and analyzed for each concentration level (10, 100, and 500 ppb). The concentration for each individual sample was calculated based on the equation of the regression line and the values of the areas corresponding to the tramadol peaks (21). The obtained values were processed statistically and the value of the coefficient of variation (CV) was calculated.

Accuracy of the method

Three standard solutions of tramadol with different concentrations (table II) were used in order to evaluate the accuracy of the method (22, 23). For each concentration, 3 series of determinations were performed, and the degree of recovery was calculated with the relationship:

$$\%\ Recovery = \frac{Calculated\ Concentration\ Value}{Nominal\ Concentration} x100$$

TABLE II.
The solutions for the accuracy study

	3xLLOQ	50%	ULOQ
TMH (ppb)	30	250	350

Time stability of the analyzed samples concentrations

The stabilities of TMH and IS in plasma were assessed by comparing the areas measured over 7-60 hours of storage at room temperature with the initial peak area (20, 22).

RESULTS

In order to establish the optimal parameters of the method, preliminary tests were conducted. We selected the chromatographic column (Hypurity™ Elite Hypersil C18, 100 x 3 mm, 3 µm) after assessing three types of chromatography columns. The mobile phases recommended by the specialized literature consist of a mixture of methanol or acetonitrile with buffer solutions such as phosphate, triethylamine, acetate, and formic acid with a pH between 2.5 and 7.3.

The results obtained in the study of the specificity of the method are shown in table III.

The chromatograms corresponding to the compounds considered for the evaluation of interferences are presented in first figure.

TABLE III
The study of the specificity of the method

	Standard	Retention time	Area at LLOQ		Area at ULOQ
	TMH	1.75	283675		38572
	MT	1.71	9913		22079
Sample	**Retention time**	**Area at LOQ**	**Blank area**	**Calculated conc.**	**% Interference**
S1	1.75	0	0	0.00	0.00
	1.71	0	0	0.00	0.00
S2	1.75	0	0	0.00	0.00
	1.71	0	0	0.00	0.00
S3	1.75	0	0	0.00	0.00
	1.71	0	0	0.00	0.00
S4	1.75	0	0	0.00	0.00
	1.75	0	0	0.00	0.00
S5	1.71	0	0	0.00	0.00
	1.75	0	0	0.00	0.00
S6	1.71	0	0	0.00	0.00
	1.75	0	0	0.00	0.00

The results obtained in the study of the interferents are shown in table IV.

The obtained results for the study of the residual effect on the column, are reproduced in table V.

The data obtained at the stability of the minimum limit of quantification (LLOQ) are shown in table VI, where S/N is the ratio between the recorded signal (S) and the background noise (N).

TABLE IV.
The interference study caused by several concomitant analytes.

Compound	**Specific transition (m/z)**		**Areas ULOQ**	**Areas LLOQ**	**% Interference (compared to LLOQ)**
MT (IS)	268.1 → 121.18		44074		
Interferences	Specific transition (m/z)	Specific transition (det)	Areas (det)	Concentration (ng)	
	284.1→ 116.1	–	–	–	
Compound	Specific transition (m/z)		Areas ULOQ	Areas LLOQ	**% Interference (compared to LLOQ)**
TMH	268.1 → 121.18		44074		
Interferences	Specific transition (m/z)	Retention time (det)	Area (det)	Concentration (ng)	
Desmethyltramadol	250.1 → 58.1	–	–		

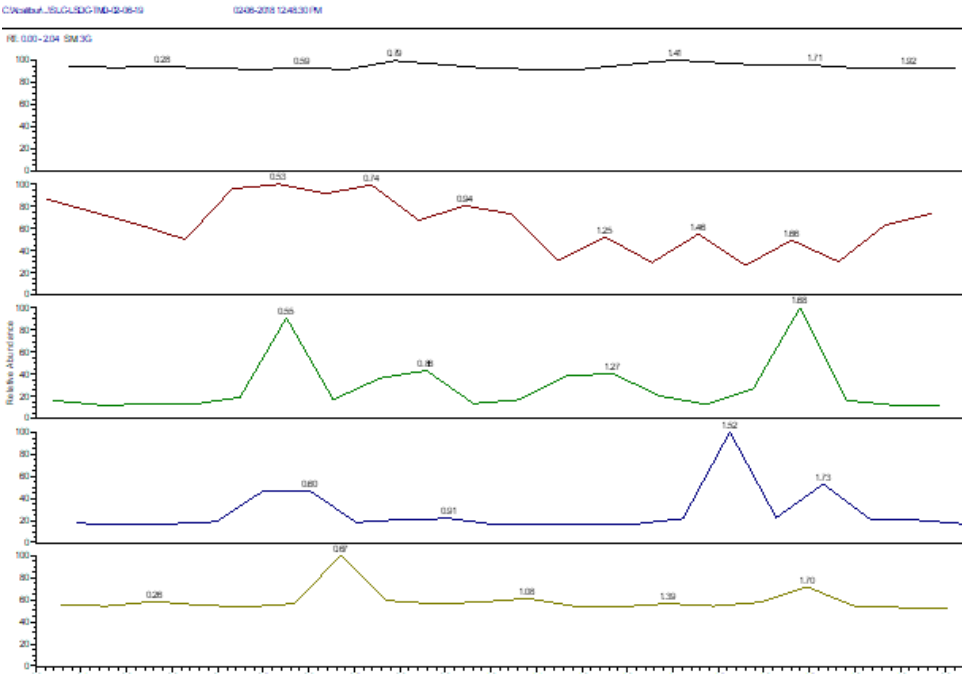

Fig 1. The chromatograms for metoclopramide, famotidine, granisetron, pantoprazole and fentanyl

TABLE V.
The residual effect on the column

Series	Sample	Areas	Residual effect (%) (against LLOQ)
TMH	Sample at ULOQ	**283675**	**0.0**
	Blank	**0**	
	Sample at LLOQ	**10337**	
MT	Blank	0	**0.0**
	Internal standard	44776	

TABLE VI.
Determination of the lower limit of quantification (LLOQ)

Name	Sample	S/N	Areas	Signal ratio LLOQ/BS
TMH	Sample at LLOQ	712	18151	0
	Blank sample	0	0	

The calibration curves obtained, through the graphical representation of the areas as a function of the tramadol concentration in the sample, are shown in figure 2.

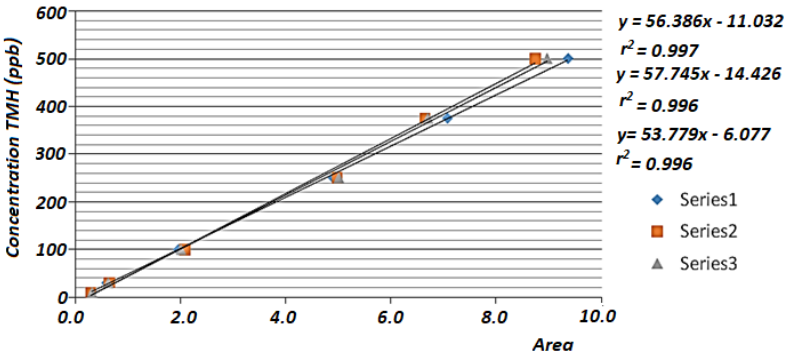

Fig. 2. Calibration curves for TMH

The experimental values obtained in the study of the precision of the method for the determination of tramadol hydrochloride by LC-MS/MS, are presented in table VII.

The graphical representation of the individual deviations for tramadol hydrochloride is shown in figure 3.

TABLE VII.

The precision of the method

TMH concentration (ppb)	10	100	500
Series	**S1**	**S2**	**S3**
day 1	93.45	96.27	103.78
day 1	98.92	93.28	90.90
day 2	107.63	91.74	99.17
day 2	109.74	99.40	103.19
Average	102.43	95.17	99.26
Standard deviation	7.60	3.39	5.94
CV	7.42	3.56	5.98

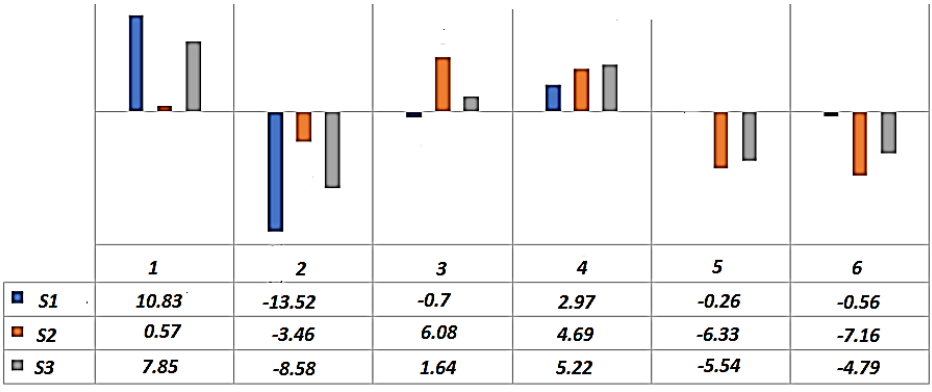

Fig. 3. Individual deviations of the calculated concentrations *vs.* theoretical concentrations of TMH

TABLE VIII.
Accuracy of the method

Theoretical Concentration (ppb)	Series	Area AS*	Area IS	Areas ratio AS/IS	Calculated Concentration (ng/mL)	Recovery (%)	RSD%
500	S1	235166	38973	6.03	327.07	93.45	1.25
100	S2	64454	33770	1.91	96.27	96.27	
10	S3	12068	32327	0.37	10.38	103.78	

***Mean of three replicates (n=3) *AS=analyzed solution *IS=internal standard**

The experimental values and calculations performed in order to evaluate the accuracy of the method for determining tramadol hydrochloride by LC/MS are presented in table VIII.

The results obtained in the tramadol stability study are presented in figure 4.

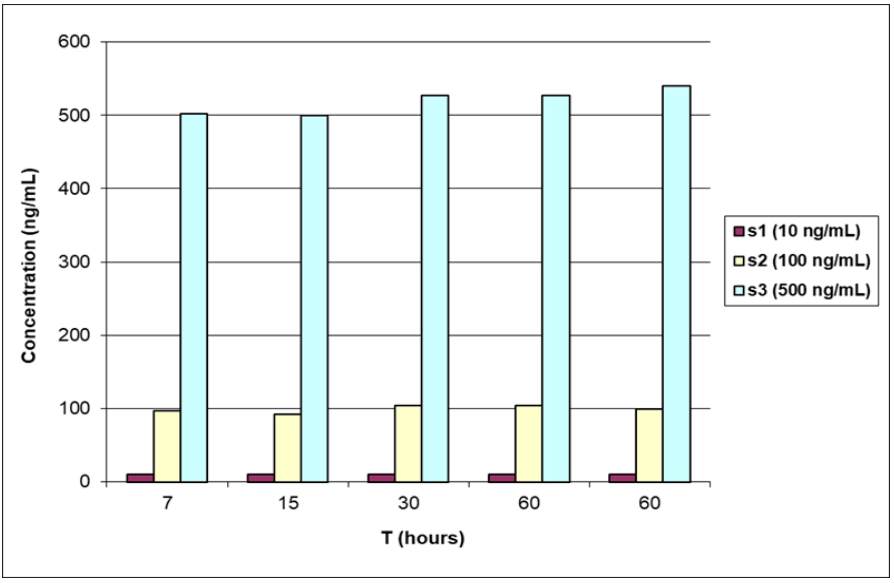

Fig. 4. Time stability of tramadol concentrations

DISCUSSION

The development of a practical chromatographic method for the determination of tramadol in human plasma is needed for clinical use. In our study, we used the MS/MS detection for tramadol because it possesses high sensitivity and selectivity. Ultraviolet detection from biological fluids is not suitable because of low sensitivity and selectivity (8). The LC separation of tramadol from a mixture using ultraviolet or fluorescence detection requires surfactants such as triethylamine and sodium dodecyl sulphate (9). These surfactants cause the ionic suppression of analytes in MS/MS analysis. The convenient ratio of the two solvents (formic acid : methanol) which provides good resolution was found

to be 40:60 (v/v). By changing this ratio, a decrease in peak area and height was found, and this would lead to a decrease in the sensitivity of the method. Other authors have used a mixture of acetonitrile - 0.1% formic acid 20 : 80 (v/v), as a mobile phase (8). The mobile phase consisted of methanol and a nonvolatile phosphoric acid salt solution was used by Ardakani *et al*. (24). The methods that are using a mobile phase with phosphate buffer are not suitable for MS/MS analysis.

In our experiments we followed the possibility of a simple procedure of processing samples, involving only deproteinization with 5% trichloroacetic acid. Other authors used for protein precipitation acetonitrile and methanol (25), or ethyl acetate (24). Using this procedure, the recoveries of tramadol in the present method were more than 89.9% while Ardakani *et al*. (23) obtained a recovery percentage of 80.8% and Tanaka *et al*. 85% (25). The run time for LC separation was shorter (5min) than in other studies (25, 26).

Analyzing table IV, it was observed the absence of signals that can be associated with interferents, and if they are present, then they are found in a concentration lower than 20% of the lower limit of quantification. Figure 2 showed that no interference occurred, due to the presence of the following substances: metoclopramide, granisetron, fentanyl, pantoprazole, and famotidine, administered simultaneously with tramadol.

In the column residual study, no residual signals were found for tramadol. For the control samples, no residual samples were found, after introducing the samples at the lower limit of quantification into the analysis (table V), as well as after using the samples at the upper limit of quantification.

The signals at the LLOQ for tramadol show a signal (S) to background noise (N) ratio of 712. On the other hand, the LLOQ value it must be a maximum of 5% of the ULOQ. In our case, it has 2% of the upper limit of quantification (table VI).

The calibration curve in human plasma of tramadol was linear over the concentration range of 10-500 ppb. Tanaka *et al*. reported a concentration range between 12.5-1600 ng/mL. The lower limit of quantification of tramadol was 10 ppb.

According to the data obtained and presented, the proposed analysis method is precise because the value of the coefficient of variation did not exceed 15% for the quality control samples, the maximum value being 7.42% (table VII). Other authors reported higher CV values for the precision study than in our case (12, 25).

The recovery of TMH was found to be in the range of 93.45-103.78%. These results show the proposed method is accurate. Complete data are shown in table VIII.

Analyzing the data in figure 4, the stability of the tramadol concentrations in the analyzed samples can be seen for 60 minutes.

CONCLUSIONS

We validated and applied a reverse phase LC method for the separation and quantitative determination, by mass spectrometry, of tramadol hydrochloride. The method was linear over the concentration range of 10-500 ppb tramadol hydrochloride. The value of the lower limit of quantification was at most 5% of the upper limit of quantification. The precision of the method estimated by repeatability and intermediate precision was below the 2% limit. The proposed method of analysis is accurate because the calculated concentration does not exceed 15% of the target values of the control samples. The shorter runtime allows the analysis of more sam-

ples. The method developed can be applied in drug analysis and control laboratories, toxicology laboratories, and for monitoring the concentration of tramadol in the blood. The developed method will be applied to evaluate the toxicity of tramadol on the human body as well as to evaluate the intensity of pain.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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EXPRESSION OF CYTOSKELETAL PROTEINS IN GLIOBLASTOMA CELL LINE

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EXPRESSION OF CYTOSKELETAL PROTEINS IN GLIOBLASTOMA CELL LINE (Abstract): The overexpression of vimentin is linked to tumors with low differentiation, metastatic diseases, and reduced overall survival. The precise function of transgelin, an actin-binding protein, in the development of cancer is still debatable. Nevertheless, its participation in the mobility, differentiation and programmed cell death of malignant cells has been well-established. **Material and methods**: To investigate transgelin and transgelin expression, we used glioblastoma U87 cell line 2D culture. We conducted immunofluorescence staining to examine cultured cells. **Results**: the studied proteins were co-expressed in the studied cell line. **Conclusions**: How transgelin is expressed in cancerous cells is similar to the expression of vimentin. This similarity could suggest that transgelin may serve as an indicator of the level of aggression in cancer. **Keywords**: VIMENTIN, TRANSGELIN, CANCER, CELL CULTURE.

Glioblastoma is the most common brain tumor, with a poor prognosis and survival rate. Despite the complex approach, including surgery, radiotherapy and adjuvant chemotherapy, most of the patients develop relapse of the disease in the first ten months after initial treatment (1, 2, 3).

Vimentin is a type III filament characteristic of mesenchymal cells coded at the level of chromosome 10p13 (4). In the adult body, it is found exclusively in specific cell types, namely fibroblasts, glial cells, endothelial cells and lymphocytes. Its roles include participation in the maintenance of focal adhesions, potentiation of lamellipodia formation through CARMIL2, and migration through interaction with actin (5, 6). Although they do not seem to be directly involved in cell motility, numerous studies have demonstrated that some intermediate filaments of the cytoskeleton, such as vimentin, affects the epithelial to mesenchymal transition (EMT), as well as on neoplastic cell invasion and migration (7- 9).

In neoplastic cells, vimentin seems to potentiate their migratory character by influencing cytoskeleton dynamic in EMT; its overexpression is correlated to poorly differentiated tumors metastatic disease and shorter overall survival (10 -12).

Originally named SM22alpha, trans-

gelin belongs to the calponin family, being an actin binding protein with multiple roles in cell dynamic and cell cycle. In the embryonic stage, the functions of transgelin are podosome formation and smooth muscle differentiation. In the adult, transgelin is found in the cytoplasm of fibroblasts and some epithelial cells, having a role in the regulation of smooth muscle fiber contraction, cell migration in response to inflammation, the formation of podosomes in smooth muscle cells and the reorganization of the cytoskeleton during cell differentiation (13, 14).

In the context of carcinogenesis, the role of transgelin is controversial, although its participation in the processes of migration, differentiation and apoptosis of neoplastic cells is known(15). Data from previous research cannot establish a pattern of transgelin's expression in cancer; studies performed on tissue samples and cell lines exert contradictory results, as transgelin being overexpressed in colorectal (16), lung (17) and hepatocellular cancer (18).

In other ways, there are some evidence of transgelin being under expressed in prostate(19), esophageal (20) and cervical cancer (21) .

The role of transgelin as a tumor marker cannot be discussed as long as it may found in significant amounts in normal tissue. To fully comprehend the role of transgelin in the spread of cancer, its function must be studied thoroughly. This will allow for an evaluation of its potential role in determining the prognosis of the disease. The exact mechanism through which transgelin operates on cancer cells remains unclear, but it is believed that it results from a modification in the expression of genes that regulate cell mobility (22).

MATERIAL AND METHODS

We investigate vimentin and transgelin co-expression in glioblastoma U87 cell line.

Cell cultures

To investigate transgelin and transgelin expression, we used a glioblastoma U87 cell line cultured in 2D system. The cells were provided by the University of Bergen, Norway with the support of Mr. James Lorens.

Cell line was cultured in DMEM/F12 medium supplemented with 10 μg/mL of insulin 5% horse serum, 20 ng/mL of EGF, 0.5 μg/mL of hydrocortisone, 100 ng/mL cholera toxin, 100 U/mL of penicillin and 100 μg/mL of streptomycin (Sigma-Aldrich, St. Louis, MO, USA). We used 96-well culture plates which were incubated at 37°C for seven days.

Immunofluorescence

We performed fixation of the plates with 4% paraformaldehyde for 1 h then we washed them with PBS at room temperature for 5 minutes. A solution containing 0.3% saponin, 0.3% Triton X 100 and 0.3% digitonin was used for permeabilization.

The process of permeabilization was exclusively conducted on cytoplasmic targets, namely vimentin and transgelin. Primary antibodies, vimentin (Monoclonal Mouse anti-vimentin Clone V9) (1:25) and transgelin (rabbit SM22 Alpha (1:25) were diluted with Dako antibody diluent and left to incubate for 72 hours at a temperature of 4°C. Afterward, cells that were labeled for vimentin and transgelin underwent two washes with PBS, each lasting for five minutes. Following this, they were subjected to a 24-hour incubation with Alexa Fluor 647 goat anti-mouse (1:200) and FITC anti-rabbit (1:200) before being observed. To protect the dyes, they were incubated

overnight at a temperature of 4°Celsius with Prolong (R) Gold antifade with DAPI.

Analysis

In order to acquire images in digital format at 20x, the TissueFAXS 4.2 software (provided by Tissue Gnostic) and the Zeiss Axio Observer Z1 microscope were utilized.

The analysis of the markers under investigation was conducted using Histo Quest 6.0 software, which expresses the average of the "sum intensity" of the fluorescent signal for each cell.

Research ethics. The research was conducted with the authorization the Institutional review board (IRB) approval from Regional Institute of Oncology (No. 108/ 29.03.2023).

RESULTS

To evaluate the levels of transgelin and vimentin expression, a glioblastoma cell line was utilized.

We conducted immunofluorescence staining to examine cultured cells.

The stained material was captured through digital imaging fluorescence microscopy. In the examined cell line, both transgelin and vimentin were simultaneously expressed, as shown in first figure.

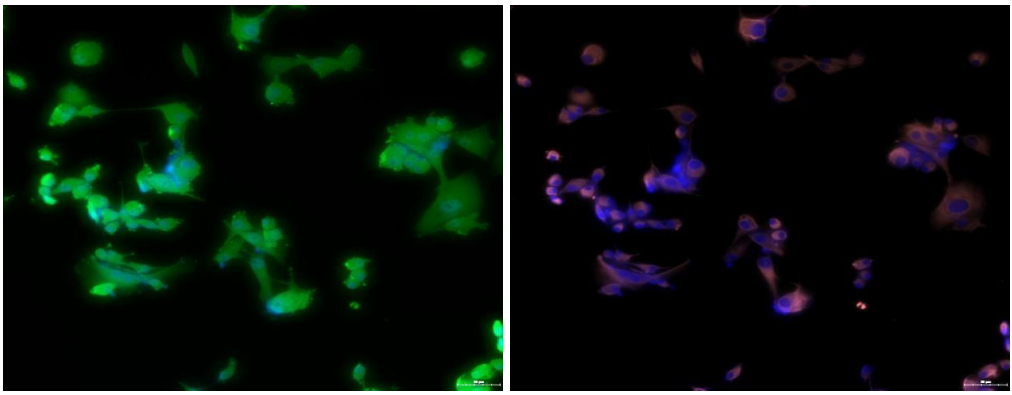

Fig. 1. Transgelin (green) and vimentin (pink) co-expression in U87 glioma cells. Pictures were acquired at 20X.

DISCUSSION

As a component of an extensive research project, this particular section focuses on transgelin and vimentin co-expression in a malignant cell line. It is an essential fragment of the project as a whole, and its findings will contribute significantly to the overall conclusions.

The focus of our research is on the expression of transgelin in an in vitro 2D culture system in relation with the expression levels of vimentin. Our findings reveal that transgelin is co-expressed with vimentin in malignant brain cancer cells.

Studies conducted in the past have demonstrated that inhibiting the function of vimentin in mice leads to the suppression of smooth muscle development and impediments in the process of scarring (6, 23). In recent years, vimentin has been recognized as a marker of EMT, a complex process that is often related to cancer spread. This is of particular importance in the context of cancer because the acquisition of a mobile

and invasive phenotype is associated with the development of metastases, which is the primary cause of death for cancer patients (8).

The involvement of vimentin in cancer cells infiltrating their surroundings is a subject of interest in the field. Understanding the role of this protein in the process of cancer cell invasion could have significant implications for the development of treatments, as its role in chemoresistance is already established (24).

In presented data, the expression of vimentin is explained by the invasive, aggressive character of glioblastoma cells. Nevertheless, a two-dimensional cell culture model would not be able to exhibit the identical progression pattern as a prototype in vivo. The primary outcome deduced from results provided should center on the co-expression of vimentin and transgelin, with both serving as possible indicators of an invasive and aggressive cellular behavior.

The represented results may vary from cell line to cell line and could be also different from in vivo studies; glioblastoma is a very heterogenous pathology, with cellular and molecular diversity not only between tumors but also within the tumor (25). Co-expression of transgelin with a well-known poor prognosis factor as vimentin may indicate a potential similar role in cancer biology. Results may also vary from type of cell line or used tissue samples, as shown before. Immunofluorescence transgelin staining sustain previous research of transgelin being expressed in aggressive cancers (26). The expression of transgelin and also its functional role appear to contrast across different tumor cell types and stromal tissues and may be potentially modified due to various factors such as microenvironmental conditions, gene expression or even cancer progression (27).

CONCLUSIONS

As cancer pathology continues to be a significant global issue, there is a strong conviction that fresh insights into the conduct of cancer with regard to invasion and metastasis are crucially required.

The expression of transgelin in cancer cells appears to resemble that of vimentin, implying that it may function as an indicator of cancer aggressiveness.

Despite conflicting data in the literature, our study of transgelin expands the knowledge base. Specifically, we aim to enhance our comprehension of the part transgelin plays in the invasion and spread of cancer, with potential implications for treatment modalities in clinical settings. Additional research is required to validate the co-expression and correlation of vimentin and transgelin with cancer metastasis.

CONFLICT OF INTEREST AND FUNDING

The authors have officially stated that there is no conflict-of-interest present in their scientific research. Additionally, they have confirmed that they received funding specifically by “Grigore T. Popa” University Medicine and Pharmacy, Iasi, Romania Grant No. 12444 from 27.06.2017. Cell lines were provided by Mr. James Lorens from University of Bergen, Norway.

STATEMENTS

Conceptualization: Elena Daniela Semen, writing original draft: Elena Daniela Semen; data acquisition and interpretation: G. Luta, manuscript revision: Elena Daniela Semen, Manuela Ciocoiu, I. Prutianu; supervising : Manuela Ciocoiu.

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BLOOD PRESSURE LOWERING EFFECTS OF CENTRAL TNF-α BLOCKADE DEPENDS ON FUNCTIONAL LEPTIN SIGNALING

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BLOOD PRESSURE LOWERING EFFECTS OF CENTRAL TNF-α BLOCKADE DEPENDS ON FUNCTIONAL LEPTIN SIGNALING (Abstract) **Background**: Obesity is a major independent risk factor for the development and progression of arterial hypertension. Leptin-mediated sympathoexcitation is a common phenomenon in obesity. Since leptin leads to the synthesis of Tumor Necrosis Factor (TNF)-α in the central nervous system, we hypothesized that the pathological activation of the sympathetic nervous system in obesity-associated hypertension may be mediated by central leptin-related TNF-α mechanisms. **Material and methods:** We compared the long-term effects of TNF-α inhibition on mean arterial blood pressure, heart rate, baroreflex sensitivity and sympathetic tone in animals with a functional leptin signaling (i.e. lean Zucker rats - LZR) and in animals insensitive to leptin (i.e. obese Zucker rats - OZR). **Results:** central inhibition of TNF-α in normotensive LZR significantly lowered mean arterial blood pressure, decreased sympathetic activity and improved baroreflex sensitivity but not in the OZR group. **Conclusions:** These findings suggest that a functionally central leptin-TNF-α signaling plays a key role in mediating the central sympathetic outflow and may represent a promising approach to ameliorate the pathophysiology of obesity related-hypertension. **Keywords:** OBESITY, LEPTIN, TNF-α, HYPERTENSION, BAROREFLEX SENSITIVITY.

Arterial hypertension is a significant contributor to cardiovascular morbidity and mortality worldwide, and the activation of the sympathetic nervous system has been shown to play a key role in its pathogenesis (1-4). Obesity is a major independent risk factor for the development and progression of arterial hypertension, as obese individuals often have excessively high levels of renal and muscle sympathetic nerve activity due to pathological sympathoexcitation (2, 3, 5). The excessive activation of the sympathetic nervous system has been demonstrated as a crucial mechanism in the development of obesity-induced hypertension. By counteracting the activation of the sympathetic nervous system, pharmacological and non-pharmacological sympatholytic therapies have been shown to lower blood pressure in obesity-associated hyper-

tension (3-7). Leptin-mediated sympathoexcitation is a common phenomenon in obesity, with various central mechanisms such as the melanocortin system or phosphoinositide 3-kinase (PI3K) signaling pathways playing a key role (6, 8). Additionally, TNF-α-leptin mediated mechanisms may also contribute to the pathological activation of the sympathetic nervous system, as leptin leads to the synthesis of TNF-α in the central nervous system, which subsequently causes sympathoexcitation and high levels of arterial blood pressure (9-11). Peripheral TNF-α may also contribute to an increased sympathetic activity in obesity-associated hypertension, as shown in both clinical and experimental studies (10). Furthermore, sympathetic activation and hypertension in such physiopathological settings are associated with impaired baroreflex control of heart rate. At the level of the nucleus tractus solitari, leptin impairs baroreflex control of heart rate in response to increase in arterial pressure (12). Moreover, central TNF-α impairs baroreflex sensitivity and increases arterial blood pressure and heart rate (13). Thus, our aim was to test the hypothesis that the pathological activation of the sympathetic nervous system in obesity-associated hypertension is mediated by central leptin and TNF-alpha. Therefore, we compared the cardiovascular responses to TNF-α blockade with etanercept in animals with a functional leptin signaling and in animals insensitive to leptin. To investigate whether leptin-TNF-α induced sympathoexcitation is driven by central or peripheral mechanisms, we administered etanercept both peripherally and centrally.

MATERIAL AND METHODS

All procedures were performed considering the reduction of animal distress and minimizing as much as possible the number of the animals for statistical significance. Six adult male obese Zucker (*fa/fa)* rats and six littermate lean Zucker rats (28-32 weeks of age) were used. Rats were housed in a temperature (21-23°C) and humidity-controlled environment with a 12 h light/dark cycle, with *ad libitum* access to food and water. All procedures involving surgical intervention were conducted under isoflurane anesthesia (2-3%). All experiments were performed in accordance to the European Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No. 123, Strasbourg, 1985) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Research Ethics Committee of the "*Grigore T. Popa*" University of Medicine and Pharmacy, Iași.

Continuous Blood Pressure Recording

For continuous measurement of blood pressure a telemetric system was used as previously described (14). Briefly, the body of the telemetry transmitters (*TRM54PB, Millar, Inc., Houston, TX, United States*) was placed intraabdominally and secured to the abdominal wall. The tip of the pressor sensor was inserted into the femoral artery and advanced into the abdominal aorta below the emergence of the renal arteries. At the end of the implantation procedure all the incisions were closed with non-absorbable sutures (*3-0 Prolene; Ethicon, NJ, United States*) and antibiotic prophylaxis administered intraperitoneally was continued for 72 hours.

Continuous Recording of BP Waveform

Using a PowerLab 16/35 acquisition system (*ADInstruments, Bella Vista, NSW,*

Australia), 24-h blood pressure waveforms from implanted telemeters were acquired continuously at a sampling frequency of 2000 Hz . TR181 smartpads (*Millar, Inc., Houston, TX, United States*) were used for both blood pressure signal acquisition and wireless charging of the implanted telemeters. Daily mean arterial pressure (MAP) and heart rate (HR) were calculated from all 24-hrs individual cardiac cycles identified using LabChart® built-in BP module (*ADInstruments, Bella Vista, NSW, Australia*) as previously described (14).

Peripheral and central inhibition of TNF-α

Peripheral inhibition of TNF-α was attained by daily intraperitoneal administration of Enbrel® in doses of 5 mg/kc as reported by others to have a proper TNF-α inhibition (15). For central TNF-α inhibition we have used osmotic minipumps (Alzet model 2002, pumping rate ± 0.5μL/hr) connected to a stainless steel 23 G canula which was inserted into right lateral cerebroventricle using a stereotactic system (*Stoelting Co, IL, USA*). Continuous intracerebroventricular administration of Enbrel® was made in doses of 10 μg/kg/day which were reported in other studies to have a proper central TNF-α inhibition (16). Briefly, after isoflurane anesthesia, the head of the rat was gently fixed into stereotactic apparatus and a longitudinal incision was made on the middle line of the skull. A small hole was drilled at the following coordinates: bregma -0.84 mm; lateral + 1.8 mm; depth + 4.3 mm and the 23 G stainless steel canula was inserted into the right lateral cerebroventricle. The osmotic minipump was inserted subcutaneously and connected to the infusion cannula via the catheter tube to deliver etanercept into the right lateral cerebroventricular. After anchoring the canula to the cranium with dental cement, the incision was closed with stainless steel clips (*Stoelting Co., Wood Dale, IL, United States*).

Estimation of Sympathetic Activity

As we previously described (17), the original daily BP signal sampled at 2000 Hz was analyzed in the frequency domain using the implementation of the LabChart® software based on the Fast Fourier Transform algorithm. Power spectra were calculated for all artifact-free segments ~1 min in duration, overlapping by 50% and windowed using a Hamming function and finally averaged to generate daily BP spectra. Analysis of the direct BP signal was preferred over extraction of cardiac cycle-related variables such as the systolic BP as it avoided the inherent issues related to resampling for unequally spaced time-series (17). Since the frequency band between 0.25 and 0.75 Hz (low frequency, LF) contains BP oscillations originating from sympathetically driven variations in arterial vascular tone (18), the power in this band was integrated and expressed as percentage of the total power below the HR (0.01-3 Hz).

Time-domain analyses

The sequence technique was used to calculate spontaneous baroreflex sensitivity from the slope of the linear regression functions between SBP and the subsequent PI of the following 3rd, 4th and 5th heartbeat , as we have previously described (17). Up and down sequences of at least three intervals with changes in SBP of >1 mmHg and in PI of >0.5 msec were analyzed only if the correlation coefficients were > 0.85.

Statistical Analysis

Results are expressed as mean ± SE. One-way repeated measures ANOVA followed by Dunnett's multiple comparison test were used (Prism 6.01, GraphPad Software) to indicate the presence or ab-

sence of statistical differences between groups.

RESULTS

Responses to peripheral inhibition of TNF-α

As shown in Fig. 1, obese Zucker rats (OZR) are hypertensive while lean Zucker rats (LZR) are normotensive. Although there is a significant difference between mean arterial pressure (MAP) in LZR *vs.* OZR (105 ± 3 mmHg *vs.* 116 ± 3 mmHg), peripheral TNF-α inhibition did not induce any changes in either group. Heart rate (HR) significantly differs, LZR showing an increased heart rate when compared to OZR as shown in figure 2. No difference of baroreflex sensitivity (BRS) between LZR and OZR was observed during the control period. Baroreflex sensitivity in LZR increased after intraperitoneal administration of etanercept, but this effect did not reach statistical significance. No changes in baroreflex sensitivity were observed in the OZR during peripheral blockade of TNF-α as shown in figure 3. As shown in figure 4, the degree of the estimated sympathetic activity is higher in OZR compared to LZR during control period and no other significant changes were observed during peripheral inhibition of TNF-α.

Responses to central inhibition of TNF-α

The MAP significantly decreased in LZR during central inhibition of TNF-α achieved by continuously intracerebroventricular administration of etanercept. No significant changes were observed in the obese group, the values of the MAP being comparable to those from control period (fig. 1). HR was maintained approximately at the same levels during the entire period of continuously intracerebroventricular administration of etanercept for both groups (fig. 2). In the LZR group, the BRS was significantly higher during central TNF-α inhibition. Conversely, in the OZR the BRS was slightly lower at the end of the intracerebroventricular administration of anti-TNF-α (fig. 3). The activity of the sympathetic nervous system significantly decreased during central TNF-α inhibition but only in the LZR group (fig. 4).

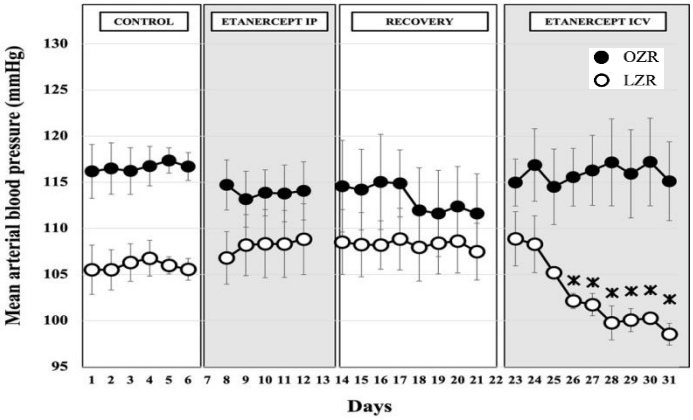

Fig. 1. Mean arterial blood pressure responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; *P <0.05 *vs.* Recovery (days 20-21).
OZR – Obese Zucker Rats; LZR-Lean Zucker Rats

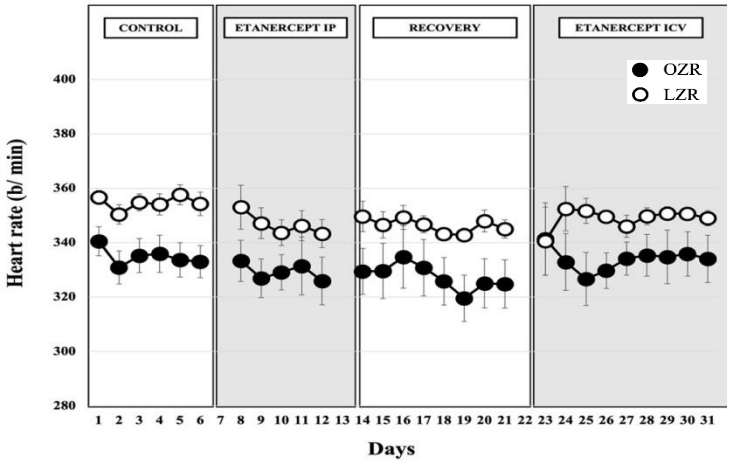

Fig. 2. Heart rate responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; OZR – Obese Zucker Rats; LZR-Lean Zucker Rats

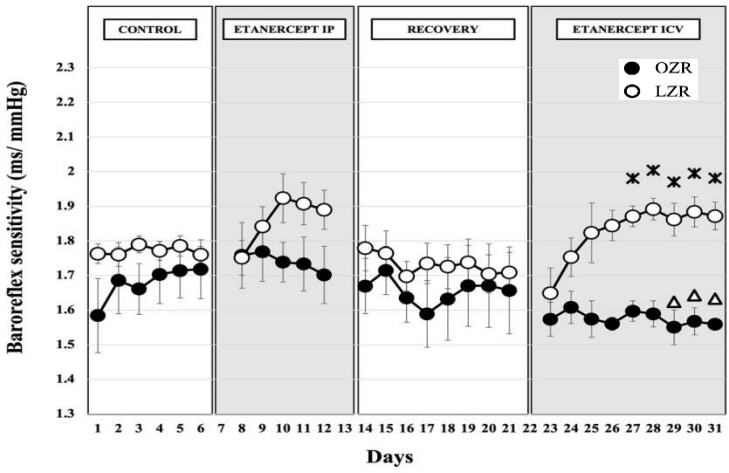

Fig. 3. Baroreflex sensitivity responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; In LZR (o) *P <0.05 *vs.* Recovery (days 20-21); in OZR (•) P < 0.05 *vs.* Recovery (days 20-21). OZR – Obese Zucker Rats; LZR-Lean Zucker Rats

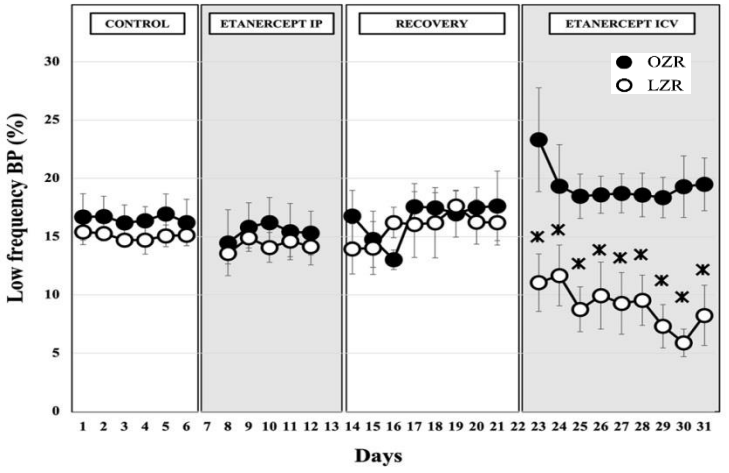

Fig. 4. Sympathetic nervous system activity responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; In LZR (o) *P <0.05 *vs.* Recovery (days 20-21); OZR – Obese Zucker Rats; LZR-Lean Zucker Rats

DISCUSSION

The major findings of the present study are: 1) central inhibition of TNF-α in normotensive LZR, with functional leptin signaling, significantly improved BRS, decreased sympathetic activity and lowered MAP and 2) in the hypertensive OZR which are insensitive to leptin, TNF inhibition fails to lower sympathetic tone, did not improved BRS and did not lower MAP.

A state of low-grade chronic inflammation is considered a feature of obesity and is characterized by secretion from adipose tissue of various inflammatory adipokines such as resistin, monocyte chemoattractant protein-1, leptin, interleukin 6 (IL-6) and TNF-α (19-21). Leptin was shown to be involved in mediating inflammatory cytokines through the activation of dendritic cells, monocytes and macrophages (22-25). Experimental studies have demonstrated a close relationship between leptin and TNF-α production through various pathways such as phospholipase D1 (26), p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK) (27) or activation of human B cells via Janus activation kinase 2 (JAK2)/ signal transducer and activator of transcription 3 (STAT 3) signaling pathways (28). In the OZR, the TNF-α adipose expression starts around 3 to 4 weeks of age when usually the animals are already obese and insulin resistant (29, 30). The level of TNF-α expression is correlated with the degree of adiposity and the associated metabolic disturbances (30). Since systemic inflammation is known to impair cardiovascular homeostasis, we hypothesized that peripheral TNF-α may represent the linking mechanism between inflammation and cardiovascular dysregulation in obesity related-hypertension. Although OZR have high levels of circulating and tissue TNF-α (31,32), in our study its peripheral inhibition did not improved cardiovascular parameters since BRS, MAP or vascular myogenic sympathetic tone did not change. These results suggest that peripheral TNF may be involved in hypertensive organ damage, systemic inflammation, endothelial dysfunction, rather than in the physiopathology of sympathetic obesity-related hypertension.

When centrally administrated in normal rats, leptin was shown to increase sympathetic outflow through pathways within ventromedial hypothalamus which result in high levels of plasma norepinephrine and epinephrine concentrations (33). Other experimental studies have shown that intracerebroventricular infusion or microinjection of leptin into the arcuate nucleus induces sympathetic activation to various territories such kidneys or lumbar nerves and subsequent increase of arterial BP and alteration of BRS (34-36). Additionally, Bin Yu *et al.* have shown that increased concentrations of central leptin and TNF-α play a key role in the development of obesity-related hypertension (37). Moreover, in obese mice, Han et coworkers have shown that central leptin-induced TNF-α production was associated with high levels of diurnal blood pressure values, suggesting that this mechanism may contribute to obesity-related hypertension (9). Also, central TNF-α inhibition over the short-term reduced arterial blood pressure and heart rate suggesting that leptin-TNF-α interplay may play a critical role in the physiopathology of obesity hypertension (9). Also, in a model of renovascular hypertension, central TNF-α inhibition reduces blood pressure, restored baroreflex sensitivity and sympathetic tone (38). Taken together, these results strongly support the hypothesis that central leptin-TNF-α may play a key role in obesity-related hypertension.

The results from our study suggest that over the long term, leptin-TNF-α mediated central sympathetic activation is functional under normal conditions and is responsible for maintaining a basal physiologic sympathetic tone, as shown in LZR. This mechanism might become hyperactive in obesity when leptin increase and lead to subsequent sympathoexcitation and hypertension. This hypothesis is supported by the results from OZR with an altered leptin signaling mechanisms in which central TNF inhibition did not lower arterial blood pressure or sympathetic tone. On the contrary, in LZR with a functional leptin signaling, TNF-α inhibition significantly improved BRS, decreased sympathetic tone and lowered MAP. Although is still a matter for further investigations, our results suggest that central leptin-TNF-α signaling may represent a promising approach to ameliorate the physiopathology of obesity related-hypertension.

CONCLUSIONS

These findings suggest that a functionally central leptin-TNF-α signaling plays a key role in mediating the central sympathetic outflow and may represent a promising approach to improve the pathophysiology of obesity related-hypertension.

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CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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IMPLICATIONS OF CHRONIC DISEASES AND ACADEMIC STRESS ON STUDENTS' QUALITY OF LIFE

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IMPLICATIONS OF CHRONIC ILLNESS AND ACADEMIC STRESS ON STUDENT QUALITY OF LIFE (Abstract): The present study had two main aims, namely to identify differences between the quality of life of students with chronic illness compared to that of students without significant health difficulties and to identify significant predictors among academic stressors. **Materials and methods:** Data were collected between May and July 2023 using an instrument consisting of The World Health Organization Quality of Life-BREF and Academic Stress Scale from 257 students, of whom 52 had a diagnosis of chronic illness and 202 did not. **Results:** there were significant differences according to the presence or absence of chronic illness in psychological health [$t(255) = -2.569$, $p = 0.011$], physiological health [$t(255) = -5.502$, $p < 0.01$] and social relationships [$t(255) = -2.640$, $p = 0.009$]. Among subjects with a chronic illness, two significant predictive models were identified, the first is composed of the predictor self-perceptions ($F(1, 54)=23.62$, $p<.01$), and the second from the predictors self-perceptions and time limits ($F(2, 54)=16.27$ $p<.01$). Among subjects without a chronic illness three statistically significant predictive models were identified, the third strongest being made up of self-perceptions, pressure to perform and time limits ($F(3, 201)=40.00$, $p<.01$). **Conclusions:** the quality of life of students with chronic illness is significantly lower compared to that of students without chronic illness. In addition to the fact that students' quality of life is impacted by academic stress driven by self- perceptions and time limits, that of students without chronic illnesses is additionally impacted by pressure to perform. **Keywords:** QUALITY OF LIFE, CHRONIC ILLNESS IN STUDENTS, ACADEMIC STRESS.

The prevalence of chronic disease among students is increasing, with this population facing a number of particular challenges in the university environment (1, 2). The best-known definition of quality of life is one that focuses on a person's subjective perception of their position in life in the context of culture and value system where they are inserted, which also involves their goals, perspectives, standards and concerns (3). As a result, quality of life can be impacted by many factors, including chronic illness and academic stress.

Chronic illness causes negative effects on students' quality of life as well as their academic performance (4), compared to students without chronic medical condi-

tions who perform better on both variables (5). Moreover, unlike their healthy peers, students with chronic illness are more likely to receive state aid and are less likely to graduate and find employment (6).

In the study conducted by authors Wilson and Spencer (7), 95% of students indicated that their chronic illness had at least a moderate impact on the psychological dimension of quality of life, with 81% estimating that they felt anxious or depressed at least once a month because of their health condition.

Accepting the limits imposed by the body on academic performance causes students, in addition to the need to reformulate goals and standards, frustration and disappointment (8). In addition to sometimes losing control over their own bodies, chronically ill students also experience feelings of losing control over their social lives (9). Thus, in general, students with a chronic condition not only have a lower health-related quality of life, but they also report higher levels of loneliness and isolation (5, 6).

Stress, academic stress in particular, has been identified as having serious implications for students' physical and mental health (10). In the present study, we aim to extend the literature by identifying the link between various academic stressors and quality of life among students with chronic illness.

In the face of these problematic situations, students with chronic illnesses tend to use maladaptive coping mechanisms, such as dissembling, ignoring and humiliating, and ignoring symptoms, which ultimately magnify their difficulties (8, 11). Such trends observed within the literature are a wake-up call for the need for adaptation on the part of universities in relation to the services offered to students with chronic illnesses in order to truly support them in a way that suits them (12).

Through this study we aimed to identify whether there are differences in the quality of life of students with chronic illness compared to students without significant health difficulties and to see the extent to which academic stressors predict quality of life for both students with chronic illness and those without significant health difficulties.

MATERIALS AND METHODS

The data was collected between May and July 2023, and the invitation was sent to students at three universities in the North-East region of Romania. The study received ethical approval from the ethics committee of the Faculty of Psychology and Educational Sciences in Iasi (number 595/12.05.2023).

Participants

A total of 260 students participated in this study and completed the questionnaires through an online survey platform (Google Forms). Of these, 3 people were excluded due to the fact that 2 had a chronic emotional illness and the other did not wish to declare a diagnosis of somatic illness. Of the remaining 257 participants, 52 had a diagnosis of chronic illness and 202 declared themselves clinically healthy. The mean age of the participants was 27.82 ± 9.09. Of the participants 33 were male and 224 females. In terms of education, 139 were pursuing a bachelor's degree, while 118 were pursuing a master's degree. Regarding the background, 67 were from rural areas and 190 from urban areas. In terms of residence, 47 lived in a dormitory, 2 in a host family, 82 rented, 37 with parents and 89 in their own home. The majority, 104, said they were in a relationship, 67 married and 86 singles. Of the total of 257, 15.6%

were following a doctor- prescribed treatment, 2.7% were self-medicating and 81.7 were not following any treatment. Chronic diagnoses included chronic renal failure, hypothyroidism, psoriasis, obesity, autoimmune thyroid, epilepsy, multiple sclerosis, asthma, etc.

Instruments

The first part of the assessment instrument aimed at collecting demographic data: university, faculty, educational cycle, year of study, gender, age, background, marital status, form of residence, health status, diagnosis of chronic disease, treatment. The second part included several questionnaires:

The World Health Organization Quality of Life-BREF (WHOQOL- BREF) (3) is a self-assessment tool for quality of life that can be applied to both people with and without a chronic illness. The 26 questions assess people's perceptions of four domains: physical health (Cronbach's $\alpha = 0.81$), psychological health (Cronbach's $\alpha = 0.86$), social relationships (Cronbach's $\alpha = 0.73$) and environment (Cronbach's $\alpha = 0.79$).

Academic Stress Scale (13) assesses through 18 items the level of stress felt by students in relation to the following four dimensions: pressures to perform (Cronbach's $\alpha = 0.59$), perceptions of workload and examinations (Cronbach's $\alpha = 0.61$), self-perceptions (Cronbach's $\alpha = 0.77$) and time restraints (Cronbach's $\alpha = 0.57$).

Statistical analyses

The variables of the present research do not differ statistically semi-significantly from a normal distribution, as the absolute value of the skewness coefficient is not greater than 1 (Labăr, 2008). Differences in quality of life dimensions according to the presence or absence of chronic disease were tested using t-tests for independent samples. To identify which stressors are significant predictors of quality of life according to the presence or absence of chronic illness, we used linear regression with the split file function activated.

RESULTS

Analyzing the first table we can see that there are significant differences according to the presence or absence of chronic disease in psychological health [$t(255) = -2.569$, $p = 0.011$], physiological health [$t(255) = -5.502$, $p < 0.01$] and social relationships [$t(255) = -2.640$, $p = 0.009$], but not in environment [$t(255) = -1.937$, $p = 0.054$]. Thus, students without a chronic disease have a better quality of life in the dimensions: psychological health, where they have a mean equal to 21.85, compared to students with a chronic disease who have a mean of 19.91; physiological health where they have a mean equal to 26.18, compared to the mean of 21.84 obtained by those with a chronic disease; and social relationships, where they have a mean of 11.12, compared to the mean of 10.02 obtained by students with a chronic disease (tab. I).

When the correlations between quality of life and academic stressors are analyzed among subjects diagnosed with a chronic illness, we observe that all correlations are significant and negative (tab. II), as follows: for the predictor pressure to perform ($r=-.45$, $p<.01$), for workload perceptions ($r=-.39$, $p<.01$), self-perceptions ($r=-.55$, $p<.01$) and for time limits ($r=-.50$, $p<.01$). Among people without a chronic illness, quality of life correlates negatively with pressure to perform ($r=-.46$, $p<.01$), workload perceptions ($r=-.33$, $p<.01$), self-perceptions ($r=-.56$, $p<.01$) and time limits ($r=-.42$, $p<.01$).

TABLE I.
T-tests for independent samples

Quality of life dimensions	Chronic disease	n	M	SD	t	df	Mr
Psychological health	Present	55	19.91	4.83	-2.569	255	0.011
	Absent	202	21.85	4.99			
Physiological health	Present		21.84	5.64	-5.502	255	0.000
	Absent		26.18	5.06			
Social relations	Present		10.02	3.07	-2.640	255	0.009
	Absent		11.12	2.66			
Environment	Present		25.04	4.91	-1.937	255	0.054
	Absent		26.45	4.76			

TABLE II.
Correlations between predictors and criterion

Chronic disease		Quality of life	The pressure to perform	Perceptions work volume	Self-perceptions	Time limits
Present	Quality of life	1				
	The pressure to perform	-.458**	1			
	Perceptions work volume	-.397**	.653**	1		
	Self-perceptions	-.555**	.484**	.337*	1	
	Time limits	-.501**	.423**	.680**	.460**	1
Absent	Quality of life	1				
	The pressure to perform	-.463**	1			
	Perceptions work volume	-.334**	.583**	1		
	Self-perceptions	-.562**	.477**	.318**	1	
	Time limits	-.423**	.554**	.597**	.437**	1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Among subjects with a chronic disease, two significant predictive models were identified (tab. III), the first is made up of the self-perceived predictor ($F(1, 54)=23.62$, $p<.01$), and the second one from the predictors self-perceptions and time limits ($F(2, 54)=16.27$ $p<.01$), the model with the highest predictive power being the second one ($R^2 =.36$). The inclusion of the predictor time limits brings an additional information (Change $R^2 =.07$) statistically significant (Change $F(1, 52)=6.47$, $p=.01$). Thus, model 2 explains 36.1% of the variance of the quality of life variable, 7.7% more than model 1, this increase being statistically significant ($p =.01$). Within model 2, the predictor with the highest influence on the criterion is self-perceptions ($\beta=-.41$), followed by time limits ($\beta=-.31$). The effect of significant predictors is negative, when their score increases by one unit, the quality of life score decreases by -2.03 points for self-perceptions and by -1.33 points for time

limits (tab. IV).

In contrast, among subjects without chronic disease, three statistically significant predictive patterns were identified (tab. III). The first consists of the variable self-perceptions (F(1, 201)=92.13, p<.01), the second of the predictors self-perceptions and pressure to perform (F(2, 201)=57.16, p<.01), while the third consists of self-perceptions, pressure to perform and time limits (F(3, 201)=40.00, p<.01). The model with the highest predictive power is model 3, which explains 36.8% of the variance of the quality of life criterion, 1.2% more than model 2, the increase being statistically significant (p = .04).

TABLE III.

Summary of hierarchical models

Chronic disease	Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
						R Square Change	F Change	df1	df2	Mr F Change
Present	1	.555	.308	.295	13.030	.308	23.629	1	53	.000
	2	.620	.385	.361	12.405	.077	6.479	1	52	.014
Absent	1	.562	.315	.312	12.483	.315	92.136	1	200	.000
	2	.604	.365	.359	12.053	.050	15.516	1	199	.000
	3	.614	.377	.368	11.964	.012	3.968	1	198	.048

TABLE IV.

Regression coefficients for direct effects of stressors on quality of life

Chronic disease		Model	B	Std. Error	Beta	t	Mr	Collinearity Statistics	
								Tolerance	VIF
Present	1	(Constant)	99.539	4.997		19.920	.000		
		Self-perceptions	-2.743	.564	**-.555**	-4.861	**.000**	1.000	1.000
	2	(Constant)	110.929	6.531		16.985	.000		
		Self-perceptions	-2.034	.605	**-.412**	-3.361	**.001**	.788	1.269
		Time limits	-1.330	.523	**-.312**	-2.545	**.014**	.788	1.269
Absent	1	(Constant)	107.858	2.480		43.497	.000		
		Self-perceptions	-2.705	.282	**-.562**	-9.599	**.000**	1.000	1.000
	2	(Constant)	116.423	3.234		35.996	.000		
		Self-perceptions	-2.124	.310	**-.441**	-6.862	**.000**	.773	1.294
		The pressure to perform	-1.045	.265	**-.253**	-3.939	**.000**	.773	1.294
	3	(Constant)	119.728	3.614		33.130	.000		
		Self-perceptions	-1.976	.316	**-.410**	-6.250	**.000**	.730	1.370
		Time limits	-.601	.302	**-.138**	-1.992	**.048**	.654	1.528
		The pressure to perform	-.789	.293	**-.191**	-2.695	**.008**	.625	1.601

In Model 3, the predictor with the highest influence on the criterion is self-perceptions (β=- .41), followed by pressure to perform (β=-.19), and the last predictor is time limits (β=-.13). The effect of significant predictors is negative, when their score increases by one unit, the quality of life score decreases by -1.97 points for self-perceptions, by -.78 for pressure to perform and by - .60 points for time limits (tab. IV).

DISCUSSION

The present study had two main objectives, namely to identify differences between the quality of life of students with chronic illness compared to students without significant health difficulties and to identify significant predictors of academic stressors. The results obtained agree with the literature, with students without chronic illnesses having significantly better quality of life than students with chronic illnesses on the dimensions of physical health, psychological health and social relationships. The only dimension for which differences were not significant was environment.

Due to the effects of chronic diseases on the body, such as pain, discomfort, the fact that the extent of physical health is more affected in chronically diagnosed students than in healthy students is not surprising (14). However, it is possible that students with chronic illnesses may have lower scores on the psychological dimension of quality of life as a result of the sensitivity exhibited by the body and psyche to the demands of the educational environment (15). Overall, the literature highlights that levels of happiness and subjective well-being in students with chronic illnesses are lower than in other students, partly due to lower levels of satisfaction of autonomy and competence needs (16).

According to our results, chronic illness can also have different effects on romantic relationships, or friendship, on the extent of relationships with others in general. Sometimes, especially when romantic partners have academic or professional responsibilities, the effects are irreparable, with relationships failing to withstand the impact of illness or treatment (17). In terms of friendships, often close friendships prior to illness drift away, or even disappear, while people known during treatment become close (17).

In terms of academic stressors, they all correlate negatively with quality of life. Among students with chronic illnesses, self-perceptions and time constraints were identified as significant predictors, while for students without chronic illnesses a significant predictor was also pressure to perform. It appears that the quality of life of students without chronic illnesses is negatively impacted, in addition to perceptions of self-confidence to succeed academically and professionally, by time limits for completing various tasks and by stress caused by parental expectations, competition with peers and criticism from teachers. The latter stressor is not a significant one for students with chronic illnesses and does not impact on their quality of life.

The present study has a number of limitations, the most important of which is the small number of subjects diagnosed with a chronic disease as opposed to healthy participants. Also, quality of life needs to be related to other variables besides academic stressors, such as coping strategies, or support from others. Future studies could also investigate whether the list of differences in environmental dimension is a reproducible result and what the possible explanations are.

CONCLUSIONS

One of the most important findings is that the quality of life of students with

chronic illnesses is significantly lower compared to students without chronic illnesses for the dimensions of physical health, psychological health and relationships with others. In addition to the fact that students' quality of life is impacted by academic stress driven by self-perceptions and time limits, that of students without chronic illnesses is additionally impacted by pressure to perform.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflict of interest. This research received no external funding.

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POSTMORTEM MACROSCOPIC DIAGNOSIS IN HYPOTHERMIA

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POSTMORTEM MACROSCOPIC DIAGNOSIS IN HYPOTHERMIA (Abstract): Accidental hypothermia fatalities are the result of extreme external environmental temperature exposure. These are an area of interest for research due to their complex features of diagnosis of certainty and the mechanisms involved in thanatogenesis. Our study aims to evaluate the macroscopic features associated with cold stress in a large group of accidental hypothermia fatalities. **Materials and methods:** The study comprised a retrospective analysis of 107 accidental hypothermia fatalities, diagnosed between 2007 and 2021, in the Forensic Medicine Institute Iași. **Results:** Variable reddish to purplish color cadaveric lividity, scrotum retraction (60% of males), intense red blood color (60.7% of cases), and skin lesions secondary to cold exposure, such as cold erythema or acute pernio (49.05% of cases), frostbites (49.7% of cases), and immersion foot (12.1% of cases) were the main macroscopic findings registered during autopsy. In addition, fetal position of the body and paradoxical undressing were other autopsy gross features identified in 12.1% and 9.3% of cases, respectively. The gross exam of the internal organs revealed Wischnewski spots (44.8%), pulmonary subpleural petechiae (11%), and Puharevici's sign (53.3%). Statistical analysis revealed a significant correlation between frostbites degree and temperature at hospitalization, between Wischnewski spots and core temperature, or alcoholaemia and traumatic injuries. **Conclusions:** Although the macroscopic features related to accidental hypothermia death are often non-specific, inconstant or even negative, their interpretation in the context of forensic investigation data, such as cold exposure evidence, social conditions, and histopathological data may contribute to hypothermia death diagnosis. **Keywords:** HYPOTERMIA, POSTMORTEM DIAGNOSIS, MACROSCOPY.

Hypothermia is the result of external thermal aggression, secondary to exposure to low temperatures (1, 2). This is defined by a state in which the body's temperature regulation mechanism is overcome by a stress factor, represented by an important decrease of environmental temperature, which settles at a core temperature below 35°C, at approximately 2°C below normal temperature (1). The loss of body heat, from the perspective of external and internal factors, is the result of the difference between the metabolic rate of the sum between physical exercise and the physical

parameters of temperature decrease (radiation, convection, and conduction) (3, 4).

Accidental hypothermia is a cause of death in at least 1,500 persons/year in the United States, while it ranges between 0.13 to 6.9 cases/100,000/ year in European countries and New Zealand (5).

Socioeconomic status, environmental factors, associated comorbidities, or alcohol and drug consumption are other factors that support hypothermia-related deaths (1, 5, 6). Depending on severity, hypothermia is divided into mild hypothermia (35-32°C core temperature), moderate hypothermia (<32-28°C core temperature), severe hypothermia (<28°C core temperature), and profound hypothermia (<24°C core temperature) (7). In the mild form, the patient shows obnubilation, lack of movements coordination, dysarthria, myosis, cold and pale teguments (8). In the moderate form of hypothermia, associated with death in approximately 20% of cases, the altered state of consciousness sets in, the teguments become cold, added to bradypnea, bradycardia, hypotension, and the occurrence of Osbourne J wave, while the cold sensation disappears (8). In progression, ventricular fibrillation, disseminated intravascular coagulation, hyperkalemia, acidosis, and coma occur in severe form of hypothermia, leading to a mortality rate >50% (8).

Death by hypothermia is associated with a series of frequent non-specific macroscopic and microscopic changes, which may lead to difficulty in diagnosis, in medico-legal practice (5, 9).

A fetal body position may be adopted by victims of hypothermia, but it may be absent in cases of alcohol consumption, or paradoxical undressing may be encountered, due to the hypothalamus failure of body temperature control or to the peripheral vasodilation and false perception of heat sensation (1, 9). These are associated with a series of external and internal macroscopic changes identified during the medico-legal necropsy, such as cadaveric lividity, skin lesions induced by prolonged exposure to cold (joints cold erythema), scrotal retraction, along with cardiac, gastric, pancreatic, renal, and/or muscular hemorrhages (1, 9, 10).

Although indicative in nature, macroscopic changes, corroborated with forensic investigation data and the results of the microscopic histopathological examination, may direct the diagnosis towards death by hypothermia in medico-legal practice (11).

In this context, the purpose of the present study is to investigate the macroscopic changes noted during the necropsy examination in a group of hypothermia victims, as well as the correlation between these changes and epidemiological and biochemical data.

MATERIALS AND METHODS

The medico-legal files of 107 cases having hypothermia circumstances of death, registered between 2007-2021, from the archive of the Institute of Forensic Medicine Iași, were re-examined and included in our study group. In all cases, 10 mL blood samples were collected from the femoral vein to determine the ethanol concentration. Additionally, 50 mL urine sample was collected to determine the alcoholuria.

The macroscopic lesions identified in these patients were statistically investigated by IBM SPSS v.26 program, using Mann Whitney and Spearman tests. p value <0.05 was considered as statistically significant.

Our study had the approval of the Research Ethics Committee of "Grigore T.

Popa" University of Medicine and Pharmacy, Iasi, no. 17275/20 August 2019, consistent with the Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects.

RESULTS

Among the total number of 107 cases of death by hypothermia analyzed in our study, 81 cases (76%) were male and 26 (24%) were female, the average age being 57.95±1.47 years. Most cases (68.2%) inhabited in rural areas and only 31.8% of cases inhabited in urban areas. Low socioeconomic status was registered in 18.7% of women victims, while alcoholism and severe meteorological conditions represented the predisposing factors of hypothermia in men, in 24.3% and 9% of cases, respectively (fig.1). Additionally, the death occurred at domicile in 10.3% of women and in hospital in 22.4% of men, respectively (fig. 2).

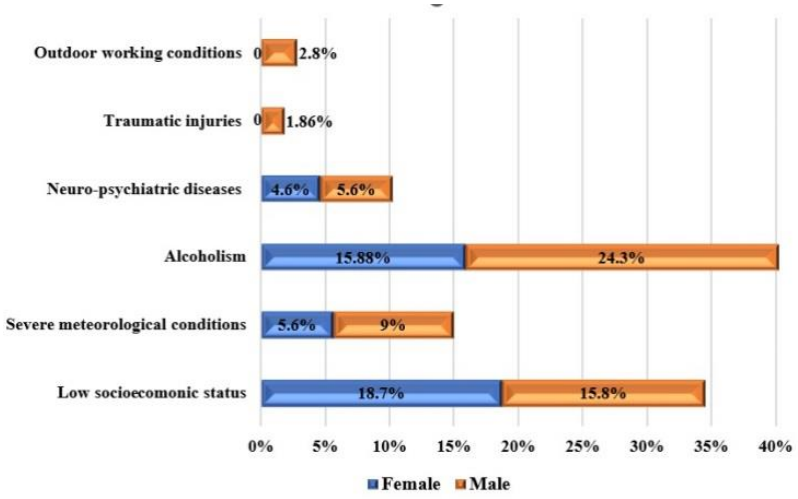

Fig. 1. Distribution of factors contributing to death by hypothermia according to gender

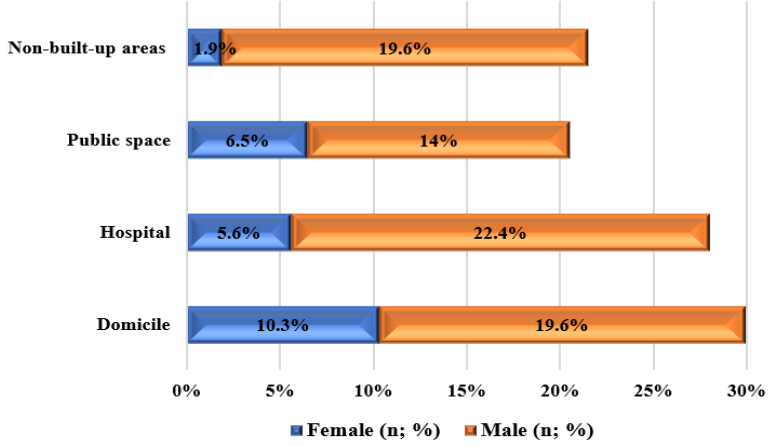

Fig. 2. Distribution of cases in relation to place of death according to gender

External exam

90.6% of cases included in the study group were found fully clothed and the phenomenon of paradoxical undressing was registered only in 9.3% of cases. 30% of cases presented a variable hospitalization period (from 1 to 30 days), the temperature at hospital admission varying between 19.5°C and 32.6°C. In these cases, death occurred due to cardio-respiratory complications (25.6%) or multiple organ failure (MOF) (25.6%). Three cases (2.8%), all men, were found frozen.

The external examination also revealed retraction of the scrotum (Puparev's sign) in 60% of men, while the other signs detected were: fetal position in 12.1% of cases, intense reddish cadaveric lividity in 31.1% of cases, cold erythema in 49.05% of cases, predominantly detected in the lower limbs (34.15%), different degrees of frostbites in 49.7% of cases, classified from first up to fourth degree, according to their severity, and immersion foot in 12.1% of cases, with variable rates among the victims genders (figs. 3-9).

Fig. 3. Reddish-pink cadaveric lividity in a case of hypothermia

Fig. 4. Knee frostbite of 3rd degree in a case of hypothermia

Fig. 5. Cold erythema in a case of hypothermia

Fig. 6. Immersion foot in a case of hypothermia

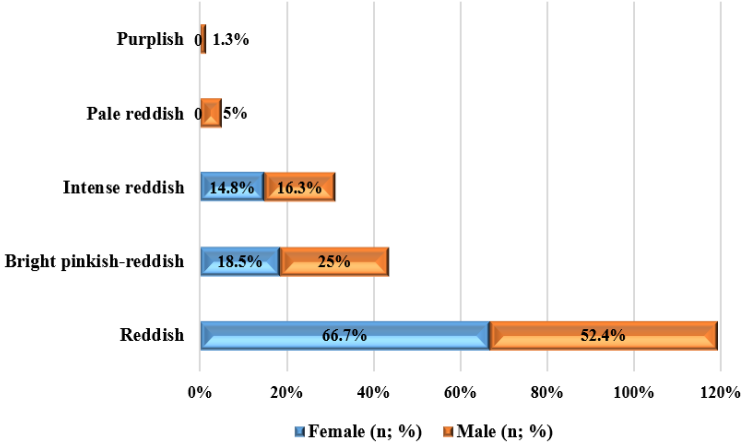

Fig. 7. Distribution of cadaveric

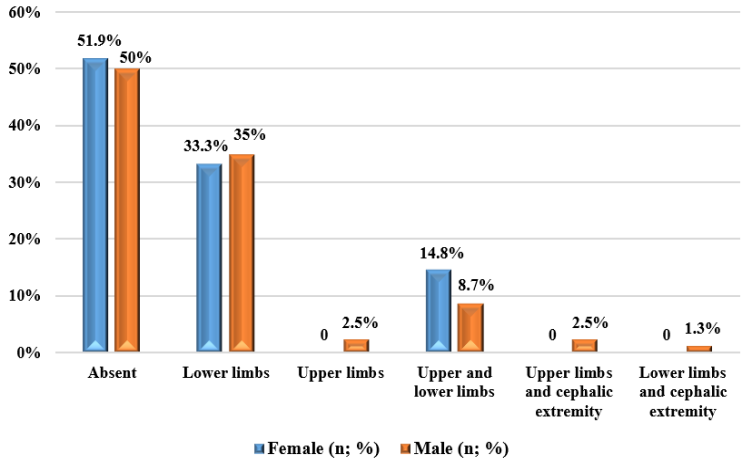

Fig. 8. Distribution of cold erythema according to gender

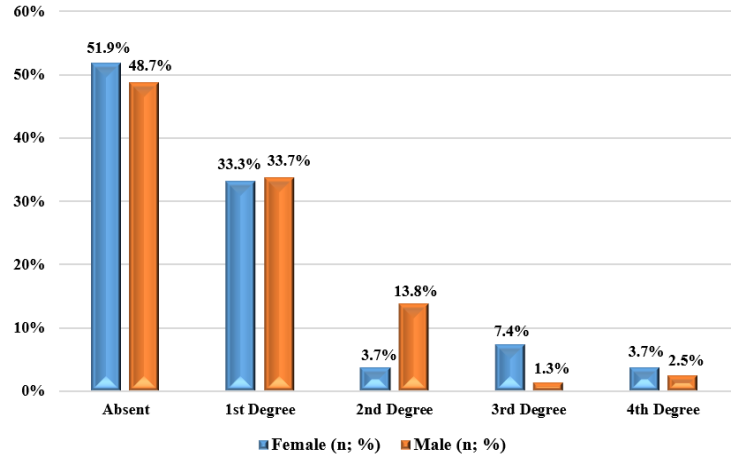

Fig. 9. Frostbite degree according to gender

In addition, Spearman's test indicated a significant correlation ($p<0.05$) between the frostbite degree and the temperature at hospitalization, with a higher degree corresponding to the lowest temperatures.

Internal exam

The internal examination of the corpses also revealed a series of injuries of the internal organs secondary to exposure of the body to low temperatures, such as gastric hemorrhagic erosions (Wischnewski spots) in 44.8% of cases, stomach retraction (Puharevici's sign) in 53.3% of cases, pulmonary subpleural petechiae in 11% of cases (figs. 10 and 11), pancreatic hemorrhages in 18.7% of cases, and intense reddish blood color in 60.7% of cases. Macroscopically identified Wischnewski spots, partially confirmed by histopathological examination, were correlated with a more

pronounced drop in body temperature (mean value of 22.42°C).

Simon's hemorrhages (of the anterior aspect of the lumbar intervertebral discs) and hemorrhages of the major psoas muscle or of the paravertebral muscles were not highlighted in order to preserve the body integrity as much as possible, if other signs of hypothermia have been noticed.

Fabrikantov spots (hemorrhagic areas in renal pelvis) were absent in 99.1% of cases, and no information was available for 1 case, as postmortem hemicorporectomy was registered.

Independent samples T-test revealed significant differences between cases with Wischnevski spots and those without it, in terms of temperature at hospitalization. Thus, cases diagnosed with Wischnevski spots had a significantly body lower temperature (mean temperature 24.15±2.79°C) compared to cases without this sign (mean temperature 26.17±2.91°C).

The internal examination also revealed the presence of pulmonary edema in 54.9% and bronchopneumonia in 17.6% of cases. Steatosis, diagnosed in 71.4% of cases, along with liver cirrhosis (6.5% of cases) and pancreatic hemorrhagic lesions (6.5% of cases) were among the most common macroscopic lesions noted in the digestive tract and its accessory organs.

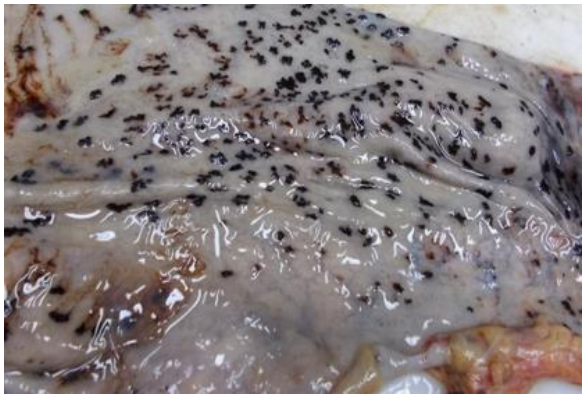

Fig. 10. Stomach Wischnewski spots in a case of hypothermia

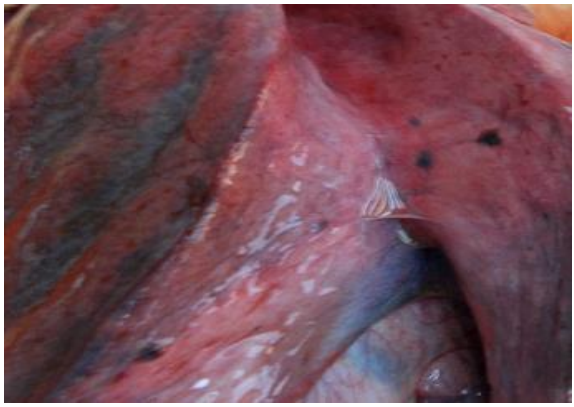

Fig. 11. Subpleural petechiae in a case of hypothermia

Alcoholaemia was positive in 42% of cases, with a range between 0-4.65g/L, its higher values being noted in men, while alcoholuria was positive in 24.3% of cases.

Traumatic injuries have been registered in 72 cases (67.3%), 62 cases (86.1%) of them having passive lesions, while 10 cases (13.9%) having non-fatal assault lesions.

The non-parametrical Mann Whitney test showed that the alcohol level was significantly higher in cases with injuries of violence produced by active force, followed by same-level falls (MR = 41.65), compared to cases with injuries produced by passive trauma (falls) (MR = 29.55) [U = 158,500; p = 0.036]. Additionally, the data showed that alcoholuria was significantly higher in cases with injuries produced by active impact followed by same-level fall (MR = 31.33) compared to cases with injuries produced by passive impact (fall) (MR = 19.53) [U = 69,000; p = 0.006] and for cases with injuries produced by active force followed by same-level fall (MR = 18.17) compared to cases without injuries (MR = 11.92) [U = 43,500; p = 0.041].

Regarding complications during hospitalization, most cases had neurological complications (46.2%), followed by MOF (25.6%), and cardio-respiratory complications (25.6%). Regarding the neurological complications of the encephalopathy type, we identified the following subtypes: post-anoxic encephalopathy (6 cases), ethanolic encephalopathy (1 case), toxic-ethanolic encephalopathy - polyneuropathy (1 case).

Following the application of the non-parametric Mann Whitney test to verify significant differences, it was found that the temperature at admission was significantly lower for MOF complications (MR = 5.72) compared to cases with cardio-respiratory complications (MR = 10.70) [U = 6,500; p = 0.032] and for neurological complications (MR = 10.44) versus cases with cardio-respiratory complications (MR = 17.60) [U = 17,000; p = 0.037], where MR represents the mean of the ranks and U represents the Mann Whitney Test.

DISCUSSION

The diagnosis of death by hypothermia is achieved by correlation of the autopsy data with the forensic investigation data on the spot and with the histopathological exam, by excluding some alternative causes of death and analyzing the circumstantial records, without means of estimation of the severity of ante-mortem cold exposure (11). The macroscopic data resulting from the external and internal examination of the corpse can provide important information, which points towards the diagnosis of death by hypothermia in medico-legal practice.

Cold erythemas and frostbites, mainly located on the extremities (earlobes, fingers and toes, or nose tip) or unclothed skin surfaces are the main skin lesions noted in hypothermia victims (12), in agreement with our findings. These become permanent changes if the freezing process is leading to cellular water crystallization in an extensive manner (12). Moreover, frostbitten skin areas must be surgically removed in people who survive low temperatures exposure, as they are territories devoid of vitality (13). Moreover, first-degree frostbites were mainly registered in our study, being detected in more than a third of cases of both genders in our study group.

Immersion foot sign is commonly related to prolonged exposure to moisture and classically occurs after more than 12 hours of cold-water exposure, in hypothermia fatalities (14). Sudden immersion in cold water may lead to skin's rapid cooling,

followed by uncontrollable hyperventilation and a concurrent rise in cardiac output and blood pressure, precipitating pre-existing cardiovascular pathology in susceptible individuals. The lack of cold-water habituation, advanced age, and pre-existent cardiovascular pathology were the most significant factors that led to a fatal end in a victim diagnosed with immersion foot, in our study group (14, 15).

The cadaveric lividity is represented by pink/bright red postmortem coloring of the teguments on their sloping sides due to the antemortem binding of oxygen to hemoglobin and its postmortem diffusion through the skin (16, 17). These observations are also supported by the results of our study, as the tints of lividity color were mainly intense in the investigated cases.

Core cooling of the body occurs regardless of the position adopted, but studies show that the decrease of core temperature occurs faster in the upright position (by approximately $1.24^{o}C \pm 0.07^{o}C$) than in the supine position (by approximately $1.02^{o}C \pm 0.06^{o}C$), including a significantly lower thermogenesis threshold temperature in the upright position compared to the recumbent position (18). In our study, we observed that victims found in the prone position managed to survive long enough to be admitted to the hospital, where rewarming and resuscitation was attempted, with a mean admission temperature of $25.58^{o}C$.

Regarding the blood color, the victims included in the study group presented an intense reddish coloration (60.7%), our data agreeing with other literature reports (1, 16).

Retraction of the scrotum and testicles was observed in 60% of the male cases, the Puparev's sign being present in 35.5% of the cases that did not survive long enough to be hospitalized. These findings are also supported by the literature mentioning that the Puparev's sign (wrinkled scrotum with testicles lifting in the direction of inguinal canals) occurs with a frequency of 38% and is part of the category of the first signs that appear in the setting of hypothermia along with cutis anserine (goose bumps), Shter's sign (pose of shivering person), cold erythema, Rayski's sign (frost of the eyelashes, with icicles in the openings of the nose and mouth), and Puharevici's sign (an empty stomach with folded mucous membrane covered with vitreous mucus) (1, 19, 20).

Wischnewski spots are hemorrhagic lesions as areas of 1mm-2cm diameter, black-brown in color, dispersed on the surface of the gastric mucosa, but without a pathognomonic value for hypothermia diagnosis (21, 22). According to literature data, these are observed in hypothermia victims in a range between 40-100% (9, 21, 22). Similar data were obtained in our own research, as the identification of Wischnewski spots was registered in 48 cases (44.8%) of our study group, although our observations could lead to underdiagnosis considering the marked postmortem autolysis, which did not allow a conclusive macroscopic interpretation of the stomach surface. Wischnewski spots identification during the autopsy guided the coroner to the death cause, especially in circumstances where the investigation data were inconclusive or insufficient.

Fabrikantov spots, as hemorrhagic areas due to kidney hypoxia have been identified only in 0.9% of cases in our research. The registered differences by comparison with a report of positive Fabrikantov spots in 75.1% of a group 460 victims of hypothermia (23) may be attributed to marked autolysis and putrefaction or to the therapy

applied during hospitalization.

Regarding macroscopic pancreatic hemorrhages and pancreatitis signs, these have been observed in 20 cases (18.7%) in our study, data consistent with those of a report with 5 cases (3.49%) in a study conducted on 143 victims, being correlated with time of cold exposure (24).

A severe form of hypothermia that can lead to death is that resulting from exposure to low temperatures in association with the consumption of ethanol, since alcohol acts as a strong peripheral vasodilator and a central anesthetic. These effects lead to a supplementary heat loss that is not counterbalanced by a corresponding increase of thermogenesis (3, 5, 25). Additionally, by increasing the blood flow to the skin and extremities, alcohol induces a false warmth sensation, while heat loss increases. The ethanol consumers may have a higher rate of hetero-aggressive behavior, which in combination with toxicological and traumatic factors, and may influence the exposure of victims to extreme temperatures, either due to the loss of consciousness or the inability to leave the cold environment. Moreover, according to the results published in the international data flow, a variable percentage, between 33% and 73% of hypothermia cases, are complicated by the consumption of ethanol (6, 10, 16). In this sense, it is already recognized that people who fall asleep under the action of ethanol, while being hypothermic, will succumb due to extreme hypothermia.

This study had some limitations given by the difficulty in discrimination between pre-existing macroscopic lesions and non-specific injuries induced by hypothermia, the incomplete information regarding the circumstances of death and time of cold stress exposure, along with the difficulties of external and internal lesions evaluation during autopsy exam in the context of postmortem autolysis.

CONCLUSIONS

The forensic specialists play an important role in the establishment of the death diagnosis by hypothermia, following the corroboration of the forensic information resulting from the on-site investigation and the witnesses' inquest, the information during the necropsy (external and internal examination of the body), the toxicological information, requiring final histopathological confirmation.

While, in most cases, due to the short survival time in the hostile temperature conditions, there is a lack of macroscopic features that could suggest or guide the cause of death to hypothermia, the internal body examination may provide important clues for diagnosis. Following frostbite erythema observed in external exam, Wischnewski spots, detected by internal examination, have the greatest practical value in establishment of the diagnosis of death by hypothermia.

The intensity of the macroscopic changes of the internal organs depends on the difference between the victim's body temperature and the ambient or the water temperature (in case of immersion) and the period of cold exposure, so the absence of macroscopic lesions can be justified by a short interval of time to death by a sudden drop in body temperature, making the postmortem diagnosis of hypothermia death difficult, especially if there is no suspicion and appropriate circumstances.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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METFORMIN'S IMPACT ON SERUM IMUNOGLOBULINS AND BRONCHOALVEOLAR LAVAGE CELLS IN MICE WITH COMPLICATED SEVERE ASTHMA

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METFORMIN'S IMPACT ON SERUM IMUNOGLOBULINS AND BRONCHOALVEOLAR LAVAGE CELLS IN MICE WITH COMPLICATED SEVERE ASTHMA (Abstract): **Background:** Severe asthma represents an important burden of mortality and morbidity in asthma being a clinical condition that has the risk of evolution to acute lung injury (ALI). Based on the anti-inflammatory and airway anti-remodeling effect of Metformin we presumed that it will promote beneficial effects in severe asthma complicated to ALI. Our study aimed to evaluate the effects of Metformin on a murine model of severe asthma complicated with ALI by assessing the modulatory effect of Metformin of plasma IgE levels and on differential cell count from bronchoalveolar lavage fluid. **Materials and methods**: There were three groups of BALB/c mice with ovalbumin (OVA)-induced asthma followed by ALI induction which consisted in OVA sensitization (days 0 and 7) and challenge (days 14, 15, 16 and 17) followed by lipopolysaccharide (LPS) intratracheal instillations (days 15 and 17) treated 30 min before OVA, in days 14-17: positive control group for disease (OVA+LPS), Dexamethasone treatment group (DEXA + OVA + LPS) and Metformin treatment group (OVA + LPS + METF). Measurement of OVA-specific IgE levels was made and bronchoalveolar lavage fluid (BALF) was collected in order to apply differential cell count. **Results**: administration of Metformin decreased the airway macrophage infiltration with no modulatory effect on airway neutrophilic infiltration. **Conclusions**: Metformin has the potential to influence the physio-pathological changes that occur in asthma followed by ALI. However, new studies to explore the specific mechanism by which Metformin influences the inflammatory and immune response in asthma/ALI overlap are needed. **Keywords**: METFORMIN, SEVERE ASTHMA, ACUTE LUNG INJURY, LIPOPOLY-SACCHARIDE.

INTRODUCTION

Asthma is a respiratory disease characterized by airway chronic inflammation and remodeling. It has a high prevalence being estimated that 334 million people worldwide are suffering from asthma (1). Severe asthma proposes a significant challenge in clinical practice considered to affect a small proportion of asthmatic patients but accounts for a significant burden of morbidity and mortality correlated with asthma (2). Available data show that severe asthma is involved in increasing the risk of pneumonia which is the most common cause for acute lung injury. Therefore, asthma is a condition that influences the risk of progression to acute lung injury (3, 4).

Acute lung injury (ALI) consists in a common respiratory disease defined by acute lung inflammation having as main pathological characteristics inflammatory cell infiltration and high permeability pulmonary edema (5). Although the therapeutic strategies of this disease are complex, the mortality and morbidity levels are high (6).

Metformin (METF), a drug used as first line agent in diabetes type 2 (7). Recent studies have highlighted its pleiotropic pharmacodynamic profile (8) and its beneficial involvement in both bronchial asthma and ALI based mainly on its anti-inflammatory and airway anti-remodeling effect (9-11).

The aim of our study was to evaluate the effects of Metformin on a murine model of severe asthma complicated with ALI by determining IgE plasmatic concentrations and evaluating the differential cell count from bronchoalveolar fluid (BALF).

MATERIAL AND METHODS

Study Design

The experiment was carried on 18 mice (females BALB/c, weighing 17-23g) from "Cantacuzino" Institute in Bucharest. Experimental site was in CEMEX facility of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, under normal conditions of temperature (22±3°C), humidity (55±5%), 12 hours day/night cycles of and with water and food *ad libitum*. Ethical approval (No. 335/17.07.2023) was obtained from The Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași, Romania.

Animals, divided into 3 groups of 6 animals each, were treated intraperitoneally, once a day, as follows: OVA+LPS group (positive control for disease) with saline solution (0.5 ml/mouse); OVA+LPS+DEXA group (DEXA group; positive control for treatment) with Dexamethasone (1 mg/kg); OVA+LPS+METFgroup (Metformin group) with Metformin (250 mg/kg).

Induction of severe allergic asthma and lung inflammation was realized with OVA(GRADE V, Sigma-Aldrich, St. Louis, MO, USA), through sensitization procedure on days 0 and 7 through intraperitoneal once a day administration of 0.2 ml/mouse OVA/aluminium hydroxide solution(Sigma-Aldrich Chemie GmbH, Deutschland) (12),and followed by the challenge procedure on days 14, 15, 16, 17, with OVA 1% aerosols (10 ml/mouse) and lipopolysaccharide (LPS) (lipopolysaccharide from Escherichia coli 0127: B8, Sigma-Aldrich, St.Louis, MO, USA) intratracheal administration on days 15 and 17 (13).

Measurement of plasma IgE levels

Blood was collected by intracardiac puncture in 0.5ml microcentrifuge tubes and the serum was separated from whole blood

by centrifugation at 3.000 rpm at 4°C for 10 min and immediately frozen at −80°C. Total IgE levels were measured with enzyme-linked immunosorbent assay (ELISA) using OVA-specific IgE using a Legend Max Mouse OVA Specific IgE ELISA kit (Biolegend, USA). ELISA plates were read with an Infinite 200 PRO M Plex Tecan 314 plate reader (Tecan, Austria) and Magellan 7.4 software (Tecan, Austria).

Bronchoalveolar lavage fluid (BALF) and differential cell count

BALF collection was through an incision in the trachea which was exposed and then cannulated and began 24 hours following the last challenge with OVA, under ketamine and xylazine general anesthesia. The BALF collection process involved three successive infusions-aspirations of 0.5 ml warm (37°C) PBS through the tracheal cannula, totaling 1.5 ml. The fluid was kept on ice until centrifuged at 250 g for 5 min at 4˚C. The supernatants were collected and transferred to Eppendorf tubes, centrifuged at 10,000 g for 15 min at 4˚C, aliquoted as 150 µl samples into other tubes and stored at -80˚C until assessment of cytokines. Following vortex mixing, 5 µl of the residual cell pellet from the 250 g centrifugation were used to prepare slides that were next stained with May-Grünwald (Sigma-Aldrich, 1.01424, USA) and Giemsa (Sigma-Aldrich, 1.09204, USA). Using an optical microscope (Leica DM6 B, Germany) with a dry objective lens (40x), ten microscopic fields were examined to determine the mean cell count per field. Additionally, in order to differentiate neutrophils, eosinophils, lymphocytes, and macrophages, 200 cells were examined with an immersion objective lens (100x), using the same optical microscope.

Statistical Analysis

Data is presented as means ± standard error (SE). The statistical analysis was done using *SPSS* software. The assessment of statistically significant differences between groups was made using parametric tests (One-Way Analysis of Variance (ANOVA) followed by the Holm-Sidak method for multiple parametric comparisons), or nonparametric tests (Kruskal-Wallis One-Way ANOVA on ranks). P-values less than 0.05 were statistically significant.

RESULTS

Plasma Ig E levels

The serum levels of OVA-specific IgE were evaluated in all three groups. The highest plasma IgE levels were detected in DEXA group followed by the levels induced by Metformin administration, but without significant statistically differences between the treated groups and the positive control group for disease (Kruskal-Wallis One-way ANOVA on ranks: H = 3.500 with 2 degrees of freedom; p=0.174; ns).

Analysis of cell count in Bronchoalveolar Lavage Fluid (BALF)

Figure 1 shows the microscopic characteristics of inflammatory cells from BALF.

Intratracheal instillation of LPS resulted in a significant predominance of neutrophilic infiltration observed in the OVA+LPS group, confirming the potency of the ALI-induction experimental model. Treatment with Metformin did not alleviate the neutrophilic infiltration, without no significant statistical differences between the OVA + LPS + METF group and the

OVA+LPS group (p>0.05). The treatment with Dexamethasone did not exhibit an effect on the neutrophilic infiltration either, with no statistically significant differences noted between the DEXA group and the OVA+LPS group (p>0.05). Treatment with METF resulted in a decrease in the total number of macrophages compared to OVA + LPS group (p<0.05), but did not induce a significant statistical difference compared to DEXA group (p>0.05). Compared to OVA + LPS group the number of lymphocytes from DEXA group and METF group was not significant statistically different (One-way ANOVA: F(2,15) = 0.162 p = 0.852; ns). The assessment of eosinophils in BALF did not provide any significant statistical differences between groups (One-way ANOVA: F(2,15) = 0.678 p = 0.525; ns).

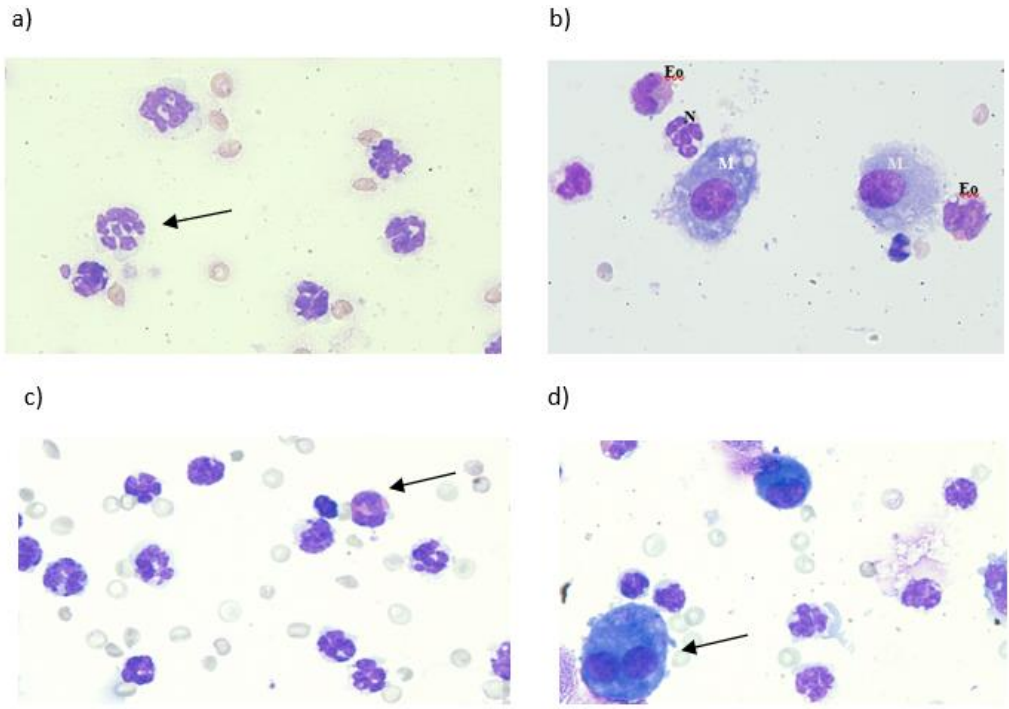

Fig. 1. Differential cell counts on optical microscopy, x100 immersion objective a) OVA + LPS group: neutrophils, hyper-segmented neutrophil (arrow); b) OVA + LPS +DEXA group: alveolar macrophages (M), neutrophils (N) and eosinophils (Eo); c) OVA + LPS +METF group: neutrophils and an eosinophil (arrow); d) OVA + LPS +METF group: neutrophils and two alveolar macrophages, one binucleated (arrow); OVA + LPS: positive control for disease; OVA + LPS + DEXA: Dexamethasone treated group; OVA + LPS + METF: Metformin treated group.

In each group, the differential number of cells represented by eosinophils, neutrophils, lymphocytes and macrophages was counted (fig. 2)

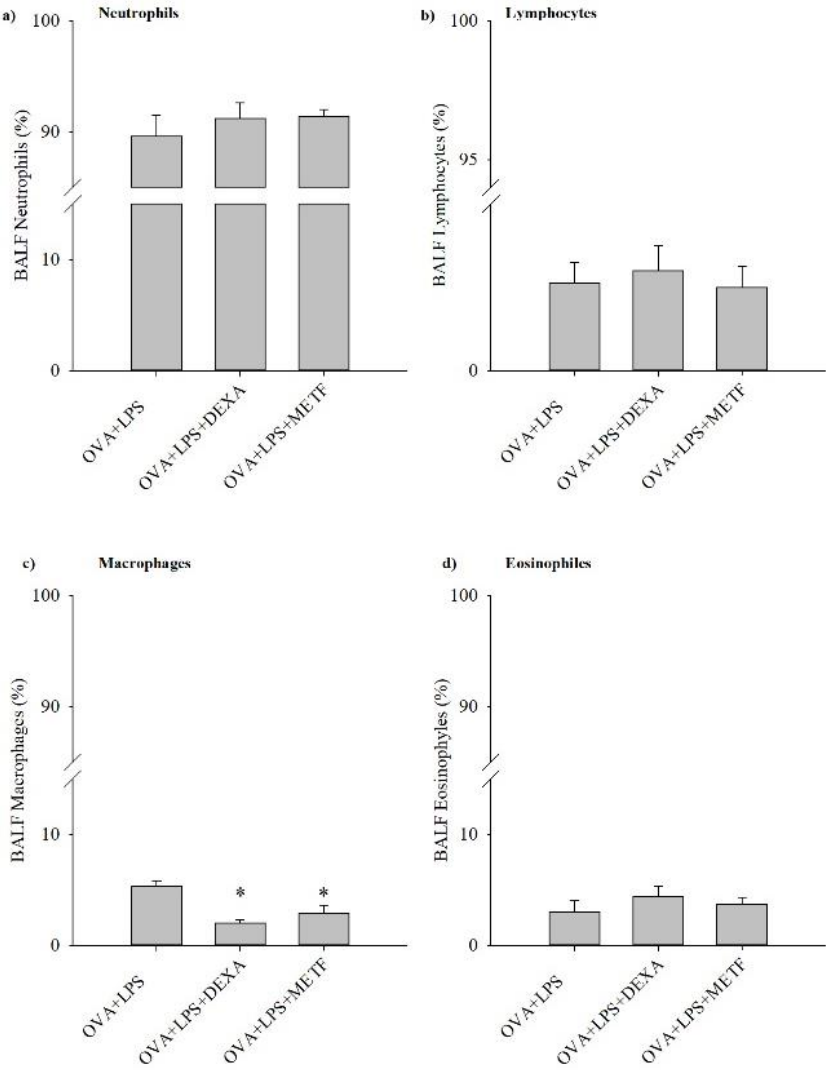

Fig. 2. Cell differential count in bronchoalveolar lavage fluid in percentages for (a) neutrophils; (b) lymphocytes; (c) macrophages; (d) eosinophils. Values are expressed as mean ± standard error; n=6. One-way ANOVA: * $p < 0.05$ *vs.* OVA+LPS group; followed by the Holm-Sidak method for multiple comparisons. OVA + LPS: positive control for disease; OVA + LPS + DEXA: Dexamethasone treated group; OVA + LPS + METF: Metformin treated group.

DISCUSSION

The results of our research highlighted the rich predominant neutrophilic airway infiltration which confirms the authenticity of the experimental model. It is considered that the neutrophilic airway infiltration

represents a defining feature of ALI which promotes epithelium and endothelium lesions through the release of cytotoxic molecules (14). Our study shows that the total neutrophils count induced by LPS was not influenced by the administration of Metformin nor by Dexamethasone. The results showing the eosinophilic and lymphocytes airway infiltration in all three experimental groups are sustaining the potency of the induced experimental model.

Data from literature shows the alleviating effect of Metformin on the airway neutrophilic infiltration in a mice model of ALI. The research published by Yu L *et al.*, points that Metformin is efficient in decreasing neutrophilic airway infiltration through inhibiting myeloperoxidase activity (15). Thus, our study presents contradictory results, this aspect could be interpreted in the context of the complexity of the animal model, being a severe asthma ALI overlap which leads to the idea of other possible underlying mechanisms of Metformin in this respiratory pathology. However, another study indicates that Metformin is efficiently in reducing neutrophilic infiltration in LPS-induced ALI in diabetic mice exposed to methylglyoxal MGO, being proposed the scavenger MGO activity of Metformin. The particular point of this study is that Metformin by itself in diabetic mice which were not exposed to MGO did not influence the neutrophilic infiltration induced by LPS (14).

Our statistical analyze on BALF cell count revealed that Metformin was efficient in decreasing the macrophage airway infiltration induced by LPS administration. Our results are confirmed by another study which points the effect of Metformin in reducing macrophage infiltration in an experimental model of ALI by suppressing LPS-induced endothelial cell pyroptosis mediated by SIRT-1 activation (16). Data show the essential role of macrophage in inflammatory respiratory diseases like allergic asthma, ALI and chronic obstructive pulmonary by initiating immunity and host defense (17, 18). In ALI the macrophages are involved in the modulation of the inflammatory response and also in the process of repairing damaged tissue (19). Based on the important role of macrophages in alveolar and lung environment the regulation process of macrophage function is proposed as therapeutic target in inflammatory respiratory disease (18).

Regarding the role of Metformin in modulating Ig E levels in respiratory diseases the published data show that Metformin has the ability to reduce OVA-specific Ig E in chronic asthma in mice (9). Our study consisting in severe asthma complicated with ALI revealed no modulatory effect of Metformin nor Dexamethasone on OVA-specific IgE levels.

Our research did not show significant differences between the potency of Metformin and Dexamethasone treatment. An interesting result is the rich neutrophilic infiltration in DEXA group. These findings can be approached based on the fact that in steroid resistant asthma phenotype Dexamethasone proven to have an impaired function in inhibiting IL-8 production which leads to persistent neutrophilic inflammation (20). Also, our results are consistent with another study that reveals that Dexamethasone treatment fails to reduce neutrophilic airway inflammation in a murine model of asthma (21).

The research direction of our study is valuable considering the clinical studies

which aim to explore the potential of Metformin treatment in reducing asthma exacerbations and acute lung severity in diabetic patients. The clinical data have controversial results, being shown the positive effect of Metformin treatment in reducing the risk of asthma exacerbation in diabetic patients (22), but also there are studies that reveal that Metformin treatment increased the risk of exacerbation asthma in diabetic patients (23).

CONCLUSIONS

Our research revealed the potency of Metformin in reducing the airway macrophage infiltration, a therapeutic target that could be influenced in severe asthma complicated with ALI. The results open the necessity of following experimental research in order to assess the therapeutic effect of Metformin in asthma-ALI overlap.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest.

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CORRELATION BETWEEN BREAST ARTERIAL CALCIFICATIONS ON MAMMOGRAPHY, CHOLESTEROL LEVELS AND CARDIOVASCULAR RISK

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CORRELATION BETWEEN BREAST ARTERIAL CALCIFICATIONS ON MAMMOGRAPHY, CHOLESTEROL LEVELS AND CARDIOVASCULAR RISK (Abstract): The **objective** of this study is to evaluate the correlation between arterial calcifications observed on mammograms and factors such as age, smoking status, body mass index, personal history of cardiovascular conditions, cholesterol levels (total cholesterol, LDL, HDL), and diabetes mellitus. **Materials and methods**: This retrospective study comprised a cohort of 1102 women, aged between 32 and 85, who underwent mammography at the "Sf. Spiridon" County Clinical Emergency Hospital Iasi from January 2018 to December 2020. Every woman underwent a routine procedure of bilateral four-view mammography. The patient's clinical and laboratory data were retrieved from the hospital's internal database. We selected in a random manner 115 age-matched women who did not have arterial calcifications in order to form the control group. **Results:** Out of the patients who received mammography, 95 cases (8.6%) showed the presence of breast arterial calcifications (BAC). As compared to control group, a significantly proportion of women with breast arterial calcifications were found to have a high body mass index (76.84%), elevated total cholesterol levels, cardiovascular diseases (21 cases, 22.10%), diabetes mellitus (15 cases, 15.78%), history of smoking (26 cases, 27.36%). **Conclusions**: The presence of breast arterial calcifications (BAC) on mammography is strongly linked to older age, elevated cholesterol levels, smoking, and diabetes mellitus. These patients also have a personal history of cardiovascular events, indicating a strong connection between BAC and an increased risk of cardiovascular problems. This correlation suggests that detecting BAC on mammography can serve as a marker for identifying women who are at a higher risk for experiencing cardiovascular events. **Keywords:** MAMMOGRAPHY, ARTERIAL CALCIFICATIONS, CARDIOVASCULAR RISK.

Mammography is a widely utilized imaging technique for detecting breast calcifications. During screening mammography, breast arterial calcification (BAC) is often detected. BAC refers to the benign and incidental medial calcification of the

mammary arteries. The formation of these calcifications is believed to occur through a complex process involving vascular inflammation, cellular damage, and subsequent mineralization of the arterial wall (1, 2, 3). It is becoming more and more evident that the presence of BAC during routine mammographic screenings could be a sign of increased risk for cardiovascular disease (CVD). Recent research indicates that the detection and severity of breast arterial calcifications can offer valuable insights into an individual's cardiovascular well-being and risk factors (4, 5). For instance, multiple studies have indicated a correlation between breast arterial calcifications and an increased risk of developing cardiovascular disease (6, 7, 8, 9).

Research on the relationship between breast arterial calcifications on mammography and systemic markers of cardiovascular risk, such as cholesterol levels, is gaining momentum. Studies have found a correlation between elevated cholesterol levels and a higher likelihood of developing breast arterial calcification. Previous studies have discovered a correlation between elevated total cholesterol levels in women and the presence of breast arterial calcifications on mammography. Furthermore, certain studies have found a connection between the existence of breast arterial calcifications and elevated triglyceride and low-density lipoprotein levels. It is evident that breast arterial calcifications can serve as a valuable indicator of the overall atherosclerotic process, giving clinicians an extra means of evaluating cardiovascular risk (10, 11, 12, 13).

In addition, the presence of breast arterial calcifications has been associated with a higher risk of cardiovascular events, including myocardial infarction, stroke, and cardiovascular death. It has been observed that patients with breast arterial calcifications tend to have a greater amount of coronary artery calcification and atherosclerosis, which in turn raises their chances of experiencing negative cardiovascular outcomes (14, 15, 16, 17, 18).

There is compelling evidence indicating a connection between breast arterial calcifications observed on mammography, cholesterol levels, and the potential for cardiovascular disease. Studies have shown that certain cardiovascular risk factors, including hypertension, smoking, and obesity, can potentially raise the risk of developing breast arterial calcifications (19, 20, 21). According to a systematic review conducted by Osman *et al.*, it was discovered that women with hypertension have a higher likelihood of having breast arterial calcifications on mammography compared to women without hypertension (22).

The existing evidence strongly indicates a connection between breast arterial calcifications detected on mammography, cholesterol levels, and cardiovascular risk. However, further research is required to gain a comprehensive understanding of the intricate relationship between these factors.

This experimental research aims to investigate the potential of mammography as a screening tool for cardiovascular disease, with a specific focus on postmenopausal women.

MATERIALS AND METHODS

Study Design

This retrospective study involved a total of 1102 women, aged 32-85, who underwent mammography at "Sf. Spiridon" County Clinical Emergency Hospital Iasi from January 2018 to December 2020. The patient's clinical history and laboratory

data were retrieved from the hospital's internal database. The study recorded information on various cardiovascular risk factors, such as hypertension, diabetes, smoking status, total cholesterol levels, diabetes mellitus, and personal history of cardiovascular diseases.

The study group consisted of 95 women who were found to have arterial macro and microcalcification through mammography out of the 1,102 cases analyzed.

The control group consisted of 115 women without arterial calcification, who were age-matched with the patients.

The weight of each patient was divided by their height squared to calculate their body mass index (kg/m^2).

Mammography

All women underwent standard bilateral four-view mammography, with mediolateral oblique (MLO) and cranio-caudal (CC) views, using between 2018 - 2019 a screen-film mammography system Senographe DMR+ and in 2020 a digital unit Senographe Pristina (General Electric Healthcare, Milwaukee, Wisconsin, USA). Mammographies were reviewed on dedicated screens, with a magnifying glass, looking for linear, parallel high intensity opacities (calcifications) within the arterial walls, on either of the two views. Mammography evaluated the presence of calcifications, type (macrocalcifications > 1 mm or microcalcifications < 1 mm), the number of involved arteries, and the distribution. Arterial calcifications can be easily identified due to their distinct pattern on mammography. They appear as linear, parallel high-intensity opacities within the arterial walls (fig. 1).

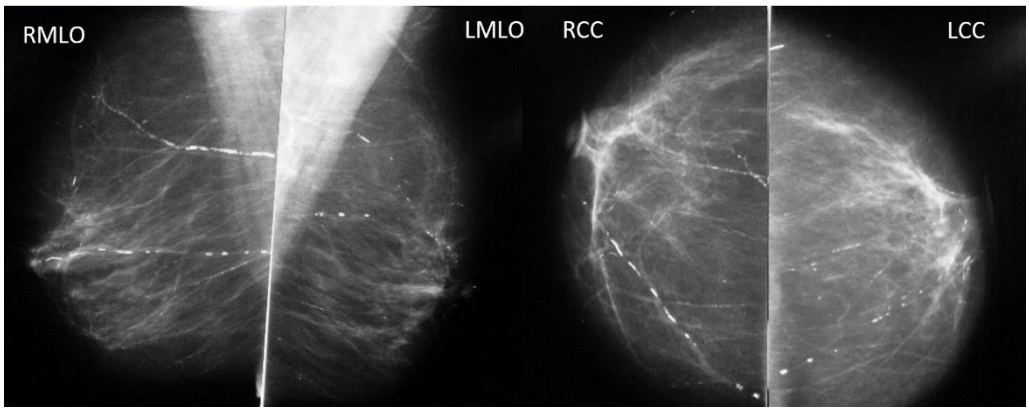

Fig. 1. Screen-film bilateral mammography, MLO and CC views - bilateral arterial calcifications (linear, parallel, high intensity opacities within the arterial walls)

Ethical Considerations

The study was approved by the local Institutional Ethics Committee in keeping with the principles of the Declaration of Helsinki. Data were collected anonymously from the electronic medical records without active patient participation.

Statistical Analysis

The continuous variables were reported as the mean and standard deviation (SD) and the categorical variables were expressed as percentages. Correlations were assessed using Spearman and Pearson coefficients of correlation. Variables between

study groups were compared using a one-way ANOVA test. P-values < 0.05 have been accepted as statistically significant.

The statistical analyses were performed using *Statistical Package for Social Sciences for Mac version 29* (SPSS Inc., Chicago, IL, USA).

RESULTS

Out of the 1,102 cases that were examined, a small percentage of 95 patients (8.6%) displayed the presence of arterial macro and microcalcification on mammography, specifically BAC (+) cases. The average ages of the BAC (+) and BAC (-) groups were 65.7±5.2 years and 59.4±6.3 years, respectively (p < 0.001). In the 60-69 age group, the highest percentage of BAC (+) was observed, reaching 64.21%. The postmenopausal periods for the two groups were 9.3±2.6 years and 6.4±1.9, respectively (p=0.04). Most of the BAC (+) cases were observed in menopausal women (90 cases, 94.73%, p < 0.05).

In the group with breast arterial calcifications, the mean body mass index (BMI) was higher (28.79 kg/m2) compared to the group without arterial calcifications (26.86 kg/m2, p<0.05).

The study revealed a clear link between age and the presence of breast arterial calcifications, with older patients being more likely to have calcifications. Additionally, a slight association was observed between BMI and calcifications, suggesting that higher BMI may be related to calcifications, although it is not a significant predictor (fig. 2).

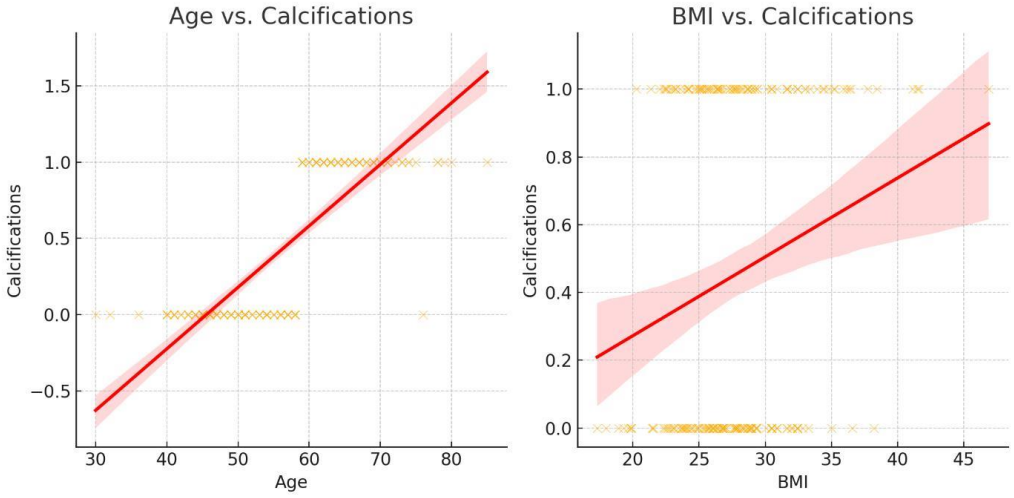

Fig. 2. Scatter plots and regression lines correlating, age, BMI and the presence of breast arterial calcifications.

Further research was conducted on the correlation between breast calcifications and obesity. Out of the individuals with arterial calcifications, only 22 cases (23.15%) had a BMI below 25 kg/m2, indicating a normal weight.

The first table provides a summary of the relationship between the presence of arterial calcifications on mammography and BMI in both, the BAC (+) and BAC (-) groups.

TABLE I.
Relationship between the presence of arterial calcifications and the BMI in BAC+ and BAC- groups

BMI (kg/m2)	< 25 (normal)	25.1-30 (overweight)	> 30.1 (obese)	> 25 (overweight + obese)
Patients, BAC +	22 (23.15%)	41 (43.16%)	32 (33.69%)	73 (76.84%)
Patients, BAC -	35 (30.43%)	55 (47.83%)	25 (21.74%)	80 (69.57%)

The presence of arterial calcifications on mammography was associated with higher mean total cholesterol (256 mg/dL) and LDL cholesterol levels (162 mg/dL) in women, compared to those without breast arterial calcifications (182 mg/dL and 125 mg/dL respectively). Additionally, BAC (+) cases with breast arterial calcifications had a mean HDL cholesterol value of 40 ± 8 mg/dL, while BAC (-) had a mean HDL cholesterol value of 52 ± 10 mg/dL.

The study found a significant difference regarding age, BMI, cholesterol levels (total cholesterol, LDL, HDL), in the study compared with the control. This information is presented in table II.

TABLE II.
Relationship between age, BMI, cholesterol levels and breast arterial calcifications

Characteristics	BAC + (n = 95)	BAC - (n = 115)	p-value
Mean age (years)	65.73 ±5.21	49.38±6.31	< 0.0001
Mean BMI	28.79±5.06	26.86± 3.83	< 0.002
Total Cholesterol (mg/dL)	256± 42	182± 32	< 0.001
LDL Cholesterol (mg/dL)	162 ± 32	125 ± 26	< 0.001
HDL Cholesterol (mg/dL)	40 ± 8	52 ± 10	< 0.05

Our study revealed a notable disparity in the occurrence of calcifications in the breast arteries between women who smoke (27.36%) and non-smoking women (14.78%) with similar calcium deposits ($p < 0.05$).

There was a higher prevalence of cardiovascular diseases (22.10%, $p < 0.05$) and diabetes mellitus (15.78%, $p < 0.05$) among women with breast arterial calcifications compared to those without calcifications on mammography. In contrast, the group without calcifications had a lower incidence of cardiovascular conditions (6.95%) and diabetes mellitus (4.34%). There appears to be a strong correlation between these abnormalities.

The study also included 27 patients diagnosed with cirrhosis. Among them, 10 (10.52%) showed arterial calcifications on mammography, while 17 (14.78%) did not have these lesions. The statistical analysis showed that there was no significant difference between the two groups ($p= 0.343$, NS). There is a negative weak correlation between cirrhosis and breast arterial calcifications, as indicated by the data. It is worth mentioning that the weak correlation is affected by the age of the patients, particularly due to the fact that only 11 of them were over the age of 55.

Associated lesions worth mentioning were fibroadenomas (4 - 4.21% cases in the study group and 6 - 5.21% in the control

group and malignant tumors (23 cases - 24.21% and 17 - 14.78% respectively).

DISCUSSION

The findings of this study reveal a noteworthy connection between BAC and an increased likelihood of an elevated cardiovascular risk. This information derived from mammography suggests that arterial calcifications can indicate a higher risk of cardiovascular problems compared to the general female population, as it suggests that other arteries could also be affected. Although breast arterial calcifications are situated in the media of the arteries rather than the intimal atherosclerotic calcifications found in other arteries, they still pose a considerable risk (23). In addition, BAC on mammography is strongly linked to older age, elevated cholesterol levels, a history of smoking, and diabetes mellitus. This information highlights the dual benefits of detecting arterial calcifications on mammography. It not only serves as a screening tool for breast cancer but also helps identify women at a higher risk for cardiovascular events.

There is a notable correlation between the occurrence of BAC and age. The age group between 60 and 69 years had the highest frequency of observed calcifications among all the patients included. The findings align with the current body of research, indicating that mammography is more likely to detect arterial calcifications in older women (18).

There is a random association between BAC and fibroadenomas and malignant tumors, which are commonly found in the general population. It is quite common for individuals aged 15 to 35 to be diagnosed with fibroadenomas, which are non-cancerous growths. On the other hand, woman over the age of 40 are more prone to being diagnosed with malignant tumors.

This study highlights the coincidental correlation between the presence of BAC and fibroadenomas, which can be attributed to their high prevalence in the general population.

CONCLUSIONS

The underreporting of arterial breast calcification is a common occurrence, as it is generally considered to be a benign condition. However, its association with cardiovascular conditions emphasizes the need for a comprehensive evaluation of patients. Additional research is needed to confirm whether mammography can effectively serve as a screening tool for cardiovascular disease, especially in postmenopausal women.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest. This research received no external funding.

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THE ESSENTIAL ROLE OF COMPUTED TOMOGRAPHY IN NEUROENDOCRINE TUMORS MANAGEMENT

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THE ESSENTIAL ROLE OF COMPUTED TOMOGRAPHY IN NEURO-ENDOCRINE TUMOR S MANAGEMENT (Abstract): Neuroendocrine tumors (NETs) are a diverse group of neoplasms that arise from neuroendocrine cells throughout the body. Their diagnosis and characterization can be challenging due to their variability in pattern and location. This study **aims** to provide an analysis of NETs demographics, computed tomography (CT) pattern, and correlations among certain characteristics determined after CT examination. **Materials and methods**: Data were collected retrospectively from a cohort of 74 patients with abdominal neuroendocrine tumors and their metastases, diagnosed and followed up using abdominal and pelvic CT from January 1, 2015, to December 31, 2023. We studied variables like age, gender, tumor location, margins, enhancement pattern (appearance on noncontract enhanced, arterial, and portal venous phases), homogeneity, presence of calcifications, lymphadenopathy, and liver metastasis. **Results**: The patient cohort with mean age 65.78 years, contains predominantly females (n=42, 56.8%). The majority of primary tumors were located in the pancreas (n=38, 63,63%) (more specifically in the pancreatic head n=19, 34.54%), and small bowel (n = 10, 18.18%). Most tumors exhibited hypervascularization in the arterial phase (n=68, 91.89%) and only few lesions (7-9.45%) contained calcifications. Liver secondary lesions (31.08%, n=23), and lymphadenopathies (36.5%, n=27 cases) were frequent in the selected cases. **Conclusions**: The demographic data suggest a higher prevalence of NETs in older adults, with a slight female predominance. Males were more likely to have tumors located in the head of the pancreas, while females had a higher incidence of hepatic metastasis. Patients with NET have a high incidence of liver metastases, which requires careful abdominal CT follow-up to evaluate both local and distant extension. CT provides an accurate localization and characterization of NETs but also vital information for staging, prognosis, and correct treatment planning. **Keywords:** COMPUTED TOMOGRAPHY, NEUROENDOCRINE, MANAGEMENT, TUMOR.

INTRODUCTION

Neuroendocrine tumor s (NETs) represent a wide and heterogeneous spectrum of diseases that vary in their primary tumor sites, functional status, and aggressive behavior (1).

They are derived from embryonic neural crest tissue (enterochromaffin cells) and

found in various locations: endocrine glands such as the pituitary, adrenal medulla, and parathyroid glands, as well as endocrine tissue nestled within glandular structures like the thyroid or pancreas, and cells within the exocrine structures like the endocrine cells lining the digestive and tract (2). The gastrointestinal tract (55-67%), the respiratory system (25%) (3), and the pancreas (1-2%) (1, 4) are the primary sites of neuroendocrine tumor development.

NETs are mainly sporadic but may occur in a familial context of autosomal dominant inherited syndromes such as MEN1, MEN2, Von Hippel-Lindau disease, neurofibromatosis type 1 and multiple tuberous sclerosis (4, 5, 6, 7, 8).

Computed tomography (CT) is the most frequently used technique for primary tumor localization, staging, therapeutic monitoring, and follow-up of patients with NETs. Triple-phase intravenous contrast-enhanced CT (early arterial, portal-venous, and equilibrium phases) is usually performed. In primary NETs, the average sensitivity of CT is 73%, a better sensitivity (75%) is attained in detecting extrahepatic metastases, and 80% is reached for liver metastases (9, 10, 11).

MATERIALS AND METHODS

This is a single-center, observational, retrospective study. The patients were selected by searching internal databases of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, to identify patients who underwent abdominal and pelvic contrast enhanced CT (CECT) from January 1, 2015 to December 31st 2023, for suspicion of NET or for evaluation treated NETs and we depicted 74 patients.

Abdominal and pelvic CT was performed with a 64-detector Incisive Philips CT scan; All patients ingested 1,500 ml water in one hour to distend the stomach and small bowel, and underwent colonic cleansing preparation followed by a 1500-2000 ml water enema before the procedure.

An abdominal and pelvic noncontrast enhanced scan was performed, then we injected intravenously up to 120 mL of 370 I/mL Iomeprol (Iopamiro 370 mg/mL), with an automatic injector, with the speed of 3 - 4 ml/second, followed by 50 ml of saline chaser; we measured with bolus tracking technique the densities during contrast injection in the descending aorta above the diaphragm; when the density was over 120 HU, the scanning started with a delay of 7 seconds for the arterial phase, 60–70 s for the portal venous phase, and 3-4 minutes for the equilibrium phase (11,12,13).

We studied variables like age, gender, tumor location, margins, enhancement pattern (appearance on non-contrast enhanced, arterial, and portal venous phases), homogeneity, presence of calcifications, lymphadenopathy, and liver metastasis.

Descriptive statistics were calculated for all variables. Frequency distributions were analyzed for categorical variables. Pearson and Spearman correlations were performed to identify relationships between continuous and categorical variables, respectively. Statistical significance was set at $p<0.05$. Our institutional review board approved the study.

RESULTS

The cohort comprised 74 patients diagnosed with NETs, who had a mean age of 65.78 years (SD 12.49), an age range of 32–88years, and a slight female predominance (56.8% female, 43.2% male). The median age for both male and female patients is relatively similar. The majority of

patients fall within the 58–74 years age range, reflecting that in our study most of the patients were middle aged and elderly. This demographic profile aligns with existing literature, suggesting that NETs predominantly affect older adults, with a slight predominance in females.

The majority of newly diagnosed primary tumor s (n=55) were located in the pancreas (n=35, 63.63%). The most common locations for primary tumor s were found in the pancreatic head (n=19, 34.54%), and small bowel (n = 10, 18.18%). Other notable locations include the pancreatic body and tail (n=9, 16.36% each), mesentery and stomach (n=4, 7.27%). Rare locations include appendix, cecum and adrenal gland (n=1, 1.81% each). This distribution highlights the pancreas and small bowel as primary sites for NETs in our study, emphasising the importance of searching for these organs when a neuroendocrine tumor is suspected.

The median age for NETs localized in the pancreatic head was around 70 years, while patients with tumor s localized in the pancreatic body (median age 55), small bowel, or stomach tend to be younger (both with a median age of 60).

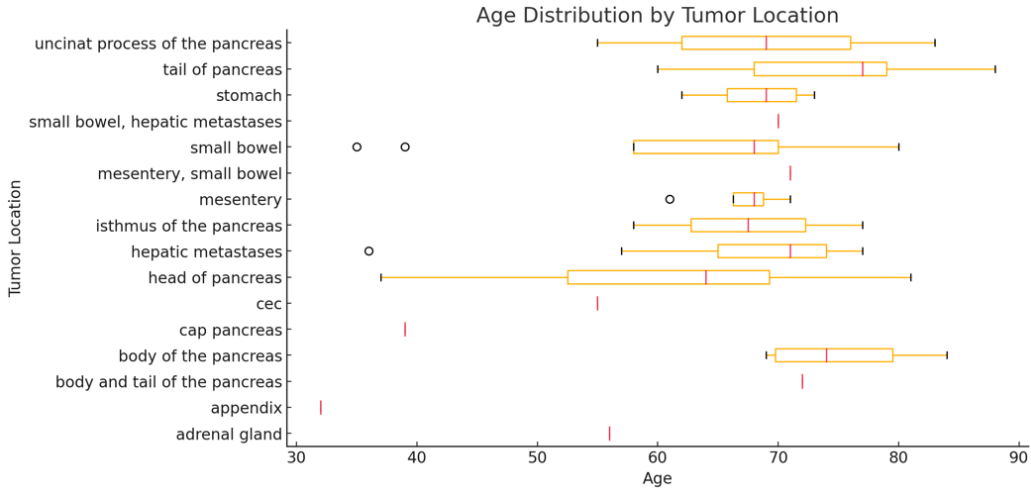

Fig. 1. Age distribution of patients across different tumor locations

Adenopathy, which is a critical factor in staging and prognosis, was observed in 36.48% of patients (n=27), while local invasion was detected in 21.6% (n=16), reflecting the aggressive nature of some tumor s and necessitating a treatment approach.

The most frequent primary tumor s associated with adenopathy's had the following locations in descending order: pancreatic head (n=9, 12.16%), mesentery (n=4, 5.4%), small bowel (n=3, 4.05%), stomach (n=2, 2.7%).

Liver secondary lesions were found in case of surgically treated followed-up patients in 19 (25.67%), but also in newly diagnosed primary tumor s. These data are similar with ones in the literature, highlighting that the liver is a common site for metastatic spread in NETs. All the tumor s located in the mesentery have hepatic secondary lesions, emphasizing the need for

carefully monitoring these patients for potential liver metastasis. Also, 11 patients out of 19 with secondary liver lesions had adenopathies present. Patients with hepatic metastasis have a slightly higher median age (70 years) compared to those without hepatic metastasis (68 years). Elderly were significantly associated with the presence of hepatic metastasis ($p<0.05$).

Various density patterns were observed in noncontrast enhanced, arterial, and venous, phases (fig. 2). On the non-contrast enhanced phase, the majority of tumor s are izodense (n=51, 68.92%), with only 20 (27.03%) hypodense tumor s, and 3 (4.05%) hyperdense tumor s. Most tumor s exhibited hypervascularization in arterial phase (hyperdense, n=68, 91.89%; izodense, n=2, 2.70%; hypodense, n=4, 5.41%). The majority of tumor s (87.8%) showed important, homogeneous enhancement in the arterial phase, while other patterns like discrete enhancement (4.1%), peripheral enhancement (2.7%), and progressive enhancement (2.7%) were rare. A high amount of tumor s remains hyperdense (n=54, 72.97%) in the portal venous phase; 9.47% were hypodense (n=7) and 17.57% izodense (n=13). 62 tumors (83.78%) had well-defined margins while 16.22% (n=12) have ill-defined margins, these last ones having also important enhancement; therefore, the hyper vascular lesions were more likely to show local invasion ($p<0.01$).

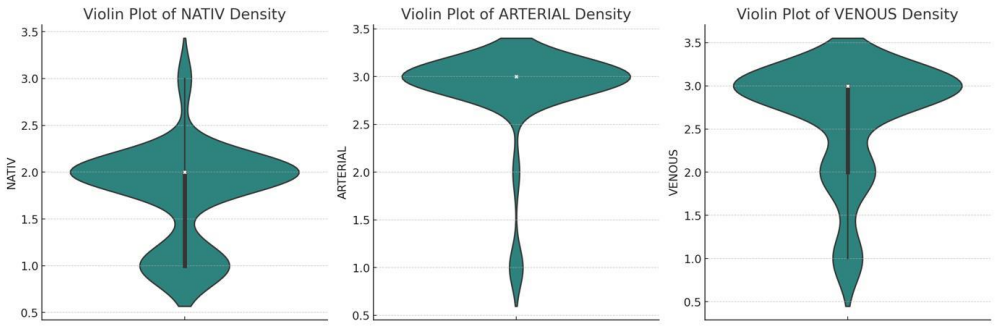

Fig. 2. Violin plot showing the correlation between NETs densities in noncontrast enhanced, arterial and portal venous phase

Less lesions were homogenous 28 (37.84%), and most of them were heterogeneous (n=46, 62.86%), with a strong positive correlation between heterogeneous tumor s and poorly defined tumoral margins ($p<0.001$). There is a borderline significant difference ($p= 0.051$), which potentially suggests that age might be linked to homogeneity, this potential trend can be explored further with a larger sample size. The elder age might be linked to tumor heterogeneity. Inhomogeneous tumors are more likely to be associated with hepatic metastasis compared to homogeneous tumors ($p< 0.05$).

Calcifications were present only in 7 cases (9.45%), with a majority of 67 patients (90.55%) showing no calcifications. Calcifications are present in 11.76% of tumor s located in the pancreatic head and in 11.11% of tumor s located pancreatic tail or small bowel. A higher percentage (50%, 2 of 4 cases) of tumor s located in the mesentery had calcifications. The presence of

calcifications can be associated with certain NETs locations (pancreatic, small bowel, mesentery) and might influence the choice of imaging modality and interpretation of results.

DISCUSSION

The characterization of this cohort highlights several critical aspects of NETs. The demographic data suggest a higher prevalence in older adults, with a slight female predominance. Males were more likely to have tumor s in the head of the pancreas, while females had a higher incidence of hepatic metastasis. The majority of patients are aged between 55 and 75.

Imaging features on CT scans revealed distinct patterns among the tumor s. Enhancement patterns were particularly notable, with 91.89% of tumor s showing significant enhancement in the arterial phase, which is indicative of the vascular nature of NETs.

The majority of tumor s were located in the pancreas (63.63%), specifically the head (19%). Other common sites included pancreatic tail and the small bowel. This distribution highlights the pancreas as the primary site of interest for NETs, emphasizing the importance of these locations in diagnostic and therapeutic strategies.

Adenopathy (a critical factor in staging and prognosis) was observed in 36.5% of patients, while local invasion was detected in 21.6%, reflecting the aggressive nature of some NETs, necessitating a differentiated treatment approach.

The high incidence of hepatic metastasis (25.67%) aligns with the known behaviour of NETs to metastasize to the liver, impacting patient management significantly and suggesting a need for regular liver monitoring. Older age is associated with the presence of hepatic metastasis in patients with neuroendocrine tumor s, which might suggest that metastatic disease is more common as patients' age increases. In the noncontrast enhanced phase, most tumors were izodense (68.9%), while in the arterial phase, a majority were hyperdense (91.9%). The venous phase also showed a high percentage of hyperdense tumor s (73.0%). These enhancement patterns reflect the dynamic vascular nature of NETs, which can be leveraged in both diagnostic imaging and interventional procedures.

Homogeneity and limits of the tumor s were also assessed, with 62.86% being heterogeneous and 83.78% well defined. Calcifications were observed in 9.45% of patients.

These imaging characteristics are crucial for an accurate diagnosis and can influence treatment. Neuroendocrine tumors are sometimes correlated with diabetes and various infections (13, 14). We are looking forward to apply artificial intelligence and machine learning in the diagnosis and management of gastroenteropancreatic neuroendocrine tumors (15).

CONCLUSIONS

The gender-based differences in tumor location highlight potential underlying biological mechanisms warranting further investigation.

The detailed analysis of enhancement patterns across different CT phases provides valuable insights into the vascular properties of these tumor s. These imaging features not only aid in diagnosis but also have potential implications for treatment, particularly in planning surgical or interventional procedures.

High incidence of liver metastatic involvement necessitates frequent liver monitoring in case of patients diagnosed with

NETs, since early detection of metastasis can significantly impact management and outcomes.

CT imaging not only aids in the accurate localization and characterization of NETs but also provides essential information on metastatic spread and associated adenopathy. and provide vital information for an accurate staging, prognosis, and treatment planning.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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WIDE AWAKE LOCAL ANESTHESIA NO TOURNIQUET (WALANT) IN HAND SURGERY - A RETROSPECTIVE STUDY ON 400 CASES

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WIDE AWAKE LOCAL ANESTHESIA NO TOURNIQUET (WALANT) IN HAND SURGERY - A RETROSPECTIVE STUDY ON 400 CASES. (Abstract): The study **aimed** to evaluate and confirm the efficacy and safety of WALANT anesthesia in hand surgery. **Materials and methods**: A retrospective analysis was conducted on 400 hand surgeries performed under WALANT between January 2020 and December 2023. Diseases included carpal tunnel syndrome (CTS), Dupuytren disease (DD), trigger finger (TF), also tendon injuries, phalangeal and metacarpal fractures, and tumor excisions. Data collected included patient demographics, anesthetic dosage, onset time, dilutions, intraoperative incidents (pain, bleeding, discomfort), complications, surgical duration, hospitalization, patient satisfaction (VAS), and functional outcomes (DASH and MHQ scores). Statistical analysis was performed using *SPSS version 29.0*, with regressions (ANOVA) and Pearson correlation coefficients. **Results:** The mean hospitalization time was 3.5 hours, with 95% of patients discharged the same day. Complications were low (5%), and functional outcomes improved significantly, with DASH scores improving by 66.7% on average. No cases of digital necrosis or other vascular complications occurred. **Conclusions**: WALANT is a safe, effective, and resource-efficient technique for hand surgery. It optimizes hospital resources, enhances patient outcomes, minimizes costs, and supports sustainable healthcare. **Keywords:** LOCAL ANESTHESIA, WALANT, HAND SURGERY, LIDOCAINE, EPINEPHRINE.

INTRODUCTION

The advancement of hand surgery, orthopedic surgery, and various other surgical disciplines has been markedly enhanced by the integration of minimally invasive anesthetic techniques, such as WALANT (Wide Awake Local Anesthesia No Tourniquet) (1). In the early 2000s, Donald Lalonde pioneered the use of a combination of local anesthesia (1% lidocaine) and epinephrine (1: 100,000) to develop WALANT for vasoconstriction, creating a bloodless surgical field without the need for general anesthesia or tourniquets (2). This technique allows

real-time functional testing while keeping patients awake, improving precision, safety, and recovery times (3). While initially popularized in hand surgery for pathologies like tendon injuries, carpal tunnel syndrome and fractures, it has proven effective in a variety of other surgical domains (4). Hand surgeries relied on general or loco-regional anesthesia in combination with a tourniquet, a practice associated with risks such as pain, nerve damage, and prolonged recovery. The WALANT technique, utilizing a solution of 1% lidocaine with epinephrine (1: 100,000), provides local anesthesia and a bloodless surgical field, while eliminating the complications and risks associated with tourniquet use and the need for additional sedation required with other anesthetic methods (2-5).

Early concerns about the safety of epinephrine in hand surgeries were alleviated through studies demonstrating its safety at low concentrations, leading to WALANT's global acceptance (5, 6). Lalonde *et al*. was initially cautious about using epinephrine in hand surgeries due to concerns over necrosis from intense vasoconstriction. He indicated the use of phentolamine as an antidote (7, 8). This type of local anesthesia is indicated and effective in hand surgeries, including both acute trauma cases and chronic pathologies, as well as in palliative hand surgery (9, 10). It has also shown significant advantages in orthopedic surgery, including clavicle fractures, distal radius fractures, ankle fractures, metatarsal fractures, and certain types of joint procedures (11). WALANT's localized approach also reduces risks associated with systemic anesthesia, making it a safer option for patients with comorbidities. Most patients can be safely discharged within a few hours of the procedure, with minimal postoperative care required due to the rapid recovery associated with WALANT, improving hospital efficiency and patient satisfaction (11-13).

By eliminating the need for anesthesia personnel, extensive monitoring, and recovery units, it has significantly lowered the cost of surgeries (14). As a green surgical technique, WALANT contributes to sustainable practices by reducing medical waste and resource utilization. Its impact on modern surgical practices across hand surgery, orthopedics, reconstructive surgery, and palliative care highlights its versatility and transformative potential (15). Due to the fact that this study was conducted during the COVID-19 pandemic, specific challenges emerged regarding patient care and safety (16). In this context, the adoption of WALANT has emerged as the preferred, and often the sole feasible approach for patient management, establishing it as a gold standard in hand surgery anesthesia (17-19). A retrospective study was conducted on 400 hand surgeries performed under WALANT between January 2020 and December 2023. All surgeries performed under WALANT anesthesia.

MATERIALS AND METHODS

A retrospective study was conducted on 400 hand surgeries performed under WALANT between January 2020 and December 2023. All surgeries were done at Plastic and Reconstructive Surgery Clinic from the Emergency County Hospital "Sf. Spiridon" of Iasi. On admission, the informed consent was obtained from all patients in the study group. The ethics committee of the hospital has granted approval for the reporting of the present study. The inclusion criteria for the study group were as follows: patients over 18 years of age undergoing hand surgery with indication of local anesthesia - carpal tunnel syndrome,

trigger finger, Dupuytren disease, tendon injuries or tenolysis, metacarpal and phalangeal fractures, patients who consented to this type of anesthesia, patients with no known allergies to lidocaine or epinephrine, ability to remain awake and tolerate the procedure. Exclusion criteria were: patients under 18 years old, patients who did not consent for this type of anesthesia, pathologies including vascular damage involving microsurgery or intricate vascular techniques, patients with psychiatric or neurological conditions impairing their ability to remain calm during surgery, patients with significant cardiac conditions contraindicating the use of epinephrine. The recorded data were collected from the medical records (observation sheet and the hospital's electronic information system) of hospitalized and treated patients. In the studied group, were registered: epidemiological data (age, gender, urban or rural residence) comorbidities, diagnosis, the amount of anesthetic used, the dilutions used, waiting time until its onset, intraoperative incidents/accidents (pain, bleeding, doctor/patient discomfort), duration of the surgery, functional outcomes (with DASH scale) measured at 6 weeks and 3 months, complications, hospitalization time and patient satisfaction level using VAS scale. In all instances, surgical procedures were conducted under local anesthesia using a solution of 1% lidocaine with epinephrine in a concentration of 1: 100,000 or 1: 200,000. Sodium bicarbonate (8.4%) was not used for buffering in any case. Pain during the procedure was objectively monitored through vital signs (blood pressure, heart rate) and subjectively through patient feedback, describing pain as non-existent, minimal, tolerable, or intolerable. Patient comfort was assessed both by tracking vital signs and through their subjective response.

Statistical analysis. The data were analyzed using *SPSS Version 29*, with the following statistical techniques: descriptive statistics for demographic data, anesthetic use, and hospitalization time, chi-Square Tests to analyze differences in complication rates between different types of surgeries, paired T-Test for comparing preoperative and postoperative DASH scores, ANOVA for comparing costs and outcomes across different surgical procedures, regression Analysis to identify predictors of postoperative functional outcomes, including age, comorbidities, and anesthetic volume

RESULTS

The gender distribution in the research cohort is represented by 55% male patients with a male/female = 1/1.22 The age of the patients ranged from 18 to 85 years, with a mean age of 50.6 years. The analysis showed that 70% of patients came from urban areas, consistent with findings in WALANT literature, where urban settings dominate due to the technique's accessibility in outpatient centers. However, the remaining 30% rural representation in the data reflects a notable proportion of trauma cases, which may be associated with labor-intensive occupations common in rural regions. The largest proportion of patients was aged 31-50 years (40%), followed by 51-70 years (32.5%), reflecting the prevalence of working-age adults in the study cohort. Carpal tunnel syndrome (CTS) was the most frequently treated condition (29%), followed by flexor and extensor tendon injuries 26,75%), metacarpal and phalangeal fractures (18.5%), trigger finger (TF) (12%), Dupuytren's disease (DD) (7%), and other surgeries (6.75%) (tab. I).

TABLE I.
Demographic data of the studied group

Pathology	18-30 y	31-50 y	51-70 y	71-85 y	Male	Female	Urban	Rural	Total	(%)
Tendon injuries	5 (1.25%)	42 (10%)	45 (8.75%)	15 (2.5%)	58 (14.5%)	49 (12.25%)	72 (18%)	35 (8.75%)	107	26.75%
CTS	7 (1.75%)	50 (12.5%)	44 (11%)	15 (3.75%)	62 (15.5%)	54 (13.5%)	80 (20%)	36 (9%)	116	29%
fractures	10 (2.5%)	38 (9.5%)	20 (5%)	6 (1.5%	48 (12%)	26 (6.5%)	40 (10%)	34 (8.5%	74	18.5%
TF	5 (1.25%)	12 (3%)	24 (6%)	7 (1.75%)	23 (5.75%)	25 (6.25%)	27 (6.75%)	21 (5.25%)	48	12%
DD	2 (0.5%)	10 (2.5%)	11 (2.75%)	5 (1.25%)	16 (4%)	12 (3%)	14 (3.5%)	14 (3.5%)	28	7%
Other	2 (0.5%)	8 (2%)	6 (1.5%)	11 (2.75%)	13 (3.25%)	14 (3.5%)	15 (3.75%)	12 (3%)	27	6.75%
Total	31 (7.75%)	160 (40%)	130 (32.5%)	59 (14.75%)	220 (55%)	180 (45%)	248 (62%)	152 (38%)	400	100%

CTS= carpal tunnel syndrome, TF= trigger finger, DD= Dupuytren disease, y=year

Patients were instructed to inform the surgeon if they experienced any pain or discomfort, even if minor. Initially, 0.3 to 0.5 mL of anesthetic was injected just beneath the dermis. Once the patient no longer reported pain, the remainder of the anesthetic was administered by advancing the needle to a depth of approximately one cm. The total anesthetic volume did not exceed 20 mL for CTS and 15 mL for each affected digital ray in cases of DD. The volume of anesthesia used and its dilution were key factors influencing both the quality of the surgical field and patient satisfaction (fig.1). Procedures that required higher volumes of anesthesia (e.g., DD) often used higher dilutions, which led to a lower incidence of complications such as intraoperative bleeding. For instance, the 1: 200,000 dilution of epinephrine provided an optimal balance between pain control and vasoconstriction, ensuring effective hemostasis during the surgery while maintaining patient comfort, confirming that the 1: 200,000 dilution allowed for rapid onset and reduced intraoperative bleeding. The reduced anesthesia volume (10-14 mL) used in the cohort group aligns with the WALANT principle of minimizing invasiveness without sacrificing efficacy (fig. 1).

For CTS cases, 10 mL the anesthetic solution was injected on each side near the incision site, and the remaining 10 mL was administered into the surrounding skin area where hypoesthesia was already present. This method was similarly employed in DD cases, with the anesthetic being injected around the planned incision sites. In TF cases, no more than 1 mL of anesthetic solution was injected into each affected phalanx. Throughout the anesthetic administration, patients were conscious, cooperative, and did not receive any sedatives. The onset of anesthesia occurred within 6 to 15 minutes (tab. II).

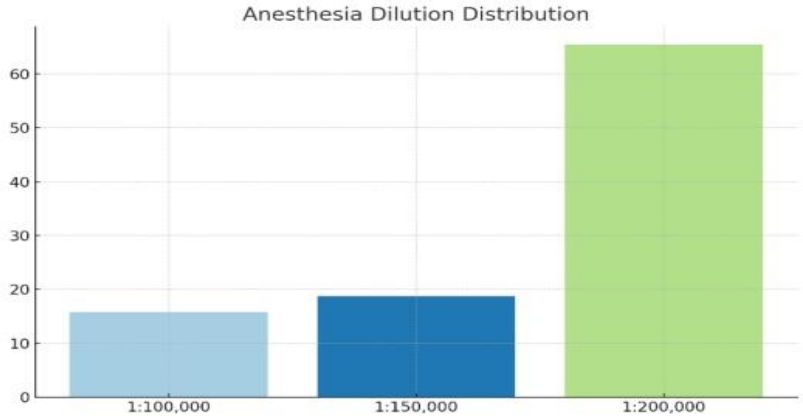

Fig. 1. Anesthesia dilution distribution

TABLE II.

Anesthesia installing time (minutes)

Range	Number	Percentage	Mean Total Group (Max / Min)	Other Metrics
< 10 min	34	8.5%		TF: 7.84 (10/5)
10-14 min	207	51.67%	13.93 (20/8)	Tendon injuries: 11.2 (14/7) CTS: 11.41 (15/8), Other: 12.31 (20/5)
15-20 min	159	38.75%		DD: 16.41 (20/8), Fractures: 14.82 (20/8)

TF-trigger finger; DD- Dupuytren's disease

Key finding from the study is the reduced hospitalization time. Percentage of patients were discharged within 3-4 hours post-surgery (fig. 2).

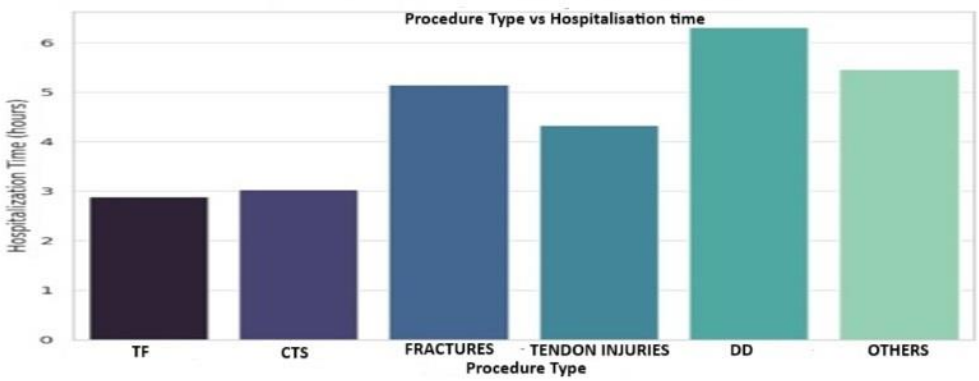

Fig. 2. Procedure type *vs.* hospitalization time

The amount of anesthetic solution used was 10-14 mL in almost half of the cases (49%), with a mean volume of 14.61 mL across the entire group. Subgroup analysis revealed that Dupuytren disease required the highest mean anesthetic volume (18.92 mL) but still under 20-25 mL how literature recommend, while trigger finger required the lowest (4.13 mL) (tab. III). These findings indicate that even if there are more complex procedures, such as those for Dupuytren's disease, which typically necessitate higher volumes of anesthetic to ensure adequate analgesia and hemostasis, lower volume with higher dilution can have good results (tab. IV).

TABLE III.

Anesthetic solution (mL)

Range	Number	Percentage
< 10 mL	37	9.25%
10-14 mL	196	49%
15-19 mL	134	33.5%
>= 20 mL	31	7.75%

TABLE IV.

Mean values by group (anesthetic solution)

Group	Mean (Max/Min)
Total Group	14.61 (20/2)
TF	4.13 (5/2)
CTS	15.43 (20/14)
Fractures	17.33 (20/15)
Tendon Injuries	8.53 (10/6)
Dupuytren's Disease	18.92 (20/10)
Other	11.37 (12/10)

Calculating the Pearson correlation coefficients and their p-values based on the cohort patients collected data the study revealed: Anesthesia Volume *vs.* Onset Time - Correlation coefficient (r): 0.99, p-value: 0.011. There is a strong positive correlation between anesthesia volume and onset time, meaning higher anesthesia volumes are associated with longer onset times. Anesthesia volume vs. satisfaction - correlation coefficient (r): -0.998, p-value: 0.002. There is a very strong negative correlation, indicating that higher anesthesia volumes are associated with lower patient satisfaction (fig.3). Dilution Level vs. Satisfaction. Correlation coefficient (r): -0.994, p-value: 0.006. There is a strong negative correlation, suggesting that higher dilution levels are associated with higher patient satisfaction. These results are statistically significant (p-value < 0.05), supporting the hypothesis that lower anesthetic volumes and more diluted solutions can lead to shorter onset times and higher patient satisfaction (fig. 3).

Hospitalization time differed significantly by procedure type, with shorter stays noted for simpler procedures like carpal

tunnel syndrome ($p < 0.001$). The absence of a tourniquet and the localized anesthesia significantly reduced the discomfort commonly associated with traditional methods. In terms of complications, the study recorded a no accidents or incidents during surgery. There were no instances of digital necrosis or vascular complications, demonstrating the safety of WALANT in this cohort. The absence of significant intraoperative bleeding or the need for epinephrine reversal agents, such as phentolamine, further supports the effectiveness of WALANT as a reliable and safe anesthetic technique.

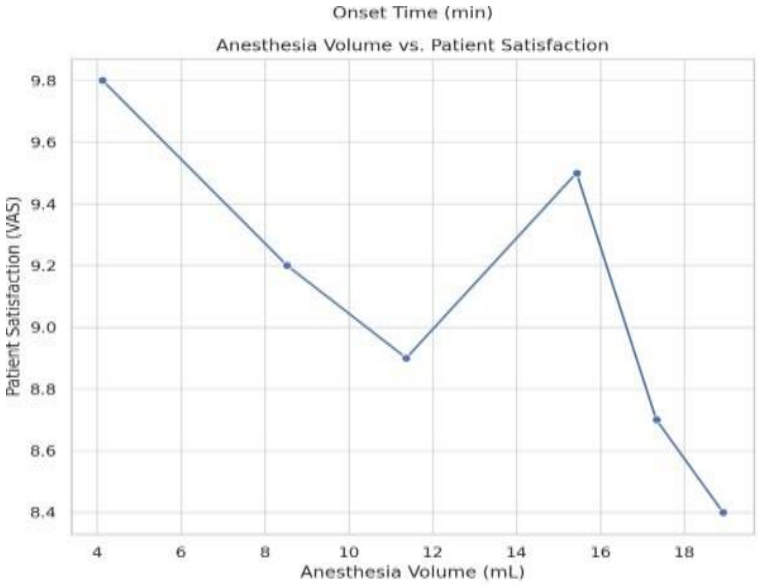

Fig. 3. Anesthesia volume *vs.* patient satisfaction

Patients in the older age groups, particularly those aged 51-70 and 71-85 years, experience longer hospital stays. This increase in hospitalization time can be attributed to several factors. Older patients are more likely to have multiple comorbidities, such as diabetes or cardiovascular diseases, which can complicate both surgery and recovery. Additionally, age-related physiological changes, such as reduced tissue healing capacity and a higher likelihood of postoperative complications, may necessitate prolonged monitoring and care (fig. 4).

Patient satisfaction was assessed using a Visual Analog Scale (VAS) and DASH scores, at 3 weeks and 6 months with results indicating high satisfaction rates. The average VAS score for pain during the procedure was low, ranging from 0 to 2, demonstrating that the anesthesia provided adequate pain relief. Furthermore, satisfaction was correlated with the volume and dilution of the anesthetic. Higher volumes of anesthesia, especially when diluted appropriately, contributed to better pain control and comfort during the procedure. Notably, patients reported less discomfort and quicker recovery times when larger dilutions (greater than 1: 200,000) of lidocaine with epinephrine were used, resulting in a bloodless surgical field with minimal pain (tab. V).

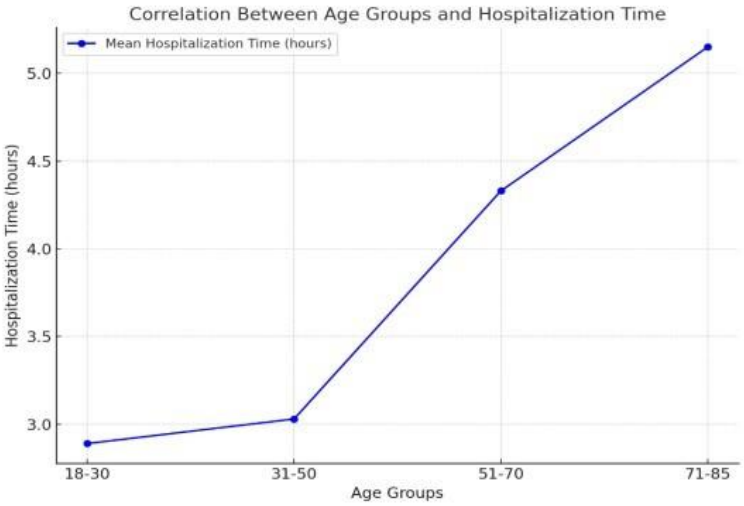

Fig. 4. Correlation between age groups and hospitalization time

TABLE V.
Functional outcomes of surgeries using WALANT anesthesia

	Number of Patients	MHQ Score (3 weeks)	MHQ Score (6 months)	DASH Score (3 weeks)	DASH Score (6 months)
CTS	116	65±5,2	85.4 ± 4.5	38±3.1	6.3 ± 2.1
TF	48	67±4,3	83.2 ± 5.1	32±5.2	7.1 ± 2.9
Fractures	74	58±6.7	79.5 ± 6.0	45±4.3	12.4 ± 3.5
Tendon Injuries	107	62±5.5	81.3 ± 5.6	40±2.2	10.2 ± 2.8
DD	28	59±6	76.8 ± 7.3	41±3.7	14.7 ± 4.0
Other	27	57±3.9	78.5 ± 6.4	47±2.5	13.1 ± 3.2

DASH-Disabilities of the Arm, Shoulder, and Hand score; MHQ- Michigan Hand Questionnaire = Trigger finger, DD= Dupuytren disease CTS= Carpal tunnel syndrome

DISCUSSION

The application of WALANT, particularly in trauma-related surgeries, compared to its predominant use for elective procedures in the literature. These differences likely reflect the demographic and clinical characteristics of the patient population, demonstrating WALANT's versatility in addressing both degenerative and trauma-related conditions (19, 20).

A significant reduction in hospitalization time, with the majority of patients being discharged within 3-4 hours, compared to the 4-6 hours of hospitalization typically reported in WALANT literature. Studies like Lalonde (2005) and Fitzcharles-Bowe (2007) suggest that WALANT procedures usually require about 4-6 hours of observation (21, 22). This study highlights the potential for even shorter hospital stays, indicating that using lower anesthetic volumes can streamline

recovery and enhance discharge efficiency without compromising patient safety.

As it for demographics, this cohort, with 40% of patients aged 31-50, contrasts with the older demographic typically reported in WALANT studies, where patients are usually aged 50-55 years (Fitzcharles-Bowe, 2007; Casal *et al*., 2011) (23, 24). The younger patient population in this study likely reflects a higher proportion of trauma-related surgeries, such as tendon injuries and fractures, which are less commonly explored in WALANT studies focused on elective procedures. This finding underscores WALANT's versatility in handling both degenerative and trauma-related conditions, extending its application beyond what is usually seen in the literature.

The study used significantly lower anesthetic volumes (10-20 mL), compared to the 20-40 mL often reported in other WALANT studies (e.g., Martin *et al*., 2005) (25). These reduced volumes were sufficient for adequate pain control, aligning with WALANT's principle of minimizing invasiveness. This result contrasts with studies by Lalonde (2005) and Kalle *et al*. (2010), which employed larger volumes of anesthesia and possibly had higher complication risks (26). Moreover, study highlighted that higher dilution of local anesthetic contributed significantly to both the safety and effectiveness of the procedure. The 1: 200,000 dilution of epinephrine used in the study group was more diluted than in many studies, where concentrations typically range from 1: 100,000 to 1: 150,000. This higher dilution allowed for better pain management and reduced the risk of complications such as excessive vasoconstriction and intraoperative bleeding (27). The surgery for carpal tunnel syndrome (CTS) tends to have shorter recovery times (Lalonde, 2005; Foucher *et al*., 2015), this study demonstrated that WALANT is equally effective for more complex procedures, such as metacarpal and phalangeal fractures and Dupuytren's disease (DD), which require higher anesthesia volumes (26). However, even these complex surgeries benefited from higher dilution (1: 200,000), which allowed for minimal complications and quicker recovery (28-30). The study's safety profile, with no accidents or complications, is consistent with the low complication rates reported in the literature, though it exceeds other studies that recorded rare complications (31). The absence of digital necrosis, vascular complications, or the need for reversal agents further supports the effectiveness and safety of WALANT when lower volumes and higher dilutions are used. This emphasizes the safety of WALANT, especially in more complex surgeries, where other studies report a higher likelihood of complications (32).

CONCLUSIONS

Considering the numerous advantages of the WALANT technique, including its rapid onset of anesthesia, efficiency, and safety profile, as well as the wide range of surgical procedures it facilitates, it represents a highly effective alternative to classical anesthesia techniques. WALANT is associated with minimal risks, significantly reduced waiting and hospitalization times, and eliminates the need for preoperative fasting or complex monitoring. Moreover, it contributes to increased patient satisfaction by providing a comfortable and awake surgical experience with

faster recovery. Given these benefits, WALANT could be regarded as a gold standard for elective or emergency hand surgery procedures.

CONFLICTS OF INTEREST AND FUNDING

All the authors declare no funding received and no conflict of interest.

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PARTIAL SPLENIC EMBOLIZATION-MINIMALLY INVASIVE ALTERNATIVE TO SURGICAL SPLENECTOMY IN THROMBOCYTOPENIC HYPERSPLENISM. A SINGLE CENTER EXPERIENCE

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PARTIAL SPLENIC EMBOLIZATION-MINIMALLY INVASIVE ALTERNATIVE TO SURGICAL SPLENECTOMY IN THROMBOCYTOPENIC HYPERSPLENISM. A SINGLE CENTER EXPERIENCE (Abstract): Partial splenic embolization (PSE) is a minimally invasive procedure that replaced radical surgical intervention of splenectomy for the treatment of severe cytopenia, mainly life-threatening thrombocytopenia secondary to hypersplenism in advanced liver disease. **Materials and methods:** This is an observational, retrospective and analytical study of 15 patients undergoing PSE from "St. John" Emergency Hospital Bucharest, admitted to Gastroenterology Clinic for several complications of liver cirrhosis, during May 2013-July 2019, while the patients' survival rate was followed up until November 2024. **Results:** Our results confirm once again that PSE is a reliable nonsurgical technique used to improve the consequences of hypersplenism associated with portal hypertension in chronic liver disease, especially in liver cirrhosis. Generally, the postembolization course was uneventful; non-severe complications developed and were properly managed by conservative treatment. **Conclusions:** A significant improvement of platelet number was obtained in all patients, allowing better outcome for each case, especially if a surgical intervention is scheduled. **Keywords:** PARTIAL SPLENIC EMBOLIZATION, THROMBOCYTOPENIA, LIVER CIRRHOSIS.

INTRODUCTION

Liver cirrhosis is an important public health issue due to its several complications related to portal hypertension that accompanies the advanced stages: variceal bleeding, hepatic encephalopathy, hepatorenal syndrome and sometimes hepatopulmonary syndrome (1, 2). Splenomegaly is a sign of portal hypertension and is responsible for hypersplenism development as an overactive splenic function of destruction the blood cells. Historically, the term "hypersplenism" was first mentioned in a paper of Anatole Chauffard in 1907, and after that in 1955, the German physiologist Morawitz defined hypersplenism related to liver diseases, while the German surgeon Denecke described the triad of hypersplenism: spleno-

megaly, peripheral cytopenia, normal or hyperplastic bone marrow (3,4). Among all consequences of hypersplenism, thrombocytopenia is the most relevant one in chronic liver disease for the biological coagulation status, because it enhances the coagulopathy of cirrhotic patient (5). Hepatic dysfunction together with severe decrease of platelet count are the major factors that potentially make the cirrhotic patients a poor surgical candidate. Thus, any attempt to increase the level of circulating platelets gained a lot of interest. Considering the per operative risks of surgical splenectomy in patients with advanced liver disease, PSE became widely used instead, being safer on one hand, and on the other hand, more efficient due to preservation of immunological role of spleen (6, 7, 8). The increase in platelet ratio is related to the volume of spleen targeted for embolization, which has been demonstrated to correlate also with the severity of post-procedural complications (9, 10). It has been stated that almost 50 % of splenic volume needs to be devitalized in order to obtain an efficient improvement of thrombocytopenia (11, 12). In fact, the goal of procedure is to achieve approximately 50-70% of spleen embolized. Major and minor complications can develop after PSE, but the majority of them are non-severe and controllable through conservative therapy, post-embolization syndrome being the most frequent encountered (13,14).

MATERIALS AND METHODS

This retrospective study involved 15 patients with pancytopenia or only with thrombocytopenia, 14 patients with chronic liver disease and one patient with a hematological condition, who underwent PSE. The goal of the study is to identify certain relationships between biological parameters pattern, especially increase in platelet or leucocyte number, the occurrence of complications and the prognosis of patients. In order to assess if the postprocedural increase in the blood indices was significant, it was crucial to choose the right statistical test.

The procedure was performed using Seldinger technique, that is percutaneous catheterization of a peripheral artery, the common femoral artery in these cases, after local anesthesia. A 5 Fr catheter was inserted into the celiac trunk via femoral artery, and after that selective catheterization of splenic artery was achieved using a 2,8 Fr microcatheter and placing it as distal as possible from the hilum to obtain the embolization of segmental splenic branches (figs. 1, 2). The material used for embolization was a gelatin foam and the procedure required contrast medium injection and fluoroscopic control.

A subgroup of patients was followed up over a period of 11 years, thus enabling the assessment of survival rate for this certain group. The remaining subjects were lost from observation due to lack of presentation at scheduled appointments.

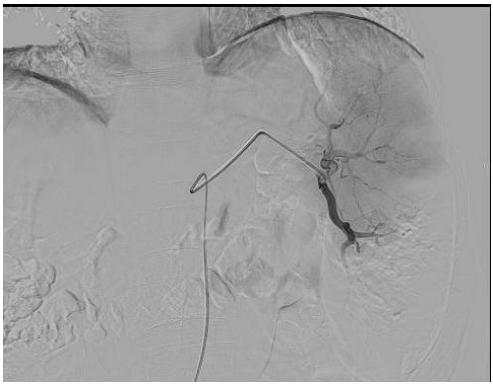

Fig. 1. Supraselective catheterization of the inferior terminal branch of the splenic artery.

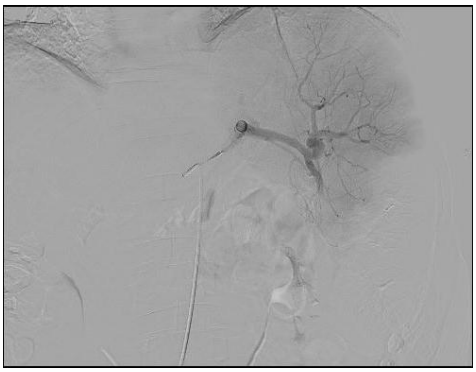

Fig. 2. Injection of embolic material was performed into the inferior branch of splenic artery.

The analysis process included several stages: data collection from patients electronic or paper files; data processing; testing the normal distribution for each continuous variable (Shapiro-Wilk; histograms); using the right statistical test (t-test, Wilcoxon, Mann-Whitney, Pearson/Spearman, Chi-square); graphic representation of biological parameters dynamics through box plot, linear graphs and scatterplots; results interpretation depending on statistical significance ($p< 0.05$) and clinical relevance.

We used different tools: Microsoft Excel for initial data analysis; SPSS / R / Jamovi / Python (pandas, scipy, matplotlib) for advanced statistical analysis; GraphPad Prism / Tableau / ggplot2 for data visualization (optional).

A preliminary step before applying statistical significance tests (parametric and nonparametric) consisted of verifying the normality of data distribution for each analyzed variable. This step is of extremely importance because the parametric test (as t-test for paired samples) involves a normal distribution of differences, while the nonparametric test (as Wilcoxon test) doesn't imply this condition. So, for each of these three variables-thrombocytes, hemoglobin and leukocytes- we applied **Shapiro-Wilk** test, considered adequate for small samples, for assessing whether the differences pre-postprocedural follow a normal distribution.

The results of this test will determine the subsequent choice of statistical comparison method (tab. I).

TABLE I.
Normality Test-Shapiro-Wilk

Variable	Normality (p Shapiro-Wilk)	Conclusion
Thrombocytes	$p < 0.05$	Abnormal distribution
Leukocytes	$p > 0.05$	Normal distribution
Hemoglobin	$p > 0.05$	Normal distribution

Based on Shapiro-Wilk test, we used: for thrombocytes nonparametric *Wilcoxon test* for paired samples, for leukocytes parametric paired *t-test* and for hemoglobin parametric paired *t -test.*

The use of Shapiro-Wilk test ensures accurate selection of the statistical tool, avoiding misinterpreting due to incorrect assumptions of data distribution.

For each variable (thrombocytes, hemoglobin and leukocytes), we stated the following hypothesis:

- **Nule Hypothesis (H0):** there is no significant statistical difference between pre and post-treatment values.
- **Alternative Hypothesis (H1):** there is a significant statistical difference between pre and post-treatment values.

RESULTS

We focused on different characteristics of the patients, including demographic, clinical and biological data.

The age of the patients was between 28 and 78 years, with a median age of 56.8+/- 13.8 years per patient. Males 'age ranged between 38 and 72 years with a median age of 57.7+/- 13.1 year per patient, while females have ages between 28 and 78 years with a median of 56.2+/- 15.1 per patient. According to Shapiro-Wilk test (p= 0.914), the distribution of variable "Age" indicates a normal distribution of subjects.

The majority of study participants, 14 patients suffered from chronic liver disease, with viral (hepatitis virus B, B+D or hepatitis C) or alcoholic etiology, while only one patient exhibited a hematologic disease, which in fact had an "off labeled" indication for PSE and did not respond in a favorable manner after the procedure.

Considering the statistical methodology presented earlier, we obtained the following results:

Thrombocytes:

- Shapiro-Wilk test showed an abnormal distribution (p = 0.004).
- The nonparametric Wilcoxon test was used.
- The result is statistically significant (p = 0.041), indicating a **significant difference** between pre and post intervention values, with a postprocedural increase of platelet level.

Leukocytes:

- The distribution of differences is normal (p = 0.10).
- The parametric paired t-test was used.
- The result is statistically significant (p = 0.002), showing a **significant difference** between pre and post intervention levels, with a postprocedural increase of leukocytes

Hemoglobin:

- The distribution of differences is normal (p = 0.98).
- The paired t-test exhibits a significant difference (p=0,328), suggesting a **slightly increase**, but non-significant, of hemoglobin after PSE.

The fluctuations of hematological parameters pre and post embolization are illustrated in the third figure.

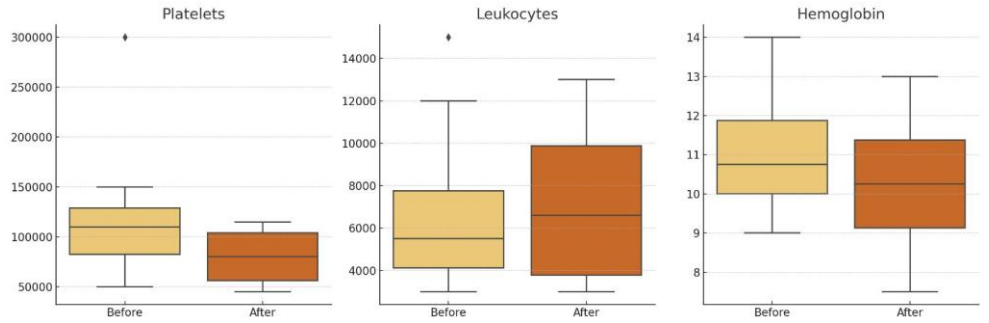

Fig. 3. Fluctuations of hematological parameters pre and post embolization

Interpretation of data based on box-plot graphs from third figure.

Thrombocytes

- distribution pre and post are hetero-

genous, but post-intervention a significant median increase is observed.
- there are extreme values (outlier), but the general trend is of **increasing.**

Leukocytes
- postintervention, median and dispersion rise significant.
- the distribution is larger, but without evident extremes - **increased systemic response** after PSE.

Hemoglobin (Hb)
- slightly, but evident increase after PSE.
- the variability is moderate, but distributions are similar; postprocedural curve is "higher"-suggesting moderate, but constant therapeutically efficiency.

TABLE II.

Essential statistical descriptive data for thrombocytes, leukocytes and hemoglobin, before and after PSE, as well as the differences between these values (Post *vs.* Pre).

	Medium level	Median	Mode	Std. Dev.	Min.	Max.	Q1	Q3
Thrombocytes, Pre	57.733,33	47.000,00	22.000,00	38.662,40	22.000,00	188.000,00	40.000,00	63.000,00
Thrombocyte, Post	95.500,00	72.000,00	56.000,00	66.639,49	38.000,00	303.000,00	56.000,00	121.500,00
Thrombocyte, Post *vs.* Pre	**37.766,67**	**25.000,00**	**34.000,00**	**27.977,09**	**16.000,00**	**115.000,00**	**16.000,00**	**58.500,00**
Leukocytes, Pre	3.437,47	3.300,00	2.500,00	1.439,43	1.570,00	6.600,00	2.500,00	4.400,00
Leukocytes, Post	7.775,00	6.575,00	2.600,00	4.444,29	2.600,00	16.900,00	4.400,00	10.400,00
Leukocytes, Post *vs.* Pre	**4.337,53**	**3.275,00**	**100,00**	**3.004,85**	**1.030,00**	**10.300,00**	**1.900,00**	**6.000,00**
Hemoglobin, Pre	10.61	11.10	11.10	2.03	6.60	12.90	9.50	12.50
Hemoglobin, Post	10.91	11.10	11.10	1.53	8.40	13.00	9.90	11.80
Hemoglobin, Post *vs.* Pre	**0.31**	**0.00**	**0.00**	**-0.50**	**1.80**	**0.10**	**0.40**	**-0.70**

Thrombocytes

The mean level of platelets increased from 57.733/μL preprocedural to 95.500/μL post procedural, with a mean difference of +37.766/μL, thus indicating a substantial clinical improvement. The median increased from 47.000 la 72.000/μL, confirming an upward trend of the response. The increase is further supported by the higher Q1 and Q3 values, indicating a global distribution towards higher post-treatment values. However, the elevated standard deviation (66.639) after procedure suggested a high dispersion, possible reflecting heterogenous responses depending on the patient or on the disease severity (tab. II).

Leukocytes

The mean level of leukocytes doubled after PSE (from 3.437 to 7.775/μL), while the mean difference was +4.337/μL, showing a favorable response. Although the median has moderately increased (from 3.300 to 6.575/μL), a high dispersion is evident (SD=4.445), and the extreme post-treatment values varies between 2.600 and 16.900/ μL (tab. II).

Hemoglobin

Unlike the other parameters, hemoglobin remained at a steady state; the mean exhibited a slight increase from 10,61 g/dL la 10,91 g/dL, with a mean difference of +0,31 g/dL, considered clinically non-significant. The median doesn't change at

all (11,10 g/dL), while the quartiles had minimal modifications, indicating a hematological stability (tab. II).

Influence of gender and age over the hematological changes after PSE

There is no linear relationship between age and platelet number variation. However, older patients tend to have a reduced hematological response. The age group 50-70 years presented a more stable increasing in platelets and leukocytes level. Hemoglobin levels stayed steady, independent the age group or gender, confirming the stability of this parameter after the procedure. The differences in the increase of the platelets do not seem extremely visual between males and women (fig. 4).

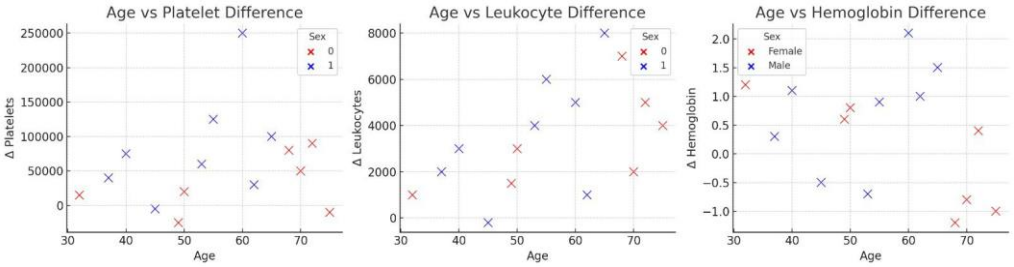

Fig. 4. Visual comparison of postprocedural response depending on age and gender

The differences between males and females regarding the hematological response post PSE are visible for thrombocytes and leukocytes, but no definitely conclusions can be made without supplementary statistical tests.

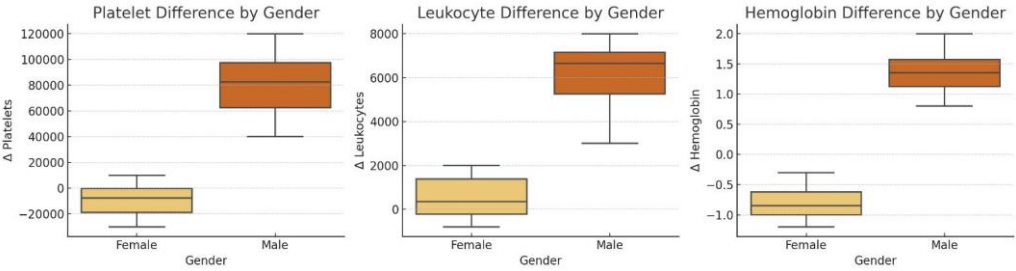

Fig. 5. Post procedural differences for thrombocytes, leukocytes and hemoglobin: females *vs.* males

TABLE III.

Summary of relevant correlations (Pearson):

Relation	Pearson Coef	Interpretation
Age-Difference Thrombocytes	-0.250	Weak negative correlation
Age-Difference Leukocytes	-0.075	No relevant correlation
Age-Difference Hemoglobin	-0.064	No relevant correlation
Sex-Difference Thrombocytes	+0.153	Weak positive correlation
Sex-Difference Leukocytes	+0.210	Weak positive correlation
Sex-Difference Hemoglobin	-0.174	Weak positive correlation

Both age and gender seemed to have only marginal influence over the post procedural fluctuations of hematological parameters in this study group. The relationships showed a weak statistical significance.

The association between the embolized splenic volume and post PSE platelet count

This analysis aims to evaluate the relationship between the embolized splenic volume and the increase in the platelet count post partial splenic embolization, in a sample of 15 patients.

The hypothesis tested is that embolizing a higher percent of spleen, the increase in thrombocytes count raised simultaneously. A simple linear regression was performed, the dependent variable was the change in platelet count post PSE, while the independent variable was the percent of embolized splenic volume. The analysis included all 15 patients (n=15).

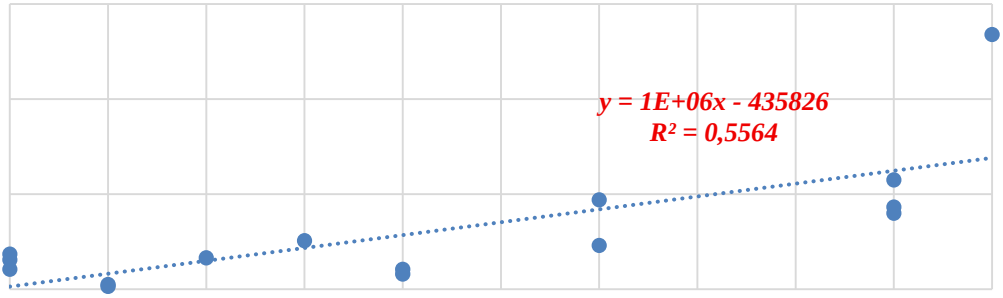

Fig. 6. Correlation between splenic infarction ratio and the platelet increase ratio after PSE

Results

The R-squared (R^2) value: 0.556 → approximately 55.6% of platelet variation could be explained through the percent of embolized volume.

The adjusted R-squared: 0.522 → adjusted for the number of observations and predictors, that indicated a moderate-strong relation.

Standard error of the estimate: 46.059,26 → indicates mean deviation of the actual values from the predicted values.

ANOVA / F Test: 16.31 / Associated p-value (F significance): 0.0014 → statistically significant

($p < 0.05$), confirming that the regression model is globally significant.

Regression Coefficients

Intercept (constant term): - 435.826 (p = 0.058) → significant negative, indicating that when the percent of embolization is zero, the model estimates a decrease of platelet level.

Coefficient for embolization percent: 1.353.123 (p = 0.0014) → significant positive, suggesting that for each unit of increasing the percent of embolization, it is estimated an increase with approximately 1.353.123 of platelet count post partial splenic embolization.

Confidence intervals (95%)

For the coefficient "percent of embolization": [629.203, 2.077.043] → the interval doesn't include the value "zero", confirming the statistical significance.

The results suggested a positive correlation and statistically significant between the percent of embolized splenic volume and the increase of platelet count after PSE. Thus, a more extensive embolization

seemed to be associated with a favorable hematological response, especially regarding the platelet count.

The survival analysis through Kaplan-Meier method in a group of 15 patients

The survival analysis is an essential tool in clinical trials for estimation of the survival rate or of the time to a relevant event (disease progression, complications). Kaplan-Meier method allows the assessment of cumulative survival probability over time, taking censored data into account. The goal of this study is to describe the survival distribution in a group of 15 patients, using data expressed in months.

Methodology

Study population: 15 patients.

Variable time is expressed in months (interval: 0-109 months).

The survival rate was analyzed using Kaplan-Meier method.

Censoring was applied to the patients who did not finish the follow-up period.

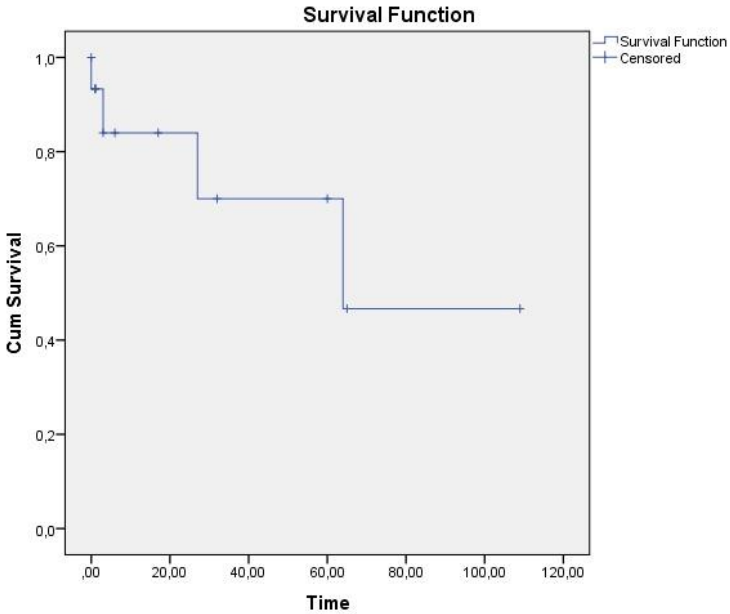

Fig. 7. The survival probability as a function of time (months)-Kaplan-Meier analysis

Kaplan-Meier graph showed a progressive decrease of cumulative survival probability on a 9-year period of time (109 months).

At 6 months, the estimated survival is approximately 73.5%.

At 64 months (over 5 years), the survival decreases to 40.8%.

The survival curve flattened out after approximately 65 months, suggesting that there are no events after this point.

Descriptive statistics

Median survival time was 64 months (confidence interval C.I. 95%: 0 – 134.1 months), indicating that 50% of patients survived at least 5 years and 4 months.

The estimated mean time of survival was 61.8 months, with a standard error of

15.04 months (C.I. 95%: 32.3 - 91.3 months).

Percentiles: percentile 50% (median): 64 months / percentile 75%: 6 months that suggests that a quarter of patients suffered an event in the first 6 months.

Survival table

The majority of events took place in the first 60 months, with only 5 events in the whole follow-up period.

After 60 months, only 3 patients remained under observation, and the curve indicated a stabilization of survival in 40% of patients.

These results demonstrated a median survival rate of over 5 years, reflecting a favorable outcome for certain patients, but also a significant risk of early events during the first month's post intervention.

The survival differences by gender: Kaplan-Meier analysis

Methodology

Study population: 15 patients.

Variable time is expressed in months (interval: 0-109 months).

Event: death or major complication

Censoring was applied to the patients who did not experience an event during the follow-up period. Patient gender: 0-females, 1-males

Statistical test for comparison: Log-Rank (Mantel-Cox)

Results

Survival estimations based on gender

Females (gender = 0):

Mean time of survival: 46.19 months (C.I. 95%: 7.34 - 85.05)

Median time of survival: 27 months (C.I. 95%: 1.18-52.81). Percentile 75%: 3 months.

Males (gender = 1):

Mean time of survival: 64.5 months (C.I. 95%: 63.81 - 65.19)

Median time of survival: 64 months (C.I. 95% unknown due to lack of sufficient events). Percentile 75%: 64 months.

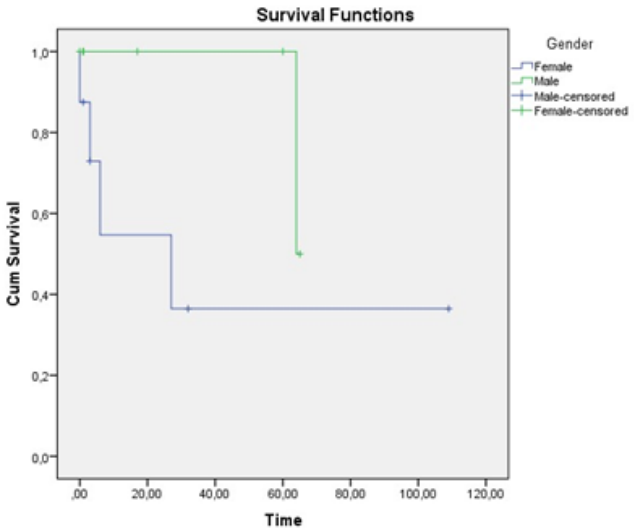

Fig. 8. Kaplan-Meier function of survival based on gender

Kaplan-Meier curve indicated a higher survival in males, with a flattened curve around 64 months and a decreased survival in females starting from the first months, with a significant reduction in the first 30 months.

Test Log-Rank. Chi-square = 1. 941 and p = 0.164 shows the difference is not statistically significant with $p < 0.05$, but suggested a possible trend in males. The results showed a clinically relevant difference but statistical non-significant regarding the survival between males and females in the study group. Males tend to have a longer survival (mean: 64.5 months *vs.* 46.2 months), with a double median comparing to that observed in females (64 *vs.* 27 months).

DISCUSSION

Splenic embolization was first introduced in early seventies by Maddison who used it for treating a refractory esophageal variceal bleeding in liver cirrhosis, and after that, the technique was improved by Spigos who imagined only a partial embolization in order to minimalize the associated complications (15, 16). Over the decades, the use of splenectomy has declined and its place has been gradually taken by this minimal invasive technique.

Our study focused on the results of PSE on hypersplenism determined by portal hypertension in liver cirrhosis. Among all the components of pancytopenia, thrombocytopenia plays a central part in cirrhotic patients. Thrombocytopenia increases a patient's risk of spontaneous bleeding, and in addition to the severity of liver dysfunction quantified through Child-Pugh classification, may contribute to make a planned surgical intervention inadvisable (18, 19). Our survey included a patient with alcoholic liver cirrhosis with severe thrombocytopenia, who was diagnosed with urothelial adenocarcinoma. Due to extremely low platelet number, the surgical intervention for associated malignancy was contraindicated. Performing PSE, the platelet level increased considerably post procedure, allowing to schedule a surgical intervention for urothelial cancer.

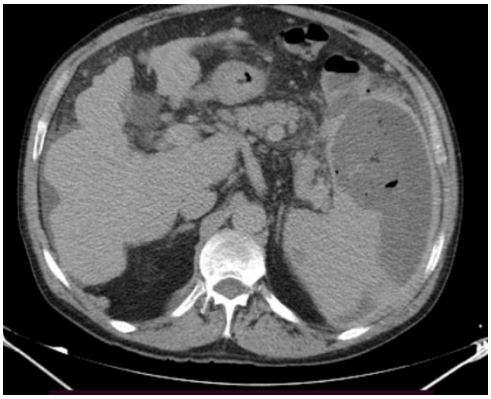

Fig. 9. Computed tomography of spleen after embolization; 50% of spleen infarcted.

Considering the case particularities, we observed different responses comparative with the whole group in two cases accompanied by autoimmune condition: either in a case with thrombotic thrombocytopenic purpura who presented a lack of response in terms of platelet increasing or a liver cirrhosis due to hepatitis C virus complicated with cryoglobulinemia, that also did not show a significant increase in thrombocyte count.

Data from literature suggested that increasing the platelet count depends on the volume of infarcted spleen which is also a predictive factor for postprocedural complications development (10, 17, 18, 19). Almost 50% of spleen needs to be infarcted to obtain a sustained increase in platelet count (20). According to the data present-

ed, we also find similar percent of infarctization in our survey (fig. 9).

Regarding the complications, we observed that postembolization syndrome consisting of fever, nausea and left upper quadrant pain was almost universal present, but without severe consequences and carefully managed with standard treatment including antibiotics. Antibiotic prophylaxis was demonstrated that it reduces the severity of complications (21, 22, 23).

Other relevant complications were acute renal failure induced by contrast medium (two patients) and splenic abscess, despite antibiotic prophylaxis. In the last case, the patient ultimately needed surgical intervention.

This analysis supported the data from literature, regarding the increase in platelet and leukocyte count which were significant, both mean and median, suggesting that the embolized spleen limited the destruction of blood cells (24, 25).

The study has several limitations: one hand the sample size is small, consisting of 15 patients, and on the other hand the study is observational and cannot demonstrate causality. Although there is a positive trend between the embolization volume and the leukocytes and hemoglobin level, the relationship with the embolization procedure is not statistically significant.

CONCLUSIONS

The findings in our survey are consistent with the data from literature regarding the improvement of hematological indices, the occurrence of complications and the relationship between embolized spleen volume and the success of the technique. Partial splenic embolization is safe when the total embolized volume does not overcome 70% of spleen volume. Periprocedural antibiotherapy is necessary in order to limit complication development. The survival rate after a period of 9 years of follow-up for a subgroup of patients remained significant due to favorable outcome of partial splenic embolization, improving the overall biological status.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research

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THE ROLE OF PREDICTIVE BIOMARKERS IN THE PROGNOSIS OF DENTAL CARIES IN CHILDREN

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THE ROLE OF PREDICTIVE BIOMARKERS IN THE PROGNOSIS OF DENTAL CARIES IN CHILDREN (Abstract): Dental caries (DC) in children constitutes a significant public health concern. **Aims:** to identify predictive factors associated with the development of DC in children, targeting the identification of relevant predictive models and their subsequent application in the design of personalized preventive therapy. **Materials and methods:** Our observational cohort study was conducted on a batch of 398 children. The research group (L_1) included 132 children affected by dental caries, while the control group (L_0) consisted of 266 caries-free children. Indicators of caries experience, oral hygiene status and biomarkers from oral fluid (OF) were evaluated. To identify predictive factors for the development of DC, a multivariable linear regression model (linear Mod) was constructed. The study was conducted in accordance with ethical standards. **Results:** Multivariable linear regression analysis revealed the interaction of a complex set of risk factors (elevated levels of *Streptococcus mutans* and tumor necrotic factor alpha - TNF-α) and protective factors (secretory immunoglobulin A - sIgA, transforming growth factor - TGF-β1, $25(OH)D_3$, and Ca/Pi ratio) in OF. **Conclusions:** The multivariable linear regression model exhibited strong explanatory power, underscoring the relevance of salivary biomarkers in the personalized assessment of caries risk. The incorporation of predictive biomarkers holds significant potential to improve prognostic accuracy and to optimize targeted preventive strategies for dental caries in pediatric populations across various age groups. **Keywords:** DENTAL CARIES, CHILDREN, SALIVARY BIOMARKERS, PREDICTION, PREVENTION.

INTRODUCTION

The scientific basis for caries risk assessment, prevention, and patient-specific treatment requires ongoing development, precise definition, and continuous validation (1). Caries risk assessment plays a critical role in enhancing pediatric dental care and serves as a foundational element in the design of preventive and minimally invasive treatment strategies (2). Given the preventable nature of dental caries, the early identification of cariogenic risk has become a central objective in contemporary pediatric dentistry. Predictive models incorporating clinical, behavioral, socioeconomic, and biological parameters - such as *Streptococcus mutans* (SM) and secretory immunoglobulin A (sIgA) - facilitate per-

sonalization of preventive approaches (3), selection of appropriate interventions (both non-invasive and invasive) and implementation of tailored clinical monitoring protocols (4), thereby contributing to the reduction of severe dental injuries (3).

The recent advancement of artificial intelligence and machine learning technologies has enabled the development of increasingly accurate predictive tools capable of identifying complex interactions among various risk factors (5). Predictive models are rapidly being applied to the prediction of DC in children (6, 7). However, the current precision of these models, their susceptibility to bias and their clinical applicability remain insufficiently evaluated, highlighting the need for rigorous validation and alignment with standardized methodological guidelines, in order to ensure their reliability and clinical relevance (7, 8, 9). In this context, the investigation of predictive factors for the onset of dental caries remains highly relevant, emphasizing the clinical benefits, methodological challenges and perspectives for implementation in pediatric dental practice.

Objectives. To identify predictive factors associated with the development of dental caries in children, in order to inform the construction of robust predictive models and support their integration into the design of personalized preventive strategies tailored to different pediatric age groups.

MATERIALS AND METHODS

A prospective observational cohort study was undertaken to address the objectives of this research. A total of 398 conventionally healthy children aged between 3 and 15 years were included and distributed into two proportional groups. The research group (L_1) comprised 132 children with carious lesions (33.2%, 95% CI: 29-38), while 266 caries-free children (66.8%, 95% CI: 62-71) formed the control group (L_0). The selected juvenile population was divided per age groups according to the target groups recommended by the World Health Organization (WHO) (7): 3, 6, 12 and 15 years old. Out of the total 398 children body, 98 were included in the 3 y.o. age group (24.6%, 95% CI: 20-29), 90 children in the 6 y.o. group (22.6%, 95% CI: 20-29), 102 children in the 12 y.o. group (25.6%, 95% CI: 21-30), while 108 subjects were selected for the 15 y.o. batch (27.1%, 95% CI: 23-32). The study subjects were evenly distributed by gender, with 199 girls (50.0%, 95% CI: 45-55) and 199 boys (50.0%, 95% CI: 45-55). Among all examined children, 196 (49.2%, 95% CI: 44-54) were from urban areas, while 202 children (50.8%, 95% CI: 46-56) resided in rural areas.

Data collection was carried out by applying tools in accordance with the questionnaires for assessing oral status proposed by the WHO (WHO Oral Health Questionnaire for Children, 2013) (10) and the "Chairside Guide for Caries Prevention and Management" proposed by World Dental Federation (9). The children oral hygiene status was assessed by estimating the OHI index (oral health index) according to Green and Vermillion. The level of caries experience was evaluated using the indices DMFT/dmft and DMFS/dmfs. For the early and accurate detection of carious lesions and demineralized enamel areas, the conventional clinical examination was supplemented by the use of a device based on Transillumination technology (with a 780 nm wavelength light), the *DIAGNOcam* (*KaVo Dental GmbH), following the recent algorithm proposed through the consensus*

report on clinical recommendation for caries diagnosis (11). In order to achieve a complex and personalized prediction of DC, clinical predictive factors (oral hygiene status and caries experience indices), oral immune biomarkers from oral fluid, and the results of caries risk assessment using *Cariogram Software* were analyzed (12).

For the biochemical study, OF samples were collected in the morning under fasting conditions (*à jeun*). Unstimulated OF (5 mL) was obtained from participants by passive spitting directly into sterile collection tubes, subsequently transported to the Scientific Biochemistry Laboratory of "Nicolae Testemițanu" State University of Medicine and Pharmacy.

The biochemical analysis of oral fluid included the determination of oral cavity immune biomarkers: secretory immunoglobulin A (sIgA), tumor necrosis factor-alpha (TNF-α), transforming growth factor beta-1 (TGF-β1), cathelicidin (LL-37), as well as biomarkers involved in calcium-phosphate metabolism and the mineralization of dental hard tissues: primary vitamin D_3 metabolite - $25(OH)D_3$ and the calcium-to-phosphate ratio (Ca/Pi), for all children included in the study.

To verify the reliability of DC prediction, a follow-up clinical examination was conducted after one year, including the reassessment of caries experience indices.

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of "Nicolae Testemițanu" State University of Medicine and Pharmacy.

Data were processed using the open-source software RStudio, version 2024.09.1+394 (https: //www.rstudio.com). The source code used for data preprocessing and analysis is available and can be provided upon request,

For the description of numerical variables, basic descriptive statistical analysis was applied: minimum and maximum values, arithmetic mean and standard deviation, median (Me), and interquartile range (IQR). These measures of central tendency and dispersion provided a detailed and accurate characterization of the distribution of each variable, facilitating the identification of outliers, potential skewness, and deviations from normality. To compare two independent groups in terms of quantitative variable distribution, the non-parametric Mann-Whitney-Wilcoxon test was used, due to its robustness.

For categorical variables, both absolute and relative frequencies were calculated, accompanied by 95% confidence intervals (CI) for the proportion estimates. This approach allowed for a solid statistical characterization of category-level distributions.

To evaluate the predictive factors for the occurrence of new caries one year after the initial examination, a multivariable linear regression model was constructed, including residual analysis.

Statistical analysis was performed using a conventional significance level (α) of 0.05, with Hochberg correction applied for multiple testing (*p-adjust*). The Fisher Test (F) was used to validate the predictive model.

RESULTS

The results of the assessment of the level of DC involvement are presented in first table. The caries experience indicators at the beginning of the study for the children in the research group (L_1) recorded values of 4.74 ±2.65, $p < 0.001$ for the

DMFT/dmft ± SD index and 8.06 ± 4.9, 95% CI: 7.2 - 8.9, $p < 0.001$ for the DMFS/dmfs ± SD index. The average number of demineralized enamel areas detected using the *DIAGNOcam* device was 0.77 (SD = 1.59; 95% CI: 0.50 - 1.00), $p < 0.001$. The children in the control group were free from dental caries.

TABLE I.
Values of the studied indicators in children with dental caries and in caries-free children

Variables	L_1: children affected by dental caries, n=132[1]	95% CI[2]	L_0: caries-free children, n=266[1]	95% CI[2]	Statistical Test[3]	p-value[3]	q-value[4]
DMFT/dmft	4.74 (2.65) 4.00 (3.25) 1.00 13.00	4.3, 5.2	0.00 (0.00) 0.00 (0.00) 0.00 0.00	NA, NA	35,112	<0.001	<0.001
DMFS/dmfs	8.06 (4.91) 8.00 (7.00) 1.00 24.00	7.2, 8.9	0.00 (0.00) 0.00 (0.00) 0.00 0.00	NA, NA	35,112	<0.001	<0.001
Demineralized enamel areas	0.77 (1.59) 0.00 (0.00) 0.00 8.00	0.50, 1.0	0.00 (0.00) 0.00 (0.00) 0.00 0.00	NA, NA	21,812	<0.001	<0.001
OHI	1.57 (0.63) 1.60 (0.93) 0.30 2.70	1.5, 1.7	0.43 (0.36) 0.30 (0.40) 0.00 1.50	0.39, 0.47	32,855	<0.001	<0.001
Cariogram, %	51.37 (13.17) 53.00 (19.50) 12.00 86.00	49, 54	77.58 (13.58) 82.00 (18.00) 39.00 96.00	76, 79	3,171	<0.001	<0.001
1.n (%); Mean (SD), Median (IQR), Minimum Maximum, 2.CI = Confidence Interval, 3. Wilcoxon rank sum test, 4. Hochberg correction for multiple testing							

The oral hygiene status of the children included in the study was assessed using the Oral Hygiene Index (OHI) by Green and Vermillion. The OHI ± SD value in children affected by DC was significantly higher (OHI = 1.57±0.63, $p < 0.001$) compared to subjects without carious lesions OHI = 0.43 ± 0.36. Hence, the oral hygiene status of subjects in group L_1 was classified as satisfactory, while that of children in group L_0 was considered good.

The complex and personalized prediction of DC using the *Cariogram Software* revealed a moderate caries risk in children with carious lesions, with the chance of avoiding new carious cavities being 53.00 (Me, IQR = 19.50), compared to caries-free children, whose chance of avoiding new carious cavities was 82.00 (Me, IQR = 18.00), indicating a low caries risk. The differences between the two groups (L_1 and L_0) recorded statistically significant values (Wilcoxon rank sum test = 3.171, $p_{adjusted} < 0.001$).

In the present study, salivary biomarkers considered by several authors (13-16) to be relevant in predicting DC were evaluated: the number of SM, and the levels of sIgA, TNF-α, TGF-β, LL-37, vitamin D_3 ($25(OH)D_3$), and the Ca/Pi ratio. Table III presents the comparative results of OF biomarker assessments in

children affected by DC (L_1, n = 132) and caries-free children (L_0, n = 266).

The number of SM in saliva was assessed in all children included in the study using the *Saliva-Check Mutans Kit (GC).* A positive test result, indicating a high SM count in saliva (> 5×10^5 CFU/mL), was observed in 53.0% of children in group L1 and in only 8.6% of subjects in group L0 ($p < 0.001$), suggesting a higher risk of developing future carious lesions for L1 subjects. A low SM count in saliva (< 5×10^5 CFU/mL), associated with a lower caries risk, was found in 47.0% of children affected by DC and in 91.4% of caries-free subjects ($p < 0.001$).

To verify the reliability of DC prediction, a follow-up clinical examination was conducted after one year to assess the indices of caries experience. Subsequently, our registrations revealed statistically significant differences between groups L_1 and L_0 for most of the indicators presented in table II. Specifically, the mean values of the variables DMFT/dmft, DMFS/dmfs, new carious lesions, and secondary caries showed a statistically significant increasing trend over time in the at-risk subjects ($p < 0.001$, $q < 0.001$). Only the number of demineralized enamel areas did not display a statistically significant difference ($p = 0.2$, $q > 0.9$). It is also noteworthy that the median distributions in the control group children remained constant at 0.00 in most cases, suggesting that only a subset of subjects develop significant changes, which supports the importance of individualized caries risk assessment.

TABLE II.

Caries experience indices in children assessed at the 1-year follow-up evaluation

Variables	L_1: children affected by dental caries, n=132[1]	95% CI[2]	L_0: caries-free children, n=266[1]	95% CI[2]	Statistical Test[3]	p-value[3]	q-value[4]
DMFT/dmft_2	6.64 (3.67) 6.00 (6.00) 1.00 16.00	6.0, 7.3	1.27 (2.07) 0.00 (2.00) 0.00 8.00	1.0, 1.5	31,923	<0.001	<0.001
DMFS/dmfs_2	12.38 (7.89) 10.00 (12.00) 1.00 48.00	11, 14	2.22 (3.88) 0.00 (4.00) 0.00 16.00	1.8, 2.7	31,825	<0.001	<0.001
Secondary caries	1.47 (1.96) 1.00 (2.00) 0.00 12.00	1.1, 1.8	0.03 (0.24) 0.00 (0.00) 0.00 2.00	0.00, 0.06	26,247	<0.001	<0.001
New carious lesions	2.33 (2.22) 2.00 (4.00) 0.00 10.00	2.0, 2.7	1.23 (2.00) 0.00 (2.00) 0.00 8.00	0.99, 1.5	23,204	<0.001	<0.001
Demineralized enamel areas _2	0.64 (1.43) 0.00 (0.00) 0.00 8.00	0.39, 0.88	0.47 (1.23) 0.00 (0.00) 0.00 6.00	0.33, 0.62	18,481	0.2	>0.9

1.n (%); Mean (SD), Median (IQR), Minimum Maximum, 2.CI = Confidence Interval, 3. Wilcoxon rank sum test, 4Hochberg correction for multiple testing

The obtained results demonstrate that longitudinal monitoring through repeated evaluations can reveal subtle changes or significant progressions that might be overlooked in a single-point assessment. The statistically significant differences in caries experience indicators between groups L_1 and L_0 emphasize their importance in monitoring DC. Furthermore, the use of statistical tests (Wilcoxon test and Hochberg correction) strengthens the validity of the identified differences.

The multivariable linear regression model (*linearMod*), developed to identify predictive factors for the occurrence of new caries one year after the clinical evaluation and assessment of salivary biomarkers, demonstrated a high explanatory ability. The coefficient of determination was $R^2 = 0.7724$, with an adjusted $R^2 = 0.7396$, indicating that approximately 74% of the variability in the number of new caries is explained by the set of predictors included in the model. The F-test was statistically significant ($F = 23.56$, $p < 0.001$), confirming that the model is globally valid and predictive (tab. III).

TABLE III.

Results of the multivariable linear regression analysis for predictors of new carious lesion development

Variables	Beta	t-value	95% CI[1]	p-value
(Intercept)*	0.4692	0.281	-2.8141, 3.7525	0.8
DMFT/dmft	-0.3491	-1.07	-0.9895, 0.2913	0.3
DMFS/dmfs	0.0162	0.331	-0.0802, 0.1126	0.7
OHI	0.4757	3.13	0.1766, 0.7748	0.002
Demineralized enamel areas	0.2486	3.46	0.1072, 0.3899	<0.001
Cariogram	0.0146	1.11	-0.0113, 0.0406	0.3
Streptococcus mutans (SM)	1.5283	5.10	0.9387, 2.1179	<0.001
sIgA	-0.0073	-5.16	-0.0100, -0.0045	<0.001
TGF-β1	-0.7610	-2.52	-1.3546, -0.1673	0.012
TNF-α	0.0836	3.10	0.0306, 0.1366	0.002
$25(OH)D_3$	-0.0175	-3.76	-0.0267, -0.0084	<0.001
LL-37	0.0161	0.541	-0.0424, 0.0747	0.6
Ca/Pi	-2.5544	-7.95	-3.1866, -1.9223	<0.001
1CI = Confidence Interval				

*The term (Intercept) represents the model constant (β_0), which is the mean value of the dependent variable (Y) when all predictors (independent variables) are equal to zero (or belong to the reference category in the case of categorical variables).

The results of the multivariable linear regression analysis suggest that the occurrence of new carious lesions in children is influenced by both, risk and protective factors that are clinically and biologically measurable.

I. Significant factors associated with an increase in the number of new carious lesions:

- The presence of *Streptococcus mutans* at elevated concentrations ($> 5 \times 10^5$ CFU/mL) ($\beta = 1.5283$, $p < 0.001$) proved to be the strongest predictor, associated with an average increase of 1.53 new lesions

compared to children with low SM levels.

- Oral Hygiene Index (OHI) (β = 0.4757, p = 0.002) - each 1-point increase in the oral hygiene score is associated, on average, with ~ 0.48 new carious lesions, indicating a significant impact of poor oral hygiene.
- Number of demineralized enamel areas detected with DIAGNOcam (β = 0.2486, p < 0.001) - a one-unit increase in the number of demineralized areas predicts the occurrence of approximately 0.25 new carious lesions, confirming the role of these early lesions as indicators of caries disease progression.
- TNF-α level (β = 0.0836, p = 0.002) - an increase in the concentration of this pro-inflammatory mediator is associated with a higher risk, suggesting the involvement of local inflammation in the pathogenesis of dental caries.

II. Significant factors associated with a reduction in the number of new carious lesions (protective factors):

- sIgA level (β = - 0.0073, p < 0.001) - each 1 mg/dL increase in secretory immunoglobulin A is associated with an estimated reduction of ~ 0.0073 new carious lesions, confirming the role of immune protection in caries prevention.
- TGF-β1 (β = - 0.7610, p = 0.012) - shows a substantial protective effect, suggesting a potential influence on tissue repair processes and immune response regulation.
- 25(OH)D_3 (β = - 0.0175, p < 0.001) - a strong protective factor, with adequate vitamin D_3 levels being associated with increased resistance of hard dental tissues and a reduced risk of lesion development.
- Ca/Pi ratio (β = - 2.5544, p < 0.001) - the strongest protective factor in the model, highlighting the importance of mineral balance in OF for maintaining enamel integrity.

III. Non-significant predictors (p > 0.05) :

- DMFT/dmft, DMFS/dmfs, Cariogram score, and LL-37 showed no significant associations with the development of new carious lesions, which may suggest either a limited influence in a multivariable context or a potential masking effect by other predictors included in the model.

DISCUSSION

A plethora of factors have been identified as contributing to the development of tooth decay, including but not limited to: genetic predisposition, poor health performance, malnutrition, specific dietary habits, the presence of microorganisms such as streptococci, fluoride and/or vitamin D deficiency, excessive sugar consumption or other factors such as age, gender and place of residence of children. The results of our study confirm the multifactorial nature of DC, highlighting that both clinical and biological factors significantly contribute to the development of new carious lesions. The Oral Hygiene Index (OHI) proved to be a significant predictor, which is consistent with previous studies showing a strong correlation between poor oral hygiene and increased incidence of DC (17). The number of demineralized enamel areas detected using DIAGNOcam was also positively associated with the occurrence of new lesions, confirming the importance of early identification and monitoring of incipient lesions, as emphasized in the new chairside guides for caries prevention (9) and highlighted in various studies (16, 18).

Elevated levels of TNF-α in OF proved to be a significant predictor associated with increased risk of DC, suggesting the involvement of this pro-inflammatory cytokine in the progression of the disease (16). This may be driven by the accumulation of cario-

genic dental biofilm. It has been shown that salivary TNF-α levels of children with Streptococcus mutans strains $\geq 10^5$ CFU/L, are significantly increased (19).

At the same time, elevated levels of sIgA, TGF-β1, 25(OH)D_3, and a Ca/Pi ratio >1.0 in oral fluid had a protective effect. This is supported by the literature, which highlights the immunoprotective role of sIgA (13, 14), the anti-inflammatory function of TGF-β1 (20), the role of vitamin D_3 in enamel mineralization (16, 21-23), and the relevance of the Ca/Pi ratio in maintaining the integrity of hard dental tissues (2, 16, 23).

The absence of significant associations for DMFT/dmft, DMFS/dmfs, the Cariogram score, and LL-37 suggests that, although these variables may be useful for the general assessment of dental or immune status, they did not have an independent significant influence within the applied multivariable model.

Hence, our results emphasize the need for an integrated approach to caries prevention in children, combining oral hygiene control, early detection of incipient lesions, optimization of salivary immune status, and maintenance of proper mineral balance.

Overall, the multivariable linear regression model suggests that caries risk is determined by an imbalance between microbial factors and immunological biomarkers, with major influences from *Streptococcus mutans*, the Ca/Pi ratio, and sIgA. Our findings support therefore, the integration of salivary biomarkers into a complex predictive model for caries risk assessment in children, in order to ensure higher accuracy with improved sensitivity and specificity in the prognosis of dental caries in pediatric populations.

CONCLUSIONS

The development of dental caries in children is determined by an imbalance between risk factors (elevated levels of *Streptococcus mutans* and TNF-α) and protective factors (optimal levels of sIgA, TGF-β1, 25(OH)D_3, and a Ca/Pi ratio > 1.0) present in oral fluid. The multivariable linear regression model endorsed high explanatory ability, confirming the utility of salivary biomarkers in the personalized prediction of caries risk. The integration of predictive biomarkers can enhance the accuracy of prognosis and the effectiveness of personalized strategies for dental caries prevention in children of different ages.

CONFLICT OF INTERESTS AND FUNDING

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DIETARY PREDICTORS OF KIDNEY STONE RELAPSE AND COMPOSITION: ASSOCIATIONS WITH PASTA, MILK, AND ANIMAL PROTEIN INTAKE

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DIETARY PREDICTORS OF KIDNEY STONE RELAPSE AND COMPOSITION: ASSOCIATIONS WITH PASTA, MILK, AND ANIMAL PROTEIN INTAKE (Abstract): Dietary factors are known to influence the risk of nephrolithiasis recurrence, yet the specific contributions of refined carbohydrates (such as pasta), milk consumption (as a proxy for dietary calcium), and animal protein intake remain underexplored. **Materials and methods**: We analyzed data from 144 adult patients monitored between 2023 and 2025 at a clinical nutrition unit. Dietary exposures included weekly intake of pasta and milk (servings/week) and total animal protein (grams/week). The primary outcomes were lithiasis relapse (yes/no) and stone composition (calcium oxalate, uric acid, or mixed/other). Group comparisons were performed using the Mann-Whitney U or Kruskal-Wallis tests, followed by Benjamini-Hochberg False Discovery Rate (FDR) correction. Multivariate logistic regression was applied to identify independent dietary predictors of relapse and stone type, adjusting for age, sex, and residential environment. Random Forest models were used for exploratory prediction analyses. **Results**: Median pasta intake was higher among patients with relapse compared with non-relapsed individuals (1.0 vs 0.0 servings/week; $p = 0.012$). This association remained significant after FDR adjustment. Milk consumption differed significantly across stone types ($p = 0.013$; FDR-adjusted significant), with the highest values observed in calcium-oxalate stones. Animal protein intake showed a non-significant trend toward higher values in uric-acid stone formers. In multivariate analysis, pasta intake was an independent predictor of relapse, while milk and animal protein did not reach significance. The Random Forest models achieved modest predictive performance (accuracy = 0.53 for relapse; 0.68 for stone type classification). **Conclusions**: Higher pasta intake was independently associated with an increased risk of lithiasis relapse, and milk consumption differed by stone composition. These findings support the hypothesis that specific dietary patterns may influence nephrolithiasis outcomes, although the predictive value remains limited. Larger prospective studies are warranted to validate these associations and to refine personalized nutritional counselling strategies for stone prevention. **Keywords:** LITHIASIS, DIETARY FACTORS, PASTA INTAKE, MILK CONSUMPTION, ANIMAL PROTEIN, RELAPSE, KIDNEY STONES.

INTRODUCTION

Dietary and metabolic factors are recognized as crucial determinants of nephrolithiasis risk and recurrence (1-3). Despite advances in metabolic assessment and imaging, the incidence of lithiasis continues to rise globally, particularly among individuals with metabolic syndrome and type 2 diabetes mellitus (T2DM) (3, 7). This persistent increase underscores the need for a clearer understanding of modifiable nutritional contributors to stone formation.

Among dietary components, refined carbohydrates, dairy products, and animal protein have each been implicated in the complex metabolic mechanisms underlying lithogenesis (4-6, 8-11). Refined carbohydrate consumption, especially from pasta and starch-based foods, has been associated with insulin resistance, weight gain, and altered urinary excretion of calcium and oxalate (8). Conversely, milk provides dietary calcium that can reduce intestinal oxalate absorption but may simultaneously elevate urinary calcium concentrations (4, 9). High intake of animal protein contributes to an increased dietary acid load, lowering urinary pH and citrate levels while raising uric acid excretion, thereby promoting uric-acid stone formation (2, 10, 11).

However, few studies have simultaneously examined the relative contributions of these three key dietary elements—pasta, milk, and animal protein—to both lithiasis relapse and stone composition. Moreover, the available literature presents inconsistent findings, partly due to methodological heterogeneity and the limited integration of nutritional, metabolic, and predictive modeling approaches.

In this context, our study investigates the association between pasta intake, milk consumption, and animal protein intake with two major clinical outcomes: lithiasis relapse and stone composition. We further explore whether these dietary factors retain predictive value in multivariate models and in machine learning analyses. By focusing on specific, quantifiable dietary exposures within a well-characterized patient cohort, this research aims to clarify potential nutritional predictors of stone recurrence and composition, offering a basis for individualized dietary counseling and prevention strategies.

MATERIALS AND METHODS

This prospective observational study was conducted between 2023 and 2025 at a private nutrition and dietetics practice in Iași, Romania. The study enrolled patients diagnosed with obesity, type 2 diabetes mellitus (T2DM), and lithiasis, who met rigorous inclusion and exclusion criteria (12-17). All participants received detailed information about the study's objectives and procedures and provided written informed consent prior to enrollment, in accordance with ethical standards for human research. The study fully complied with the principles of the Declaration of Helsinki and received approval from the Institutional Ethics Committee (18).

Inclusion criteria required participants to be over 18 years of age, with a confirmed diagnosis of lithiasis documented through abdominal ultrasonography. Participants were also required to demonstrate the ability and willingness to attend all scheduled follow-up visits as outlined in the study protocol.

Exclusion criteria included the presence of acute complications, a current or past history of oncological disease, or contraindications to the imaging procedures used in the study (2, 19). Additional exclusion factors comprised pregnancy or breastfeeding, severe psychiatric disorders that could

interfere with protocol adherence, and refusal to participate.

Data collection involved participant screening and inclusion, obtaining informed consent, and collecting demographic and clinical information. Baseline anthropometric measurements were performed, medical documentation was reviewed, and participants completed standardized dietary behavior questionnaires (20).

The composition of kidney stones was determined by infrared spectroscopic analysis, performed at the Michel Daudon Center in Iași, which operates within the Urology Clinic of the Dr. C. I. Parhon Hospital, Iași. This method enabled precise identification of calcium oxalate, uric acid, and mixed stones, ensuring analytical consistency across all collected samples.

Statistical analysis was performed using R version 4.4.2. A significance level of $p < 0.05$ was adopted. Descriptive statistics were calculated for the cohort—means ± SD for continuous variables and counts (percentages) for categorical variables. For univariate comparisons between relapse groups, the Mann-Whitney U test was applied to continuous variables, and the Chi-square test was used for categorical variables. To compare continuous dietary variables across stone-type categories, the Kruskal-Wallis test was used.

To control for multiple testing across the dietary comparisons (three dietary factors × two outcomes), the Benjamini-Hochberg false discovery rate (FDR) procedure was applied. Multivariable logistic regression models were fitted with relapse (yes = 1, no = 0) as the dependent variable, and age, sex, urban residence (urban = 1), and the three dietary factors as independent predictors. Missing numeric values were imputed with cohort medians before regression analyses.

Secondary logistic regression models contrasted uric acid versus calcium oxalate stone composition as the outcome variable. For exploratory predictive modelling, we implemented Random Forest classifiers with stratified 70/30 train-test splits. Model performance was evaluated using overall accuracy and classification metrics computed on the held-out test set.

RESULTS

Between 2023 and 2025, a total of 867 individuals were evaluated at a private nutrition and dietetics practice in Iași, Romania, and 144 patients who met the eligibility criteria were included in the study. The mean age of participants was 52.25 ± 11.90 years. The sex distribution comprised 74 males (51.4%) and 70 females (48.6%). Regarding residence, 90 participants (62.5%) were from urban areas and 54 (37.5%) from rural areas.

Relapse status was available for 143 patients, and complete dietary information for the three factors (pasta, milk, and animal protein) was obtained for 142 patients. Complete data for both diet and relapse were available for 141 patients, as were datasets combining dietary and stone-type information.

Univariate analysis showed that patients who experienced relapse reported a higher pasta intake (median = 1.0 servings/week) compared with those without relapse (median = 0.0 servings/week), with a Mann-Whitney $p = 0.01225$.

Milk consumption did not differ significantly between relapse groups ($p= 0.32605$).

There was no significant association between animal-protein intake and relapse status: relapsed patients consumed a median of 110 g/week, whereas non-relapsed patients consumed 80 g/week (Mann-Whitney $p = 0.28031^{*}$) (tab. I).

No significant differences in demographic characteristics (age, sex, or urban versus rural residence) were observed between relapse and non-relapse groups.

Stone-composition analyses. Analysis by stone type (oxalate, uric acid, other) revealed significant variation in milk consumption, with calcium-oxalate stone formers showing the highest intake (Kruskal-Wallis p = 0.01260) (2, 9, 21).

Differences in animal-protein intake across stone-type groups approached statistical significance (p = 0.07928*), as uric-acid stone patients tended to consume more animal protein.

Pasta intake did not differ significantly between stone types (p = 0.14542*).

After applying the Benjamini-Hochberg false discovery rate (FDR) correction across the six dietary tests (three dietary factors × two outcomes), pasta consumption remained significantly associated with lithiasis relapse (adjusted p ≈ 0.0378).

Similarly, the association between milk consumption and stone type also remained significant (adjusted p ≈ 0.0378), confirming the robustness of both findings (tab. I).

Associations involving animal-protein intake lost statistical significance following FDR adjustment.

TABLE I

Univariate and multivariable analyses of dietary factors

Dietary factor	Relapse (median or trend)	P-value	Adjusted P-value (FDR)	Stone type (median or trend)	P-value	P-value (FDR)	Observations
Pasta (servings/week)	Relapse = 1.0; No relapse = 0.0	0.01225	0.0378	Oxalate = 0.5; Uric acid = 1.0; Other = 0.5	0.14542	-	Independent predictor of relapse
Milk consumption (per Serving / week)	-	0.32605	-	Higher in oxalate stones	0.01260	0.0378	Not significant for relapse
Animal protein (g/week)	Relapse = 110; No relapse = 80	0.28031	-	Oxalate = 90; Uric acid = 120; Other = 100	0.07928	-	Trend for uric acid stones, not significant after FDR

Multivariable logistic regression analyses, adjusted for age, sex, and urban residence, demonstrated that pasta consumption was an independent predictor of relapse. Neither animal protein nor milk intake were statistically significant predictors of relapse in the fully adjusted model.

A separate binary logistic regression comparing uric acid versus calcium oxalate stone composition revealed a positive association between animal protein intake and uric acid stones; however, this association lost statistical significance after application of the FDR correction (2, 10).

Additionally, the data indicated that patients with higher milk consumption tended to have elevated urinary calcium levels, which correlated with the formation of calcium-oxalate stones.

TABLE II
Predictive modeling with random forest

Outcome	Test Accuracy	Key Predictors	Observations
Relapse (binary)	0.53	Pasta intake	Model showed limited discrimination
Stone type (multiclass)	0.68	Milk intake	Moderate performance
Feature Importance	-	Pasta (relapse), Milk (stone type)	Consistent across models

The Random Forest classifiers trained with demographic and dietary information reached a 0.53 test accuracy for binary relapse prediction and 0.68 test accuracy for stone-type classification (multiclass outcome) based on the results (tab. II). The feature importance rankings showed that pasta and milk emerged as the leading predictors for their respective outcomes in both studies (7, 22).

DISCUSSION

This study demonstrates that specific eating habits-particularly pasta consumption, milk intake, and animal-protein consumption-are associated with the risk of lithiasis recurrence and stone composition. In a cohort of 144 patients, we identified a novel and statistically significant association between pasta intake and stone relapse, together with a robust relationship between milk consumption and stone composition (oxalate versus uric acid). Although animal-protein intake showed a biological tendency toward uric-acid stone development, this association did not remain statistically significant after FDR adjustment.

Our findings are consistent with the broader literature linking dietary patterns and nephrolithiasis. Dietary calcium derived from dairy products has repeatedly been shown to protect against calcium-oxalate stones in large cohorts and randomized clinical trials, primarily through intestinal binding of oxalate and the subsequent reduction in urinary oxalate excretion (4, 10, 23, 24). Therefore, the assessment and monitoring of active metabolic lithiasis must take these nutritional influences into account (12).

A high animal-protein intake is a classic risk factor for uric-acid lithiasis, increasing urinary acidity and urate excretion while reducing urinary citrate levels (2, 10, 11, 24). In our data, uric-acid stone formers tended to consume more animal protein; however, the limited sample size and semi-quantitative nature of dietary gram estimation likely reduced statistical power, leading to the loss of significance after FDR correction.

The association between pasta consumption and stone relapse represents an innovative signal of this study. Pasta, a refined carbohydrate with a high glycemic load, may contribute to lithogenesis through insulin resistance and associated changes in urinary uric acid and pH-mechanisms known to promote both uric-acid and mixed-type stone recurrence (3, 8, 13). While previous large-scale studies have investigated global dietary patterns and beverage types (25-27), very few have isolated pasta intake as an individual variable. Our findings suggest that frequent pasta consumption may serve as a proxy

marker for refined-carbohydrate, low-fiber dietary patterns associated with increased recurrence risk. Future mechanistic research should investigate whether pasta consumption directly alters urinary chemistry through metabolic effects.

The observed associations align with established biological pathways, which reinforces their physiological plausibility. Consumption of refined carbohydrates such as pasta induces postprandial hyperinsulinemia and, over time, insulin resistance, both of which influence urinary acidification and uric-acid metabolism. Milk provides calcium that binds intestinal oxalate, reducing its absorption, urinary oxalate concentration, and calcium-oxalate supersaturation (9,21). Population-based evidence further indicates that calcium-rich diets are associated with a lower recurrence risk across diverse populations (28). Conversely, animal-protein intake contributes to dietary acid load, lowering urinary citrate and increasing uric-acid excretion, thus fostering an environment conducive to uric-acid stone formation (2,10,11,24). The prevalence of oxalate lithiasis remains particularly influenced by environmental and dietary factors that modulate these metabolic pathways (2).

Our findings support the application of individualized dietary counseling in lithiasis management. Specifically, patients should be advised to (i) reduce refined-carbohydrate intake and favor low-glycemic, fiber-rich sources; (ii) obtain calcium primarily from milk and dairy products, rather than supplements consumed at inappropriate times; and (iii) moderate animal-protein intake, particularly in individuals prone to uric-acid stones. However, given the limited predictive power of diet-only models for relapse, comprehensive management should combine nutritional guidance with urinary biochemical monitoring and broader lifestyle interventions (24). Recent studies comparing type 1 and type 2 diabetic patients with nephrolithiasis underscore the necessity of tailoring management to metabolic profiles (7). Likewise, longitudinal assessments of renal function following nephrectomy highlight the long-term benefits of integrated dietary control on kidney health (17). Stone composition should therefore remain a central determinant in designing preventive and therapeutic strategies (6).

Despite the strengths of the present work, several limitations must be acknowledged. The observational and retrospective nature of the study precludes definitive causal inference and leaves room for residual confounding. The semi-quantitative dietary data introduce potential recall bias, and estimates of animal-protein intake (grams/week) may be imprecise. Although the overall cohort size provided adequate power to detect moderate associations, subgroup analyses by stone type were smaller and may have been underpowered. Finally, results from this single-cohort study may not be generalizable to populations with distinct dietary patterns, genetic backgrounds, or stone-formation rates.

CONCLUSIONS

In this cohort, pasta intake emerged as an FDR-significant correlate of stone relapse, while milk consumption was significantly associated with stone composition, showing patterns consistent with the protective physiology of calcium-oxalate stone formation. Animal-protein intake demonstrated a possible biological link with uric-

acid lithiasis, although statistical support was limited after adjustment.

Overall, these findings highlight that dietary factors alone cannot reliably predict stone recurrence, yet they identify promising avenues for further investigation into metabolic and dietary mechanisms underlying lithiasis. The results support the value of personalized nutrition counseling as an integral component of comprehensive stone-prevention strategies.

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EFFECTS OF COVID-19 ON THE DIAGNOSIS OF BRAIN METASTASES. A RETROSPECTIVE ANALYSIS

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EFFECTS OF COVID-19 ON THE DIAGNOSIS OF BRAIN METASTASES. A RETROSPECTIVE ANALYSIS (Abstract): Delays in cancer diagnosis and treatment can significantly impact patient survival and quality of life. The COVID-19 pandemic intensified these challenges by limiting access to medical services and postponing essential oncologic interventions. This study **aimed** to evaluate the effects of such delays on the diagnosis and management of brain metastases in neuro-oncology patients during the pandemic period. **Materials and methods**: A retrospective analysis was conducted on 100 patients who underwent surgical treatment for brain metastases at the Department of Neurosurgery, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iași, Romania. Patients were divided into two groups: the pre-COVID group (2018-2019, n = 57) and the COVID group (2020-2022, n = 43). Demographic, clinical, and pathological data were collected from medical records. Tumor volume, anatomical location, and histopathological characteristics were compared between groups. **Results**: The mean age of patients was 57.9 years, with a female-to-male ratio of 1.17: 1. The most common primary tumors were breast (27%), bronchopulmonary (20%), and gastrointestinal cancers (18%). Brain metastases were predominantly located in the supratentorial region (80%), especially in the parietal and occipital lobes. The median tumor volume was higher in the COVID group (16.8 cm^3) compared with the pre-COVID group (12.1 cm^3), suggesting delayed diagnosis and disease progression during the pandemic, although the difference did not reach statistical significance ($p = 0.11$). **Conclusions**: The COVID-19 pandemic contributed to diagnostic delays and increased tumor burden in patients with brain metastases, reflecting reduced access to timely healthcare. While demographic characteristics remained similar between periods, larger tumor volumes observed during the pandemic indicate that healthcare disruptions had tangible effects on disease progression. Continued research is necessary to assess the long-term impact of these delays on treatment outcomes and survival, as well as to develop adaptive strategies to ensure continuity of oncologic care during future healthcare crises. **Keywords:** COVID-19, BRAIN, METASTASES, TUMOR.

INTRODUCTION

Delays in cancer treatment can have significant implications for patient survival and quality of life. One of the most concerning consequences of such delays is the increased risk of mortality and disease

recurrence. Studies have demonstrated that timely diagnosis and early initiation of treatment are crucial for improving patient outcomes (1,2). When treatments are postponed, cancer has time to progress, which may render subsequent interventions more complex and less effective (3, 4, 5, 6). This situation underscores the importance of early interventions and close monitoring of disease progression.

Beyond biological risks, treatment delays can impose a substantial financial burden on patients. This may translate into additional long-term costs if the disease advances and requires more intensive and expensive therapies. Patients may face not only the financial strain of medical expenses but also economic losses caused by their inability to work or maintain daily activities. Thus, the consequences of delayed treatment extend beyond the medical sphere, affecting the socio-economic well-being of patients and their families.

The COVID-19 pandemic has amplified these challenges, placing significant strain on healthcare systems worldwide. Many institutions were forced to postpone elective cancer surgeries and reduce the use of systemic therapies as medical resources were reallocated to address the public health emergency. These measures have raised concerns about the long-term consequences of delayed treatment for cancer patients (2). Although it is evident that postponement negatively affected clinical outcomes, the lack of comprehensive data makes it difficult to precisely assess the impact of such delays on survival and disease progression.

Therefore, the need for high-quality data to evaluate the effects of treatment delays in oncology has become increasingly evident. Without such information, it is impossible to quantify the real impact of COVID-19 related preventive measures on cancer patients and on the healthcare systems that serve them. A deeper understanding of these effects could guide the development of more effective, patient-centered public health policies, ensuring timely interventions and optimal resource management.

Looking forward, the pandemic has served as a period of reflection for the medical community. It is essential for healthcare systems to reassess how they allocate and manage resources and to develop strategies that minimize delays in essential treatments. This requires not only better coordination among healthcare professionals but also a patient-centered approach that allows for the continuation of care even in times of crisis. Hence, future healthcare systems must be more flexible, adaptable, and better equipped to respond to emerging challenges, thereby ensuring improved outcomes for cancer patients.

In this context, our study focuses on the impact of COVID-19 in the field of neuro-oncology, emphasizing potential delays in the diagnosis of brain metastases. These metastases often present acutely, leading to new or progressive neurological deficits, symptoms of increased intracranial pressure, or seizures (7, 8, 9).

Delays in managing these conditions can have serious consequences, leading to a more severe decline in patients' health status. For example, delayed diagnosis may allow the disease to advance to a stage where therapeutic interventions become more complex and less effective, particularly in the context of heterogene-

ous molecular subtypes of brain metastases (3, 4, 5, 6). Many of these cases may require more sophisticated support and intensive management, which not only increases patient risk but also adds additional strain to the healthcare system.

During the pandemic, healthcare priorities were restructured, and resources were heavily burdened by the need to respond to the COVID-19 public health crisis. This situation led to delays in diagnosis and treatment, as well as decreased access to essential services for patients with neuro-oncological conditions. Therefore, it is crucial to analyze how these delays have affected clinical outcomes and to identify strategies for minimizing their negative impact, ensuring the efficient continuation of care.

Our study aims to provide a detailed analysis of these delays and to explore the so-called "therapeutic windows" that may arise in neuro-oncological treatment pathways. A comprehensive understanding of these aspects will not only contribute to improved management of patients in the future but will also aid in formulating more effective health policies that can meet patient needs during times of crisis. Investigating the impact of COVID-19 in neuro-oncology is essential not only for ensuring patient recovery but also for strengthening healthcare systems overall, enabling them to respond to emerging challenges with adaptable and effective solutions.

MATERIALS AND METHODS

We conducted a retrospective study of patients who underwent surgical treatment at the Department of Neurosurgery, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iași, Romania, during 2020-2022, and compared these findings with data from the pre-pandemic period (2018-2020).

Patients with newly diagnosed brain metastases were included, and the origin and type of primary tumors were recorded. Patients with multiple primary tumors who presented new manifestations of cerebral metastases were also included, as well as those with a syndrome of cancer of unknown primary origin.

We excluded patients with any diagnosis other than brain metastases. Patients with recurrent tumors were excluded, as were those unable to undergo MRI examination.

Patients were classified according to their access to medical care in:

- those treated during the pre-pandemic period with normal, unrestricted access (2018-2019; pre-COVID group), and
- those treated during the pandemic period with limited access to healthcare (2020-2022; COVID group).

The study analyzed 100 patients with brain metastases (57 in the pre-COVID group and 43 in the COVID group). Clinicopathological data were collected from electronic medical records and included demographic information and morphological characteristics of the cerebral metastases (location and cytopathological features).

The cytopathological examination was performed on intraoperative smears obtained from small biopsy samples collected during surgery. A small specimen (1-2 mm^3) was placed on the edge of a clean, dry, labeled glass slide and crushed gently using another labeled slide, applying sufficient pressure to spread the tissue into a

thin film on both slides. Both slides were immediately stained with 1% toluidine blue. In all cases, the cytological diagnosis confirmed the presence of brain metastases.

RESULTS

The analysis provides a detailed overview of the characteristics of patients with brain metastases included in the study, revealing valuable information about both their demographic profiles and disease progression.

A total of 100 patients were included, of whom 57 were treated before the pandemic and 43 during the COVID-19 period. This division allowed for comparison of potential differences in management and outcomes between the two periods.

The mean age of the patients was 57.9 years, with most individuals in their sixth and seventh decades of life. This suggests a higher incidence of brain metastases among middle-aged and elderly adults, consistent with the fact that such oncological complications typically occur at advanced disease stages in patients with long-standing primary malignancies. The median interval from initial cancer diagnosis to the development of brain metastases was approximately 34.42 months, indicating a substantial disease trajectory before cerebral involvement became clinically significant.

Gender distribution showed a female-to-male ratio of 1.17:1, indicating a slight predominance of women in the study population.

Regarding the patients' origin, nearly half came from rural areas (48%), while the remainder resided in urban areas (52%).

Educational level revealed a diverse profile: 62% of patients had completed secondary education, 36% held higher education degrees, and 4% had only primary education.

Overall, these data help outline a complex demographic and clinical profile of patients with brain metastases, including age, sex, education, and access to care.

The most common primary tumor was breast cancer (27%), followed by bronchopulmonary cancer (20%), and gastrointestinal tumors (18%) (fig. 1).

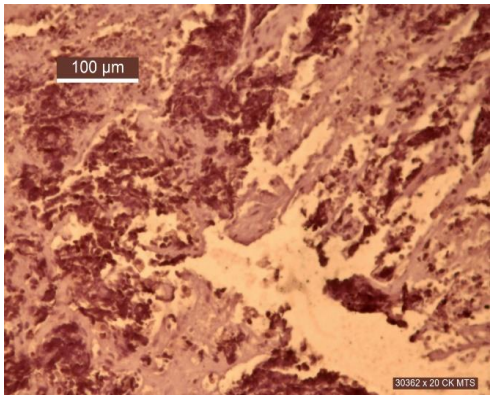

Fig. 1. Undifferentiated bronchopulmonary carcinoma metastasis.
(Immunodetection with cytokeratin, ×200)

In detail, among the gastrointestinal tumors, 5 patients were identified with esophageal cancer, 10 with colorectal cancer, 1 with pancreatic cancer, and 1 with peritoneal cancer. Other patients presented different types of malignancies: 7 were diagnosed with malignant melanoma, and 9 had renal tumors (fig. 2).

A total of eight patients were classified as having cancers of unknown primary origin. This classification was necessary because these patients died during the COVID-19 pandemic, and the cause of

death was attributed to the virus, preventing accurate determination of the tumor's primary site.

These findings highlight the diversity of primary tumors that can lead to brain metastases and provide an overview of the distribution of cancer types within this patient group (fig. 3).

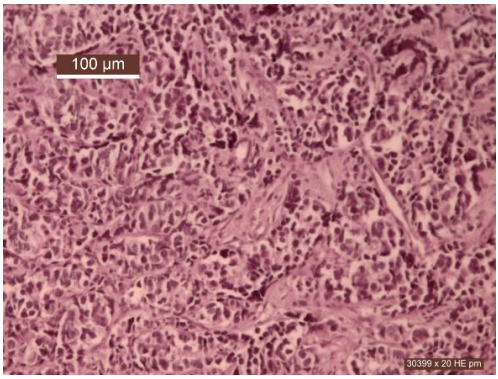

Fig. 2. Metastasis of moderately differentiated prostatic adenocarcinoma in a 68-year-old man. Preserved tubular structure is observed.

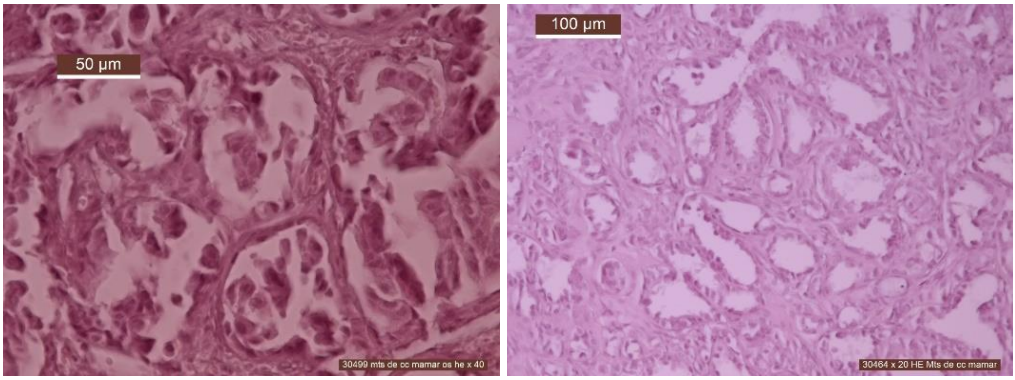

Fig. 3. Ductal carcinoma in a 60-year-old woman (HE staining, ×400). Tubular carcinoma in a 68-year-old woman (HE staining, ×200).

According to the results obtained, the distribution of brain metastases by anatomical location showed a predominance in specific brain regions. Thus, metastases were identified in the cerebellum in 20% of cases, in the right parietal lobe in 20%, in the left parietal lobe in 30%, in the right occipital lobe in 20%, and in the left occipital lobe in 10% of cases.

Regarding the distribution of brain regions, metastases were more frequently located in the supratentorial area (80%) compared with the infratentorial area (20%). This predominance in the supratentorial region is an important observation, as different areas of the brain can influence the symptomatology and clinical progression of patients with brain metastases.

These data indicate a higher tendency for metastases to localize in the parietal and occipital regions, which may have implications for treatment planning and follow-up strategies in such patients.

In this study, the majority of patients (92.6%) underwent surgical resection of brain metastases. This surgical intervention constituted an essential component of patient management, reflecting an aggressive therapeutic approach aimed at controlling metastases within the central nervous system (CNS).

From an oncological history perspective, 64% of patients had a previously diagnosed malignant neoplasm and were under regular surveillance without evidence of brain metastases before the study period. However, in 36% of patients, the brain metastasis represented the first manifestation of malignancy. These patients developed cerebral metastases without prior cancer symptoms; among them, 25 belonged to the pre-COVID group and 18 to the COVID group, suggesting a relatively balanced distribution between the two-time frames.

Analyzing the metastasis patterns, in 49% of patients the brain metastasis was the only secondary lesion identified outside the primary tumor, whereas in 51% of cases, brain metastases coexisted with metastases in other organs. This finding indicates a more aggressive systemic progression of the disease in the latter group.

The mean volume of brain metastases was 19.7 cm^3, with significant interindividual variability (±18.1 cm^3), the median being 13.5 cm^3, and ranging from 0.3 to 69.4 cm^3. When comparing the pre-COVID and COVID groups, the mean tumor volume was higher in patients treated during the pandemic, with a median of 16.8 cm^3, compared to 12.1 cm^3 in the pre-COVID period. Although this difference was not statistically significant ($p = 0.11$, Mann-Whitney U test), it may suggest a potential impact of the pandemic on early diagnosis and management of these patients.

These findings provide a complex overview of the clinical and pathological characteristics of patients with brain metastases, highlighting aspects related both to disease history and to management across different time periods (pre-COVID vs. COVID).

DISCUSSION

In malignant tumors, delayed initiation of treatment is a critical factor that significantly worsens prognosis. Studies show that even a four-week delay can have a measurable impact on morbidity and mortality among cancer patients, regardless of the treatment modality (1, 2). Such delays adversely affect all therapeutic options, though the degree of impact varies: the risk of disease progression increases by an estimated 6-8% for surgical interventions, 9% for radiotherapy, and 13% for systemic therapies such as chemotherapy and immunotherapy (1, 8, 9).

Before the COVID-19 pandemic, treatment delays in oncology were primarily determined by two categories of factors: patient-related and systemic/institutional (1, 2). Among patient-related factors, the most common obstacle was lack of financial resources, affecting approximately 28% of cases. This difficulty reflects challenges in covering the costs of treatment, transportation, and accommodation in specialized centers (3). Additional factors, such as distance from treatment facilities (12.7%), dependence on others for support (9%), and delayed presentation due to symptom neglect (16%), also contributed to treatment postponement (2, 6).

From a medical standpoint, delays of-

ten occurred when patients were initially diagnosed outside major specialized centers, where access to modern equipment and multidisciplinary teams was limited (4,7). In such cases, the time required for diagnosis and referral to an appropriate facility prolonged treatment initiation, negatively influencing prognosis and survival outcomes (3, 5).

During the COVID-19 pandemic, the impact of these factors intensified, amplifying treatment delays among oncology patients (2, 8). Pandemic restrictions including reduced treatment capacity in cancer centers, reallocation of medical resources to COVID-19 care, and isolation measures-further limited access to therapy (2, 8). As a result, delays caused by the pandemic compounded existing healthcare challenges, exacerbated the prognosis for many patients with malignant diseases (1, 2, 8).

The reduction in the number of surgical procedures and oncologic therapies, as well as the rescheduling and postponement of cancer treatments, became a global concern during the pandemic (6, 7). These disruptions affected most medical centers worldwide and interfered with supply chains, including the availability of medical personnel, which decreased by up to 79% in some institutions (6). This reduction in human resources had a major impact on hospitals' capacity to maintain standard treatment flow for oncology patients (8, 9).

However, the impact of COVID-19-related disruptions on cancer patients was not uniform. The final effect of these restrictions varied depending on multiple factors, including age, comorbidities, type of treatment required, and disease stage (5, 6). For instance, elderly patients or those with multiple comorbidities faced a higher risk of complications due to delayed care, while patients with aggressive tumors were particularly affected by postponement of essential therapies such as surgery and systemic treatments (3, 7).

These pandemic-induced changes highlighted the vulnerability of healthcare infrastructure during global crises and underscored the urgent need for adaptation strategies to ensure continuity of essential care for patients with severe conditions like cancer (1, 2, 8). The pandemic forced many oncology centers to reprioritize cases by severity and to develop innovative solutions to minimize the negative effects of restrictions on cancer patients (2, 9).

CONCLUSIONS

The present study provides relevant data on the impact of the COVID-19 pandemic on the diagnosis of brain metastases, contributing to a better understanding of how this global health crisis influenced the evolution and management of cancer cases involving the brain. Comparative analysis of patients diagnosed before and during the pandemic revealed no significant demographic differences between the two groups, suggesting that age, sex, and area of residence were not influenced by the pandemic period.

However, a statistically significant difference was observed in the median tumor volume, which was higher in patients diagnosed during the pandemic. This increase in tumor size suggests that diagnostic delays-caused by reduced access to health-care services during the pandemic-led to patients presenting with more advanced disease stages and larger brain lesions. Such situations complicate treat-

ment planning, as larger and more advanced tumors typically require more complex interventions and are associated with a less favorable prognosis.

To fully assess the long-term impact of diagnostic delays on treatment outcomes and survival among patients with brain metastases, this study will continue. The future objective is to analyze in greater detail how diagnostic delays, determined by pandemic-related constraints, affected treatment efficacy and overall disease progression.

Continuing this research will enable a more comprehensive understanding of the pandemic's consequences on oncologic care and provide valuable insights for optimizing healthcare system responses for similar crises in the future.

CONFLICT OF INTEREST AND FUNDING

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LAPAROSCOPIC APPROACH IN RECTAL CANCER SURGERY: A RETROSPECTIVE SINGLE-CENTER STUDY ANALYSIS OF CLINICAL OUTCOMES

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LAPAROSCOPIC APPROACH IN RECTAL CANCER SURGERY: A RETROSPECTIVE SINGLE-CENTER STUDY ANALYSIS OF CLINICAL OUTCOMES (Abstract): Along with total neoadjuvant therapy (TNT), surgical resection is the cornerstone of rectal cancer treatment. Minimally invasive techniques include: laparoscopic, robotic, transanal total mesorectal excision and transluminal local excision. In this study, we evaluated the feasibility and outcomes of laparoscopic surgery for rectal cancer in a single-center cohort. **Materials and methods:** We conducted a retrospective analysis of consecutive patients, who underwent surgical treatment for primary rectal cancer at a tertiary institution. A total of 99 patients were included and divided in two groups: laparoscopic (66 patients) and open (33 patients). Clinical and perioperative variables analyzed included age, sex, body mass index, comorbidities, tumor location, surgical technique, length of hospital stay, and postoperative complications. **Results:** The mean age was 68.9 years. Laparoscopy was performed in 66.7% of the patients (conversion to open resection - 15.1%) and was applicable across all age groups. Approximately half of the cases were cancers of the upper rectum. Patients underwent low anterior resection in 61.61%, 24.24% underwent abdominoperineal resection, 14.14% underwent resection with colostomy, and all cases were elective. The median length of hospital stay was 10 days in the laparoscopic group and 11 days in the open surgery group; however, no significant differences were observed between the two groups (p=0.287). According to the Clavien-Dindo classification, for the laparoscopic group, the complications were distributed as grade I (n = 12), grade II (n = 5), and grade III (n = 4); for the open group, grade I (n = 5). Anastomotic leakage (AL) was present in <10 % of cases. **Conclusions:** Rectal cancer is a complex disease that presents oncological, anatomical and functional challenges, which are addressed using a variety of surgical modalities. Our study demonstrates that the laparoscopic approach in rectal cancer is feasible regardless of age and tumor location, and showed a trend toward shorter hospital stay, the difference was not statistically significant. **Keywords:** RECTAL CANCER, LAPAROSCOPIC SURGERY, MINIMAL INVASIVE SURGERY, OPEN SURGERY, RADIOTHERAPY, LARS.

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide. According to World Health Organization data, approximately 1.4 million new cases were reported globally in 2012, with nearly one-third arising in the rectum (1). Advanced age (≥ 80 years) is widely recognized as a major risk factor for CRC, and the growing number of older patients presents new challenges in surgical management (2). Over the past several decades, laparoscopic surgery has been increasingly adopted for the treatment of colorectal cancer and is currently recommended by guidelines (3). Compared with open surgery, laparoscopic approaches are associated with reduced surgical trauma, less postoperative pain, decreased intraoperative blood loss, faster recovery of bowel function, and shorter hospital stays (4-6). Accumulating evidence indicates that minimally invasive techniques, most notably laparoscopic and robotic surgery, achieve long-term oncologic outcomes comparable to those of open surgery. Nevertheless, questions remain regarding their influence on the technical quality of total mesorectal excision (TME) and the adequacy of oncologic clearance. Variations in reported TME specimen quality between laparoscopic and robotic approaches have been described in the literature, raising concerns about the potential implications for local recurrence and postoperative functional outcomes (7).

MATERIALS AND METHODS

This study is a retrospective observational analysis of patients who underwent surgical resection for rectal cancer at a single tertiary referral center, Sf. Ioan Emergency Hospital, Bucharest, between January 2023 and September 2025. The initial dataset included 165 patients (25 needed emergency surgery, 14 had incomplete data and 27 lost to follow up). A total of 99 consecutive patients were included in the analysis. Patients were divided into two groups according to the surgical approach: laparoscopic surgery (n = 66) and open surgery (n = 33). Ethical approval was obtained from the Ethics Committee of the St. John Emergency Hospital (Ref. No 2023-1542). Eligible patients had a histologically confirmed diagnosis of rectal adenocarcinoma and underwent elective curative-intent resection.

The choice of surgical approach was based on surgeon preference and patient-related factors. All patients were managed in accordance with the European Society for Medical Oncology (ESMO) clinical practice guidelines for colorectal cancer applicable at the time of treatment (8). Therapeutic strategies were determined on an individual basis during multidisciplinary tumor board meetings, involving colorectal surgeons, radiation oncologists, medical oncologists, pathologists, and radiologists. The indication for neoadjuvant therapy was established according to the tumor stage and radiologic assessment. Patients selected for preoperative treatment received long-course chemoradiotherapy consisting of either continuous infusion 5 of fluorouracil (5-FU) or oral capecitabine administered concurrently with pelvic radiotherapy to a total dose of 50.4 Gy. Surgical resection was typically planned within 6-10 weeks after completion of chemoradiotherapy; preventive loop ileostomy was performed in selected cases.

RESULTS

Data collection. Clinical and perioper-

ative variables were obtained from a prospectively collected institutional database dedicated to patients with CRC. Clinical, demographic and pathological data were extracted from a prospectively maintained institutional database and reviewed retrospectively. The collected variables included age, body mass index (BMI), tumor location, type of anastomosis, surgical approach, overall survival, and perioperative outcomes.

Surgical Technique. All patients underwent TME according to standardized oncologic principles. TME emphasizes complete resection of the mesorectum along the embryologic planes to minimize recurrence (11). Laparoscopic procedures were performed using a conventional multiport technique, whereas open resections were conducted via midline laparotomy. The type of anastomosis (hand-sewn, mechanical, or no anastomosis) was selected based on the tumor location and intraoperative findings. Three types of surgery were performed in our study:

- For the upper rectum: resection with anastomosis/colostomy;
- For mid-rectal resection with anastomosis/colostomy/abdomino-perineal resection (APR);
- For low rectum: resection with anastomosis (including intersphincteric resection (ISR) in selected cases) or APR.

Preventive loop ileostomy was performed in 10.10% of the patients included in the study.

Statistical analysis. The primary outcomes were perioperative and pathological results according to surgical approach. Secondary analyses evaluated the distribution of surgical approach across age groups, obesity status, tumor location, and anastomotic technique. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using standard statistical software.

A total of 99 patients who underwent rectal cancer surgery were included in the analysis. The median age was 68.9 years. The proportion of laparoscopic procedures increased slightly with advancing age, accounting for 61.5% of surgeries in patients younger than 60 years and approximately 70% of procedures in patients aged 70 years or older. Obesity was another parameter studied. We noted similar proportions of non-obese (57.58% in the laparoscopic and 54.55% in the open group) *vs.* obese patients (42.42% in the laparoscopic and 45.45% in the open group) in the groups. No statistical difference was observed between the two groups ($p=0.83$). Patients' characteristics are presented in table 2. The potential relationship between type 2 diabetes mellitus and postoperative complications was evaluated. No statistically significant association was observed between diabetes status and complication occurrence ($p = 0.440$). Among patients without diabetes, 24 experienced postoperative complications, compared with five patients in the diabetic group. These findings suggest that, within this cohort, diabetes was not associated with an increased risk of postoperative morbidity. Similarly, the distribution of surgical approaches did not differ according to the diabetes status ($p = 0.99$). Laparoscopic surgery was performed in 50 non-diabetic and 16 diabetic patients, while open surgery was performed in 25 non-diabetic and 8 diabetic patients.

The median age was 71.5 years in the laparoscopic group and 69.0 years in the open surgery group. No statistically sig-

nificant difference was observed between the two cohorts (p = 0.401). These findings indicate that chronological age did not significantly influence the selection of surgical technique in this cohort, supporting the comparability of the two groups with respect to baseline demographic characteristics. Two-thirds of the patients were treated laparoscopically (66.7%). Laparoscopy was performed across all age groups, including patients aged ≥70 years. No major imbalance in distribution of obesity between the laparoscopic and open groups. Laparoscopic surgery was feasible across different age groups and body habits, including in elderly patients. We noted that age > 70 years did not preclude the use of laparoscopic surgery. For upper rectal lesions, we performed resection with anastomosis in most cases (78.7%), whereas for low rectal lesions, APR was predominantly performed (83.3%), followed by ISR (intersphincteric resection) in 4 cases (16.7%). For mid-segment lesions, resection with anastomosis was predominantly performed (71.4%). We performed anastomosis in 61.61% of the patients (tab. II).

TABLE I.

Patients'characteristics in the study

	Laparoscopic group (n=66)	**Open group (n=33)**	p
Age, years (mean=68.7)			0.8642
<60	8 (61.5%)	5 (38.5%)	
60-69	20 (62.5%)	12 (37.5%)	
70-79	31 (70.5%)	13 (29.5%)	
≥80	7 (70.0%)	3 (30.0%)	
Sex Male Female	 41 (62.12%) 25 (37.88%)	 21 (63.63%) 12 (36.37%)	0.30
Diabetes Mellitus type 2 Absent Present	 50 (66.7%) 16 (66.7%)	 25 (33.3% 8 (33.3%)	0.44
BMI category Non-obese (<30 kg/m2) Obese (>30 kg/m2)	 38 (57.58%) 28 (42.42%)	 18 (54.55%) 15 (45.45%)	0.83
LOS ≤7 days 8-14 days > 14 days	 7 (10.6%) 49 (74.24%) 10 (15.16%)	 2 (6.06%) 26 (78.78%) 5 (15.16%)	0.287

TABLE II.
Type of surgeries performed for rectal cancer in the study

Type (Rectal localization)	Upper	Mid	Low
Resection with anastomosis	37 (78.,7%)	20 (71.4%)	4 (16.7%)
Resection with colostomy	10 (21.3%)	4 (14.3%)	0 (0%)
Abdominoperineal resection (APR)	0 (0%)	4 (14.3%)	20 (83.3%)

The distribution of anastomotic technique differed according to surgical approach. Mechanical anastomosis was the most frequently performed technique overall and was more commonly associated with laparoscopic surgery (33 cases, 75.0%) compared with open surgery (11 cases, 25.0%). Similarly, hand-sewn anastomosis was performed predominantly in the laparoscopic group (12 cases) *vs.* the open group (5 cases). In contrast, non-restorative procedures were more frequently observed in the open surgery cohort, accounting for 55.3% of such cases compared with 44.7% in the laparoscopic group. This suggests a relatively higher proportion of definitive stoma procedures among patients undergoing open resection. Overall, restorative procedures were more frequently performed laparoscopically, whereas open surgery was relatively more common in cases requiring non-restorative management. However, these findings should be interpreted with caution in light of potential selection bias, as patient selection criteria - including clinical presentation, disease severity, and intraoperative findings - likely influenced both the choice of surgical approach and the type of reconstruction performed.

The median length of hospital stay was 10 days in the laparoscopic group (n = 66) and 11 days in the open surgery group (n = 33). No statistically significant differences were observed between the two approaches (p = 0.287). Patients in the laparoscopic group had a slightly shorter median hospital stay than those undergoing open surgery. No statistically significant association was observed between tumor location and the type of surgical approach (p = 0.2195). Although upper tumors were the most frequent in both groups, laparoscopic procedures were more commonly performed overall. The effect size was small (Cramer's V = 0.211), indicating a weak association.

Upper rectal cancer was predominant in both groups (45.4% for the laparoscopic group *vs.* 51.5% for the open group). Despite the lesion, our preferred surgical approach was minimally invasive (tab. III).

Early postoperative complications were related to surgery: anastomotic leakage (AL), urinary bladder necrosis, surgical site infection (SSI), and general: pneumonia and renal failure. We used the Clavien-Dindo (CD) classification (9), and the results are shown in table IV. In the laparoscopic group, the complications were distributed as grade I (n = 12), grade II (n = 5), and grade III (n = 4). In eight cases of the laparoscopic group, anastomotic leakage occurred and was treated with conservative treatment in five cases, one case benefited from radiologic drainage control, and two cases were reoperated. The overall leakage fistula rate was 8.08%. For the open group, we noted only grade I (n = 5) complications.

TABLE III.
Distribution of patients relative to anatomical segments of rectum and surgical approach

	Laparoscopic surgery	Open surgery
Upper rectum	30 (45.4%)	17 (51.5%)
Mid rectum	16 (24.2%)	12 (36.3%)
Low rectum	20 (30.4%)	4 (12.2%)

TABLE IV.
Postoperative complications

Complications after Clavien Dindo (CD) classification	Laparoscopic	Open
CD I SSI (surgical site infection) Pneumonia Renal insufficiency *Clostridium difficile* colitis	SSI - 9 (6 perineal wound) Pneumonia - 2 Renal insufficiency - 1	SSI - 2 Pneumonia - 1 *Clostridium difficile* colitis - 2
CD II Anastomotic leakage - conservative treatment	5	
CD IIIa Radiologic drainage	1	
CD IIIb Anastomotic leakage - 2 Operation Urinary bladder necrosis	AL -2 1	

Late complications were anastomotic stenosis: four cases (two in the laparoscopic and two in the open group), parastomal hernia: 2 cases, bowel obstruction: 1 case, morbidity related to colostomy: prolapse - 1 case, stenosis - 1 case, rectourethral fistula: 1 case.

DISCUSSION

This study evaluated the use of laparoscopic surgery for rectal cancer in a real-world, single-center cohort, with particular attention paid to patient age, surgical complexity, and operative decision-making. The results demonstrated that laparoscopic rectal cancer surgery was widely applied across different age groups, body habitus, and tumor locations, supporting its feasibility in routine clinical practice. Certain aspects inherent to laparoscopic surgery, such as pneumoperitoneum, prolonged operative time, and prolonged Trendelenburg positioning, may increase the risk of postoperative complications, particularly in elderly patients, who often have reduced physiological reserves compared to young-

er individuals (10). Following the successful implementation of laparoscopic techniques in colon cancer, several large multicenter randomized trials, including the COLOR II and COREAN studies, have demonstrated that laparoscopic rectal cancer surgery achieves local recurrence and disease-free survival rates comparable to those of open surgery (11, 12). Although the initial results of the ACOSOG Z6051 and ALaCaRT trials did not establish noninferiority of laparoscopy based on pathological endpoints, longer-term analyses have revealed no significant differences in two-year disease-free survival or overall survival between laparoscopic and open rectal cancer resections (13, 14).

The technical difficulties related to colorectal laparoscopic surgery are related to narrow operative field in the pelvis and limitations related to instrument mobility. Studies have shown conversion rates between 12 and 30% (15). Our data revealed a conversion rate of 15.1%. A pooled analysis of 14 randomized controlled trials reported that laparoscopic rectal resection achieved rates of positive circumferential resection margin (CRM), incomplete mesorectal excision, and positive distal margin comparable to those of open surgery, fulfilling the predefined criteria for noninferiority (16). In line with these results, the more recent LASRE trial further demonstrated comparable rates of complete mesorectal excision, negative CRM, clear distal margins, and lymph node retrieval between laparoscopic and open approaches, reinforcing the concept that minimally invasive surgery can meet established oncologic standards in rectal cancer treatment (17).

A notable finding of this study is the frequent use of laparoscopy in elderly patients. Although advanced age is often associated with increased comorbidities and reduced physiological reserve, laparoscopic surgery was performed in our study in nearly 70% of patients aged ≥ 70 years, including those aged ≥ 80 years. This suggests that chronological age alone was not considered a limiting factor for minimally invasive rectal cancer surgery in appropriately selected patients. Similar data were reported by Peltrini *et al.*, who found that age does not affect outcomes after laparoscopic rectal cancer surgery in the elderly (18). The distribution of surgical approaches according to obesity status further supports the adaptability of laparoscopic techniques. Obesity, commonly regarded as a technical challenge in rectal surgery, did not appear to significantly limit the use of laparoscopy in this cohort. Also, we did not find a significant association of obesity with postoperative complications (p=0.98). A recent meta-analysis by Gutierrez *et al.* concluded that obesity does not affect the 90-day morbidity rate or short- and long-term oncologic results in patients who underwent surgery for mid-and lower rectal cancer (19). In our study, the distribution of surgical approaches did not differ according to diabetes status (p = 1.000). The absence of a significant association in the present study may therefore reflect the relatively small sample size, the moderate prevalence of diabetes in the cohort, or effective perioperative optimization of comorbid conditions.

According to literature, protective ileostomy is usually performed in patients with high risk of AL (male sex, diabetes, neoadjuvant therapy, distance from anal verge < 5 cm) (20). We performed loop ileostomy in 10 patients and no complications related to stoma were noted. The median LOS was 10 days in the laparoscopic group and 11 days

in the open group. No significant differences were observed between the two groups (p=0.287). Although the laparoscopic cohort showed a slightly shorter median hospitalization, the difference was not significant. These findings are broadly consistent with randomized trials such as COLOR II and COREAN, which reported faster postoperative recovery parameters in laparoscopic groups, including shorter hospital stay (11, 12). Nevertheless, unlike those large controlled studies, our real-world cohort did not demonstrate a statistically significant reduction in hospitalization duration. This may reflect the relatively limited sample size, potential selection bias, or heterogeneity in patient complexity.

Regarding postoperative complications, we did not find a significant association with diabetes (p=0.439). Consequently, although diabetes was not identified as a significant predictor of complications in our analysis, larger studies are needed to better clarify its role in postoperative outcomes after rectal cancer surgery. These results differ from several reports in the literature, which suggest that diabetes may contribute to higher rates of postoperative complications, including surgical site infections, delayed wound healing, and anastomotic leakage following colorectal surgery (21). The anastomotic patterns observed in this study provide additional insight into surgical decision-making. Laparoscopic surgery was more frequently associated with restorative procedures, including both mechanical (33 cases in the laparoscopic *vs.* 11 cases in the open group) and hand-sewn anastomoses (12 cases in the laparoscopic *vs.* five cases in the open group). In contrast, open surgery was more commonly selected for non-restorative procedures, particularly in patients with low rectal tumors. The role of laparoscopy in rectal cancer surgery has been extensively debated, particularly following the initial results of randomized trials, such as ACOSOG Z6051 and ALaCaRT, which failed to demonstrate non-inferiority with respect to pathological endpoints. However, subsequent analyses have shown comparable disease-free and overall survival between laparoscopic and open surgery (13, 14). In this context, the present study adds real-world evidence supporting the continued use of laparoscopy as a viable and adaptable surgical approach. The findings of the present study reinforce the concept that laparoscopic surgery remains the foundational minimally invasive technique and should be regarded as a reference standard when evaluating newer surgical modalities.

Postoperative complications are an important aspect to be discussed. AL was present in <10% of the cases, all of which belonged to the laparoscopic group. Interestingly, only two patients needed reoperation. Treatment consisted of peritoneal lavage, drain placement near the anastomosis, and protective loop ileostomy in one case, and for the second patient it was opted for dismantling the anastomosis and end colostomy. The most frequent severe complication of rectal surgery is AL, with a reported prevalence of 2-5% (22), but there are significant variations. We did not find any cases of AL in the open group because of the small number of cases that underwent open low anterior resection and the subclinical leaks that led to late postoperative stenosis. There were four cases of anastomotic stenosis: two in the laparoscopic group and two in the open group. Our data aligns with literature (23-26).

Limitations: This study has several limitations. Its retrospective design and

relatively small sample size limit the ability to draw definitive conclusions regarding oncologic superiority or long-term outcomes. In addition, the choice of surgical approach was influenced by surgeon preference and case complexity, introducing potential selection bias. Nevertheless, this study reflects real-world practice and provides valuable insights into the feasibility of laparoscopic rectal cancer surgery in a broad patient population.

CONCLUSIONS

In this single-center retrospective cohort study, laparoscopic rectal cancer surgery was feasible across a broad range of patient ages, body habitus, and tumor locations, including elderly patients and anatomically challenging cases. Although open surgery remained more frequently selected for non-restorative procedures and low rectal tumors, laparoscopic approaches were widely applied in routine clinical practice. In appropriately selected patients and when performed by surgeons with substantial expertise, laparoscopic proctectomy appears to be a feasible operative approach that may achieve acceptable oncologic outcomes. However, given the heterogeneity of the available evidence, further confirmation through adequately powered randomized controlled trials is warranted to more clearly define the relative roles of open and minimally invasive techniques, particularly with respect to pathological quality measures and long-term survival.

CONFLICT OF INTEREST AND FUNDING

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CONGENITAL IDIOPATHIC TALIPES EQUINOVARUS: CURRENT CONCEPTS AND TREATMENT

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CONGENITAL IDIOPATHIC TALIPES EQUINOVARUS: CURRENT CONCEPTS AND TREATMENT (Abstract). Clubfoot is a congenital malformation that has a global distribution. Despite recent advances, its etiopathogenesis still remains unknown. Although surgical treatment was preferred in the past, the actual gold standard for treatment is conservative (the Ponseti method). Nevertheless, surgery can still be an option in a small percent of cases. As clubfeet are prone to relapses, their prevention with the use of the foot abduction orthosis (FAO) is important. **Keywords**: CONGENITAL TALIPES EQUINOVARUS, CLUBFOOT, PONSETI, FOOT DEFORMITIES.

Idiopathic congenital clubfoot or talipes equinovarus is a complex, three-dimensional rigid deformity of the foot and ankle, that affects 1 in 1,000 newborns in Romania, males being affected twice as frequently as females (1). About 50% of patients have a bilateral involvement. This deformity is characterized by the presence of four pathological deviations: *varus, cavus, adductus* and *equinus*. True idiopathic clubfeet should be differentiated from syndromic clubfeet, associated with genetic syndromes (e.g. Down syndrome and Larsen syndrome), neurological diseases (spina bifida), myopathies, amniotic band syndrome or teratological malformations. A common similar but benign deformity is the postural clubfoot, a condition determined by prolonged, in utero mispositioning of the feet, which is completely or almost completely correctable at the initial examination and requires no other surgical or conservative treatment except for passive mobilization and stretching.

Many classifications have been proposed in the past, but their reproducibility wasn't satisfactory. The most frequently used classifications are the Dimeglio and the Pirani scores. Both are based on physical examination and they were found to have a good inter-observer reliability and reproducibility after an initial learning phase (2-4). The Dimeglio classification relies on grading the passive correction of the following parameters: equinus, varus,

derotation of the calcaneus-forefoot block and the adduction of the forefoot. Upon adding up the points attributed to each deformity, clubfoot can be classified in soft-soft feet (benign), soft-stiff feet (moderate), stiff-soft (severe) and stiff-stiff feet (very severe). The Pirani score, which relies on assessing six signs, evaluates and scores both the hindfoot contracture (posterior crease, empty heel, rigid equinus) and the midfoot contracture (medial crease, curvature of the lateral border, the position of the head of the talus). The final grading is obtained by adding the hindfoot and forefoot scores. As the Dimeglio score evaluates the reducibility of the foot and the Pirani score evaluates the foot aspect, they could be used at the same time when evaluating the results and monitoring the treatment (5). *The International Clubfoot Study Group* (ICFSG) score, which is more complex and measures the treatment results by scoring the morphological, functional and radiological evaluation of the feet, also proved to have a good inter-observer reliability (6, 7).

ETIOPATHOGENY

Although many theories (8-10) have been proposed so far, the etiology remains unknown. The mechanical theory, described in the past by Hippocrates, suggested that the deformity develops due to increased intrauterine pressure during development, but it is not accepted anymore as the appearance of clubfoot has been observed at 14 weeks in a uterine cavity with abundant amniotic fluid (11). The genetic component appears to be significant, as suggested by the incidence variations among different populations: 1-2: 1,000 in Caucasians and higher in Polynesians (12-14). Other theories were related to the abnormal development of connective tissue and bones (15, 16), neurological (17-19) or vascular anomalies (20). Maternal smoking (21, 22) and early amniocentesis leading to vascular disruptions (23) increase the risk for clubfoot. The pathological modifications of clubfeet have all been well described and it is beyond the purpose of this article to review them extensively. These include: the abnormal development and malposition of the foot bones; contracture and fibrosis of soft tissues (ligaments, tendons, muscles); vascular anomalies; abnormal innervations of the peroneal muscles. All of them can be present in various degrees in patients with clubfeet.

DIAGNOSIS

The ultrasonographical diagnosis of clubfoot can be established at a mean gestational age of 22 weeks (14 to 35.6) with a positive predictive value of 83%, but the accuracy rate varies in different studies and the need for karyotyping is still debated (24). At present, the diagnosis is still clinical, based on the presence of the four typical deformities: varus, equinus, adductus and cavus. The severity and rigidity of these pathological modifications vary from one patient to another. In very severe cases, the presence of a medial plantar and a posterior retrocalcaneal crease can be noted. The circumference of the calves and the length of the feet should also be noted because calf hypotrophy is always present on the affected side. Radiological evaluation was used as a preoperative and postoperative assessment method when the treatment of clubfeet was surgical. Nowadays, radiological examination is not routinely performed in newborns due to the absence of ossification of the foot bones at that age. In addition to that, Ippolito et al. (25) demon-

strated that the talocalcaneal angle, a common radiological parameter used to assess the hindfoot, was misleading in 75% of the patients which were investigated, and it didn't reflect the true relationship between the two bones. Another issue is radiation exposure of infants. Taking into consideration the fact that radiographs are performed near radiosensitive organs and that the current treatment is mainly conservative, relying mostly on palpation of the bones in the foot, after measuring the radiation dose utilized in the assessment of clubfoot, Radler et al. (26) do not recommend taking radiographs while using the Ponseti method.

TREATMENT

The goal of treatment is to correct the four deformities and to obtain a plantigrade, functional, pain-free foot that allows standing and normal walking. Many treatment methods have been used so far and they can be divided into two groups, surgical and conservative.

Although preferred in the past decades, the surgical treatment was almost completely replaced by the conservative one. However, in certain cases such as resistant forms, very stiff types of clubfeet or residual deformities, it remains the only available option. It was usually preceded by passive mobilization or stretching sessions followed or not by immobilization, to increase foot flexibility and correct as much as possible the deviations. If results were not satisfactory and there was little progress, surgical treatment was indicated. The proper age for surgery used to vary from surgeon to surgeon, depending on the age and the foot dimension. The surgical interventions used for the treatment of clubfeet can be divided into three main types: soft tissue releases, tendon transfers and bone procedures, the last ones being reserved for older children. The most frequently used were the posteromedial approach (Turco), the posterolateral approach (Metaizeau) and the circumferential approach (Cincinnati), the latter allowing an excellent visualization. The soft tissue surgery included: lengthening of the Achilles tendon, posterior capsulotomy of the ankle and release of the subtalar joint, lengthening of the flexor hallucis longus, flexor digitorum longus, tibialis posterior tendon, capsulotomy of the talonavicular joint, partial or total release of the interosseous ligament, release of the calcaneocuboid joint and calcaneofibular ligament. The extent of release used to vary among surgeons. The corrected position of the foot was thereafter temporarily maintained by some form of internal fixation, usually a Kirschner wire passed through the talonavicular joint, followed by immobilization in a cast for 6 to 12 weeks.

Since Ponseti (11) described his method, it has become the most utilized method of treatment throughout the world. It consists of two phases: the initial correction phase and the maintenance phase. The correction phase is mainly conservative and consists of weekly sessions of manipulation and immobilization of the foot to gradually correct each of the four deformities (cavus, varus, adductus and equinus). It has a mean duration of 5 to 7 weeks depending on the stiffness of the clubfoot and the doctor's experience. The cavus is the first deformity corrected by supinating the foot and applying pressure on the first metatarsal head to realign the forefoot with the hindfoot. The adductus and the varus deformity are corrected together by performing a progressive abduction, while applying counterpressure on the head of the talus. The equinus is the

last deformity to be corrected: a percutaneous Achilles tenotomy under local anesthesia is performed after the foot can be abducted more than 60º and if dorsiflexion is less than 15º-20º. The procedure is followed by immobilization in a long leg plaster cast for 3 weeks. The removal of the last plaster marks the beginning of the maintenance phase, which consists of wearing a foot abduction orthosis for the next 4 years (fulltime during the first 3 months and 14 to 16 hours a day until the age of 4-5 years). The FAO maintains the affected feet in 60˚-70º of abduction and 10º-15º of dorsiflexion. Non-compliance with wearing the FAO was found to be associated with recurrence (27). Factors associated with non-compliance or poor-compliance are: discomfort while using the brace, low educational level of parents and low family income (28).

DISCUSSION

Satisfactory results were obtained on the short term after surgical release of clubfeet (29-31), but long term follow up studies report stiff and not fully functional feet, which needed additional surgeries (32). In a 17-years follow-up study, after evaluating foot function and patients'satisfaction, Symeonidis et al. (33) concluded that even after achieving an initial successful surgical correction, residual symptoms after surgical treatment may alter walking ability and the quality of life. Ponseti's method of treatment achieves the highest correction rate in approximately 90% of the cases as reported by different Pediatric Orthopedics Centers where the method was adopted (34-37). Complications are possible with both methods of treatment. In surgically treated patients, leaving aside the surgery-related risks, the most frequent complications are: increased stiffness of the ankle, subtalar and midfoot joints, under correction or residual deformities (forefoot adduction, persisting cavus, equinus or hindfoot deformity), talar flattening and avascular necrosis of the talus, overcorrection, in toeing gait, weakness of the plantar flexors, pain and development of arthritis (38, 39). However, after the Ponseti treatment, recurrence of one of the four deformities (usually the forefoot adductus with dynamic supination) is the most frequent complication to arise and it is correlated with the inadequate use of the foot abduction orthosis (28). Conservative treatment consisting of manipulations and immobilization in long leg plaster casts followed by bracing after the deformity is corrected can usually be effective, but with growth children don't tolerate the foot abduction braces as well. Transfer of the tibialis anterior tendon to the lateral part of the foot is preferred in these cases because it maintains the corrected position and eliminates the need for bracing (40).

The thing about clubfoot is its natural tendency to recur despite successful surgical or non-surgical correction. This is can be explained by the fact that the pathological modifications and contractures are still present in the posterior and medial aspect of the calf, ankle and foot. These relapses occur until the age of four or five, and are rare afterwards, even in only partially corrected clubfeet (41). This is the main reason for the indication of wearing the FAO for the following four years of age. In a prospective study that compared surgical treatment to the Ponseti method of treatment, Halanski et al. (42) found that despite the high recurrence rate in both groups, patients treated with the Ponseti method required less interventions for the correction of the deformity and less revi-

sion surgery. Even though this method has a high initial correction rate (90%), extensive surgery will still be required in the rest of the feet which don't respond to treatment (43, 44).

CONCLUSIONS

In our opinion, even though it has been described as a congenital malformation, clubfoot should be considered a developmental disorder due to the nature of its pathological modifications and its strong tendency to relapse. After reviewing the literature and based on our experience, we recommend the Ponseti method as the first choice of treatment. If clinicians strictly respect its principles, a high correction rate can be achieved. We consider useful weekly evaluating the foot with the Pirani and Dimeglio scores during the correction phase. The importance of the FAO cannot be overemphasized and sometimes the maintenance phase proves to be more challenging than the correction phase. As noncompliance with the bracing protocol is an important factor linked to relapses, efforts should be made to improve compliance and the brace design. Despite the high initial correction rate of the Ponseti treatment, surgery will still be necessary in those feet that don't respond to treatment and in some forms of relapsed feet.

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URINOMA: A POTENTIAL UROLOGIC EMERGENCY

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URINOMA A POTENTIAL UROLOGIC EMERGENCY (Abstract): **Aim:** A urinoma is characterized as a collection of urine outside the urinary tract as a result of disruption of the collecting system induced by a high pressure in the collecting system **Material and methods:** We reviewed the medical records of all patients with urinoma treated in the Iasi Urology Clinic between January 1, 2013 and March 1, 2017 Results**:** A total of 36 patients, (18 males and 18 females), age between 18-70 years with an average age of 51.10 years, were included. The etiology of obstruction was represented by lithiasis 29 (80.55%) cases, pelvic cancers 5 (13.88%) cases, pyelonephritis 1 (2.78%) case and trauma 1 (2.78%) case. The size of lumbar collection was between 1 cm (n=19) and 28 cm (n=1). The interval from symptom onset to presentation was: 6-10 hours - 12 (33.33%) cases; 12-24 hours - 17 (47.22%) cases, up to 5 days - 7 (19.44%) cases. The treatment was represented by the insertion of a double J stent in 29 patients, percutaneous nephrostomy in 5 (13.88%) patients. Two (5.5%) patients were treated conservatively. The post-operative evolution of patients was favorable. The ultrasound examination before discharge revealed no collection in 34 (94.44%) patients and the presence of urinoma in evident remission in 2 (5.56%) cases. Eight (22.22%) of our patients had positive urine culture with *E. coli* (n=4), *Enterococcus spp*. (n=2), *Klebsiella spp*. (n=1) and *Pseudomonas* (n=1). Six (16.66%) of our patients had infected urinoma which needed drainage of lumbar collection. **Conclusions:** Urinoma is a rare complication secondary to ureteral obstruction and it has a high risk of infection. All the cases must be investigated in hospital, double-J stent insertion and antibiotic therapy is the most frequent treatment option but some selected cases can be treated conservatively **Keywords**: URINOMA, LITHIASIS, DOUBLE-J STENT.

Urinoma is a pathological situation in which the patient presents an encapsulated collection of extravasated urine, usually located in the perirenal space but at times found in the retroperitoneal space, peritoneal cavity, pleural cavity, and even in the mediastinum (1). Urinoma is secondary to ureteral obstruction with cause a high pressure above the obstacle, but the exact mechanism is not fully understood.

The etiologies for spontaneous urinary extravasation from the upper urinary tract are: caliceal fornix extravasation and ureteral (or peripelvic) rupture. The former is caused by a sudden increase in renal pelvis pressure and backflow of urine into the

renal sinus in case of obstruction. This is considered to be a protective physiological mechanism producing decompression that may protect the kidney from high-pressure injury (1).

In patients with obstructive uropathy, there is often a sharp rise in intrapelvic pressure and the collecting system may rupture at its weakest location-the fornix. Being relatively rare, this phenomenon has not been systematically studied.

Urinomas in children are rare and secondary to obstructive uropathies, such as posterior urethral valve, pelviureteric junction, and/or vesicoureteric reflux.

The diagnosis of the urinoma, although uncommon, is important for emergency physicians because it needs a specialized urological service where to admit and to offer special treatments to the case.

The treatment of perirenal extravasation should be individualized. Conservative follow-up, endoscopic treatment, percutaneous urinary drainage, and surgical correction of obstruction have all been suggested depending on the underlying cause and amount of extravasated fluid.

MATERIAL AND METHODS

In this retrospective study, the medical records of all patients with urinoma admitted and treated in the Iași Urology Clinic between January 1, 2013 and March 1, 2017 were reviewed. Diagnosis was performed by ultrasound and fore some cases by CT scan. Data on age, presence and degree of ureterohydronephrosis (UHN), imagistic dimensions of the urinoma, etiology, time from symptom onset to presentation and treatment method were collected.

RESULTS

Between 2013-2017, 36 patients, 18 males and 18 females were diagnosed with urinoma. The age of the patients was between 18-70 years with an average age of 51.10 years. All patients had UHN, the UHN's degree was I-II (out of V) in 28 cases and III-IV in 8 patients.

TABLE I.
Dimension of urinoma measured by ultrasound

Size (cm)	No. of patients
<1	19
2	7
3	3
7	1
10	3
11	2
28	1

The etiology of obstruction was represented by lithiasis 29 (80.55%) cases, pelvic cancers 5 (13.88%) cases, pyelonephritis 1 (2.78%) case, and trauma 1 (2.78%) case (figure 1). Of this patient 17 (47.22%) had urologic history (1 stone wave lithotripsy, 6 nephrostomies and 10 ureteral stents indwell). Eight (22.22%) of our patients had positive urine culture, most encountered bacteria were *E. coli* (n=4), followed by *Enterococcus spp.* (n=2), *Klebsiella spp.* (n=1) and *Pseudomonas* (n=1). Six (16.66%) of our patients had infected urinoma which needed drainage of lumbar collection.

The interval from symptom onset to presentation was: 6-10 hours - 12 (33.33%) cases; 12-24 hours - 17 (47.22%) cases, up to 5 days - 7 (19.44) cases. The treatment was represented by the insertion of a double J stent in 29 (80.55%) patients, percutaneous nephrostomy in 5 (13.88%) patients and 2 (5.55%) patients were treated conservatively. The post-operative evolution of patients was favorable. The so-

nographic examination before discharge revealed no collection in 34 (94.44%) patients and the presence of a collection but with smaller size in 2 (5.56%) cases.

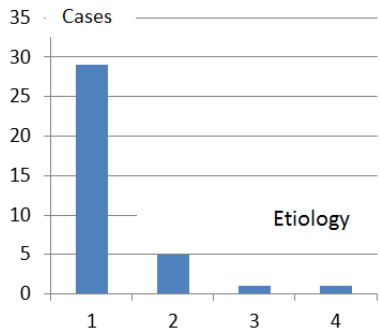

Fig. 1. Etiology of urinoma
(Legend: 1- lithiasis, 2- pelvic cancer, 3- pyelonephritis, 4- trauma)

DISCUSSION

Urolithiasis is a disease with an increased incidence and prevalence worldwide. Although rare, urinoma is one of the possible complications of urolithiasis. According to Pampana *et al.* (1) perforation could occur at any level from the calix to the bladder but it is usually seen at the fornices and upper ureter. It may lead to several serious consequences including urinoma, abscess formation, urosepsis, infection, and subsequent irreversible renal impairment. This leads to the necessity to early intervene in the presence of urinoma by performing urgent double-J stent insertion, nephrostomy tube insertion, or in some cases ureteroscopy with stone extraction.

The mean age of patients in our group was 51.10 years, which is very similar as the one of Gershman's (2) group of 108 patient which was 52.3 years. Regarding the male: female ratio in our group was 1:1but in other studies was 7:3 (2, 3).

In our study, the etiology of obstruction was represented by lithiasis (80.55%) followed by pelvic neoplasms (13.88%). Urolithiasis was also the main cause of obstruction in studies of Gershman and Almujalhem (2, 3). Rare etiologies for urinoma were also described. These include obstruction because of posterior urethral valves (5), gravid uterus (6), iatrogenic causes (7, 8), pelvic-ureteric junction (PUJ) obstruction (9), vascular extrinsic compression (10, 11), i. v. fluid administration (12), bladder carcinoma (13), retroperitoneal fibrosis, vesical-ureteric junction obstruction (2).

Most of our patients (80.55%) were evaluated in the first 24 hours of symptoms onset, but urinoma usually does not have a specific symptom, although Doehn *et al.* (14) states that one of the specific manifestations of forniceal rupture is the sudden pain relief due to the decrease in collecting system pressure. Sometimes this situation could not be differentiated from a renal colic but sometimes could mime an acute abdomen. In our study, the patients had no atipic symptoms, most of them declared lumbar pain and the first ultrasound examination diagnosed the lumbar collection.

Most of cases reported in the literature have a delayed diagnosis due to the gradual onset of symptoms, including loin pain and sepsis (when they become infected). Pain in the loin and infection are commonly thought to be the effects of pyelonephritis, and ultrasound is an appropriate first-line investigation to exclude hydro nephrosis or collections.

Contrast-enhanced CT has an increase specificity regarding diagnostic because urine flow into the fluid collection can be

documented by increased attenuation in the collection on delayed images but imaging alone is not able to distinguish between a urinoma and other types of fluid collection (15, 16).

In some cases, the diagnostic could be very difficult due to lack of symptoms. Diamond and Marshall (4) reviewed 34 patients with spontaneous urinary extravasation, and nine of these patients were treated through exploratory laparotomy for acute abdominal conditions. In this situation, imagistic evaluation of the patient is very important. According to Moak *et al.* (15) and in our case, ultrasonography represents the first line of investigation for renal colic.

The most important treatment is to deal with the underlying condition that led to the urinomas formation in the first place (16). Nephrostomy or stenting is needed in cases caused by leakage without obstruction such as in traumatic injuries or postoperative anastomotic dehiscence. If the urinoma is small, it may not need to be drained separately. The urine may reabsorb if further flow into the urinoma has been eliminated.

When obstruction of the urinary system causes the urinomas, the obstruction needs to be relieved.

Urinoma drainage is indicated in several situations. Fever or leukocytosis, suggesting that the urinoma is infected is good reason to proceed to drainage. The urinoma may need to be drained to reduce pain or pressure on adjacent structures (16).

In our study only 8 (22.22%) had positive urine cultures, most frequent bacteria were *E. coli*. This percent is high compared with results of Gershman et. al. in which case only 5.2% had a documented urinary tract infection, but lower as the percent obtained by Doehn *et al.* which was 27.4%. This author also noticed that more than half of the patients had systemic signs of

infection such as leukocytosis (54.3%) and/or elevated C-reactive protein level (61.5%).

According to Doehn *et al.* (14) urinoma is a potential urologic emergency due to the reported risk for perinephric abscess formation and urosepsis that carry high morbidity rate. The author performed in all patients, primary endoscopic therapy (internal drainage) (96.3%) or insertion of a nephrostomy tube (3.7%) and concluded that with a low-pressure system and antibiotic treatment, the outcome is excellent, and secondary complications can be effectively prevented. Therefore, minimally invasive procedures or conservative care offer excellent results, and several case reports confirm this.

We performed insertion of a double J stent in 29 (80.55%) patients, percutaneous nephrostomy in 5 (13.88%) patients with good results, taking into consideration same clinical elements (general condition, fever, risk of infection, renal function, inflammatory elements, the initial cause of UHN). No collection was revealed in 34 (94.44%) patients before discharge. The conservative treatment was applied only in 2 (5.55%) cases. In some cases, this can be an option. Kalafatis *et al.* (17) treated 35 (41%) of 81 cases in a conservative approach (bed rest, intravenous fluid, and antibiotic) and concluded that conservative management can be applied to certain patients with fornix rupture. Also, Almujalhem (3) treated conservatively 57.5% of patients and concluded that that conservative management is a valid option of treatment of noncomplicated cases of spontaneous fornix rupture.

CONCLUSIONS

In our study, we find that urinoma is a rare complication of ureteral obstruction which affect both males and females, often secondary to lithiasis and can become a potential urologic emergency, Risk of infection and evolution to abscess is high and leads to the necessity to early intervention to decompress the collecting system. Ultrasound may detect fluid collections in the perinephric or retroperitoneal areas, but is recommend contrast CT to confirm the diagnosis.

Fluid around the kidneys, ureters, or bladder may represent extravasated urine, and further imaging may be necessary to characterize injuries fully.

Double-J stent insertion is the primary endoscopic therapy with good results. Some selected cases can be treated conservatively.

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UNIVERSAL AND PARTICULAR ASPECTS OF SCIENTIFIC GROWTH AND SPIRITUALITY IN THE FORMATION OF MEDICAL RESEARCHER

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UNIVERSAL AND PARTICULAR ASPECTS OF SCIENTIFIC GROWTH AND SPIRITUALITY IN THE FORMATION OF MEDICAL RESEARCHER (Abstract): The education and growth of researchers should aim not only to teach concepts but also to form character. The process of initiation into a field requires the practice of virtues explicated in the article below. This article proposes that scientists develop their character along two planes: a vertical plane that is concerned with internal moral formation of the virtues of the scientist and a horizontal plane that is concerned with societal influence and the role that medical research plays in serving humanity. We also argue that scientists that view their formation and research practices coexisting with a belief in God and faith may enlighten and embolden their research experiences and endeavors. **Keywords:** SPIRITUALITY, VIRTUES, SCIENTIFIC FORMATION, MEDICAL RESEARCH, FAITH.

"In the religious sphere, the classical expression for wholeness is 'the peace of God' which, according to St. Paul, exceeds all nous (rational understanding), and which is able to safeguard the heart (the center of personality) and the noemata (acts of rational understanding)" (1).

Yet while there exist both supporters and skeptics of this line of inquiry, current and forthcoming empirical evidence in this field published on the connection between religion and health continues to strongly indicate a positive correlation. Prestigious universities are conducting programs of research in the field and numerous top journals have published findings that are statistically significant. Therefore, we encourage new medical researchers and social science investigators to incorporate this field in their formation and familiarize themselves with this work and further build future research on evidence that have been already published in this domain. We will go a step forward and argue in this paper that a researcher's personal and professional formation will benefit from an alliance between religion and medicine-an alliance which could generate new knowledge about how faith and religion contributes to health and healing in people (2).

LIMITATIONS IN SCIENTIFIC INQUIRY

Some of the most fundamental questions of humanity such as *what is the mean-*

ing of life or *what happens after we die* remain largely overlooked by modern scientific inquiry which privileges that which is quantifiable. Such questions do not easily lend themselves to quantitative experimentation, and we must recognize that scientific research remains powerless to provide answers to these profound questions. Recognizing the limitations of the scientific mind in obtaining a complete objective grasp on reality of truth about the world and our place in it, nevertheless, the paper endeavors to comment on the motivation, values, and assumptions in scientific inquiry and biomedical research aligned with the quest for the philosophical, moral, and religious truth about the world and what place we - the scientists and/or care takers have within it. Additionally, we will expand on the possibility that the medical scientists seek the truth and could bear witness to the truth in harmony with God's created order. In this empirical reality of current world, a spiritually attuned scientist may bring God's created order more fully to light and stretch his own horizon towards the uncreated light wherein the Creator dwells.

Many top scientists view their research practices in the light of spirituality. A notable example is Francis Collins: "*I have found there is a wonderful harmony in the complementary truths of science and faith. The God of the Bible is also the God of the genome. God can be found in the cathedral or in the laboratory. By investigating God's majestic and awesome creation, science can actually be a means of worship*" (3).

Religion and spirituality impact on medical care and health are studied across the globe (4); as an integral part of the health care system researchers should be guided in their work and constantly reflect on how their research is applied to improve the society in general and the well-being of the patients. This purpose may remind the scientist that serving humanity is also an expression of serving God.

WISDOM GUIDES SCIENTIFIC RESEARCH FORMATION

It is not enough for medical science to increase general knowledge; the purpose of science is to serve the welfare of communities. While clinical research is essential, the quality of this research is reflected directly by the strengths and weaknesses of the discipline itself. As a result, the education and formation of both clinicians and researchers should be aiming not only towards teaching concepts but also to form character. We then propose the formation of such character on two planes:

A vertical plane mainly focusing on moral formation and virtues a scientist may acquire to be a good person, e.g. justice, honesty, humility, graciousness as well as cultivating the virtues expressed in serving others, e.g. kindness and love. All these criteria are very similar with those that are cultivating and nourishing a relationship with God.

A horizontal plane mainly concerned with the way how the scientist may influence the society and the services brought to the humanity. This plane certainly aims to align the research in the way that society benefits but also appropriate boundaries to scientific quest for progress and knowledge are maintained.

RESEARCH AND VIRTUES

Those responsible for training new researchers should seek to develop not only the research skills of students but also their character. Contemporary moral philosopher Alasdair MacIntyre argues that new participants in any complex social activity, such

as scientific research, must develop character-building virtues in order to learn their craft (5). Students of novices must accept the authority of established standards of excellence in research and recognize the inadequacy of their own work in light of those standards before they can gain the capacity to criticize or further develop their standards.

The process of initiation into a field requires the practice of particular virtues. New researchers require *justice* to discern what they owe to whom. They must determine, for example, how to show their gratitude to their teachers, how to treat animal subjects of research humanely, and how to compensate human subjects appropriately. They require *courage* to take the risks necessary for good research. And perhaps the most crucial virtue for researchers is *honesty*. The entire enterprise of scientific research would collapse without the commitment to a truthful sharing of results. Consequently, the scientific community levies harsh punishments on scientists who betray this trust. The virtue of *honestry* also promotes a high regard and relentless pursuit of the truth. The truthful researcher constantly seeks to expand his or her knowledge and avoids misleading sources. Such researchers candidly evaluate their own performances, and by practicing *humility*, seek out constructive criticism.

Educators must do more than discuss virtues because the virtues are not abstract intellectual concepts. Rather, they are habits of good character and behavior; students will acquire them only through active participation in research under the close guidance of mentors who possess these virtues themselves. Research from the "positive psychology" movement indicates that under the right circumstances virtues can be taught. MacIntyre's work suggests that virtuous behavior will flourish only within communities that hold the virtues at the core of their common life (6). Therefore, academic departments must recruit and promote faculty members who exemplify virtue.

This emphasis on the moral formation of researchers is meant to supplement, not replace, the existing guidelines on ethical research. Indeed, fidelity to fundamental research statutes, such as those regarding informed consent, could be considered a virtue. However, these guidelines may not always provide a clear direction in challenging situations, and from time to time institutions will promulgate the guidelines as unethical. In response, virtuous persons must act upon their character and pursue right.

A CHRISTIAN ASCETIC TRADITION AND ETHICS IN RESEARCH

We assume that a student who embraces a research career will seek a practical pathway by which the student can develop virtuous habits of character. Lessons from literature that reflect on virtues would further allow researchers to learn from the long history of ethics (e.g., authors like Homer, Aristotle, Benjamin Franklin, and Jane Austen). Several religions consider the virtues necessary for a good and moral life; thus, researchers can enrich their understanding of the virtues by engaging with these traditions and welcoming religious perspectives in discussions about research ethics. This so called "open pluralism" can contribute to the professional development of novice researchers, particularly if they belong to one of these religious traditions (7).

To illustrate we will offer a few considerations from the ascetic tradition of the Near East. In the Platonic model of psychology, the human psyche consists of three key elements: the noetic, or intellect;

the incentive describing desires and animations; and the appetitive, the ability to satisfy bodily needs. Each element can be characterized by the virtues of justice, courage, and temperance respectively, with wisdom as the overruling principle of the human soul. A modern commentator of Christian-Platonic understanding, John Claude Larchet builds on the philosophies of early Christian thinkers such as St. John of Damascus and Maximus The Confessor. Larchet describes these powers of the human soul as vegetative (nutrition and growth), animal (aggressiveness, desire and affectivity) and reasoning (reason being the principal characteristic and that distinguishes humans from all other creatures) (8).

The power of reasoning has two important faculties: the spiritual faculty (intellect, or nous, linked to humans' noetic ability and responsible for the moral and psychological senses of consciousness) and the faculty of self - determination (8). Although it adopts the Platonic virtues, the Christian ascetic tradition, with respect to the incentive element of the soul, focuses on the virtue of love, cultivating practices that exhibit brotherly love and compassion (9). Early Christian-Platonist thinker, St. John Damascus, described the contrasting "sins" to the incentive element as including "heartlessness, hatred and lack of compassion" (10). Peter of Damaskos states that love of neighbor is highest among all virtues (11). And St. Isaac of Syria states that a person whose incentive powers are in the right order has a heart "burning with love towards the whole creation: towards men, birds, animals, demons and every creature…cannot bear to hear or see the least harm done to or misfortune suffered by creation" (12, 13). In contrast, the "passions" are barriers to manifesting love. The passions should not be understood as human emotions but rather as vices precluding obedience to the Word of God (14, 15). Examples of these passions include avarice, bitterness, resentfulness, anger, pride, jealousy, lustfulness, or what the ascetic fathers call "self-love." The Church teaches that such "passionate" persons will be preoccupied with satisfying their own desires for pleasure, wealth, and praise, and will fail to attend to or to cultivate the true virtues of compassion and mercy. Particularly relevant to research practice, those who give in to their passions will regard others as rivals or as impediments towards satisfying their self-love (9) - a pattern that is likely to erode the spirit of teamwork and undermine a research team's effectiveness and success.

An important reason for bringing to light these traditional thinkers in contemporary practice and regarding the ethical growth of researchers and scientists is that we believe a similar model can be used as an impetus towards understanding the purpose and virtuosity of performing scientific research.

UNDERSTANDING SKILLS AND WISDOM

There is a distinction between two types of knowledge - wisdom and skill- that applies to every realm of life including medical research. In this section, we will discuss the distinction between phronesis, or practical wisdom, and techne, or technical skill, as used in Aristotle's language. Phronesis is the development or internal formation of the self as someone cognizant of his or her purpose that acts with wisdom in all areas of life; techne is the production or formation of an outside object or external good (e.g., a drug, a car, a hospital). Phronesis aims at moral and ethical understanding and virtuous living, whereas techne aims at the creation of virtuous products (16).

Those who teach medical sciences or research techniques should exhibit "tech-

nical" knowledge, the product of their teachings being the skills that are imparted on their students. Teachers should further develop practical wisdom by applying and practicing virtuous behavior in their own lives so they can become role models for their students.

WISDOM - A COMMON GROUND FOR SCIENCE AND RELIGION

Since antiquity wisdom has been understood as a psychological trait. In modern times, wisdom has been variously described as involving social attitudes or behaviors, pragmatic knowledge of life, emotional homeostasis, reflection or self-understanding, value relativism and tolerance, and the acknowledgment of uncertainty with the ability to cope with it effectively. Although broadly defined, wisdom is consistently recognized as including thoughtful decision-making, compassion, altruism, and insight (17). In ancient philosophy, by contrast, wisdom was considered attainable in the context of practicing "askesis," which implies exercises of self-control, self-discipline, self-denial, and meditation guided by a mentor. Both Western and Eastern religions teach that to develop wisdom one needs to practice this self-constraint, disciplined morality, charitable concern, and compassionate actions (17).

According to Pavel Florensky, a Russian Orthodox theologian and thinker, wisdom is Divine and a direct representation of God's creative love. "Sophia is the original nature of creation, God's creative love… For everything exists truly only insofar as it communes with the God of Love, the Source of being and truth" (18). Because of this act of love, wisdom is imprinted in each one of us "in our hearts by the Holy Spirit which is given unto us' (Romans 5:5)". The medical arts seek to integrate science with this wisdom from the Holy Spirit for the benefit of patients. The purpose may not always be to cure but to help the patient throughout the process, while instilling a realistic hope or peace in individual suffering, something medical science alone cannot do. Medical disciplines therefore serve a greater purpose: to understand needs and sufferings of patients and address these problems through medical practice and research. We suggest that the training of practitioners and researchers therefore needs to address both developments of self as well as sensitivity to others.

CONCLUSIONS

As we have noted, religion and spirituality may enter the medical research sphere when researchers personally see their work as part of a Divine calling. The individual may view medical research as part of an obligation to serve humanity and thereby indirectly attain Divine grace. A researcher may decide to work towards ameliorating a disease, or works to serve an underprivileged community. These sorts of research endeavors encompass a drive to choose research endeavors prudently to bring about maximal scientific benefit, but which also contain an element of Divine service.

A medical researcher who is motivated by religious concerns may also strive to focus his activities upon a more vertical plane whereby he works to develop within himself the sort of moral character that is pleasing to God and other humans. He may, for example, practice prayers that help him stay regarding God and through that connection instill virtuous behaviors and thereby be a better servant of God.

These two dimensions *horizontal* and *vertical* that involves the formation of the researcher and we have highlighted in the beginning of this chapter are often pursued simultaneously and there may be places for

overlap. A case example is a researcher who employs his skills to introduce virtues into the medical education curricula and thereby affects physician behaviors and patient care. Another example is a researcher who examines the ways in which religion impacts patient behaviors and works to develop healthcare systems that serve the religious and spiritual needs of patients, clinicians, and researchers alike.

The content presented on this paper may enable the future researcher to render a deeper consideration of the idea that a fundamental relationship exists between rigorous scientific growth and spirituality, and to encourage further reflection and meditation on the universal and particular aspects of this perspective.

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DEMENTIA–ETHICAL AND LEGAL IMPLICATIONS IN PSYCHIATRIC MEDICAL ASSISTANCE. CASE REPORT

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DEMENTIA-ETHICAL AND LEGAL IMPLICATIONS IN PSYCHIATRIC MEDICAL ASSISTANCE (CASE REPORT) (Abstract)*:* In psychiatric pathology, patients suffering from dementia are part of the vulnerable population category. The Universal Declaration of Human Rights and the law of mental health and psychiatric disordered patient's protection brings new changes for patients that temporarily lack decisional ability, by adding the notion of conventional representative. The purpose of this research paper is to present and evaluate the necessity of conventional representation, in the case of a patient diagnosed with medium-severe stage mixed dementia. This case is representative of the many cases of dementia in clinical practice that raises many ethical and legal discussions, and for which the conventional representation may be a solution. **Keywords*:*** INFORMED CONSENT, CONVENTIONAL REPRESENTATIVE, DEMENTIA.

Dementia constitutes a class of neurodegenerative disorders characterized by persistent and progressive alteration of cognitive functions, ascending to disability and premature death. The most common cognitive functions affected in dementia are memory, attention, orientation, language, thinking and judgment (1).

Due to this impairment of the ability of logical thinking and understanding of the patient with dementia, especially in the early stages of medium and severe illness, the question is whether to fully inform the patient about the necessary investigations required for diagnosis, the application of therapeutic methods and about performing the medical interventions necessary for restoring personal autonomy. In this situation, a person with difficulties in assessing the consequences of a decision that impacts himself can benefit from the assistance of a legal or conventional representative, that can provide a valid, informed and documented consent. Conventional representatives are people with full legal capacity, designated by the patient, that have the full legal and mental capacity to assist the patient and to represent him during medical treatment.

The legal representation will be achieved through the signing of an agreement by the patient – following a model made available by the health unit, under conditions that follow the law.

If the hospitalized person was declared as lacking decisional capacity, it will have the support of the legal representative, appointed according to law. If the patient

does not have a legal representative or has not appointed a conventional representative due to lack of mental capacity, the health unit is obliged to immediately notify the guardianship authority to establish protection measures (4-6).

CASE REPORT

The case presented below relate to patient of 58-years-old, male, from a rural area, grade 3 medically retired, having secondary education, married.

Patient presents to a doctor's office, at the "Socola" Institute of Psychiatry, in Iasi, with her daughter, with symptoms manifested by partial space-time and auto and allo-psychically disorientation, bradypsychia, psycho-cognitive slowness, episodes of confusion and anxiety, hypo-amnesic fixation and evocation, spontaneous and voluntary hypoprosexia, depressed mood, psycho-emotional lability, tearfulness.

The doctor communicated to the patient and its accompanying daughter the need for hospitalization for diagnosis and treatment plan. He presents and explains, in clear language, comprehensible to the patient, the elements of informed consent, that the patient accepts by signing the consent to admission to the "Socola" Institute of Psychiatry in Iaşi.

General history

From the patient's statements, his mother deceased at 70-years-old, showing elements of dementia syndrome (space-time disorientation, stopped recognizing family members) in the later years of her life.

Chest effort angina. Myocardial stroke, chronic stage. Stage II, grade 3 essential arterial high blood pressure, high additional risk. Grade II obesity. L4-L5 operated herniated disc. Headache syndrome. Vertiginous syndrome.

Lives at home, with his wife, non-smoking, denies alcohol consumption.

Disease history

The patient first presented at the "Socola" Institute of Psychiatry in Iasi, in 2011, for depressive symptoms, manifested by depressive mood, demobilizing ideations, pessimistic, bradypsychia, bradyphrasia, bradykinesia and mixed insomnia. At that moment, he was showing a slight cognitive deterioration (MMSE=26 points). Given the symptoms, the patient receives antidepressant treatment, with neurotrophic and neuroprotective medication.

Subsequently, he is overseen through the territorial network of psychiatry, his condition being relatively good and stable. Approximately 6 months ago, the depressive symptoms began to reappear, alongside with a dementia syndrome (partial space-time disorientation, attention and memory deficits bradypsychia, paraphrasia, dyspraxia), due to which the patient presents himself, along his family, at the hospital, for clinical and therapeutic reevaluation.

Clinical and psychiatric examination

Good general condition, grade II obesity (BMI=36 kg/m^2), abdomen increased volume due to fat tissue, lumbar postoperative scar (L4-L5).

Psycho-diagnosis of expression. The patient has a cooperative attitude, hypomobile mimicry, reduced gesturing, depressive facial aspect, wrinkled forehead, omega-melancholic, lowered mouth commissures, mobile sight, intermissive eye contact with the doctor, feels the need to frequently gaze back to his daughter to receive help answering the questions or for confirmation ("the turn-head-back sign"),

low tone and intensity voice, clothing care and proper hygiene.

Cognitive functions. The patient presents hyperesthesia, with irritability and reduced tolerance to frustration, without perception disorders such as hallucinations during the examination. Attention reveals spontaneous and voluntary hypoprosexia, difficulties in focusing and evoking (incomplete auto-biography data such as "I don't remember where I had been working". "I don't know if I took my treatment" and "I don't know why I came here"). Thought reveals ideation and verbal slowness (bradypsychia), with episodes of confusion, decreased ability to abstraction, generalization and reasoning, decreased decisional capacity, impoverished thinking, auto-depreciative and demobilizing ideation, feelings of hopelessness. Low levels of imagination.

Affective and motivational functions. The patient manifests a depressive mood, tearfulness psycho-emotional lability, generalized anxiety, physical and mental asthenia, fatigue, anhedonia, apathy. Instincts highlight eating instinct disorders, with its reduction, sexual instinct disorders with decreased libido and an exaggerated defense instinct, manifested through the fear of disease.

Effector functions. His volition is manifested through hypobulia, with lack of initiative and capacity to act. Communication is difficult, with paraphrasia, answers questions with difficulty (due to the difficulty in remembering certain information). Verbal language disorders are present, such as dysphasia with verbal amnesia and paraphrasia. Motoric conduct reveals hypokinesia with slowness and episodes of psychomotor restlessness, bradykinesia. The patient presents hypnic disorders such as mixed type insomnias.

Synthesis functions. The patient is partially space-time oriented, auto and allopsychic, disease awareness is partially present, presents derealisation, depersonalization.

Psychological evaluation. Moderate psycho-cognitive deteriorative configuration with depressive manifestation in psycho-somatic and pre-involute context. MMSE=15 points (orientation –6 points, fixation memory–2 points (4 points after rehearsals), calculus=0 points, language=6 points).

Clinical explorations

Neurological examination conducted by the neurologist in the psychiatric hospital finds an intermittent L5 radicular syndrome (possibly remaining after surgery for L4-L5 disc herniation in 2013) and raises the suspicion of lacunar cortex and vascular dementia, a reason for which he calls for a CT examination. Treatment recommendations from the neurologist were: Actovegin 200 mg x 3/day, Milgamma N 1 capsule x 3/day, Cerebrolysine 10 ml 1 vial/day, 10 days, Aflanil 100 mg 1 capsule x 2/day.

Native CT examination performed in axial plain reveals: Cortical-subcortical cerebral atrophy, predominantly supratentorial. Stage 1 leukoaraiosis, ASC predominantly in the internal carotid system. Rare cerebral ischemic gaps are present. It also highlights the thickening of the mucosa in all levels of aerial paranasal sinuses. The procedure shows a mucosa polyp in the left maxillary sinus, with a diameter of approximatively 19 mm, for which an ENT consult is recommended.

The electrocardiogram recorded a sinus rhythm with a pulse rate of 83 bpm, a blood pressure of 130/90 mmHg and sequelae of lower myocardial infarction (of which the patient claims he suffered in 2011 – did not

submit supporting documents).

Blood and urine samples didn't highlight pathological changes.

Internal medicine examination established the following diagnoses: Painful chronic ischemic heart disease; Effort chest angina; Lower myocardial infarction in chronic stage; Grade II obesity; Stage 2, grade 3 arterial high blood pressure with additional risk; Cerebral hypo-perfusion in the ascendant basilar-vertebra system. Therapeutic recommendation was: Aspenter 74 mg/day, Noliprel 1 capsule/day, Nitromint 2.6 mg 2 capsules/day.

THE POSITIVE DIAGNOSIS

The positive diagnosis determined after the clinical examination and complementary investigations is: Severe dementia in medium-severe stage. This diagnosis is supported by both ICD-10 criteria as well as DSM IV.

ICD-10 criteria

Diagnosis is based on the presence of dementia (decline in memory and thinking that compromise personal activity). Memory deterioration typically affects recording, storage and playback of new information and is accompanied by a deterioration in thinking, reasoning and reduced ideation flow, spontaneous and voluntary hypoprosexia, consciousness is retained for at least 6 months. Impaired cognitive functions are usually unequally present with: memory loss, intellectual deterioration and neurological focal signs. Judgement and understanding can be relatively well kept. Associated features: hypertension, carotidal murmur, emotional lability with transitory depressive mood, explosive crying or laughter, transitory episodes of consciousness dizziness or transitory delirium. Personality is relatively unmodified or apathy or disinhibition changes may occur, increasing previous features such as: eccentricity, paranoid attitude and irritability (2).

DSM IV criteria

A. Multiple cognitive deficits, with manifestations in both sections below:

1. Memory deterioration (impairment of the ability to learn a new information or to recall a previously learned information);

2. One or more of the following cognitive disturbances:

- Aphasia (language disturbance);
- Apraxia (deterioration of the capacity to perform motor activities, despite the sensorial function being intact);
- Agnosia (inability to recognize or identify objects, despite the sensorial function being intact);
- Disturbance in the execution function (planning, organizing, sequencing, abstracting).

B. Cognitive deficits from A1 and A2 criteria both cause a significant deterioration in social and professional functioning and represent a significant decline from the previous level of functioning.

C. Neurological focal signs and symptoms (ROT exaggeration, pseudo-bulbar paralysis, walking abnormalities, decrease of muscular strength in an extremity) or laboratory data that indicate cerebral-vascular disease (multiple infarcts involving the cortex and underlying white matter), of which it is etiologically connected with the disorder.

D. The deficits do not occur exclusively during delirium (3).

TREATMENT

From 2011 to date (patient initially diagnosed with depressive disorder) he received the following treatment: Tianeptine

12.5 mg 3 capsules/day, Alprazolam 0.25 mg 2 capsules/day, Clonazepam 2 mg/day, Nicergoline 30 mg/day, MagneB6 3 capsules/day and regular cures with Cerebrolysine vial 5 mL, 1 vial/day, 10 days per month. During the hospitalization in 2016, in addition to the anti-depressant treatment, an anti-dementia therapy is initiated with Rivastigmina patch 5 mg/day. This treatment was increased after a month to 9.5 mg/day and Memantine 5 mg/day. Medication is increased step by step, with 5 mg/week until it reached 20 mg/day. Remaining are therapeutic schemas recommended by the neurologist and internist respectively, as previously mentioned.

The patient is referred for hospitalization and monitoring in the territorial psychiatry network where he belongs, recommending continuing the treatment initiated in the hospital, returning to hospitalization if necessary, as well as the beginning of procedures to establish social protective measures of which the patient benefits given the affection of personal, social and professional functioning (GAFS=28).

EVOLUTION AND PROGNOSIS

Negative prognosis factors, in this case, are: family history (mother with dementia syndrome), debut at a relatively young age (55-58-years-old), associated somatic affections (myocardial infarction, hypertension, ischemic heart disease, obesity, brain atrophy) and a relatively rapid evolution. Positive prognosis factors are represented by family involvement and support, appropriate treatment of the associated diseases as well as a good socio-economic condition.

The evolution of this case will largely depend on anti-dementia treatment which initially appears to be satisfactory, an improvement in cognitive functions and general mental state of the patient being observed during hospitalization. Generally, the evolution of patients with dementia receiving treatment is relatively stable or slowly progressive.

DISCUSSION

This case represents one of multiple cases of dementia that question the validity of informed consent to be hospitalized in a medical institution for evaluation and treatment. The dilemma that arises in this situation is if the patient with moderate-severe cognitive deterioration has the capacity to make the right choices, to participate in the process of evaluation and diagnosis communication and to accept subsequent therapeutic conduct at any time during these stages. For many patients with moderate stage dementia (MMSE=12-15), especially those with a vascular component, a variability in cognitive symptoms exists, which makes these patients to have a fluctuating decisional capacity. When this ability is integral, the patient can appoint a conventional representative that will assist him during hospitalization, informing him clearly about the legal limits of this representation.

In our case, the patient signed the informed consent, at under the law admission, without assigning a conventional representative. During hospitalization, the patient had numerous episodes of confusion with a decrease in abstraction, generalization and reasoning capacity and a reduction in decisional capacity, moments during which the patient was assisted and supported by his daughter, without bringing into discussion the signing of the conventional model to appoint his daughter as his representative. After approximately 10 days after admission, the patient stopped experiencing these episodes of confusion, com-

pletely regaining his decisional capacity. This is a typical case in which appointing a legal representative would have been useful by allowing the family to legally intervene on necessary decisions to be taken during hospitalization, in the patient's moments of confusion.

The new rules for implementing the law of mental health and protection of mentally disordered people, approved by Order No. 488/2016, entered force on May 4th, 2016, create the legal framework for the appointment of a conventional representative. Therefore, Annex 5 of the mentioned rules contains the model of the convention for these cases, clearly establishing the rights and obligations of both parties. This representation is done free of charge. The duration of the convention is agreed by both parties and both the representative and the person represented can unilaterally terminate it.

CONCLUSIONS

Thus, starting from this case report and given these new regulations, it is recommended to more carefully evaluate the decisional capacity of the patient, both during admission as well as its likelihood of evolution during hospitalization. Taking the example of our patient situation, in the cases where there is a suspicion of deterioration of the decisional capacity, besides informed consent, patients are informed of the possibility to appoint a conventional representative and they are presented with the conventional model of the present rules. By signing the informed consent and the agreement to appoint a conventional representative, the patient is protected and given the insurance that his wishes are respected, even in times of decisional incapacity.

In our case, the early diagnosis enable patient to actively and autonomously engage in decision-making, before this capacity starts to be affected by illness. This way, the information necessary to understand the symptoms is found out early, which allows taking decisions regarding the future, how medical and psychosocial nursing will be carried out and an improved quality of life.

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TRUNK AND HIP ANALYSIS DURING GAIT IN POST-DISCECTOMY, ACTIVE LUMBAR STABILIZATION

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TRUNK AND HIP ANALYSIS DURING GAIT IN POST-DISCECTOMY ACTIVE LUMBAR STABILIZATION (Abstract): This paper presents a post-discectomy rehabilitation program through active lumbar stabilization with focus on trunk and hip movement analysis during gait. Material and methods: Analysis consisted of a kinematic gait study, with kinematic point tracking and software data processing in MATLAB. The analysis was performed on a patient with L5-S1 discectomy. The purpose was to track progress and change in trunk and hip during the rehabilitation program and to establish a relationship between trunk and hip movement during gait, in regards to the patient's rehabilitation program. Results: Trunk and hip analysis during gait showed significant differences in patient hip and trunk rotational movement between the three analyzed readings of the patients. Changes were found in both left and right hip flexion-extension coupling and flexion in terminal swing. The rotational movement of the trunk is reduced in a more physiological margin from -13.4 to -1.0 anterior-posterior movement which correlates with un improved in transversal rotation. The analysis made for hip and trunk synchronization revealed an increased synchronization after the end of the functional reeducation program. Conclusions: This method allowed to evaluate changes in trunk and hip motion during gait. Tracking and visualizing the motion between trunk and hip during gait gives a better understanding of the importance of each movement and the strong link between gait phase coordination and motion angle amplitude. It gives a new perspective on the rehabilitation process that targets lumbar stabilization. **Keywords**: REHABILITATION, GAIT ANALYSIS, DISK HERNIATION.

Lumbar discectomy is one of the most common surgical treatment in lumbar disc herniation. The main goal of the postoperative rehabilitation program is to restore lower spine movement and strength of the corresponding muscles.

To our knowledge, trunk and hip kinematic data during gait has not been yet clearly defined. There is a general understanding on flexion, extension, rotational movements and synchronous phenomena that occur between trunk and hip during gait. There are few studies (1, 2,3) that describe a kinematic analysis of the relationship between trunk movement and hip/pelvis movement during gait. In a kin-

ematic study (3), overlapping trunk and hip curves results in a common area. This common area describes the time frame where trunk and hip, align. Shape, size and other detail aspects of the area are important to determine imbalanced movements and muscle realignment need. Also, the trunk and hip coordination in gait phases is important as de-synchronization affects gait performance as well as pathological kinematic angles (4). It is important to note that this type of analysis does not have a scientific study reference yet, but has the foundation of what is already known in terms of trunk and hip kinematics.

During the rehabilitation program, the physiotherapy specialist follows an already established protocol, making some changes to accommodate the patient's current state and health history.

Lack of objective feedback in a rehabilitation physiotherapy type program is an issue. Pain scales such as Visual Analog Scale (VAS), movement tests such as Prone Instability Test, Passive Lumbar Extension Test and Lumbar Dynamometry are current evaluation test that quantify the progress of a rehabilitation program.

MATERIALS AND METHOD

The study was conducted in the Physiokinesiotherapy and Rehabilitation Center of "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

The patient studied was a 37 years old male, of height 1.8 m and of weight 70 kg. He came to the center for rehabilitation after having a lumbar discectomy surgery at L5-S1 level 4 weeks prior. The rehabilitation protocol for our patient consisted of a four-stage program of active lumbar stabilization(ALS) exercises (5). The four stages are muscle re-education, static stabilization, dynamic stabilization and functional activities. In total, for our patient, the rehabilitation program consisted of 30 sessions set throughout a period of 4 weeks.

Gait analysis was performed 3 times. First evaluation was made at the start of the rehabilitation program to assess the initial patient's gait and will be referred to as A1 in the study. The second time was after 15 sessions, referred to as A2, and the third time at the end of the rehabilitation program referred to as A3.

To perform the gait analysis, the patient was asked to walk at a normal walking speed rate covering a distance of 10 m, back and forth for 10 times. Body landmarks were established for trunk, pelvis, hip, and knee. Four digital cameras were positioned to record from the front, back, left and right. Video analysis was performed using DLTdv3 digitizing toolbox for MATLAB® (6) to track the landmarks. General functions in MATLAB® where used to obtain gait angles for each gait segment, plotting values and data comparison. We used MATLAB® version R2007b with license number 336.240.

Values were obtained for speed, step length, duration of gait phases and motion angles at each gait segment. The angles were plotted to obtain kinematic gait graphs.

Regarding these kinematic gait graphs, the overlapping area mentioned earlier occurs during gait between the Terminal Stance and Initial Swing phases (3). During gait, this is the interval where the pelvis/hip and the trunk align. In normal gait and at normal walking speed the trunk rotates counter clockwise with forward leg movement, all while the pelvis and hip rotate in the opposite direction. The coor-

dination of trunk and hip movement is important for an optimal and efficient gait.

By analyzing these areas, our focus was to find a relationship between trunk and hip kinematics with respect to gait phases and follow progress throughout the rehabilitation process. This was also a method of quantifying the link between trunk and hip movement during gait.

RESULTS AND DISCUSSION

For a better understanding of our patient's trunk and hip movement coordination during gait we first made a general analysis of hip kinematics in sagittal plane. In figure 1 are plotted the left hip angles during gait for each of the three-gait analysis made. Of interest to the study was the Pre-swing phase timing during gait and differences in angle amplitudes.

The red line corresponds to A1, before rehabilitation started, the light blue line corresponds to A2 and the dark blue line corresponds to A3. There is a considerable difference in the graphs showing a clear change of patient's gait at hip level (fig. 1).

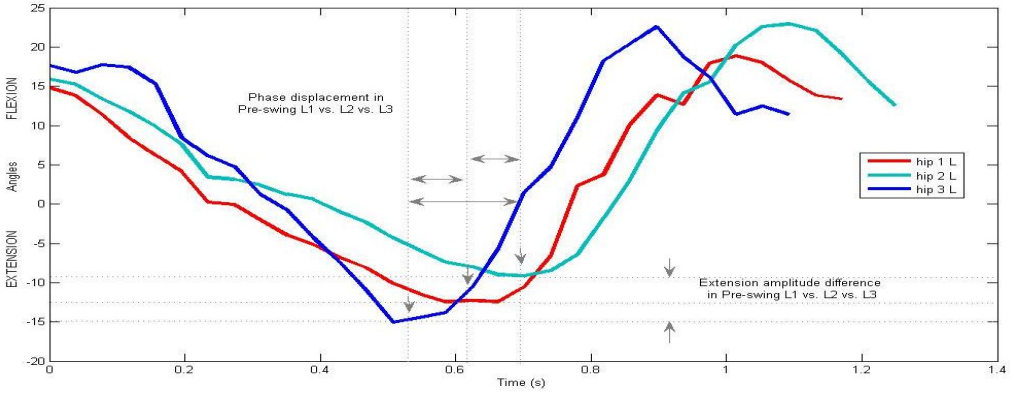

Fig. 1. Comparison between first, second and third gait analysis of hip movement angles in sagittal plane (left side)

At initial contact in A1the hip flexion was 15°, half the value of the average normal (7). During terminal stance, the hip reaches a maximum of 13° of extension and then continues with a flexion of approximately 19° during mid-swing, ending the cycle with a decrease in flexion to 18° in terminal swing. The values found are significantly lower than the normal averages (7) and describe a deficit in movement amplitudes during gait, both in flexion and extension of the hip. In A2 flexion at initial contact increases to 16°, extension decreases at 9° in pre-swing and flexion increases at 23° in terminal swing. At this stage, values get closer to normal range except for extension in pre-swing. In A3, flexion at initial contact increases to 18°, extension increases at 15° in pre-swing and flexion remains at 23° in terminal swing. A3 exhibits values that are in normal range (7). This shows a clear progress and a good rehabilitation of our patient.

In figure 2 the dotted lines correspond to the right hip for each of the analyses and have corresponding colors to the ones from the left side. The graphs for the right side are reversed so that they match the left side

for a better comparison. This comparison was made to illustrate if there were any differences in values or coordination of gait phases between left and right hip.

In A1 there is a difference in pre-swing phase timing between left and right (marked with red arrows). This de-synchronization is also present in A2 but the difference between the phase timing is smaller than in A1(phase timing is marked with light blue arrows).

In A3 there is a clear synchronization between left and right (marked with dark blue arrows). This shows that left and right hip match in phase timing and have very similar motion amplitudes. To get more into detail, in pre-swing, when the pelvis drops it is stabilized by a complex set of muscles (rectus abdominals, external oblique, iliopsoas, low back extensors, hip extensors and hip flexors). If these muscles from one side do not work in synch with the ones from the opposite side than it translates on the graph in a difference in amplitude and timing of gait phase.

In figure 3 are plotted the left trunk angles in sagittal plane during gait for each of the three-gait analysis made. For an easy reference, we kept the colors corresponding the each of the gait analysis.

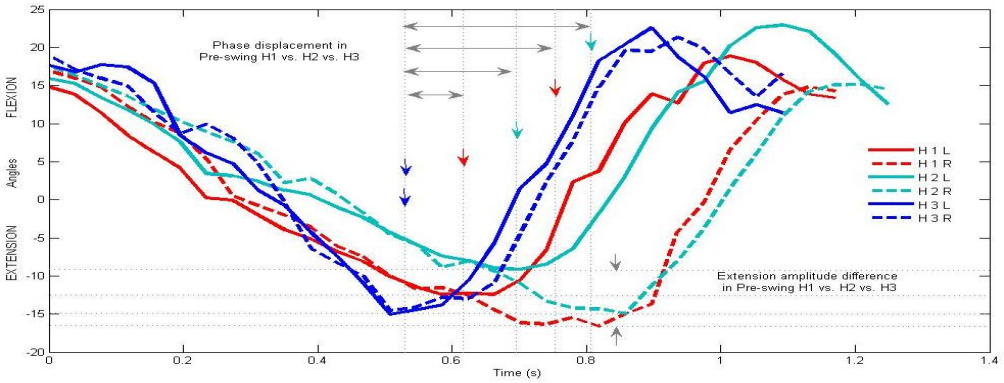

Fig. 2. Comparison between first, second and third gait analysis of hip movement angles in sagittal plane (left and right side)

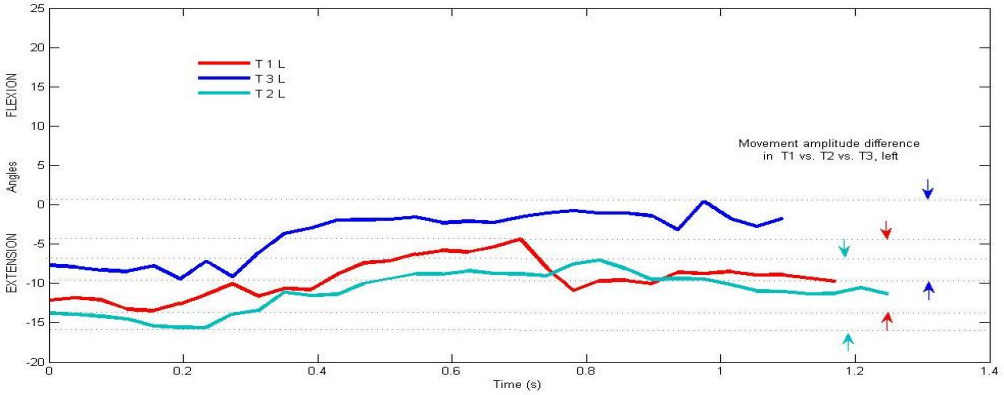

Fig. 3. Comparison between first, second and third gait analysis of trunk movement angles in sagittal plane (left side)

In A1, trunk remains in a constant hyper-extension with values varying with gait phases. The trunk oscillates in sagittal plane with values raging in the interval of (-13, -4). At initial contact trunk is extended at -13°, which is more than double the average normal value (8).

In A2 there is still a predominance of hyper-extension of the trunk but not as fluctuating in amplitude as before rehabilitation started.

However, in A3 trunk extension is significantly reduced. The arrows in the right in corresponding colors show the amplitude interval for trunk in each gait analysis. In A3 the trunk oscillates in sagittal plane with approximate values raging in the interval of (-10, 0). Compared to A1, at initial contact in A3, trunk is extended at approximately 7°.

In fig. 4 the dotted lines correspond to the right trunk for each of the analyses and have corresponding colors to the ones from the left side. The graphs for the right side are reversed so that they match the left side for a better comparison. A1 and A2 do not have symmetrical left versus right trunk graphs. There is also a difference in flexion moment in A1 where peak areas do not overlap. A3 shows a similarity between left and right curve with a slight bigger value of flexion on the right side.

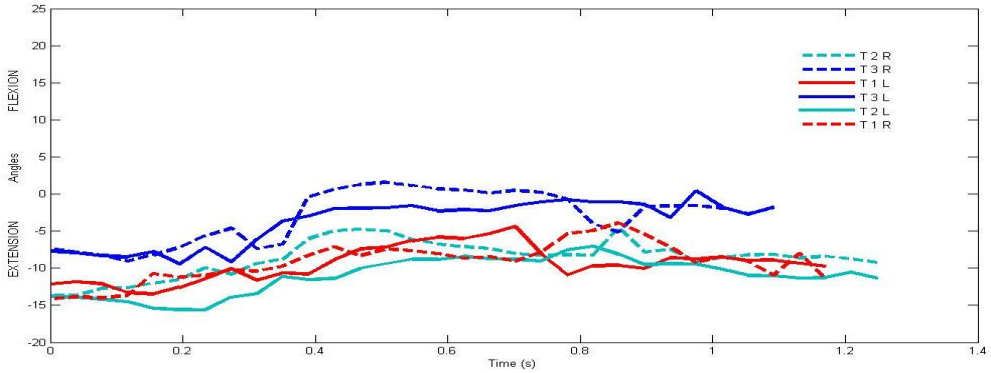

Fig. 4. Comparison between first, second and third gait analysis of trunk movement angles in sagittal plane (left and right side)

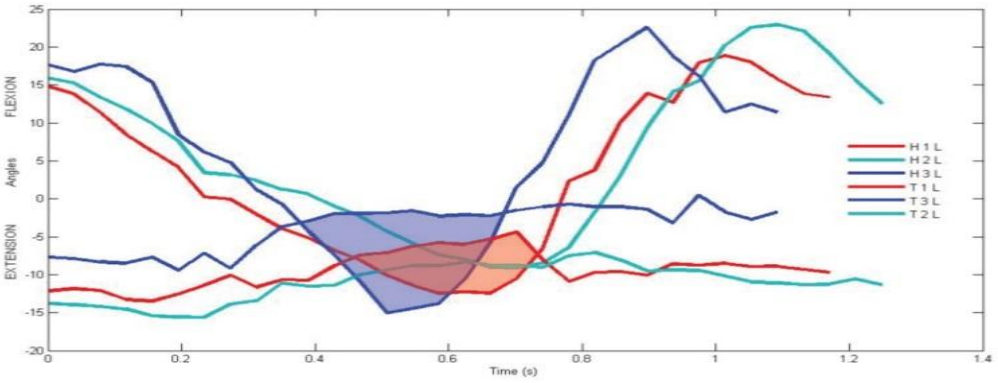

Fig. 5. Overlapping area comparison between trunk and hip movement angles in first, second and third gait analysis (left side)

In figure 5 are highlighted the overlapping areas between hip and trunk gait graphs. Each overlapping area has the color corresponding to the graphs from A1, A2 and A3 gait analysis. As explained earlier this marks the time frame where hip/pelvis and trunk align.

Overlapping area in A3 is greatest and is located at the middle of the gait cycle. This translates in a good motion synchronization. Peak of trunk curve is when trunk bends forward to move the center of mass outside the base of support. Lowest point on the hip gait graph is when hip is in maximum extension and prepares for toe off to continue in swing phase. Therefore, an increase in sagittal plane flexion of trunk would raise the curve making the overlapping area bigger. Also, a decrease of hip extension would raise the curve, making the area smaller. Ideally, there must be a balance between motions of segments.

Regarding the position of the overlapping area with respect to the gait curves, the optimum position should be in the middle of both curves. That is because maximum hip extension and trunk flexion should occur at approximately 50% of gait cycle. In A3 overlapping area occurs as normal.

In figure 6 are included the graphs corresponding to hip and trunk from right side and are highlighted the overlapping areas between hip and trunk gait graphs. For a better and clear view, only graphs from A1 and A3 are shown.

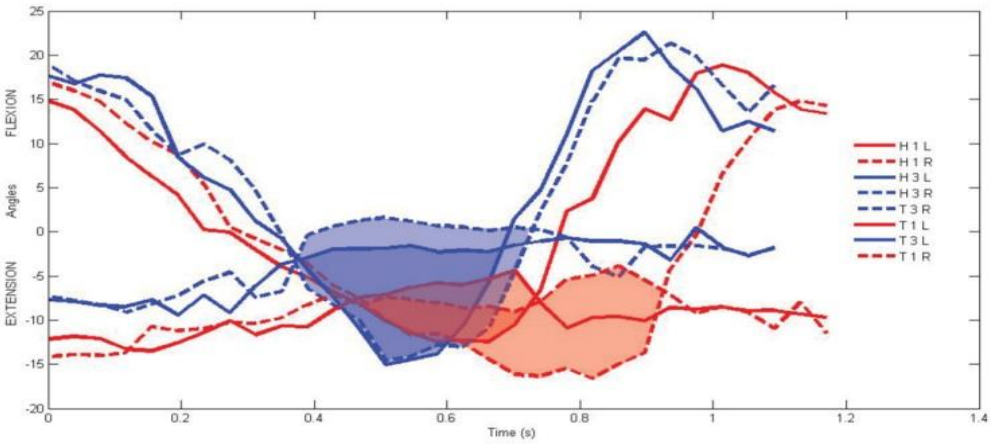

Fig. 6. Overlapping area comparison between trunk and hip movement angles in first and third gait analysis (left and right side)

After the analysis of the kinematics of trunk-hip related rotational movement we can conclude that the transvers rotational movement of the hip and trunk improved. That means that the patient could move with a hire discrepancy between the two segments because of the increased dynamic stability which promoted higher transversal rotational movement.

We found similar results in the only two articles (9, 10) from the scientific literature that covers this subject. The difference is that this studies compared normal versus pathological lower back pain (LBP) patients. They found that the LBP patients compared to healthy people had a more

rigid and less flexible pelvis-thorax coordination.

A significant difference can be seen between the left and right side in A1 overlapping areas. This translates in an overall pronounced and de-synchronized rotation of hip and trunk. Visually, the right-side area is bigger than the left side. In A3, areas are similar in shape and size. This shows a good motion synchronization and amplitude control by muscles that can lead to a decrease in stress forces on lumbar disks.

CONCLUSIONS

Performance and efficiency feedback in rehabilitation program was defined and tracked through a kinematic analysis system. We defined the relationship of trunk and hip motion during gait with respect to the gait phases that allowed for a better understanding of lower back, hip and pelvis muscle action and synchronization during the rehabilitation program. The method allowed for tracking change in trunk and hip motion during gait.

Our method of tracking and visualizing the motion between trunk and hip during gait gives a better understanding of the importance of each movement and the strong link between gait phase coordination and motion angle amplitude. Therefor this also puts a new perspective on the rehabilitation process that targets lumbar, pelvis and hip muscles showing the importance of muscle alignment and synchronization for optimum motion stabilization.

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DOES THE THEORY OF RELATIVITY APPLY TO CARDIOVASCULAR RISK? CHANGES IN THE SPECTRUM OF CARDIOVASCULAR RISK FACTORS IN ELDERLY PATIENTS – CASE REPORT

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DOES THE THEORY OF RELATIVITY APPLY TO CARDIOVASCULAR RISK? CHANGES IN THE SPECTRUM OF CARDIOVASCULAR RISK FACTORS IN ELDERLY PATIENT– CASE REPORT (Abstract): The cardiovascular system balance can be disturbed by a various number of factors, some of them modifiable and others, such as age, which cannot be influenced. In the context of aging associated with such comorbidities as diabetes mellitus and rheumatoid arthritis, we wonder if the classical cardiovascular risk factors are enough to assess the probability of cardiac decompensation, or other factors may be involved. We present the case of a 76-year-old patient known with permanent atrial fibrillation, high blood pressure, diabetes mellitus, hypodiastolic heart failure and rheumatoid arthritis who was admitted with cardiac decompensation without evident cause and despite apparent therapeutic compliance. Geriatric assessment revealed marked mnesic and cognition deficit, malnutrition risk, dependency, depression and frailty. In such patients, the role of cardiac sarcopenia and rheumatoid arthritis in the decline of the cardiac function and patient outcome must be assessed and then managed. **Keywords**: ELDERLY, CARDIOVASCULAR RISK FACTORS, SARCOPENIA, RHEUMATOID ARTHRITIS.

In the context of the increased lifespan, the diseases afflicting the elderly occupy an even more important place in the current medical practice. We are now witnessing major changes in the spectrum of cardiovascular risk factors in elderly patients. Beside age itself, several other risk factors must be considered. Frailty and its core element – sarcopenia and even cardiac sarcopenia – is one of these factors. Also, the presence of several comorbidities, known risk factors (diabetes mellitus, arterial hypertension) or "silent risk factors" (connective tissue diseases, rheumatoid arthritis), influence the outcomes of elderly patients with cardiovascular disorders.

CASE REPORT

We present the case of a 76-year-old patient with an about one-year history of permanent atrial fibrillation, grade 3 arterial hypertension (AHT), diabetes mellitus type 2, hypodiastolic heart failure and rheumatoid arthritis (RA) admitted to the Clinic of Geriatrics-Gerontology, "Dr. C.I. Parhon" Hospital Iasi. During the last

months, the patient noticed an accelerated and progressive deterioration of cardiac function, despite the apparent therapeutic compliance and without evident cause.

The patient was at home, on treatment with digitalis, diuretic, platelet antiaggregant and long-acting nitrate drugs. The treatment of rheumatoid arthritis was previously discontinued because of the appearance of side effects (iatrogenic thrombocytopenia). Also, the patient was partially bedridden.

The geriatric assessment revealed: marked mnesic and cognition deficit (MMSE=23/30), risk of malnutrition (MNA 18/30), a high level of dependency (ADL=4/6, IADL=1/8), moderate depression (GDS=7) and frailty (FRIED score=4/5).

Biologically, an inflammatory syndrome (VSH 50 mm/h), mild anemia (Hb=10.1 mg/dl), fibrin degradation products ++ and lower tract urinary infection were found.

Electrocardiogram (ECG) showed atrial fibrillation and alterations in the terminal phase in the inferolateral territory.

Chest radiograph revealed calcified aortic knob, *g*lobally increased heart size (cardiothoracic index 0.75), swelling of the left middle arch, opacification of both lung bases, pulmonary condensation syndrome more marked in the right lung, enlarged right pulmonary hilum, left fissure (fig.1), appearance obviously contrasting to the radiologic image recorded one year earlier (fig. 2).

Echocardiography showed cardiac cavities globally dilated. Also found was a systolic dysfunction with reduction of left ventricular ejection fraction (LVEF) from 56% one year earlier to 47% on current admission. An important finding was severe pulmonary arterial hypertension (PAH), previously absent.

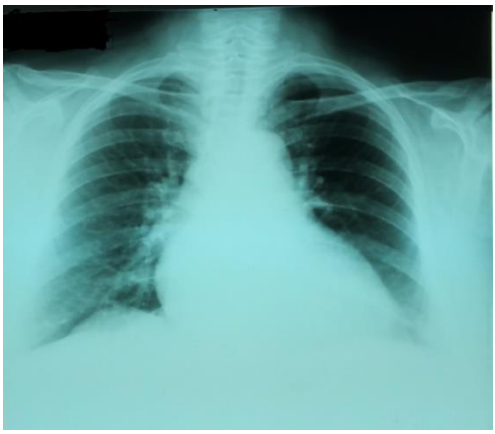

Fig. 1. Chest radiograph, obtained one year before current admission

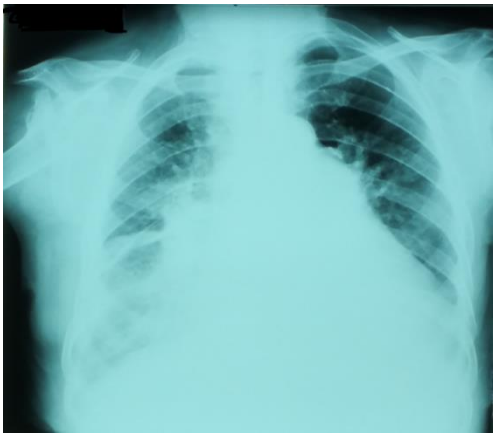

Fig. 2. Chest radiograph on current admission

The patient presented diabetes mellitus and AHT as major predictable risk factors for cardiovascular disease. However, the sudden onset of heart failure and occurrence of severe PAH in less than six months after the initial presentation despite therapeutic compliance cannot be explained by the above mentioned risk factors. This fact made us re-evaluate the cardiovascular

risk of the patient and consider the patient-specific risk factors. Thus, the impact of pulmonary embolism, cardiac sarcopenia and comorbidities, an important role being occupied by rheumatoid arthritis was explored. Geriatric assessment confirmed clinical frailty and suggested the possible presence of clinically manifest cardiac sarcopenia, especially since the patient was bedridden because of heart failure symptoms. A MRI was recommended, but the patient postponed it.

The analysis of comorbidities, corroborated with the data in the literature and clinical presentation of the patient raised the problem of the role of rheumatoid disease in the occurrence and progression of cardiac affection.

In the given situation, we felt necessary to reduce the risk of thromboembolism induced by arrhythmia and prolonged immobilization. Thus an anticoagulant therapy with calcium nadroparin was initiated. Then we focused our attention on maintaining the cardiac function by digitals therapy and the correction of edemas with diuretic therapy with intravenous furosemide followed by the oral administration of a combination of a loop diuretic and a potassium sparing diuretic (spironolactone). The symptoms of cardiac decompensation were improved, edemas reduced and the patient lost 12.5 kg after 13 days of treatment.

DISCUSSION

According to the Framingham study (1), old age together with smoking, diabetes mellitus (DM), AHT, high levels of LDL-C and respectively low levels of HDL-C are major cardiovascular risk factors. Nevertheless, the particularities of the elderly patient together with the presence of multiple associated comorbidities can significantly modify the cardiovascular risk. This fact can lead to atypical symptoms and course of cardiac diseases, as well as to an unforeseeable rapid progression. Among the elderly, heart failure affects 1% of the subjects and is responsible for about 1/5 of the annual hospitalizations in patients over 65 years old (2).

The structural and functional aging changes in the heart and blood vessels determine *per se* a significant increase in cardiovascular risk in the elderly patient (3). Thus, there is a decrease in the number of cardiac myocytes directly proportional to age. This fact determines the consecutive occurrence of compensatory hypertrophy of the remaining cells and the proliferation of the tissue matrix following the alteration of the fibroblastic function. Some molecular changes occur, with perturbation of ionic balance and abnormalities of myocardial relaxation in protodiastole (4). These structural and functional ventricular changes lead to left ventricular dilatation and reduced LVEF, markers of cardiac sarcopenia in elderly patients (5). Their onset is at the beginning of adult life and continues until old age when they stop, and a significant reduction in myocardial mass occurs, indicating the presence of cardiac sarcopenia (6). According to the recommendations of the European Working Group on Sarcopenia in Older People, sarcopenia is defined as a quantitative and functional reduction in muscle tissue (7). A frequent association between skeletal muscle loss in the elderly and heart failure was found. However, the identification of this entity at heart level raises serious diagnostic problems, because of the high difficulty of evaluation.

A special attention should be given to the presence of the RA, as its prevalence increases with the increasing lifespan of the

population. This disease affects 1% of the world population and 5% of the population over 65 years (8), being often associated with cardiovascular complications. In Romania, it is estimated that RA affects up to 3% of elderly population (9).

A series of studies have analyzed the relation between RA and cardiovascular disorder. Among these patients it was noticed an increased cardiovascular mortality, which can be attributed on one side to accelerated atherosclerosis and, on the other side, to the development of heart failure. Also, the incidence of myocardial infarction in these patients is high, and mortality within the first 30 days after an ischemic coronary event is higher. From this point of view, we can consider that the cardiovascular risk of the RA patients is comparable to that of the diabetic patients (10).

The cardiovascular disease can begin before the first symptoms of RA, with the onset of subclinical immunological changes. The pathophysiological substrate is represented, most probably, by the pro-inflammatory status. At molecular level incriminated are IL-6 and TNF-α by their relationship with insulin-resistance, as promoters of accelerated atherosclerosis (ATS) (11). At the same time, a correlation between the markers of the inflammation and the progression of ATS was noticed. High levels of C reactive protein (CRP) are considered an independent predictor of cardiovascular mortality in patients newly diagnosed with RA (10). At the same time, the erythrocyte sedimentation rate (ESR) level indicates the effect of inflammation on intima-media thickness of the vessels. It was noticed that most patients with RA present ESR levels over 40 mm/h in the first 6 months after the occurrence of heart failure (12).

The main cardiac changes in patients with RA are represented by asymptomatic diastolic dysfunction, left ventricular hypertrophy (LVH), increment in right ventricular pressure and development of PAH. An echocardiographic study (13) identified in these patients the presence of diastolic dysfunction (32.4% of the cases), systolic dysfunction (18.1% of the cases), but also of PAH in about half of the cases.

Patients with RA have twice the risk of developing heart failure, which thus becomes the main cause of mortality. In most cases, its occurrence cannot be explained by the presence of traditional cardiovascular risk factors. It was noticed that, even if LVEF was preserved in most cases, mortality at 1 year following heart failure was higher in patients with RA compared with non-RA patients (2).

There are several studies that identified a high level of association between RA and PH. The presence of the latter represents a negative prognostic factor, indicating an average survival of 2-3 years from the time of symptom occurrence (8, 14).

As to the therapeutic strategy which should be considered in the case of the association of RA with cardiovascular disease, until now the information is contradictory. Angiotensin converting enzyme inhibitors are known for their beneficial effects in the treatment of PAH (15). Certain studies (10) recommended the use of angiotensin converting enzyme inhibitors, since they improve the endothelial dysfunction. Also statins could prove useful, due to their effect against arterial stiffness. By using anti-TNF-α agents, it is possible to have benefits in improving the cardiac function in the patients with incipient heart failure (11), but further studies are necessary in this regard.

CONCLUSIONS

In our study, the development and accelerated progression of cardiac disease cannot be explained only by the presence of traditional cardiovascular risk factors, but also by a series of other factors.

Thus, frailty, malnutrition, dependency, depression, which are characteristic to the elderly patients, represent a vicious circle that acts as a factor promoting the rapidly progressive decline of cardiac function. Sarcopenia, both in the skeletal muscles and especially in the cardiac muscle, appears as a deceiving pathological entity, which creates serious diagnostic and therapeutic problems. It is imperative to consider all the existing comorbidities. Nowadays, pathological entities, once characteristic to a relatively younger patient, are associated with all ages, as is the case of RA. The association of other factors, such as cardiac sarcopenia and frailty, may modify the course of heart failure and strongly interfere with the prognosis of these patients. Intensive treatment of all particular conditions of senior patients will improve their quality of life and will reduce disabilities and dependency.

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NEUROPSYCHOLOGICAL GROUNDS IN THE AWAKE BRAIN SURGERY

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NEUROPSYCHOLOGICAL GROUNDS IN THE AWAKE BRAIN SURGERY (Abstract): Traditionally, the awake cerebral neurosurgical interventions are carried out when the primary motor areas and the areas involved in language are concerned. This procedure is also known as *mapping* (Intraoperative Brain Mapping). The intraoperative neuropsychological protocol supposes the setting of a list of steps and tasks that the patient must perform during the surgery, taking into account the lesion localization (both the cortical and the subcortical structures), the cognitive functions involved and the individual particularities of each patient. As a result of the high validity of certain tests, we propose a common minimal set of tests, which can allow a minimal standardization and replicability. **Keywords**: BRAIN AWAKE SURGERY, BRAIN MAPPING, INTRAOPERATIVE NEUROPSYCHOLOGICAL PROTOCOL.

Relatively recent archaeological findings show that cranium trepanation is not a new procedure, as we could believe. She has been successfully carried out long ago the appearance of the general anesthesia. A number of skulls discovered in Peru shows a healing percentage of 55% of 214 patients (1). From several points of view, the modern era of awake craniotomy starts more than 50 years ago, when Wilder Penfield and Andre Pasquet (1) published their reference work regarding the surgical and anesthetic aspects of the surgical interventions after the local and intermittent administration of sedatives and analgesic drugs. However, as chronological attestation, Penfield seems to be preceded in the cortical stimulation by Robert Bartholow (1) early from 1874. This one describes the case of a 30-year-old patient, Mary Rafferty, who addresses to him for a consultation, with a cranial orifice of 51 mm, most probably acquired after a scalp carcinoma. Inspired by the testing on animals of David Farrier, Bartholow (1) applies a low-intensity electric current in different areas of the exposed brain of his patient. These stimulations lead to the appearance of certain motor reactions. Several concepts highlighted by Penfield and Pasquet are still relevant.

The cognitive neurosciences benefit nowadays from a better knowledge of the neuronal grounds of the cognitive processes

and their clinical applications, mostly in the neurosurgical practice. Over the last decades, remarkable progress has been made in the non-invasive cortical mapping (magnetoencephalography, functional MRI), but also at the level of the invasive mapping (awake intraoperative cortical stimulation) (2). The main reason in using awake craniotomy is the cortical *mapping*, the observation of cortical stimulation effects on the cerebral functions and at the same time the reduction of lesions in the adjacent eloquent tissue during tumor resection. Traditionally, the awake cerebral neurosurgical interventions are carried out when the primary motor areas and the areas involved in language are concerned, but are not limited to them.

After the analysis of a numbers of articles, we were not very surprised to find out that the non-verbal functions mapping received less attention, even though the functional deficits, other than aphasia, can have an equally strong impact on patient's activities and on his quality of life. Visual-spatial and cognitive deficits are more frequently reported after the detailed and extended neuropsychological assessments. In several countries, a diagnostic of hemianopsia or unilateral spatial neglect leads to the interdiction to drive (3).

Verbal and nonverbal functions' mapping in the dominant and nondominant hemisphere - electrical brain mapping findings

Language mapping

The perisylvian areas of the dominant left hemisphere are classically described as being involved in language (4). Consequently, specific language tasks have been enforced during surgeries for the tumors located in these areas, in order to avoid permanent postoperative deficits of the written or oral language. For instance, the spontaneous speaking, counting, objects and actions naming, recognizing famous personalities, reading and written have been used for the mapping of the superior and posterior areas of the temporal lobe, inferior parietal lobe and lateral part of the frontal lobe.

Because of the intraoperative tasks, the electric mapping made possible the establishment of anatomic and functional correlations which led to an updated vision of language connections in general. Therefore, a new paradigm was proposed, based on a parallel and interactive distribution model of the network at a higher scale. This model includes an anterior path which connects the orbitofrontal, prefrontal and dorsolateral prefrontal areas with the temporo-occipito-parietal regions, by means of the inferior fronto-occipital fasciculus. This anterior path is involved in the semantic processing and the direct electrical stimulation during the denomination tests determines semantic par aphasias. Moreover, the posterior path interesting the superior longitudinal fasciculus, which connects the inferior frontal gyrus and the ventral premotor cortex with the superior temporal cortex and passes through the inferior parietal lobe, is involved in the articulatory components of language. The direct electrical stimulation of these structures causes phonemic par aphasias and dysarthria. Recent articles presenting the morbidity profile describe a percentage of 13% of the patients with dysphasia after an aggressive resection of parietal lobe gliomas (5).

Other cortico-subcortical regions of the left hemisphere are also involved in speech articulation and namely, the left additional motor area (dominant hemisphere), the anterior part of the insula and the lentiform

nucleus causing dysarthria upon electrical stimulation. These circuits converge towards a final common path in sensorial-motor primary cortex causing a dysarthria or anarthria when stimulated during speech (6). Moreover, this extended network is modulated by cortico-striate circuits, provoking language control troubles or self-regulation upon electrical stimulation. More than that, the posterior part of the inferior longitudinal fasciculus plays a crucial role in the visual recognition in the image denomination tasks, but also in lexical items tasks. A subordinate network serving to the written language was also described, comprising the additional motor area, the Exner area and the superior and inferior parietal lobe, but also the superior longitudinal fasciculus.

Language mapping in patients speaking several languages or the mapping of sentence translation has been carried out in bilingual and polyglot patients. Therefore, a sub circuit involved in language, dedicated to the change/switching has also been remarked. The control of choosing the spoken language can temporarily be affected mainly during the stimulation of the left caudate nucleus (7) (which provokes perseverations), showing at the same time its role in the executive functions, such as selection, inhibition, planning and attention processing.

Finally, the right hemisphere, described as having a "mirror" configuration is involved in prosody, the semantic processing of words and of the speech, the processing of the context and of language pragmatic abilities, beside its involvement in executive functions, mainly the attention and the working memory.

Nonlanguage mapping

With regard to the sensorial-motor functions, the classical intraoperative neurophysiological monitoring using evoked potentials or motor stimulation during the general anesthesia, allows the mapping through muscle contractions during the procedure and prevents therefore hemiplegia. But movement is not action. By opposition, the direct electrical stimulation in patients in vigil/awake phase allows the objectivisation of certain high level perseverations in the visual-spatial processing due to the mapping, not only of the motor function, but also of the somato-sensorial and visual function and even of the spatial cognition. The execution of movements in the contralateral hemi body, when the resection comes very close to the pyramidal and thalamocortical paths, brings valuable information in preserving the fine motor activity, essential for a normal life standard.

In the same train of thought, a complete hemianopsia can have a major negative impact on patient's life quality, affecting his daily activities, mainly because the development of this type of deficiency leads in most cases to the interdiction to drive. Visual pathways mapping is possible by using the direct electrical stimulation of the awake patient in case of tumors from temporal-occipital junction, through the appearance upon stimulation of the positive visual responses (phosphines or illusions such as metamorphopsia) and negative, such as visual field defects or blurred vision (8). Therefore, hemianopsia or the visual field deficits should be avoided.

Moreover, the cortical areas (mainly the right supramarginal gyrus or the superior temporal cortex) as subcortical paths (the right superior longitudinal fasciculus) serving to the spatial cognition, can be identified and preserved during the resection of

the parieto-occipital junction, asking the patient to carry out tests such as the line bisection task (the patient must draw a sign at the middle of a line and one presents to the patient a range of several lines placed in different positions as compared to the margins of the paper or of the computer screen). The cerebral structures highlighting the vestibular functions, mainly the superior temporal gyrus and the parietal-insular cortex, connected by the superior longitudinal fasciculus of the "non-dominant" hemisphere for language, can also be objectivized and spared during the intervention for the temporal-parietal lesions, using the direct electrical stimulation in the awake condition, having as result the reproducible vertigo.

Other superior cognitive functions received less attention, but they can however be tested during the awake mapping procedure (for instance, calculation tests during the left parietal resections, mainly in the left angular gyrus). The stimulations during the memorization, retention/ storage or delayed memory were also used for the mapping of the mnesic function, by means of the awake craniotomy procedure, involving the premotor areas in the dominant hemisphere, the left anterior temporal lobe or the fornix. The frontal visual field, which provokes saccades and is related to the attention and to the working memory, was also investigated. The use of a non-verbal semantic association test during the intraoperative stimulation highlighted the neural basis, accentuating the multimodal semantic processing (verbal and non-verbal).

The trans-modal (visual-verbal) association tasks were carried out by the patients during the resections of tumors located between the left dorsolateral prefrontal cortex and the temporal posterior cortex, which is connected through the inferior occipito-frontal inferior fasciculus. Emotional aspects of feeling recognition at the level of the superior part of the face, at the level of the eyes ("mirror of the soul") were also described in patients with posterior perisylvian tumors. Finally, the use of a song interpretation task in patients that were vocal singers was reported in five cases, describing dissociations between speech and melodicism outside the primary sensorial-motor area.

The need to develop a personalized protocol

We must be aware of the fact that the eloquence of the functional areas cannot be accurately anticipated only based on anatomic structures. The anatomic knowledge is necessary but not sufficient to operate with a high degree of certainty mostly in the "functional neurooncology". Therefore, neurosurgeons must benefit from the advantages of the direct electrical stimulation technique in order to develop customized protocols for each patient, rather according to the functional borders than to the tumor borders in gliomas resection. In order to support these ideas, a major anatomical and functional individual variability was proved, by using the functional neuroimagery in healthy volunteers, but also by using the direct electrical stimulation in patients with epilepsy. In a recent report, Ius et al. 2011 (9), proposed an atlas of the functional resections in patients who developed a low-grade glioma, mainly represented by the main association paths which should not be resected if one wants to avoid permanent deficits. Moreover, the authors found eloquent areas which can be compensated, mainly at cortical level. The

authors concluded that the identification of a given functional area cannot be accurately carried out before the intervention because of the plasticity, conferring a crucial role to the electric mapping for the "online" anatomical and functional correlations and the preservation of the eloquent areas during the awake brain surgery.

Furthermore, the intraoperative mapping through direct electrical stimulation is not only an instrument, but it is also considered an access door to the functional network which allows us a better understanding of the neural basis, by means of the cerebral processes. The neurophysiologists brought into discussion ethical aspects of this invasive technique, in which we must not forget that the main objective is patient's benefit through the maximal resection and the preservation of the important functions. As a consequence, it is important to find equilibrium in the number of tests strictly necessary in order to preserve patient's life quality and not enforce too many tests which can be time consuming over the intervention, inefficient and possibly harmful.

A multidisciplinary approach involving neurosurgeons, neurologists, neuropsychologists and speech therapists is crucial in order to build an adequate intraoperative protocol and practically adapted to each individual patient, with the selection, to the possible extent, of sensitive tests capable of assessing more than one cerebral function. For instance, the classical picture naming task allows an integrated, complex testing which involves visual recognition, semantic processing, lexical access/vocabulary, phonological decoding and verbal production at the same time. An accurate analysis, in real time of the possible functional deficiencies produced by direct electrical stimulation or the resection itself should be carried out by a specialist during the tumor resection.

The selection of the intraoperative tasks must be based both on the features of the patient and of the lesion. The neuropsychologist must first of all determine the hemisphere dominance. The identification of the manual dominance is not sufficient, as cases of postoperative crossed aphasia were reported. Therefore, the most important factor in assessing the cerebral dominance is the existence of certain preoperative language transformations, even subtle ones, highlighted by a detailed neuropsychological examination.

Furthermore, patient life features (job, hobby, habits) must play an important role in selecting the intraoperative tasks. As compared to a standard protocol, one must use specific tests in order to assess the individual needs of each patient. For instance, a calculation test can be necessary for a mathematics teacher, mostly when the lesion is located in the left parietal lobe; a reasoning task is important for a lawyer; a spatial cognition task is crucial for a military strategist or for a cartographer, while a synthesis and analysis and mental rotation task is absolutely necessary for a draftsman or an architect.

Lesion features (localization, volume) must also be taken into account. A lesion does not affect one specific anatomic structure only, but it can involve the entire cerebral functional network.

Preoperative psychological counseling and assessment

It is recommended to explain to the patient the intraoperative procedure and to present him to the members of the surgical team, but also to perform a neuropsycho-

logical assessment at least one day before the surgical intervention. The neuropsychological assessment aims at determining the base line of neurocognitive functions, carrying out the tests included in the intraoperative protocol, for familiarization purposes and for removing the items causing difficulties to the patient, so as not to obtain false negative intraoperative responses. The involuntary movements, but also the inhibition of the voluntary ones can create to the patient a discomfort, anxiety accompanied by vegetative manifestations and even the alteration of the vital signs (10, 11). All these manifestations without an acceptance and a good compliance of the patient, based on the information and gaining the trust of the patient can threaten the proper development of the neurosurgical procedure. Therefore, besides the neuropsychological assessment sessions, the patient is informed with regard to the neurosurgical technique and procedure to which he will be subject and one shall explain to him the importance of his cooperation – he shall be made aware of the need to have an active – participative behavior during the surgery. Moreover, he shall be informed that during the stimulation can appear movements or reactions that the patient did not want to perform or, to the contrary, that he wants to perform, but he is not able to do so. He must be assured that these incidents are transient and are caused by the direct cortical stimulation and will not have long-term consequences. As we already mentioned, it is important that the intraoperative neuropsychological protocol be customized, adapted according to the interested cerebral area, to the eventual existing deficits, so as to obtain an optimal cooperation of the patient and to take into account the individual features of the patient.

The neuropsychological assessment

It is recommended to perform an objective and reproducible neurocognitive assessment, in the preoperative and postoperative stages, not only for assessing the neurosurgical side effects on the cognitive functions, but also for adapting the eventual functional rehabilitation in accordance with the results of the neurocognitive assessment (12).

The neuropsychological protocol in the cortical *mapping* imposes the determination of the base line, by the assessment of the following functions: temporal and spatial orientation, orientation to self and others; verbal and visual memory, working and delayed memory; attention; language: fluency, comprehension and repetition, vocabulary, written form, calculation; visuospatial analysis and synthesis, mental rotation; testing the rough motor function and the fine movements; tactile, visual, auditory and proprioceptive sensitivity; executive functions, affective mood. Two neuropsychological assessment sessions are needed: preoperative and postoperative.

The intraoperative protocol

In light of the recent data, certain authors propose the drafting of a common minimal protocol, which can test not only the language, but also other cognitive functions, which can be enforced in preoperative, intraoperative and postoperative stages in the awake craniotomies (7). The objective would be to facilitate the replicability between different neuro-oncology centers.

Therefore, in order to meet a minimum standard on pre- and postoperative language testing, the protocol should include: a subjective questionnaire which can inventories

patient's plaints, handedness assessment, verbal fluency assessment / verbal spontaneity, a picture naming task with the calculation of the reaction time, a semantic fluency test, a timed semantic association task and also a timed vocabulary task. Of course, it is possible that the testing battery be extended in accordance with the variables related to the individual habits of the patient. The list of specific tasks can comprise the symbolic side of language - metaphors, repetition, vocabulary and written form.

The proposed protocol for testing the pre- and postoperative non-verbal functions can include: a subjective questionnaire which may inventorize patient's complaints also, the assessment of the information processing speed, the working memory, the executive functions (flexibility, inhibition), ideomotor and reflexive praxis and visuospatial cognition. And here appears the freedom of adding other specific tasks which will assess different aspects of social cognition or feelings, for instance.

Even though this set of tests is a proposal only, must be noted the fact that they have already been validated by favorable functional results, reported in hundreds of cases of extended resection of gliomas, carried out according to the functional limits. Besides language, it has been proven that intraoperative mapping also allowed the preservation of the complex neurocognitive functions, such as the working memory, the spatial cognition, calculation, linguistic switching in bilingual patients, trans-modal reasoning and executive functions.

CONCLUSIONS

The selection of the tests which shall constitute the intraoperative neuropsychological protocol must take into account both lesion localization and the functional areas involved, but also the subcortical association paths and the individual habits of the patient (job, hobby and customs). By the detailed assessment of the general condition of the patient, of the global cognitive functions and of the verbal skills, the psychologist has an important contribution in selecting the patients for the cortical mapping procedure, but also in its intraoperative development. The intraoperative neuropsychological protocol should contain a minimal common set of tests which would also allow a certain degree of standardization and statistical analysis, but which can be adapted to each patient with additional tests specific to the individual habits of each patient.

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NEWS

PUBO-FEMURAL DISTANCE: A MORE ACCESSIBLE TECHNIQUE FOR EARLY DIAGNOSE OF DEVELOPMENTAL HIP DYSPLASIA IN NEWBORNS

Ultrasound imaging is, and has been for some time now, the gold standard for early diagnose of developmental hip dysplasia. Although there are several techniques, they usually require an experienced pediatric orthopedic surgeon or radiologist to interpret the results. The pubo-femural distance represents the distance between the lateral aspect of the pubic bone over the acetabular incisure and the medial surface of the cartilaginous epiphysis of the femoral head, both from a neutral position and with approximately 90 degrees hip flexion, without rotation, adduction or abduction. The coronal view necessary for obtaining a correct measurement is far more easily obtained that the incidence that the other methods require, making it a more accessible technique for the less experienced practitioners, but with similar results. This method could make possible a screening program for detecting developmental hip dysplasia, without the extensive training (Teixeira SR, Dalto VF, Maranho DA et al. Comparison between Graf method and pubo-femoral distance in neutral and flexion positions to diagnose developmental dysplasiaof the hip. *Eur. J.Rad.* 84 (2015) 301–306).

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THE ACUTE SCROTUM IN CHILDREN

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THE ACUTE SCROTUM IN CHILDREN (Abstract): The acute scrotum syndrome is a medical-surgical emergency and the recognition of this condition by both healthcare professionals and the general population may result into the patients' coming in earlier for medical examination and into the preservation of the gonad in case of torsion. The purpose of this retrospective analytical research is to point out specific epidemiological aspects in pediatric patients suffering from acute scrotum, and to review the existing diagnosis and treatment options. The study included 208 patients, of whom 16 with vanishing testis and 192 with acute scrotum (torsion of testis 25.5%, torsion of the hydatid of Morgagni 68.2%, epididymo-orchitis 5.2%). The torsion of the hydatid of Morgagni occurs in boys with a mean age of 10 years and it involves both testes equally, whereas the torsion of testis usually occurs around the age of 13 and is twice more common in the left gonad. Another significant difference between the two conditions is the inflammatory syndrome, which occurs in 45.4% of the children with torsion of testis versus only 18.2% in the torsion of hydatid. Only one out of six testes torted during the neonatal period could be saved (16.6%); the gonad preservation rate was as high as 68.2% in the group of patients with testis torsion occurring outside the neonatal period. These alarming data are accounted for by the non-recognition of the severity of the condition and by the delayed surgical therapy, which occurs on the average 20 hours after the testis torsion has set in. If the asepsis and antisepsis standards are observed, patients with torsion of the hydatid of Morgagni or torsion of testis require neither fluid sampling from the tunica vaginalis for culture, nor antibiotic therapy. **Keywords**: ACUTE SCROTUM, TESTICULAR TORSION, TESTICULAR APPENDAGE, EPIDIDYMO-ORCHITIS.

The acute scrotum syndrome refers to the sudden onset of pain, swelling and other inflammatory phenomena in a hemiscrotum, which may be caused by conditions such as orchiepididymitis, torsion of testis or appendix testis, testicular hematoma due to local injury or hematocele due to adrenal hemorrhage during the perinatal period, testicular tumor, strangulated inguinal or scrotal hernia, acute scrotal edema, Henoch-Schönlein purpura. Strangulated inguinal hernia and testis torsion are surgical emergencies, whereas orchiepididymitis may only require medical therapy. Scrotal injury with hematoma or incarcerated hernia may be diagnosed according to anamnesis, whereas Doppler ultrasound scanning may differentiate the inflammatory phenomena due to orchiepididymitis from the absence of blood flow due to testis torsion (1). A detailed history, through physical examination of the genitalia and

medical imaging examinations such as Doppler ultrasound scanning, and sometimes scintigraphy or IRM, usually allow a preoperative differential diagnosis. Nevertheless, even the slightest diagnosis doubt requires surgical examination designed to rule out testis torsion as the cause of the acute scrotum and to save the gonad (2,3,4). The purpose of this research is to emphasize specific epidemiological aspects in pediatric patients suffering from acute hemiscrotum syndrome, and to review the existing diagnosis and treatment options in a regional Romanian center.

MATERIAL AND METHODS

We have conducted a retrospective analytical study on 257 patients hospitalized and treated in the Pediatric Surgery Clinic of the "Sfânta Maria" Children's Hospital of Iaşi over a period of about 3 years (January 2012 – February 2015). Our hospital is the only tertiary hospital in the Moldova area, which treats pediatric patients coming from 7 counties. The patient data were retrieved from the hospital's computerized database, after entering the disease code N44, which stands for "Torsion of Testis". The system generated 257 patients encoded with this diagnosis, from a total number of 4816 diagnoses. Only 208 of them were eligible for our research, the remaining 49 being excluded as their diagnoses did not correspond to the aim of our study. We carried out an analysis and statistical processing of the patients' demographic data, diagnosis on hospital discharge, age on the first hospitalization, number of hospitalization days, personal history, paraclinical tests and examinations, treatment method, operating technique, subsequent evolution, pathology findings. All patients were seen by at least one specialist doctor, routine complete blood counts, inflammatory tests (ESR, Fibrinogen) and urinalysis were performed. Sometimes, additional examinations were conducted, i.e. abdominal and scrotal ultrasound scanning, Doppler ultrasound scanning and even CT in uncertain cases. The best course of care adopted in our clinic recommends a surgical procedure in all the acute hemiscrotum syndrome cases, in whom suspected testis torsion cannot be ruled out by clinical and paraclinical means, or in whom the painful symptoms due to torsion of the hydatid of Morgagni do not subside within a few hours from hospitalization. The statistical analysis was conducted using SPSS for Windows and Microsoft Excel. The data were reported as means ± standard deviations. The differences between groups were analyzed using the t Student test, and the level of statistical significance was $p \leq 0.05$.

RESULTS

131 of the 208 patients included in the study were finally diagnosed with torsion of the hydatid of Morgagni, 65 with torsion of testis, 10 with acute orchiepididymitis and 2 with post-traumatic scrotal hematoma. Excluding the 16 patients who came in for congenital undescended testicle, which postoperatively proved to be vanishing testis, among the acute scrotum group the testis torsion rate was 25.5%, the rate of torsion of the hydatid of Morgagni was 68.2% and orchiepididymitis 5.2%.

After group analysis, we noted that most of the patients came from urban areas (1.33 ratio). The overall mean age of the patients was 9.66±4.47 years (Fig. 1). 44 of the 65 children finally diagnosed with testis torsion came in for acute scrotum syndrome at a mean age of 13.01±3.93 years, 5 came in immediately after birth and were diagnosed with neonatal testis torsion and 16 patients came in for congenital undes-

cended testicle at the mean age of 3.10±3.00 years; their postoperative diagnosis was vanishing testis. As concerns the mean age on consultation for acute hemiscrotum syndrome, a comparison between the 131 patients with torsion of the hydatid of Morgagni (9.82 years) and the 49 with torsion of testis (11.68 years), reveals a difference of about 2 years, which is statistically significant (p=0.001). The difference increases if we exclude from the second group the five patients who came in during the neonatal period, i.e. 9.82 versus 13.01 years. The torsion of the hydatid of Morgagni occurred on the left side in 67 patients, on the right side in 61 patients and on both sides in 3 patients, whereas the torsion of testis impaired 21 patients in the right side, 42 patients on the left side and 2 patients on both sides.

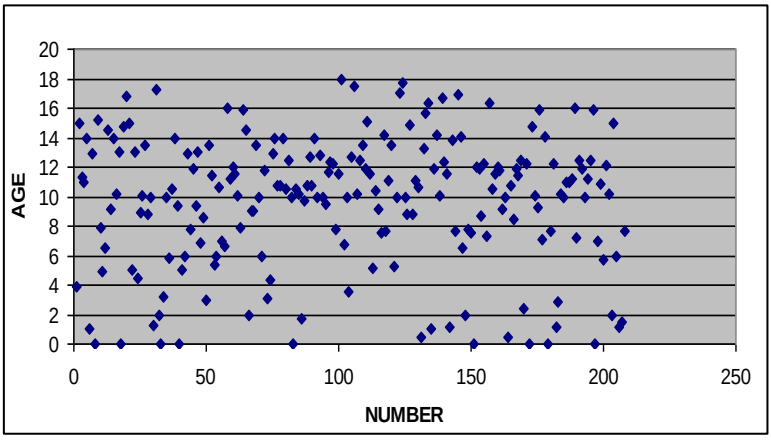

Fig. 1. Distribution of patients by age

The analysis of the personal history of the 131 patients with torsion of the hydatid of Morgagni revealed a prior injury in 7 of them and an intercurrent respiratory condition in 5 other children. Associated conditions were detected in 28 children, 14 of whom suffered from uro-genital conditions. The mean period of time elapsed between symptom occurrence and the patients' seeking medical assistance was 21 hours (between one hour and 7 days). In 44 of the patients the symptoms were edifying, and the doctor on call did not request medical imaging tests, whereas in the other 87 children an abdominal and scrotal ultrasonography was performed. The lab tests revealed an inflammatory syndrome (high leukocytosis, ESR and Fibrinogen levels) in 24 children (18.2%) and nutritional anemia in 9 of them (6.8%). Only three patients were prescribed conservative treatment consisting of bed rest, non-steroidal anti-inflammatory drugs and cold compressions on the aching spot, and they were fully recovered. The other patients underwent surgery under general anesthesia, which consisted of hemiscrotum exploration, removal of the torted hydatid of Morgagni (Fig. 2).

A biopsy of the tunica vaginalis was performed in 9 patients, and fluid was sampled in 55 other patients for culture and antimicrobial susceptibility testing. The culture was negative in all the 55 cases. After the surgery, 59 patients were given antibiotic therapy, namely cephalosporins

or beta-lactams. The postoperative evolution of four patients was marked by the occurrence of a scrotal hematoma, three of whom underwent a second surgery to remove it. Another patient needed a second surgery for postoperative scrotal abscess. The other patients recovered rapidly, and they were discharged, on the average, 2.6 days after the surgery. No pathology tests could be conducted on the removed hydatid of two of the patients due to the fact that the removed mass was too small. In all the other patients, the microscopic examination revealed signs of stasis, inflammation, hemorrhage, hemorrhagic necrosis (Fig. 3).

Fig. 2. Torsion of the hydatid of Morgagni – intraoperative aspect

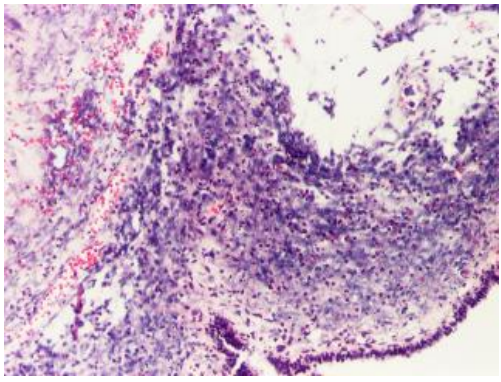

Fig. 3. Hydatid of Morgagni – haemorrhagic necrosis and leukcocytic exudate – HEx100

Three of the 44 patients with torsion of the testis who came in for acute hemiscrotum syndrome outside the neonatal period had suffered prior injury. Associated conditions were detected in 8 children, as 6 of them were also suffering from uro-genital diseases. The mean period of time elapsed between symptom occurrence and the patients' seeking medical assistance was 20 hours (between one hour and 11 days). The lab tests revealed an inflammatory syndrome in 20 children (45.4%) and nutritional anemia in 3 of them (6.8%). Surgery was performed without preoperative imaging tests in only 6 patients, whereas an abdominal and scrotal ultrasonography was performed in the other 38 patients. Since it is not readily available in our hospital, Doppler ultrasonography was conducted preoperatively only in 6 patients, and postoperatively in 10 patients. A pelvic CT scan was performed in an adolescent suspected of testis tumor, but the postoperative pathological examination set the diagnosis of old testis torsion, with total glandular necrosis (Fig. 4). The testis was surgically approached through the scrotum in all the patients, the testis was detorted (between 180° and 1080°), the spermatic cord was injected with xylocaine and, depending on the recoloring degree, the surgeon decided to keep or to ablate the gonad (Fig. 5). Orchiectomy was performed in 7 patients due to the clearly necrotic gonad (the mean period of time before going to see a doctor was 4.4 days for these patients), whereas in other two patients orchiectomy was carried out after 48 hours (on second look). In the other 35 patients, the doctors decided to keep the gonad on condition of its regular ultrasonographic follow-up, and the diseased progressed (one and three-month follow-up) in other 5 patients. As concerns the 14 patients in whom the dis-

ease progressed (31.8%), 7 of the group of 21 were living in rural areas and 7 of the group of 23 were living in an urban environment. Thus, there were no significant differences as concerns the patients' living environment. Overall, after detorting, the three-month follow-up revealed that 30 patients (68.2%) recovered. Orchidopexy was performed on the impaired side in the patients in whom the gonad was preserved, and contralateral orchidopexy was preferred for 7 patients. Two patients required a second surgery, one for the excision of a postoperative hematoma and the other for pus draining. The mean hospitalization duration was 3.7 days for the first surgery.

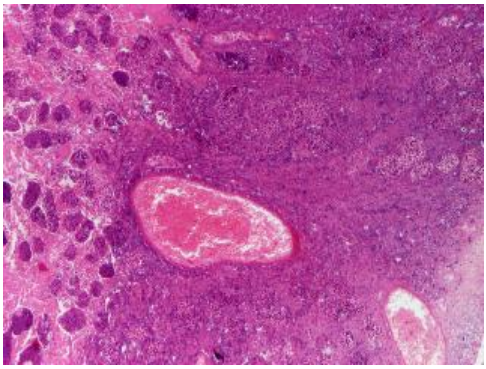

Fig. 4. Testicular torsion - haemorrhagic necrosis – Hex40

Fig. 5. Testicular torsion – intraoperative aspect

Two of the 5 patients with neonatal testis torsion, who came in immediately after birth for acute scrotum suffered from torsion on the right side, two on the left side and one on both sides; orchiectomy was performed in 2 children on the first surgical procedure as there were no doubts about the ischemic necrosis diagnosis, whereas in a patient the gonad was initially preserved but it was finally removed two days later (on second look). Contralateral orchidopexy was also performed in all these patients. The right testis of the child with bilateral torsion was removed on account of the advanced necrosis, whereas his left testis was detorted and fixed, but it later progressed to testicular atrophy. In another patient the testis was detorted and the spermatic cord was injected with xylocaine. The testis was preserved and the 3-month follow-up ultrasonography revealed the patient's recovery. Thus, only one out of six testes could be saved (16.6%).

Fluid was sampled from the testicular vaginal tunic of 29 of all the 49 patients with acute hemiscrotum syndrome due to testis torsion, and the culture was positive in 3 children younger than 6 months; 34 children were administered broad-spectrum antibiotics.

Ten patients, with a mean age of 9.97 years (two newborns, one 5 month infant and 7 children older than 10 years), with acute scrotum syndrome were diagnosed with acute orchiepididymitis intraoperatively. Fluid from the tunica vaginalis was sampled and the result was positive in five of them. All the patients were administered antibiotic therapy. The patients were examined for genitourinary tract abnormalities, detected in 3 of them, and urinary infections, present in two patients. The evolution was poor in a single child with cicatricial phimosis, recurrent urinary in-

fections and postoperative scrotal abscess, who required orchiectomy.

Two boys exhibited right vanishing testis, one suffered from bilateral torsion and 13 suffered from left testis torsion. The congenital undescended testicle was examined by inguinal approach in 9 children and by laparoscopic approach in 7; biopsy of the blind-ending deferent duct was performed in 12 patients (Fig. 6).

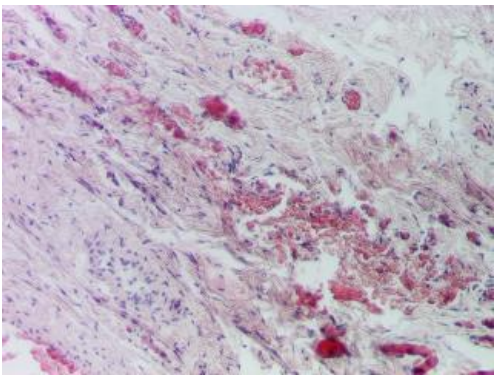

Fig. 6. Vanishing testis –connective-vascular tissue– HEx100

DISCUSSIONS

The medical technological breakthroughs achieved lately have led to the decrease of the number of surgical procedures performed in patients with acute scrotum syndrome. Different studies have proven that only 14-38% of the patients with acute scrotum suffered from testis torsion, despite the physical examination and their personal history that would indicate surgery (5). Our research revealed a 25.5% testis torsion incidence rate, a 68.2% Morgagni hydatid torsion incidence rate and a 5.2% orchiepididymitis incidence rate.

Torsion of appendix testis, more precisely of the hydatid of Morgagni and more rarely of the appendix epididymis, is the most common cause of acute scrotum syndrome in children. The hydatid of Morgagni is an embryonic remnant originating in the Müllerian duct, attached to the upper testis pole and occurring in about 90% of the patients. The symptoms evocate an acute scrotum syndrome, the pain sets in progressively, unlike in torsion of testis, and is less sharp, and is sometimes located only in the upper testis pole, where the torted hydatid may be palpated and viewed (blue dot sign). In order to avoid about two thirds of the surgical procedures that are not absolutely necessary each patient should undergo medical imaging tests, more precisely Doppler ultrasound scanning. Ultrasonography sometimes reveals the hydatid as a hypoechogenic mass, which is highly vascularized due to inflammation, and a reaction hydrocele may also occur. The Doppler ultrasound scan may reveal and quantify the blood flow in the testis and the scrotum, and may provide information about the structural integrity of the gonad. The treatment may be conservative and consist of rest, ice packs, scrotum lifting, anti-inflammatory and analgesics. If the diagnosis is uncertain, the pain very sharp or does not subside further to treatment, surgery is recommended for the removal of the necrotic hydatid (6). Unfortunately, since Doppler ultrasound scans could not be performed on the patients hospitalized in our clinic and since there were no well established therapeutic protocols, far too few children could benefit from this examination method and therefore only three of the patients with torsion of the hydatid of Morgagni received conservative treatment, which considerably increased the surgical procedure rate. Nevertheless, this is not a bad approach, since most authors recommend immediate surgery if there is even the slightest doubt

about the diagnosis, all the more so as any medical imaging technique may have false negative results and thus delay the surgical procedure that may save a torted testis (2,3).

Torsion of testis is a surgical emergency that may occur at any age and, if improperly treated, may lead to infertility and psychological impairment. The annual incidence rate of this condition is 1/4000, and about 61% of all the patients are younger than 25 years, and exhibit bimodal age distribution, namely during the neonatal period and around the age of 13 years (7). Our research supports these data, as all the patients younger than 1 year suffered from testis torsion. When comparing the mean age at which the patients came in for acute scrotum, we noted a significant difference of about 3 years between the two groups, namely the 131 patients with torsion of the hydatid of Morgagni (9.82 years) and the 44 patients with torsion of testis (13.01 years). Similar studies have also reported older age of patients with torsion of testis than of patients with torsion of the hydatid of Morgagni (8).

In children, testicular torsion usually occurs in the absence of any predisposing factor, since a prior scrotal injury or intense physical effort was only reported in 4-8% of the cases (9). In our case, a prior injury was reported only in 5.3% of the children with torsion of the hydatid of Morgagni and in 6.8% of the children with testis torsion. Other possible triggering factors are the sudden testis volume increase during puberty, congenital undescended testicle, testicular tumors, low temperatures associated with an exaggerated cremasteric reflex, presence of a long intrascrotal spermatic cord (9). Whereas appendix testis torsion seems to affect both sides equally (27 left versus 21 right), left side impairment by torsion of testis was twice more common than right side impairment, which was also reported by other literature studies (8). Another significant difference between the two conditions is the occurrence of the inflammatory syndrome in 45.4% of the children with torsion of testis versus 18.2% in those with torsion of hydatid. Other studies also suggested that leukocytosis and the NLR (neutrophil to lymphocyte ratio) are predictive of inflammatory processes and also torsion of testis (10,11).

Detorting during the first 6 hours is associated with over 90% chances of saving the testis, whereas the same procedure applied more than 24 hours from the event only saves less that 10% of the testes (12). Surgery is sometimes delayed due to lack of medical education, to children living in disadvantaged families who do not seek medical assistance, to long distances to the nearest hospital or sometimes to the failure of the first doctor who examines the child to recognize the condition. In our research, the mean period of time elapsed between symptom occurrence and the patients' seeking medical assistance was 21 hours for children with torsion of the hydatid of Morgagni and 20 hours (between one hour and 11 days) for those with torsion of testis, which explains the testicular preservation rate of only 68.2%. In our research, the patients' living environment did not influence in any way their postoperative evolution. The surgeons usually decides whether to preserve or remove the gonad during the surgical procedure, depending on the time elapsed from symptom occurrence, on the degree of torsion, on the color of the testis after detorsion, and possibly after the spermatic cord has been injected with xylocaine. If the black-purplish color persists, this indicates irreversible glandular necro-

sis and recommends removal, whereas if the testis becomes pink again, it should be preserved and orchidopexy should be done. The torted testis preservation rate varies between 58 and 75.7% in various studies (13,14). Most authors recommend bilateral orchidopexy after detorsion, in order to prevent any reoccurrence (15), but in the absence of therapeutic protocols this was not complied with throughout our study. Orchidopexy is done by fastening the testis against the scrotal wall using two or three suture points with nonabsorbable stitches crossing the tunica albuginea. Although it has been argued that this technique may trigger the production of antispermatic antibodies, this has not been accurately proven and, at any rate, the torsion reoccurrence risk seems higher (16).

Originally described by Taylor in England, in 1897, and then 50 years later by Campbell in North America (17,18), perinatal testicular torsion is rare yet controversial event as to its etiology, clinical form, management and long-term sequelae. Perinatal testicular torsion includes antenatal torsion and torsion occurring during the first 30 days after birth, and it makes up about 12% of testis torsions in children (10.2% in our research). The possible etiopathogenic theories include difficult delivery, breech presentation, multiparity, high birth weight and exaggerated cremasteric reflex (19). Prenatal torsion has minimum clinical symptoms and no discomfort, whereas postnatal torsion has significant clinical symptoms, such as pain and acute inflammatory phenomena in a previously healthy testis. Since antenatal ultrasonography does not have high sensitivity to this abnormality, antenatal torsion is usually detected on the routine newborn checkup and it is usually too late to save the gonad. Therefore, the management of this condition is highly controversial. Some authors only recommend clinical and ultrasound monitoring and avoidance of surgical procedures, whereas others suggest immediate scrotal or inguinal exploration and contralateral orchidopexy (20). According to other authors, if the torsion occurs much before the birth (the inflammatory signs on the hemiscrotum are discrete), surgery can wait and the patient may receive conservatory treatment or elective orchiectomy and contralateral orchidopexy will be done; if the torsion occurs at birth or after birth, surgery is a major emergency and is to be done even in the absence of any medical imaging tests (21). The advocates of conservative treatment rely on the low incidence of contralateral torsion and support the avoidance of any anesthetic and surgical risks during the neonatal period. Moreover, if the parents are correctly informed, they will bring the child to the emergency room on the first signs and symptoms of torsion of testis, which means that surgery may be done on time. In a study conducted on 48 cases of bilateral perinatal testicular torsion, despite the prompt surgical intervention in 46 of these patients, only 3 testes exhibited a blood flow on postoperative Doppler ultrasonography (20). In our research, only one patient exhibited signed of recovery at the three-month follow-up, and the gonad had to be removed due to advanced necrosis in all the other cases.

If the asepsis and antisepsis standards are observed, patients with torsion of the hydatid of Morgagni or torsion of testis confirmed and surgically treated require neither fluid sampling from the testicular vaginal tunic for culture, nor antibiotic therapy. Nevertheless, fluid was sampled from the tunica vaginalis of 55 patients with torsion of the hydatid of Morgagni, for culture purposes, and the results were

negative in all these cases; furthermore, 59 patients were administered intravenous antibiotic therapy. Only one patient living in a disadvantaged social environment and who did not observe the postoperative hygiene requirements developed a scrotal abscess 5 days after the surgery and required a second surgical procedure. As for the patients with torsion of testis, only 3 of the 30 samples collected were positive, all three in infants under the age of 6 months; on the other hand, 34 patients were administered broad-spectrum antibiotic therapy. In our opinion, according to these data, fluid sampling from the testicular vaginal tunica is only necessary for patients with orchiepididymitis or strong inflammatory phenomena. Prophylactic antibiotic therapy is recommended only to infants with acute scrotum who underwent surgery.

Orchiepididymitis is the most common cause of acute scrotum syndrome after puberty, with an incidence peak at the age of 40-50 years, but it may also occur in babies. In our research, the incidence rate of this condition was 5.2% versus 9% or 16% in other studies, which also included patients older than 18 years (8,22), and the disease affected two categories of patients, namely neonates and adolescents. Surgery was performed in all the patients, who were administered antibiotic therapy. The evolution was positive in 9 out of 10 cases. The most common etiology is urinary tract infection related to possible associated malformations, hematogenous infection or scrotal injury. The treatment is conservative if we are absolutely certain about the diagnosis and it consists of analgesics, anti-inflammatory drugs, ice pack and antibiotic therapy, under ultrasound scanning and biological monitoring. Nevertheless, if there is even the slightest doubt surgery is to be done in order to rule out testis torsion as a cause of acute scrotum.

CONCLUSIONS

Torsion of the hydatid of Morgagni occurs in boys at the approximate age of 10 years and it affects both testes equally, whereas torsion of testis usually occurs at the age of 13 and affects twice more often the left gonad. Another significant difference is the inflammatory syndrome, which occurs in 45.4% of the children with torsion of testis versus only 18.2% of those with torsion of hydatid. Only one out of six torted testes could be saved in the neonates (16.6%), whereas in the group of patients with torsion of testis that were not neonates, the gonad preservation rate was 68.2%. These data are accounted for by the non-recognition of the severity of the condition and by the delayed surgical therapy. Patients with torsion of the hydatid of Morgagni or torsion of testis require neither fluid sampling from the tunica vaginalis for culture, nor antibiotic therapy.

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A CASE OF POLYMYOSITIS WITH INTERSTITIAL LUNG DISEASE ONSET

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A CASE OF POLYMYOSITIS WITH INTERSTITIAL LUNG DISEASE ONSET (Abstract): Polymyositis is an idiopathic inflammatory myopathy of unknown aetiology that affect skeletal muscles causing symmetrical, proximal muscle weakness, and also other internal organs. The investigations reveal elevated skeletal muscle enzyme levels and characteristic electromyography (EMG) and muscle biopsy findings. Pulmonary involvement in polymyositis includes respiratory muscle weakness, aspiration pneumonia, interstitial lung disease, infection and drug-induced pneumonia. We expose the case of a young woman (47 years old) who presented to the Pulmonology Clinic with fever, cough, purulent sputum, discrete myalgia, being diagnosed at that moment with interstitial lung disease and treated with antibiotics, low dose of corticosteroids and symptomatic drugs. The evolution was slowly favorable for the respiratory impairment, but the patient developed exacerbated myalgia, muscle weakness, reaching even the impossibility of maintaining orthostatism, and also joint pain. Biological investigations revealed an important hepatocytolysis syndrome and also increased levels of muscle enzyme. The hypothetical diagnosis was polymyositis and to sustain this theory it was performed a muscle biopsy. The patient was transferred afterwards to the Rheumatology Clinic, in order to perform other specific investigations. In our clinic the patient maintained elevated levels of skeletal muscle enzymes and the muscle biopsy revealed polymyositis findings. Also immunological investigations objectified the presence of Jo1 antibodies. Therefore we pleaded for the diagnosis of idiopathic polymyositis, acute form. A multidisciplinary approach is needed in order to establish an accurate diagnosis and to institute a proper treatment. **Keywords:** POLYMYOSITIS, INTERSTITIAL LUNG DISEASE, MUSCLE ENZYME.

The inflammatory myopathies are a heterogeneous group of subacute, chronic, or acute acquired diseases of skeletal muscle. The muscle biopsy identifies an inflammatory infiltrate that, according to its localization and distribution, decidedly contributes to diagnosis. Specialty literature reveals three entities: dermatomyositis, characterized by a specific rash which precedes muscle weakness; polymyositis, with muscle weakness, symmetrical and diffuse, involving the proximal muscles of the neck, shoulders, trunk, hips and thighs and inclusion body myositis (1). Therefore polymyositis is an idiopathic inflammatory myopathy of unknown aetiology that evolves over weeks to months,

affecting adults, rarely children. It's pathognomonic manifestation is represented by symmetrical, proximal muscle weakness. The internal organs are also affected (2). Pulmonary involvement in polymyositis includes respiratory muscle weakness, aspiration pneumonia, interstitial lung disease, infection and drug-induced pneumonia (3). There are cited in literature several cases in which interstitial lung disease represents the onset of polymyositis, the diagnosis in those cases being much harder to establish (4,5,8).

The clinical diagnosis is confirmed by elevated levels of muscle enzyme, the most sensitive being creatine kinase. Also the biological investigations reveal an important hepatocytolysis syndrome and increased levels of lactate dehydrogenase and aldolase (2,6).

By far, the investigation which establishes the accurate diagnosis is muscle biopsy. The specific findings are represented by inflammation and multifocal lymphocytic infiltrates surrounding and invading healthy muscular fibers. In chronic stages connective tissue is increased and reacts with alkaline phosphate (6,7).

CASE REPORT

We expose the case of a young woman (47 years old) that was admitted to the Pulmonology Clinic for fever, cough and purulent sputum and discrete myalgia. The symptomatology had appeared about one month prior to admission. The patient had a history of chronic obstructive pulmonary disease, arterial hypertension and depression. She was a stay-at-home mother, did not smoke or drink and she lived in a rural household.

Clinical examination revealed an influenced general state, with depressive facies, sweaty skin. Pulmonary auscultation objectified crackles disseminated both lung fields.

Biologically the patient presented an important hepatocytolysis syndrome (ASAT=628U/l, ALAT=406U/l), elevated level of lactate dehydrogenase (1187 U/l), inflammatory syndrome (ESR= 20mm/1h, CRP= 48mg/l), with decreased levels of serum proteins (46g/dl).

Chest radiography revealed the presence of infiltrative-nodular opacities on both lung fields, without systematized condensation (fig.1). Bacteriological sputum exam was negative for BK.

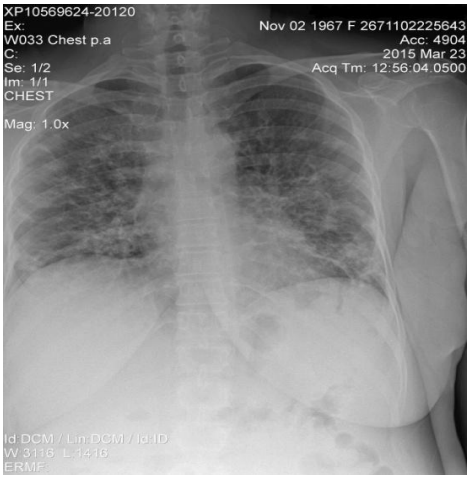

Fig. 1. Aspect of interstitial lung disease on chest radiography.

Corroborating clinical examination, radiological and biological investigations this leads to the establishing the diagnosis of interstitial pneumonia and the patient was treated with antibiotics (Ceftriaxone), low dose of corticosteroids (Prednisone 40mg daily) and symptomatic drugs (to treat fever and pain).

The evolution was slowly favorable for the respiratory impairment, but the patient developed exacerbated myalgia, muscle weakness, reaching even the impossibility

of maintaining orthostatism, and also joint pain. The hypothetical diagnosis was polymyositis and to sustain this theory it was performed a muscle biopsy. The patient was transferred afterwards to the Rheumatology Clinic, in order to perform other specific investigations.

In the Rheumatology Clinic the patient maintained elevated levels of transaminases (ASAT= 375U/l, ALAT= 351U/l), inflammatory syndrome (CRP= 26,03mg/l), increased level of lactate dehydrogenase (1670U/l) and hyposideremia (34 µg/dl). Also the value of creatine kinase was elevated (3013U/l). Anti HCV antibody and Ag HBs was negative, rheumatoid factor also negative. Urine analysis was pathologic with urine culture positive for *Klebsiella* spp.

Musculoskeletal examination objectified the impossibility of maintaining orthostatism and muscular testing revealed MRC= 3-4 for the proximal muscles of upper limbs , MRC= 1 for proximal muscles of legs and MRC= 1-2 for cervical muscle. The patient also presented pain on palpation of topographic points of the knees.

The muscle biopsy revealed polymyositis findings with inflammation and multifocal lymphocytic infiltrates surrounding and invading healthy muscle fibers (fig.2).

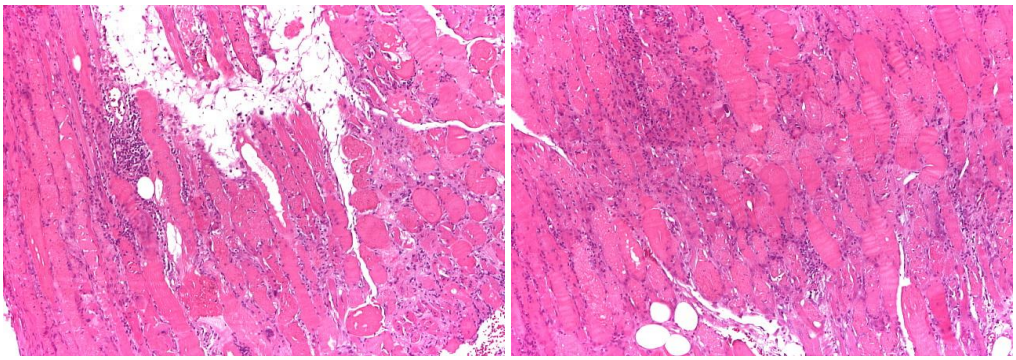

Fig. 2. Polymyositis findings at the muscle biopsy; HE, magnification x40.

Also the immunological investigations revealed the presence of Jo1 antibody, which are pathognomonic for polymyositis with interstitial lung disease onset. It was took into consideration also a paraneoplastic syndrome, but the abdominal-pelvic ultrasound was normal.

Clinical manifestations, biological and immunological investigations as well as the aspect of muscle biopsy pleaded for the diagnosis of idiopathic polymyositis, acute form.

The treatment administrated in hospital was represented by corticosteroids, first in pulse therapy for three days (Solumedrol 1g daily), then Prednisone 1 mg/kgc daily, antibiotics (Ciprofloxacin 200mg/12h), proton pomp inhibitor, symptomatic drugs and kinetotherapy.

The evolution was slowly favorable with decrease in dynamics of transaminases, lactate dehydrogenase, creatine kinase (tab. I) and slight improvement in muscle weakness. Home treatment was represented by immunosuppressive drugs (Azathioprine 100 mg daily), proton pomp inhibitor, Prednisone 75 mg daily, vitamin D and calcium. It was made the recommendation to return in the Rheumatology clinic in one month for biological investigations.

TABLE I
The evolution of biological investigations.

	03.04.2015 (Iaşi)	15.04.2015 (Iaşi)	17.05.2015 (Tulcea)	06.07.2015 (Iaşi)	17.07.2015 (Iaşi)
ALAT	351 U/L	265 U/L	24U/l	31U/l	31U/l
ASAT	375 U/L	134 U/L	33U/L	34 U/l	35U/l
LDH	1670 U/L	1217 U/L	-	686 U/l	676 U/l
CK	3073 U/L	1478 U/L	262U/L	254 U/l	97 U/l

In July the patient returned in the Rheumatology clinic with discrete myalgia, clinical examination revealing a significant improvement of muscle testing (MRC=5). Biologically the patient presented normal values of transaminases, CK and lactate dehydrogenase with decreased values (tab. I).

DISCUSSIONS

Pulmonary involvement in polymyositis includes interstitial lung disease, infection favored by respiratory muscle weakness or drug-induced pneumonia (3).

Interstitial lung disease may be the onset of polymyositis, the motive of attention. Polymyositis may have this onset mainly when the immunological investigations reveal the presence of Jo1 antibodies and clinical manifestations include arthralgia. Jo-1 antibodies represent an important marker of a clinical syndrome characterized by inflammatory myopathy, interstitial pneumonitis, arthritis, fever, Raynaud's phenomenon. This form of respiratory affection do not seem to worsen prognosis (1, 9, 10).

One of the particularities of the case that we presented is the unusual onset of polymyositis, with interstitial lung disease, which made the diagnosis much difficult to establish. Also, initially, the patient was undertreated with corticosteroids (Prednisone 40 mg daily to a patient weighing 90 kilograms), in absence of a certain diagnosis, which allowed to exacerbate the musculoskeletal manifestations reaching even the impossibility of maintaining orthostatism.

CONCLUSIONS

We presented this case report in order to highlight the idea that interstitial lung disease may be a first sign, a first manifestation of polymyositis. The clinician should remain alert to potential muscular or cutaneous symptoms and forms of connective tissue disease whenever a pathological diagnosis of nonspecific interstitial pneumonia is made.

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NEWS

PARADOXICAL SPHINCTERS IN THE ABDOMEN AND A POSSIBLE G.E.R.D. CURE

Nitric oxide molecules serve as neurotransmitters to relax smooth muscle tension in many parts of the body. They play an important role for correct smooth muscle function in unusual locations. Cells producing nitric oxide can be found in the gastric fundus, the anorectal continence organ, vesicourethraltract and also in the uterine cervix in the final trimester of pregnancy. In all these locations they serve as elements of anatomical sphincter structures that have a paradoxical function. As an example, the lower esophageal sphincter (LES) represents a variation of circular muscular occlusive mechanisms found elsewhere in the gastrointestinal tract. The LES is a double layer stretch sphincter that operates in an apparently paradoxical manner: it closes when under stretch and opens when the muscle fibers contract. All traditional forms of operative treatment of gastroesophageal reflux disease (GERD) impede the natural functioning of the stretch sphincter to a greater or lesser degree by locking it up. The cause of GERD is mainly by contraction of the esophagus brought about by the cephalad transposition of the stretch sphincter segment into the chest (an incipient axial hernia - frequently undiagnosed in early stages). The operative repositioning of the stretch sphincter segment into the abdominal cavity provides sufficient restoration of the natural topographic relationships to achieve a cure of GERD. In particular, high-resolution esophageal manometry of the lower esophageal sphincter can easily detect every functional disturbance caused by gastric plication. (Stelzner F. Paradoxical sphincters in the abdomen. *Der Chirurg*. Doi:10.1007/s00104-015-0029-5, 20.06.2015, 86:8:761-770)

Roxana Gherasim

CURRENT TREATMENT OF DIABETIC MACULAR EDEMA - LITERATURE REVIEW

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CURRENT TREATMENT OF DIABETIC MACULAR EDEMA (Abstract). Diabetic macular edema is the main cause of vision loss in developed countries among working people. Laser photocoagulation was the mainstay of treatment for many years, but it could only preserve visual acuity, and not increase it. The anti-VEGF (Vascular Endothelial Growth Factor) agents proved their efficacy in reducing macular thickness and increasing visual acuity, although a minority of patients (30 %) achieved good visual acuity (20/40). Corticosteroids were most effective in reducing macular edema, but this reduction in macular thickness did not correlate with the level of visual acuity. They were associated with secondary cataract and glaucoma. **Keywords**: DIABETIC MACULAR EDEMA, LASER, ANTI-VEGF, CORTICOSTEROIDS.

Diabetic macular edema is a complication of diabetic retinopathy which involves the central part of the retina (macula) and it is the main cause of vision loss in developed countries among working people (1). This is why the socioeconomic consequences of this disease are very important, with significant implications for patients and healthcare providers (2).

Diabetic macular edema appears after the break-down of the blood-retinal barrier. Its incidence is directly correlated with the duration of diabetes and with poor glycemic control (3).

Chronic hyperglycemia produces a multifactorial cascade of biochemical processes including increased capillary permeability, activation of cytokines and inflammation, blood flow alteration. Vascular endothelial growth factor (VEGF), especially A-isoform, is a major factor in the stimulation of inflammation and also in angiogenesis (4). Hypoxia caused by microvascular alterations stimulates the production of VEGF to improve the capillary perfusion.

Neuronal dysfunction induced by hyperglycemia can also contribute to the drop of vision (5). Early diabetic neuropathy can be evaluated by Neuropad® test (6).

The aim of this review is to present the conclusions of the most important studies in the literature regarding this condition. Included in the analysis were only the prospective, comparative and randomized trials which included laser treatment, anti-VEGF agents and corticosteroids with a minimal follow-up of 6 months.

Laser photocoagulation

Laser photocoagulation was the main-

stay of treatment for diabetic macular edema. This was the conclusion of 2 major clinical trials: Diabetic Retinopathy Study and Early Treatment Diabetic Retinopathy Study (ETDRS).

Although photocoagulation was efficient in preserving visual acuity, it did not succeed to increase it. Patients with perifoveal ischemia are not considered eligible for this kind of therapy. Laser treatment can reduce the risk of moderate visual loss (defined as a drop of 3 lines on the visual acuity chart), by 50% but only 3% of the patients presented an increase in visual acuity (7).

Recent studies proved that laser photocoagulation resulted in an over 10 letter improvement in visual acuity in 7% to 31% of the cases (8).

This type of treatment for diabetic macular edemaalso has side effects. Foveal burns, visual field defects, subretinal fibrosis were reported after photocoagulation (9).

Despite aggressive treatment, some patients suffered a severe visual loss after the laser treatment (10).

Intravitreal anti-VEGF agents

Ranibizumab was the agent most frequently used in studies. There are 9 trials investigating the effect of this anti-VEGF agent in the treatment of diabetic macular edema.

Ranibizumab for Edema of the mAcula in Diabetes (READ) 2 was the first important randomized clinical trial which compared the intravitreal injections with laser photocoagulation. After 6 months, the group treated with Ranibizumab visual acuity was significantly better than in the group treated with laser. Also, the addition of laser after Ranibizumab did not provide additional functional benefits (11).

Ranibizumab Monotherapy or Combined with Laser versus Laser Monotherapy in Asian Patients with Diabetic Macular Edema (REVEAL) was a similar study. It included 3 groups of patients who were treated with Ranibizumab, laser, or the combination Ranibizumab plus laser.

After 12 months of follow-up the results were better for the groups who received intravitreal injections in terms of improved visual acuity (12).

In the *Ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema* (RESTORE) study the patients were also randomized to laser, Ranibizumab, and combined treatment. The results were analyzed after 12 months.

The group who received Ranibizumab had the best-corrected visual acuity. After 24 months of follow-up the outcomes were similar to those after 1 year of treatment (13).

These two studies proved a statistical significant improvement of visual acuity for the Ranibizumab patients compared to those who received laser treatment, especially when the variable analyzed was the proportion of patients who gained more than 15 letters on the visual acuity chart.

Ranibizumab Injection in Subjects With Clinically Significant Macular Edema (ME) With Center Involvement Secondary to Diabetes Mellitus (RISE and RIDE) studies were identical in design. They compared 2 doses of Ranibizumab (0.3 mg and 0.5 mg) with sham. After 24 months the proportion of patients who gained more than 15 letters was higher in the 0.3 mg group in RISE study, and in the 0.5 mg group in RIDE study. Both clinical trials showed better functional outcomes with Ranibizumab than with sham (14).

Ranibizumab for Edema of the Macula in Diabetes (READ 3) is another study which compared the outcomes of a high dose Ranibizumab (2 mg) with the standard dose (0.5 mg). After 6 months no statistically significant differences in visual acuity were found between the 2 groups (15).

Another study, published only in abstract form, compared monthly intravitreal injections with Ranibizumab (0.5 mg) versus Bevacizumab (1.5 mg). After 48 weeks, no statistically significant differences were found between the groups (16).

The Diabetic Retinopathy Clinical Research Network (DRCRN) study, conducted by Elman (17), compared the efficacy of Ranibizumab plus prompt laser (3 to 10 days after the injection) or deferred laser (after 24 weeks) with sham injections plus prompt laser or Triamcinolone (Trivaris) plus prompt laser. After 1 year, the groups who received Ranibizumab had better visual acuity compared to those treated with laser or Triamcinolone. After 2 years of follow-up the proportion of patients who gained more than 10 letters was not statistically different between the Ranibizumab plus prompt laser group compared to laser group, but there was a statistically significant difference between laser and Ranibizumab plus deferred laser. The authors of the study did not have an explanation for this fact.

Bevacizumab proved its efficacy in the treatment of diabetic macular edema in several prospective studies. The most well-known is *Bevacizumab or Laser Therapy in the Management of Diabetic Macular Edema* (BOLT) study (18) which compared the intravitreal injections of Bevacizumab (1.25 mg) with laser photocoagulation. After 24 months of follow-up visual acuity was higher in the Bevacizumab group; also the proportion of patients who gained more than 10 letters was higher in the group treated with intravitreal injections.

Lam (19) compared 2 doses of Bevacizumab (1.25 mg versus 2.5 mg) for the treatment of diffuse diabetic macular edema. After 6 months, there was an improvement of visual acuity in both groups compared to baseline, but there was no difference between groups. The patients received 3 intravitreal injections during this period.

Four clinical trials investigated the combination of Triamcinolone and Bevacizumab.

Soheilian (20) compared 3 groups of patients with diabetic macular edema: the first group was treated with Bevacizumab, the second with laser photocoagulation, and the third with Bevacizumab plus Triamcinolone.

After 36 weeks, the visual acuity improved more in the bevacizumab group than the other two groups, although the difference was not statistically significant. After 24 months there were not statistically differences between the groups.

Intravitreal Bevacizumab with or without Triamcinolone for refractory diabetic maculat edema (ATEMD) study (21) included 3 groups of patients treated with Bevacizumab, Triamcinolone, and combined treatment, respectively. The outcomes were similar after 6 months.

Lim (22) studied the combination of Bevacizumab and Triamcinolone and compared it with Bevacizumab and Triamcinolone alone. No differences in best-corrected visual acuity or macular thicknessbetween the groups were found after 12 months of follow-up.

Another study, conducted by Ahmadieh (23), compared 3 groups of patients with

diabetic macular edema who did not adequately respond to laser. Group I was treated with Bevacizumab (3 injections of 1.25 mg every 6 weeks) plus Triamcinolone (a single 2 mg injection), group II received Bevacizumab (administered in the same manner as in group I), and group III received sham injection. The visual acuity improved more in the first2 groups compared to sham group, but there were not statistically differences between Bevacizumab group and the group on combined therapy.

Pegaptanib was studied only in two trials. Both studies compared this anti-VEGF agent with sham treatment.

Cunningham (24) conducted a study which compared 3 doses of Pegaptanib (0.3, 1 and 3 mg) with sham in patients who did not receive previous laser treatment. After 6 months, the first 2 groups had better functional outcomes than the 3 mg Pegaptanibor sham groups.

The second clinical trial, reported by Sultan (25) in 2011, compared the efficacy of Pegaptanib with sham. After 2 years of follow-up, visual acuity was significantly better in the treated group than in the sham group, but there was no difference in the proportion of patients who gained more than 10 letters.

Aflibercept is the newest anti-VEGF agent available and its efficacy in treating diabetic macular edema was proven by the Da Vinci trial (26). That study compared laser treatment with several doses of Aflibercept, and the functional outcomes were better for the anti-VEGF agent after 6 months. The best regimen proved to be 2 mg every 4 weeks.

The main complication after intravitreal injections is endophthalmitis. Because of the continuously increasing numbers of intravitreal injections that are performed worldwide, it has become the main cause of endophthalmitis.

Corticosteroids

Dexamethasone was studied in 2 important trials.

Haller (27) compared 2 doses of Dexamethasone (350 and 700 μg), administered as an intravitreal implant, with no treatment. After 3 months, only the patients who received 700 μg gained more than 10 letters in visual acuity. This difference was statistically significant (33% versus 12%). The 350 μg group showed a non-statistically significant improvement compared to laser.

After 6 months of follow-up there were no differences between the treated groups and the non-treated group. Dexamethasone had a maximum effect after 3 months, but the efficacy began to decline after that.

The second trial, by Callanan (28), included 2 groups of patients: one treated with laser, the other with laser plus Dexamethasone. Although in the first months better results were noticed in the Dexamethasone group, after 12 months of follow-up the authors did not find any differences between the groups.

Fluocinolone is another corticosteroid which was studied (as an intravitreal implant) for the treatment of diabetic macular edema. The *Fluoconolone Acetonide in Diabetic Macular Edema* (FAME) study (29) compared 2 doses of Fluocinolone (0.2 and 0.5 μg/day) with sham intravitreal injections in patients who had at least one laser treatment before being included in the study. After 24 months visual acuity was significantly better in the corticosteroid groups compared to sham. The differences between the 2 groups on Fluocinolone were

not significant.

Pearson (30) compared Fluocinolone with laser treatment. After 3 years, there was no difference between the groups regarding the proportion of patients who gained more than 15 letters in visual acuity.

Triamcinolone was evaluated in several comparative studies as intravitreal injections. The trials that included patients treated by laser and patients who received Triamcinolone showed better results for the laser groups (31). Ranibizumab was also superior to Triamcinolone, and the differences reached a statistical significance level.

The main complications of corticosteroids are cataract and glaucoma. Their incidence is directly dose-related. The biodegradable intraocular implants that release the corticosteroid in a controlled manner seems to have a better safety profile and an efficacy that is comparable to the anti-VEGF agents. In a recent study, published in Ophthalmology in February 2015, it is suggested that the first line of treatment should be the anti-VEGF agent for the phakic eyes and the biodegradable implant with Dexamethasone for the pseudophakic eyes.

CONCLUSIONS

In our opinion, the anti-VEGF agents proved their efficacy in treating diabetic macular edema. Ranibizumab was the most used agent in clinical trials. It is superior to laser, but the results of the different studies must be analyzed with moderation, because half of the patients will not have a driving vision.

Corticosteroids produced a more significant reduction in macular thickness compared to laser or anti-VEGF agents, but they were associated with secondary cataract and glaucoma.

Despite a wide spectrum of possibilities for treating diabetic macular edema, only a minority of patients achieve good vision (20/40), so future studies should bring new therapies for treating this condition.

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NEWS

INFLAMMATORY HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. The incidence is increasing because of the increasing prevalence of chronic liver disease, both viral and metabolic. Uncommonly, HCC occurs in patients without cirrhosis. Lymphoepithelioma-like carcinoma (LELC) is defined as a tumor composed of large undifferentiated epithelial cells with a predominant lymphoplasmacytic component. These tumors are reported to occur in several organs, including nasopharynx, salivary glands, lungs and stomach and also have been uncommonly reported in trachea, lacrimal glands, ureters, urinary badder, uterine cervix, vagina, ovaries, breast, soft tissue, and skin. Epstein-Barr virus (EBV) has been shown to be strongly associated with LELCs of the nasopharynx, stomach, salivary glands, lungs, and thymus in the form of integration of the viral genome in the tumor cell nuclei. However, this association has not been shown in LELCs of the urinary bladder, uterine cervix, or skin. Regardless of EBV status, the morphologic features and prognosis are the same, except for the uncommon sites where the prognostic information is limited. Human papilloma virus (HPV) DNA has been shown in the tumor cells of lymphoepitheliomas of the breast, and transcriptionally active HPV has been shown by in situ hybridization in LELCs of the oropharynx. There is controversial evidence of involvement of HPV in the uterine cervix; some authors report its presence, whereas others do not. The World Health Organization has recently recognized LELC, or inflammatory HCC (i-HCC), as a variant of HCC. To date, LELCs of the liver have been reported in the form of cholangiocarcinoma (n ¼ 20) and HCC (n ¼ 9), with only 1 lymphoepithelioma-like HCC (LEL-HCC) being positive for EBV. The authors studied all cases of LELC of the liver of the institution from the past 23 years, by analyzing for the expression of p16 protein or the presence of HPV. They characterized the immunophenotype of the inflammatory cells within the tumors, the epithelial differentiation pattern of the tumor cells, features of the background liver, and the viral status of the tumor cells. All of the cases were HCC that arose in noncirrhotic livers, two showed biphenotypic differentiation, and none were positive for EBV or HPV (Patel KR, Liu TC, Vaccharajani N, et al. Characterization of Inflammatory Hepatocellular Carcinoma. Arch Pathol Lab Med. 2014;138:1193–1202).

Doina Butcovan

CLINICAL AND PARACLINICAL ASPECTS OF EBSTEIN'S ANOMALY - SEVERE FORM IN NEWBORNS

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CLINICAL AND PARACLINICAL ASPECTS OF EBSTEIN'S ANOMALY – SEVERE FORM IN NEWBORNS (Abstract): Ebstein's anomaly is a rare heart malformation, with a broad spectrum of anatomic abnormalities, leading to different clinical, electrocardiographic, echocardiographic changes and a reserved prognosis. We have described a case of an infant diagnosed with Ebstein's anomaly – a severe form. **Keywords:** EBSTEIN'S ANOMALY – SEVERE FORM, PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA

Ebstein's anomaly is a congenital malformation of the tricuspid valve in which septal and posterior valves are not attached to the tricuspid annulus, having a spiroid line, shifted to right ventricle apex; and the anterior foil is usually malformed, abnormally attached or adherent to right ventricle. The prevalence of disease is of 5.2 to: 100,000 newborns, representing 0.5% of MCC (1, 2, 3).

The control studies that have been conducted so far suggest the involvement of anomaly more frequently in twins, in children with a family history of MCC and whose mothers were treated with benzodiazepines (6).

80% of patients with Ebstein's anomaly can also develop interatrial communication (7, 8) through which right-to-left shunting of blood that can occur are right-left blood, and 20%: bicuspid aortic coarctation of the aorta, aortic valve atresia, atresia or pulmonary hypoplasia, mitral valve prolapse.

The severity of hemodynamic disorders in patients with Ebstein's anomaly depends on the degree of movement and functional status of the tricuspid valve foils. In patients with minimal apical displacement of tricuspid foils, valvular function is normal; while patients with severe displacement foils or abnormal tricuspid valve attachment have an increased pressure in AD and right-to-left interatrial shunting (5).

We will discuss the clinical, electrocardiographic, echocardiographic features and therapeutic management of a newborn diagnosed with Ebstein's anomaly.

CASE REPORT

We will present the case of a male infant aged 11 days, which is hospitalized by clinical transfer with generalized cyanosis, polypnea, tachycardia and the investigation revealed a systolic murmur detected in maternity.

Family history: the infant is the third

child of an apparently healthy couple; mother aged 31 yrs. and father aged 40 yrs. There are no cases of congenital anomalies or genetic disorders in the family.

Personal physiological history: Pregnancy was assessed 5 times by the family physician; mother was admitted to hospital for premature impending birth at 32 weeks. Birth was full term, natural assisted by medical staff in the ambulance, birth weight: 3,400 g, neonatal hypothermia (35.3˚C)

Pathological personal history: Diagnosis of neonatal hypoglycemia, maternofetal infection and congenital heart malformations is made in the neonatology department.

Clinical history at admission: General poor state, cyanotic elastic skin, cold extremities, FA-3/4 cm normotensive, normal chest, symmetrical rib excursion, tachypnea 45/min, SaO_2(-):70%, SaO_2(+):98%, rough MV, without over-added rales, chest area normally conformed, tachycardic rhythmic heart sounds, AV=160/min, systolic murmur degree III / 6 in II intercostal left space. BP right upper limb: 67/47 mmHg, BP right leg 65/41 mm Hg, liver at 2 cm below costal margin, abdomen depression, bowel movements and physiological urination.

Cardio-thoracic radiography shows a global cardiomegaly ICT=0.7, normal pulmonary transparency and free cisto-diafragmatic sinuses (fig.1).

The electrocardiogram shows sinus rhythm of 140/min, electrical axis to the right (+120 degrees), right atrial overload (where P is high, sharp in DII, DIII), major block of right bundle branch (prolongation of QRS=180 msec in pericordial leads) with secondary repolarization changes, BAV degree I (PR interval elongated PR=210 msec) (fig.2).

Fig. 1. Cardio-thoracic radiography global cardiomegaly

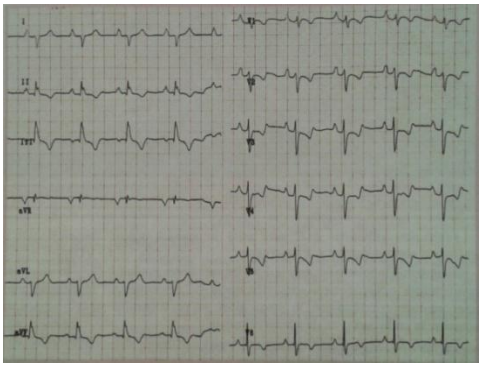

Fig. 2. Electrocardiogram RS 140/min BRD, BAV

Transthoracic echocardiography was the chosen method for the diagnosis of Ebstein's anomaly; there are right cavities with increased volume, SIV paradoxal movements. Apical 4C, there appears the apical displacement of the tricuspid septal insertion foil 5.3/5.5 mm/m^2 body surface area, VD degree of atrialization is over 80%, with compression of the left ventricle, intrarial septal discontinuity, type of oval patent foramen are bidirectional, predominantly right side. Heart failure FE: 38% (fig.3). All elements support Ebstein's anomaly diagnosis.

Genetic examination revealed a small skull (PC:-1.5 DS), FA large 4/4 cm, nor-

motensive, mild facial dysmorphia.

The infant received spironolactone during hospitalization, patient's state remaining stationary under treatment.

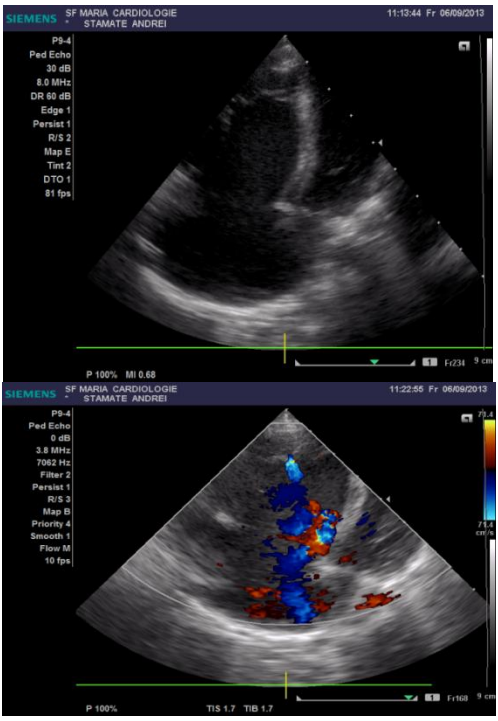

Fig. 3. Patient S.A.G.: echocardiography (four chambers view) Ebstein's anomaly severe form

The patient was transferred to the Institute of Cardiovascular Diseases in Targu Mures for possible univentricular corrections.

Tachycardia with wide QRS (possible antidriomic lead) appeared during hospitalization, with hemodynamic decompensa-tion; after a parenteral load with Amiodarone, electrical cardio version is attempted but the patient relapses in tachycardia, for this reason a beta blocker was added to the initial treatment, being kept short periods of sinus rhythm. The patient is transferred to Department of Pediatric Cardiology, where he continues treatment and held for surgery.

When was readmitted to Pediatric Cardiology Department, patient's state was relatively good, dysmorphic facies, elastic skin, generalized cyanosis, moderate hypotonia, slightly tightened vesicular murmur, intercostal and subcostal circulation. Stetacustic heart - rhythmic heart sounds, noise II duplicated, III noise present, systolic murmur degree III/6 parasternal left. Other signs: abdomen depression, liver 2-3 cm below the costal margin, no signs of meningeal irritation.

Electrocardiogram displayed a sinus rhythm of 120 bpm, axis QRS +60 degree, PQ=0.16 sec, right ventricular overload, major block of right bundle branch, without signs of terminal phase QT: 30 sec.

DISCUSSION

In Ebstein's anomaly, variability of tricuspid valve displacement causes diverse clinical, electrocardiographic, echocardiographic changes. Depending on the anatomical changes, Carpentier made this anatomical and functional classification:

- type A: minimum displacement of the septal tricuspid valve, wide, mobile anterior tricuspid valve, small atralized portion of VD, good functional VD;

- type B: displacement of the septal valve of tricuspid and posterior valve by 25 mm, wide, mobile anterior tricuspid valve, without tracking; relatively good functional VD;

- type C: anterior tricuspid valve partially attached to the VD wall, posterior tricuspid valve agenesis, severe hypoplasia of septal tricuspid valve;

- type D: all right ventricle is covered by anterior tricuspid valve, with minimum systolic opening (4,12).

Heart failure is a major factor in the subsequent management of the case. The

degree of heart failure presented by the infant is supported by the small size of VD, a significant size over 80% of VD atrialization, septal tricuspid valve hypoplasia, left ventricular dysfunction secondary to interventricular septum bulge to the left in diastole, hypoplasia of left heart HTAP (9).

Symptoms were present even at birth with generalized cyanosis, SaO2 (-): 85%, tachypnea and tachycardia; it progressively worsened due to hypoglycemia, neonatal hypothermia and maternal fetal infection.

Although electrocardiography is a classical method which is considered to be the first choice for Ebstein disease severity detection, recent studies support a close relationship between morphological changes of assessed complexes and echocardiographic modifications or nuclear magnetic resonance (10).

Cardiac arrhythmias: Paroxysmal supraventricular tachycardia, ventricular tachyarrhythmias secondary to VD and VS damage are a negative prognostic factor and ablation is indicated using radiofrequency.

In patients with severe tricuspid regurgitation, class III-IV NYHA heart failure, cyanosis, dilation or dysfunction of VD, monocuspe tricuspid valve reconstruction from anterior foil of anterior tricuspid simultaneously with DSA closure are indicated (11, 12, 13).

CONCLUSIONS

Ebstein's anomaly is one of the rarest congenital heart diseases with highly variable evolutionary spectrum.

Newborns having pronounced cyanosis, heart failure phenomena, cardiac arrhythmias, cardiomegaly with increased cardiothoracic index and severe hypoplasia of the septal tricuspid valve lead to a negative outcome in case of Ebstein's disease.

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NEWS

A SYSTEMATIC REVIEW AND META-ANALYSIS OF 18F-LABELED AMYLOID IMAGING IN ALZHEIMER'S DISEASE

Timely diagnosis of dementia removes prolonged uncertainty, unnecessary investigations, delays in initiation of symptomatic treatments, and recruitment of poorly characterized patients into research trials. Definitive diagnosis may only be achieved by histopathological examination of invasive brain biopsy or postmortem tissue, or molecular genetic testing in a minority with inherited dementia. Clinical diagnostic criteria in recent use for Alzheimer's disease (AD) often fail to differentiate accurately between AD and non-AD pathology with up to 40% of patients diagnosed with non-AD dementias identified as having pathology consistent with AD at postmortem in some series. When considering the pathophysiology of AD, it has been proposed that the presymptomatic phase is characterized by an early rise in amyloid accumulation, followed later by synaptic dysfunction, tau-mediated neuronal injury, reduction in brain volume, and finally emergence of cognitive symptoms, followed by a clinical syndrome of frank dementia. This suggests a sensitive and specific biomarker of brain amyloid deposition, such as amyloid imaging, would be a useful diagnostic tool, perhaps as an adjunct to investigation of cerebrospinal fluid (CSF) biomarkers (including amyloid and tau), cerebral hypometabolism ascertained by 18Ffluorodeoxyglucose positron emission tomography, hippocampal volume, tractography, and clinical cognitive assessments. The most widely studied radiolabeled amyloid ligand, 11C-labeled Pittsburgh compound B (11C-PiB), demonstrates high affinity and selective amyloid binding. But, disadvantages including expensive technique, a radioactive decay half-life of only 20 minutes and a scanning time of 30 minutes, limited its utility in clinical settings. Nowadays, amyloid imaging using three novel fluorine 18–labeled (18F) tracers, 18F-florbetapir, 18F-florbetaben, and 18F-flutemetamol, with a longer half-life than 11C-PIB of 110 minutes has recently been investigated in a number of studies, including phase 2 clinical trials. These agents have recently gained approval for use in clinical practice. The benefits of identifying amyloid in vivo extend to monitoring disease progression, and, with the advent of treatments targeting amyloid deposition, potentially also as a surrogate biomarker of treatment efficacy. In conclusion, this systematic review and meta-analysis has demonstrated favorable sensitivity and specificity of amyloid imaging with novel fluorine tracers in diagnosis of AD, supporting their use as an adjunct in clinical practice (Yeo JM, Waddell B, Khan Z, et al. A systematic review and meta-analysis of 18F-labeled amyloid imaging in Alzheimer's disease. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* 2015; 1: 5–13).

Doina Butcovan

DIABETES MELLITUS, CHRONIC COMPLICATION IN PATIENTS WITH ACROMEGALY: CASE REPORT AND REVIEW OF THE LITERATURE

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DIABETES MELLITUS, CHRONIC COMPLICATION IN PATIENTS WITH ACROMEGALY: CASE REPORT AND REVIEW OF THE LITERATURE (Abstract)**:** Disturbances of glucose metabolism are frequently observed in patients with acromegaly. Excess amounts of GH and IGF1 interacts with metabolic regulation, and indeed, GH hypersecretion is associated with hepatic and peripheral insulin resistance; this and also other mechanisms are involved in the development of diabetes mellitus. It can quickly improve if the levels of GH decline after the therapy. **Case report**: We present a patient of 54 years old, admitted in the clinic in 2009, with diagnosis of acromegaly. MRI scan reveals an expansive pituitary tumor 15/16/17 mm. Values of GH, IGF1 and blood glucose were much above normal. Body mass index 27, 5 kg/m^2. After 12 months of a medical treatment with somatostatin analogues, the evolution of blood parameters was favorable, but the patient has discontinued his treatment. At the hospitalization in 2012 there is a precarious control of the diabetes under oral antidiabetic agents. A new treatment has been applied: it was practiced surgical transsphenoidal ablation. At last admit, May 2014, the value IGF1 has been normal, GH has been below 1 ng/mL, and HbA1c was 5.27%. Dyslipidemic syndrome has been constantly present; the values of triglycerides and cholesterol should be a little more upper limit. **Conclusions:** Following successful treatment of acromegaly with surgery, glucose tolerance improves. Balancing type 2 diabetes, with return to normal HbA1c and the decrease in cholesterol and triglycerides values, represents the result of favorable normalization GH in our patients with acromegaly. **Keywords:** DIABETES, ACROMEGALY, TRANSSPHENOIDAL SURGERY.

Acromegaly is a condition characterized by oversecretion of growth hormone (GH) and insulin-like growth factor 1 (IGF1). In 98% of cases hypersecretion due, to a pituitary somatotrophic adenoma; in the other cases (less than 2%) it is the result of over-secretion of Growth Hormone Releasing Hormone (GHRH) from a hypothalamic lesion or from a carcinoid tumor of the gut or pancreas(1). Occasionally acromegaly is found in association with glands, pancreatic islet cells, tumors like glucagonoma, insulinoma and gastrinoma, cutaneous tumors that include multiple angiofibromas, as part of Multiple Endocrine Neoplasia syndrome (MEN) Type 1 (2).

Disturbances of glucose metabolism are frequently observed in patients with acromegaly. The correlation between acromegaly and diabetes was noted as early as 1884 by Loeb (3). Some studies have shown that the incidence of hyperglycemia and glycosuria in acromegalic patients may vary between 10-40 % (4, 5, 6) or over 60% of patients with acromegaly include diabetes mellitus, impaired glucose tolerance and impaired fasting glucose (7, 8). French Acromegaly Registry reported a 22.3% incidence of diabetes mellitus (DM) in acromegalic patients (9). The diagnosis of acromegaly is usually delayed for years, exposing patients to slowly evolving chronic complications (8), that may include: high blood pressure (hypertension) cardiovascular disease, particularly enlargement of the heart (cardiomyopathy), osteoarthritis, diabetes mellitus, precancerous growths (polyps) on the lining of colon, sleep apnea, carpal tunnel syndrome, reduced secretion of other pituitary hormones (hypopituitarism), uterine fibroids, benign tumors in the uterus, spinal cord compression, vision loss. The development of carbohydrate metabolism disorders and/or progression to diabetes in patients with acromegaly may depend on several factors, such as age and gender (10), the levels of GH (11), as well as the duration of acromegaly and duration of exposure to elevated GH levels (9,11). A further possible factor involved in the early development of diabetes is a positive family history of DM (10, 12). A final factor that may also influence the development of glucose disturbances is the specific treatment for acromegaly. Somatostatin analogues may influence glucose metabolism both by lowering insulin secretion and by lowering GH and IGF1 levels (13, 14). The average interval between the onset of acromegaly and that of DM was 9.5 years; however, simultaneous onset of acromegaly and diabetes mellitus was noted in as many as 46.8% patients (9).

CASE REPORT

We present a patient of 54 years old, admitted in the clinic in 2009, with diagnosis of acromegaly. MRI scan reveals an expansive pituitary tumor 15/16/17 mm. Value of GH has been 74ng/mL, (N: 0-3), IGF1 has been 477 ng/mL (N: 93-410) and blood glucose was 168mg/dL. Body mass index (BMI) was 27.5 kg/m². A medical treatment with somatostatin analogues was initiated.

After 12 months of Sandostatin LAR- 20 mg/28 days, the evolution of blood parameters was favorable, but the patient has discontinued his treatment. At the hospitalization in 2012 there is a precarious control of the diabetes under oral antidiabetic agents: blood glucose was 325 mg/dL, HbA1c value was 9 % and the GH increased at 104ng/mL. A new treatment with the intention of the decrease in the GH has been applied: it was practiced surgical transsphenoidal ablation. The cyto-pathological interpretation: pituitary adenomas with somatotoph cells. Evolution after surgery was favorable. At last admit, May 2014, the value IGF1 has been normal, GH has been below 1 ng/mL, and HbA1c was 5.27 %. Joint dyslipidemic syndrome has been constantly present; the values of triglycerides and cholesterol should be a little more upper limit. BMI = 27 kg/m².

DISCUSSION

Disturbances of glucose metabolism in acromegaly can be explained by the direct hyperglycemic effects of GH excess. Excess amounts of GH and IGF1 interacts with

metabolic regulation, GH hyper-secretion is associated with hepatic and peripheral insulin resistance (15). Insulin resistance is also worsened by the lipolytic action of GH generating non-esterified fatty acids that act on the liver to increase hepatic glucose production and inhibit muscle utilization of glucose. Nabarro et al. (11) have reported the results of a large cohort of 208 patients among which 48 had diabetes. They found a significant correlation between age, duration of evolution of acromegaly, and GH level with the presence of diabetes but only by univariate analysis because of the insufficient size of the cohort. Biering et al. (16) have identified age but not GH level as risk factor for diabetes. Møller et al. (17) have studied insulinresistance in acromegalic patients before and after adenomectomy. They have demonstrated that insulin resistance in acromegalic patients is relieved after adenomectomy. The precise mechanism of insulin resistance in the presence of excessive chronic GH is still not clear. GH has a short-term insulin-like effect, but chronic exposure impairs insulin response. Cross talk between GH receptors and insulin receptors have been demonstrated. Hyperinsulinism, insulin resistance, and diabetes are well-recognized cardiovascular risk factors in general population and may contribute to the increased cardiac morbidity and mortality of acromegalic patients (18-21). Clinically, therefore, diabetes in acromegaly resembles Type 2 Diabetes. Diabetic complications, such as retinopathy, are rare but can be seen occasionally. Diabetic ketoacidosis is rare. Age at diagnosis of acromegaly, body mass index, hypertension and duration of evolution of acromegaly were significant independent risk factors associated with development of diabetes. For an acromegalic patient, the risk for the presence of diabetes was increased by 4.4% by age and 12.9% by additional BMI (kg/m^2). Presence of hypertension increased the risk of diabetes by 2.5%. Prevalence of diabetes compared the studies, may be very different: The Spanish (22) and the Belgian registries (23) found a still higher incidence of 37.6 and 25.3% respectively. In the French Acromegaly Registry (9), the prevalence of diabetes was 22.3%. In a study published previously which observed the prevalence of DM in 213 patients with acromegaly, we found 24 cases of DM which represent 11.2 % (5). Diabetes was diagnosed at the same time as acromegaly in 46.8% of patients (9). For other patients, the discovery of diabetes predated the diagnosis of acromegaly with a mean duration of 5.8 years (9) or mean duration of active acromegaly before diagnosis 7.5 years as well as discrete female predominance (5, 12, 13). Although based on the clinical estimation and patient anamnesis, diabetes was present before the presumed beginning of acromegaly in 9.8% of patients (9) or in 20.8% (5). Complications are frequent and the consequences of a delayed diagnosis 5% of patients presented with arterial hypertension (5, 9) rheumatologically complications or pituitary deficiencies.

Diabetes was treated with diet, metformin alone, sulfonylureas associated with metformin, and insulin in 17.6-30% of patients (5, 9). Furthermore, after treatment of acromegaly, diabetes disappeared in 30% of the cases and treatment could be reduced in a number of other cases. Another question is the impact of different treatments of acromegaly in the evolution of diabetes. Ronchi et al. (24) have compared two somatostatin analogs (octreotide LAR versus lanreotide SR) describing a decrease in insulin resistance with both drugs, oc-

treotide LAR appearing more detrimental to diabetes than lanreotide. In a study by Colao et al. (25), where a group of patients was treated for 5 years with somatostatin analogs, no significant change was noted on the prevalence of diabetes during the study duration, although HOMA-R and HOMA-b significantly decreased. Møller et al. (17) have showed a complete reversal of insulin resistance and glucose and lipid metabolism in acromegalic patients cured by adenomectomy. In a study by Kinoshita et al. (26), glucose metabolism in patients cured by surgery was improved when patients had preserved b-cell function.

CONCLUSIONS

In our patients with acromegaly, balancing type 2 diabetes, with return to normal HbA1c and the decrease in cholesterol and triglycerides values, represents the result to favorable normalization of GH.

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NEWS

SINGLE INCISION TRANS-UMBILICAL TOTAL HYSTERECTOMY: ROBOTIC OR LAPAROSCOPIC?

The aim of this study was to compare the early surgical outcomes in patients who underwent total hysterectomy with laparoendoscopic single-site surgery (LESS-TH) versusn robotic single-site total hysterectomy (RSS-TH). Methods: Twenty-four patients who underwent RSS-TH and thirty-four patients who underwent LESS-TH were retrospectively evaluated. Patient characteristics, operation time, intraoperative data (conversions, complications, estimated blood loss, etc.) and postoperative pain scores were compared. Results: The total operation time was significantly longer in the robotic surgery group, with a time of 98.5 vs. 86 min ($p = 0.013$), while vaginal closure time was significantly higher in the laparoscopic surgerygroup ($p = 0.011$). Intraoperative outcomes and postoperative pain scores were similar in the two groups. Conclusion: RSS-TH helps surgeons to overcome the technical disadvantages of LESS-TH, particularly vaginal cuff closure, ergonomics and instrument crowding and clashing. Early surgical outcomes are comparable in the two groups, and both techniques are safe and feasible. (Akdemir A, Yildirim N, Zeybek B, Karaman S, Sendag F. Single Incision Trans-Umbilical Total Hysterectomy: Robotic or Laparoscopic? *Gynecol Obstet Invest* 2015 Jan 27. Epub ahead of print)

Radu Dănilă

ABDOMINAL RADICAL TRACHELECTOMY AS A METHOD OF PRESERVING FERTILITY IN PATIENTS WITH CERVICAL CANCER

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ABDOMINAL RADICAL TRACHELECTOMY AS A METHOD OF PRESERVING FERTILITY IN PATIENTS WITH CERVICAL CANCER (Abstract): Cervical cancer is the 4th most common type of cancer in women across EU. However, in women of reproductive age, the incidence of this pathology is high, therefore, over the last decades various strategies have been developed with the purpose of preserving fertility in oncological patients. This paper aimed at reviewing the existing literature information regarding the use of abdominal radical trachelectomy as a method of fertility sparing treatment for patients with early invasive cervical cancer injuries, without neglecting to emphasize on the surgical, obstetrical and oncological outcomes of this procedure. **Keywords:** CERVICAL CANCER, FERTILITY PRESERVING, ABDOMINAL RADICAL TRACHELECTOMY.

Apart from breast cancer, which is the most frequently encountered cancer in women, cervical cancer is the second most common malignancy in women, over 12,000 cases of invasive cervical cancer being annually diagnosed. Although the average patient age at diagnosis is around 50 years old, younger women are also being at risk of developing it, around 20% of the patients diagnosed being under their '40.

Over the past decade, several screening programs have been developed for the most frequent types of cancers in women, aiming at systematically applying a routine test in the asymptomatic population to identify people with pre-cancerous, incipient lesions, perfectly curable by appropriate treatment.

The European Union Public Health provided financial support for the development of screening programmes for cancer thereby, in many EU countries, there is a trend in the implementation of screening programs for reproductive age women, thereby, many oncological patients may be diagnosed with cervical cancer in the early-stage (FIGO IA1-IB1), an essential condition if fertility preservation is desired. In contrast to the global rate, in the EU, the incidence of cervical cancer is low due to the effective implementation of cervical cancer screening programs. The incidence

of cervical cancer is comparatively low in the EU due to the effective implementation of cervical cancer screening.

Unfortunately, the incidence of cervical cancer in our country, as well as mortality from this cause, places Romania at the top of the Eastern Europe region and European Union ranking despite the screening programs that have been implemented through the national health system in our country (1).

The treatment for cervical cancer has evolved considerably in the recent years and is now focused on preserving fertility of the oncological patients of reproductive age. Dissimilar to standard cancer treatment which may include surgery, chemotherapy, radiotherapy or a combination thereof, and aims solely at treating the condition, the radical trachelectomy represents a new surgical approach that emphasis on treating the malignant disease and retain fertility in patients wanting to conceive (2).

This paper aimed at reviewing the existing literature information regarding the use of abdominal radical trachelectomy as a method of fertility sparing treatment for patients with early invasive cervical cancer injuries, without neglecting to emphasize on the surgical, obstetrical and oncological outcomes of this procedure.

Selection of eligible patients using determinant criteria

To achieve the best oncologic results while ensuring the preservation of fertility is essential to consider a series of determinant criteria that will enable the medical team to select the eligible patient that might benefit from this surgery. The eligibility criteria for patients that undergo this procedure were firstly proposed by Roy and Plante (1998) and included aspects regarding the size of the lesion, which should be 2 cm or even smaller, diagnosed as FIGO Stage IA1, without any vascular invasion, FIGO stage 1A2 or 1B1(3). Additionally, the authors recommended at least 5 mm clear margin after the resection and proposed that any possible involvement of the upper endocervical canal, as well as any lymph node metastasis should be excluded by magnetic resonance imaging (MRI).

The outcome of this procedure depends crucially on the selection of the patients, thereby there are specific contraindications. For example, in the case of neuroendocrine and small cell tumors, this procedure may not be considered an option due to the poor prognosis and the high risk of distant metastasis, which are frequently observed in these cases (4, 5). The dimensions of the tumors, the amount of unaffected cervical stroma, as well as the possible involvement of the parametrium may be determined using ultrasonography or magnetic resonance imaging (MRI). Generally, tumors larger than 2 cm are accompanied by invasion of the vascular space, extension of the lesions towards the upper endocervix or parametrium.

Unlike magnetic resonance imaging (MRI) or computed tomography (CT), which have proven ineffective in detecting any possible lymph node metastases, sentinel lymph node mapping (SLNM) performed during pelvic lymphadenectomy appears to be the most precise way for assessing the status of the pelvic lymph nodes.

The approach depends on the tumor type, for example in the case of type IA1 tumors, without lymphovascular space invasion, a cone biopsy may be taken into consideration, whereas in the case of type IA1 tumors with lymphovascular space invasion, it is necessary to combine the cone biopsy with lymphadenectomy. On

the other hand, IB1 tumors larger than 2 cm and IB2 tumors may be highly challenging due to the risk of lymph node involvement, therefore in these cases the fertility preservation may not be an option.

Types of radical trachelectomy techniques

Radical trachelectomy, which may be approached vaginally or abdominally represents a conservative procedure that implies the resection of the cervix, parametria (tissue adjacent to the cervix), and paracolpos while the uterine corpus and fundus are preserved, thereby ensuring the ability to carry a pregnancy (6).

Vaginal radical trachelectomy (VRT), was first developed in the late '80, for the treatment of stages IA2 to IIA type of cancers, and involves the removal of the cervix, a part of the parametrium, as well as the one third of the upper part of the vagina, while preserving the uterine fundus. The procedure may be associated with pelvic lymphadenectomy. One disadvantages of this method is a higher recurrence rate, possible due the incomplete removal of the parametrium, where metastases are often confirmed by histopathological exam, as highlighted in 1996 by Benediti-Panici (7).

Abdominal radical trachelectomy (ART) is a procedure firstly described by Romania gynecologist Eugen Aburel in the early '30, but in 1997, Smith was the one who proposed this approach as a fertility-preserving method in oncological patients (8). In contrast to vaginal radical trachelectomy, the abdominal approach resembles more the traditional hysterectomy, thereby being more easily implemented in hospitals since it does not require advanced laparoscopic skills with are essential for performing VRT. Besides, due to its radical approach, the complete removal of the parametrium is more achievable.

Moreover, ART with pelvic lymphadenectomy is an alternative procedure in cases with distorted cervicovaginal anatomy in which the vaginal approach may not be feasible, or in patients who have severe cervical and upper vaginal scarring, such as those with repeated conization of the cervix.

Abdominal radical trachelectomy – surgical technique steps

The first step consists in opening the abdomen using a vertical incision on the midline. The external and internal iliac fossae lymph nodes and as well as the obturator one is removed and subsequently, frozen sections are prepared for histological examination. Like radical hysterectomy, the ureters are dissected, and the ureteric tunnels are the opened (9). The uterosacral ligaments and the parametrium must be divided. The next step consists in opening the vagina, retrace the uterus, cervix and parametrium superiorly and subsequently amputate the cervix, parametrium and vagina. Histopathological examination by frozen section is used to analyze the superior margins of the amputated cervix, as well as the margins of the still existing cervical tissue to ensure that the margins are tumor-free. These investigations are conducted to establish that after the reconstruction, there are no cancerous cells at the level of the uterus and vagina (10). If a proper margin is available after the amputation, then the surgeon may perform cerclage suture which will be inserted at the level of the cervical stump thereby ensuring future cervical competence. Further on, the occlusion of the surgical-formed cervical canal may be prevented by inserting

and fixing a catheter in the external region of the uterus. Finally, the vagina is sutures and the abdomen is closed.

The general complication rate for this intervention has been reported to be like that of radical abdominal hysterectomy. Specific complications for radical trachelectomy include cervical stenosis, amenorrhea, cerclage expulsion, an increased risk of spontaneous abortion and premature delivery (11) and Chorioamnionitis caused by disruption of the endocervical glands and reduced secretion of mucus (12). In the case of a pregnancy, patients that undergo such a procedure must deliver via classical Caesarean section. There is a minimal risk of secondary infertility since the mucous secretions are indispensable for the endocervical ascension and maturation of sperm. Additionally, the occurrence of adhesions following surgical intervention is not excluded.

The emergence of a new branch of oncology named oncofertility and oncogenetics led to the development of new approaches for pregnancy and birth monitoring consecutive radical trachelectomy with or without iliac lymphadenectomy, a procedure which is performed as a treatment for cervical cancer. In this particular situation, a uterine tranche is sutured directly to the vaginal stump and it has been demonstrated that if a pregnancy occurs, the gestational sac may be restrained through the contraction of the segmental uterine muscle at the level of the isthmus, even in the absence of the cervix.

Surgical and obstetrical outcomes after abdominal radical trachelectomy

The abdominal radical trachelectomy has the advantage of enabling the excision of even larger cervical tumors (up to 4 cm) stage IB1 or IB2. However, according to studies conducted by Wethington, the obstetric outcome after abdominal trachelectomy is less favorable (13). Furthermore, in one of the studies, conducted in two hospitals from Romania, the authors reported that 7 women from a total number of 23 patients that underwent the surgery, attempted to conceive, only 3 of them succeeding. Eventually, only one of the pregnancy was finalized with the birth of a healthy baby at term, while the other two were miscarriages (14).

Accordingly, a study regarding the reproductive and obstetrical outcomes of 114 patients who underwent a radical abdominal trachelectomy previous their pregnancy was retrospectively analyzed. The pregnancy rate in this case was 36.2%, nevertheless, 64.7% of the patients gave birth prematurely. In addition, a study conducted by Okugawa on 151 patients, revealed that pregnancy rate among the patients that tried to conceive after abdominal radical trachelectomy was 25%, 15 women from a total of 61, achieving pregnancies, which were delivered by C-section (15). It is worth mentioning that premature rupture of the uterine membranes occurred in 6 of cases.

Martínez-Chapa highlighted the crucial role that cerclage plays in delivering the baby at term (16). In fact, increasing the rate of full-term birth is one of the ultimate goals of specialists as reported by Tamauchi, since presently, only one third of the pregnancies are delivered at term (17). A study led by Okugawa, also emphasized on the necessity of preventing possible complications that may arise during pregnancy and often result in rupture of the membranes and premature delivery (18).

The patients must wait at least six months following radical trachelectomy

before attempting to conceive. Because of trachelectomy, the cervix or lower uterus becomes scarred and beside cervical stenosis, difficulties in becoming pregnant naturally may occur. This inconvenient may be over fulfilled through assisted reproductive techniques, such as intrauterine insemination, in vitro fertilization, or even embryo or oocyte cryopreservation before surgery. Given the increased risk of second trimester pregnancy loss and preterm birth, an eventual pregnancy must be followed closely, and serial ultrasound examinations will be performed to monitor cervical length (19).

In the event of a preterm delivery, the cervix opens or thins earlier than normal. In this case, antenatal glucocorticoids will be administered to ensure to fetal lung maturation (20). Vaginal delivery should be avoided because a lateral cervical tear in the short-scarred cervix may extend into the nearby uterine vessels, resulting in catastrophic hemorrhage.

When it comes to oncological outcome, a study conducted by Pareja highlighted that only 3.8% of patients presented cancer recurrence, from a total of 485 patients that underwent a procedure of abdominal radical trachelectomy (21). In 9.5% of the cases, several postoperative complications were identified, the most common being cervical stenosis. Notwithstanding, over 85% of the patients managed to preserve their fertility and 67 patients from a total of 113 who tried to conceive, manage to obtain a pregnancy (59.3%).

Nevertheless, monitoring a pregnancy after abdominal radical trachelectomy requires special attention and should involve a multidisciplinary team composed of obstetrician, surgeon, neonatologist and oncologist.

CONCLUSIONS

We may conclude that abdominal radical trachelectomy represents a fertility-sparing procedure for patients diagnosed with early invasive cervical cancer that intend to conceive.

The pregnancy rates have improved significantly over the last years, however, only one third of the pregnancy are delivered at full-term, which is why the medical management is extremely complex.

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CELIAC DISEASE: 10- YEAR EXPERIENCE IN A ROMANIAN TERTIARY CENTER

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CELIAC DISEASE: 10- YEAR EXPERIENCE IN A ROMANIAN TERTIARY CENTER (Abstract) A**im:** To evaluate the experience of a single coeliac center over a 10-year-observational period. **Material and methods:** Between January 2003 and December 2013 a total of 195 consecutive patients admitted with celiac disease were tested by multiple duodenal biopsies, anti-tissue transglutaminase and anti-gliadin antibodies, and baseline demographic, clinical, biological and immunological parameters. **Results:** Patients were divided into two major groups according to the clinical features and number of signs and symptoms present upon admission: gastrointestinal (131, 67.17%) and non-gastrointestinal (64, 32.8%). Anti-tissue transglutaminase and anti-gliadin antibodies showed seropositivity in 109/158. Histological samples were available in 152 cases, according to Marsh-Oberhuber classification 11.18% being type 0, 17.76%, type I–II, and 71.05% type III. Correlations between anti-tissue transglutaminase antibody titers and Marsh-Oberhuber classification were found to be statistically significant. Body mass index was available in 96 cases. We found that severe atrophy was predominant in patients with a BMI<18 kg/m^2. **Conclusions:** Celiac disease has an increasing prevalence and can be diagnosed at any age. Histology samples were indicative of different stages of villous atrophy. The disease prevalence is significantly higher among women. There was no statistically significant correlation between Marsh classification and BMI values. **Keywords:** CELIAC DISEASE, ANTI-TISSUE TRASGLUTAMINASE ANTIBODIES, ANTI-GLIADIN ANTIBODIES, MARSH–OBERHUBER CLASSIFICATION.

Celiac disease (CD) is a chronic multi-systemic immune-mediated entero-pathy precipitated by exposure to dietary gluten in genetically predisposed individuals (1).

Until the 1970's, the estimated global prevalence of celiac disease in the general population was just 0.03% (2). The risk for the development of celiac disease appears to increase as one lives longer. Celiac disease is being increasingly recognized in the elderly (3); 2.5 % of the elderly in Finland have celiac disease compared to 1 % in children (4, 5). It is unlikely that genes have changed, and there is evidence that gluten exposure has not either (6). In clinical and pathological terms, our understanding of the disease has considerably improved in recent decades, thus making an accurate diagnosis possible. Despite the huge interest for CD for both the general population, gastroenterologists and general practitioners according to current

knowledge there is still a lack of celiac patients cohort trials. In the past 5 years the size of CD patient population in our centre has been growing rapidly with a huge prevalence of CD among atypical nonspecific presentation and first degree relatives. We performed a retrospective review of the medical records of all patients diagnosed with CD and followed up at our hospital in the past 10 years in order to see if the trend is similar to that reported in the literature (7-10).

MATERIAL AND METHODS

The data for this retrospective cohort study were taken from a database of CD patients assessed at a Iasi town tertiary referral center between January 2003 to December 2013. The study is part of a single-center research aiming to investigate the true prevalence of celiac disease. We collected data from 147 female and 48 male patients (75.3% *vs.* 24.7%, respectively), with a mean age of 33 years, range 18 - 68 years. The yearly distribution of patients enrolled in this study is shown in figure 1.

The graph shows an increasing incidence of celiac disease due to increasing awareness and knowledge of pathophysiology, wide spectrum of clinical presentation, disease progression, and pathological mucosal intestinal features, which range from early and mild clinical forms of disease to severe villous atrophy.

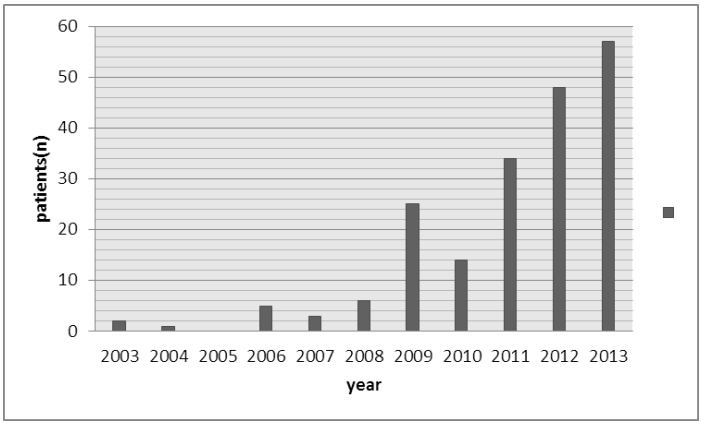


Fig. 1. Celiac disease cases over a ten-year period

Patients with serology (anti-gliadin or anti-transglutaminase) and histology (DII, DIII, bulb) performed within 3 to 6 months of each other were included in the study.

Patients were divided into two major groups categorized by their clinical features according to the number of signs and symptoms present upon admission: gastrointestinal (GI): non- specific abdominal pain, dyspepsia, bloating, flatulence, heartburn, chronic diarrhea, unintentional weight loss; and non-GI: iron deficiency anemia, dermatitis herpetiformis, osteoporosis, elevated liver enzymes, chronic fatigue, joint pain, headache, infertility, ataxia, epilepsy, aphthous stomatitis.

Patient consent was obtained before further examinations including quadrant biopsy specimens taken from the bulb, second or third part of the duodenum. Duodenal histology samples were taken in 152 (77.9%) cases. The mucosal specimens were

graded independently according to Marsh-Oberhuber classification as follows: type 0 - normal villi, type I-II - normal villi but intraepithelial lymphocytosis without (I) or with (II) hyperplastic crypts, type III a-c - varying degrees of villous atrophy with hyperlplastic crypt (1). Immunohistochimical studies were performed and CD3+IELs were counted with a 100 x flat field light microscope objective. The histological evaluation was performed in the Department of Pathology of the hospital.

Serological results were based on measuring tissue transglutaminase antibody (anti-TTG) IgA and IgG (normal range: 0–30 U/ml) and anti-gliadin (anti-gl) antibodies (normal range: 0-12U/ml). The IgG antibody tests were applied for cases of selective IgA-deficiency. Baseline serology data of 158 patients were available.

Positive diagnosis was made using a combination of findings from the medical history, physical examination, serology, and upper endoscopy with histological analysis of multiple biopsies of the duodenum. A positive CD-specific serology in patients with villous atrophy confirmed the diagnosis of celiac disease.

Data were analyzed with the SPSS Software for Windows Version 17.0 (SPSS Inc., Chicago, Illinois). Quantitative data were expressed as the number of subjects and percentages, or as medians and ranges. When appropriate, the Pearson correlation coefficients were used to assess correlations between different variables. A p-value <0.05 was considered statistically significant.

RESULTS

Celiac disease was found in 131 (67.17%) patients in the GI group, 99 (75.57%) females. The most frequent complaint was upper abdominal pain, loose stools accompanied by bloating and abdominal discomfort irrespective of bowel movement. In the non-GI group composed of 64 patients (39: 60.9% females), most of the patients (47: 24.10%) were referred to our clinic for investigations unrelated at first glance to digestive disease: chronic fatigue, joint pain, headache and low iron levels which failed to return to normal after iron supplementation.

Most of the patients had at least one prior admission and at least one other medical prescription. CD was diagnosed in 84 (64.12%) patients in the GI group, and 29 (45.31%) in the non-GI group.

There were 4 patients diagnosed with dermatitis herpetiformis (biopsy-proven) none having any digestive complaint (fig. 2).

Body mass index was available in 96 cases. We found that severe atrophy was predominant in patients with a BMI<18 kg/m^2, and Marsh I, II were common in patients with normal BMI, but there was no statistically significant correlation between Marsh type and BMI values ($p = 0.470$).

The clinical utility of serology testing: Serum IgA class anti-TTG and anti-gl were measured in 145 (74.3%) patients. Values greater than 30U/L (anti-TTG) and 12 U/L (anti-gl) were considered positive. Anti-endomysial (EmA) test results were available in a few cases thus its utility cannot be commented upon.

All patients were screened for IgA deficiency. In case of deficiency the corresponding antibodies were measured in immunoglobulin G (IgG) class.

Nine patients had selective IgA-deficiency; IgG-anti-tissue transglutaminase antibody tests were performed.

Seventy-eight out of 145 patients had anti-TTG, 5 patients had only anti-gl anti-

bodies and 52 patients both anti-gl and anti-TTG. Seropositivity was detected in 109/145 (75%) of cases.

There was a significant association between serum TTG level and clinical presentation, low levels being more common in the screen rather than symptom-detected subjects (fig. 3).

TABLE I

Demographic data on the study participants

Parameter	**Patients**, no=195
Females, %	147 (75.3%)
Age, median (range)	33 (18-68)
Main reason for disease suspicion	
Gastrointestinal symptoms[1],%	131(67.17%)
Iron deficiency anemia/low iron levels%	47(24.10%)
Extra-intestinal symptoms[2],%	64(32.82)
Family history of CD,%	14(7.17%)
IgA deficiency,%	9(6.2%)
Autoimmune disease, %	7(3.5%)
[1] abdominal pain, dyspepsia, bloating, flatulence, heart burn, chronic diarrhea, unintentional weight loss [2] iron deficiency anemia, dermatitis herpetiformis, osteoporosis, elevated liver enzymes, chronic fatigue, joint pain, headache, infertility, ataxia, epilepsy, aphthous stomatitis .	

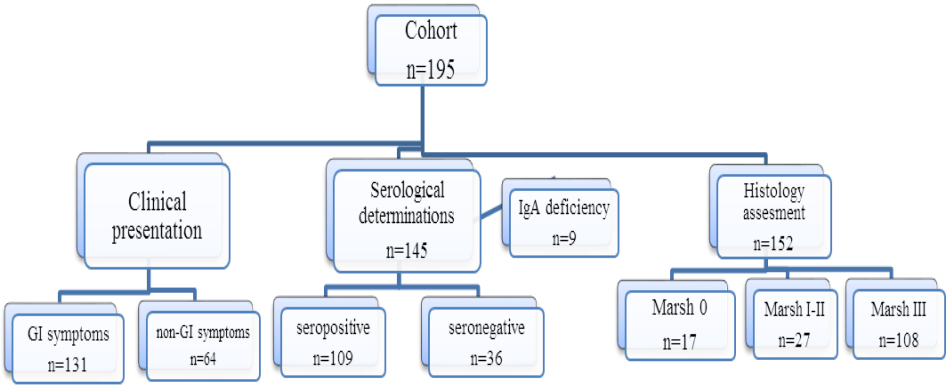


Fig. 2. Flow chart representing the features of celiac disease in our cohort

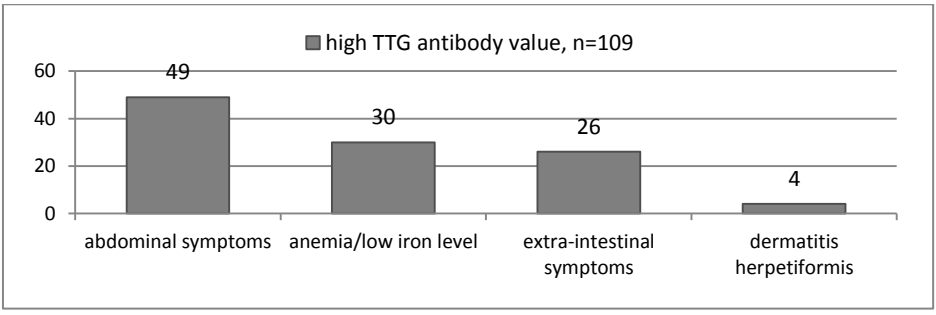


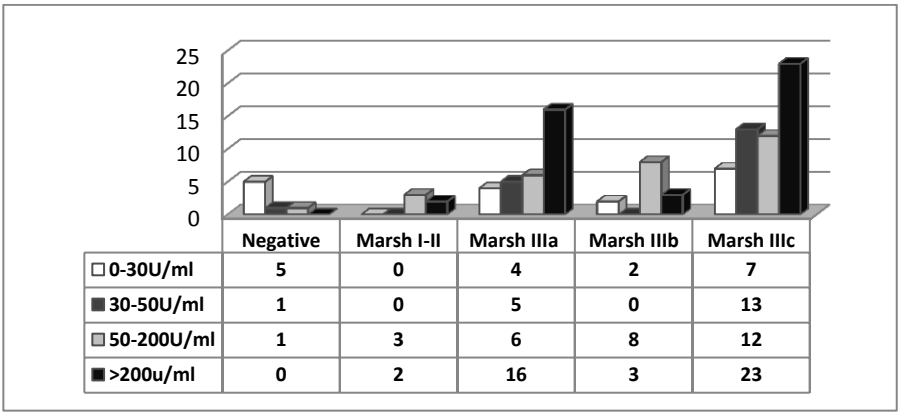
Fig. 3. Serum transglutaminase antibody levels according to clinical presentation

Duodenal mucosal histology Multiple biopsies were taken from the duodenum (DII, DIII) and histological samples were available in 152 (77.9%) patients.

Using the Marsh-Oberhuber classification we found: Marsh 0 in 17 patients, Marsh I-II in 27 patients, Marsh 3a in 35 patients, Marsh 3b in 23 patients, and Marsh 3c in 50 patients. Small bowel mucosal villous atrophy and crypt hyperplasia (Marsh III) were found in 75% of the TG positive subjects. All patients with dermatitis herpetiformis had severe villous atrophy and elevated antibody titers.

Associations between serological testing and mucosal damage: Serological and histological results were available in 132 (67.69%) patients with CD. Ninety-nine (75%) of these patients were serologically and histologically positive for CD. Negative serology and positive histological results were present in 23 patients (17%), and positive serologies with negative histology were found in 10 patients (7%).

Correlation between histological lesions and anti-TG titer was examined in 111 cases. There was a significant association between high levels of antibody and the severity of small bowel mucosal involvement (fig. 4).



	Negative	Marsh I-II	Marsh IIIa	Marsh IIIb	Marsh IIIc
0-30U/ml	5	0	4	2	7
30-50U/ml	1	0	5	0	13
50-200U/ml	1	3	6	8	12
>200u/ml	0	2	16	3	23

Fig. 4. Relation between TG2-ab and the severity of small bowel mucosa involvement

DISCUSSION

In this study we reported data using a multiparametric approach to describe the dynamics of CD over a ten-year period. We analyzed the clinical, serological, histological, and in particular cases the laboratory findings, family history and associated disease of the 195 celiac patients hospitalized in our department between January 2003 and December 2013. Female predominance was obvious in our cohort (75.3% vs. 24.7%), and this prevalence was confirmed by other clinical studies available in literature (2). The mean age of 33 years was indicative of a disease which can be identified in all age groups and not only in children as previously considered.

Serology testing showed a majority of seropositive subjects, but it is important to add that not all the patients met the serology criteria thus stating the need to perform a further duodenal biopsy. In our cohort, 23 patients proved seronegative but with a positive histology. Relying solely on antibody titers to make a positive diagnosis would leave a significant percentage of

patients deprived by a correct diagnosis. Elderly celiac patients were seronegative more often than younger celiac patients. One reason for the low antibody titers in older patients might be the sequestration of antibodies to the intestinal mucosa over time (4).

The vast majority of patients (75%) proved to have severe histological alterations (Marsh III) and the severity of mucosal damage correlated well with antibodies levels. Previous studies have suggested the presence of such correlation by showing parallel alleviation of histologic damage and serology findings (7).

Whilst current literature suggests a discriminatory role for high risk group, our data suggests that there is no single clinical feature of itself a reliable risk stratifies (8). The study had some limitations inevitably, consisting in the uncontrolled, retrospective designed, and in the fact that in some cases, the crucial histological parameters often lacked making the exact definition of CD by Marsh-Oberhuber classification impossible. A major strength in this study is the large number of patients representative of those in everyday clinical practice.

CONCLUSIONS

In our study, we found that celiac disease can be diagnosed at any age. The percentage of females was significantly higher. Histology diagnosis usually showed different stages of villous atrophy. Active screening for CD and its complications and associated diseases is increasing used. Celiac disease must be considered in the differential diagnosis of all patients with malabsorbtion symptoms despite older age in order to prevent disease complications.

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MULTIDISCIPLINARY TREATED THYMOMAS WITH FATAL OUTCOME. A RETROSPECTIVE STUDY

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MULTIDISCIPLINARY TREATED THYMOMAS WITH FATAL OUTCOME. A RETROSPECTIVE STUDY (Abstract): The **aim** of the study was to assess factors concurring to fatal outcome of patients operated for thymoma with or without myasthenia gravis. **Material and methods:** A retrospective observational study was carried out on a series of 10 patients treated for thymoma in the IIIrd Surgical Unit of "St. Spiridon" University Hospital. **Results:** Mean survival time was 4.45 years and mortality rate was 38,4%. In 8 patients III and IV Osserman stage myasthenia gravis was associated. 2 patients without myasthenia gravis were diagnosed with a locally advanced stage of thymic carcinoma. According to WHO pathological classification of thymoma, 2 cases were included in type C, those without myasthenia gravis associated and the rest in type B1 (2 cases) and B2 (6 cases). According to Masaoka classification all cases presented invasive thymoma: 6 cases type II, 3 cases type III and one case with type IV. Apart of one case in stage IV, all cases benefitted of complete surgical resection. No perioperative and early postoperative mortality was recorded. On long term follow-up the cause of death was related to aggravation of myasthenia gravis in 6 cases, to cardiac failure in 2 cases (acute myocardial infarction and constrictive pericarditis) and in 2 cases to metastatic disease (thymoma related death - 20%).**Conclusions:** Aggravation of myasthenia gravis was the first cause of death in this series in spite of complete resection and intensive immunosupressive treatment. Completeness of surgical resection is the most important prognostic factor. The histopathological type of thymoma with fatal outcome was type B and C after WHO classification. Key words: THYMOMA, MYASTHENIA GRAVIS, FATAL OUTCOME

Thymoma patients are a real challenge for the therapeutic team as the outcome can be unpredictable and the response to treatment sometimes refractory. Although most of the authors consider the association of thymoma with myasthenia gravis a good prognostic factor, because it speeds the disclosure of the thymoma in a resectable stage, we noticed that following an initial improvement, myasthenia gravis might become more aggressive and refractory to immunosuppressive treatment. Another important favorable prognostic factor is the completeness of surgical resection but the influence of pathological type of thymoma remains unclear. The aim of the study was

to assess factors concurring to fatal outcome of patients operated for thymoma with or without myasthenia gravis.

MATERIAL AND METHODS

A retrospective observational study was carried out on a series of 10 patients treated for thymoma in the IIIrd Surgical Unit of "St. Spiridon" University Hospital. From 1990 to 2010, 81 cases of thymic lesions were treated by a multidisciplinary team including neurologist, radiologist, immunopathologist, oncologist, surgeon and anesthetist. Thymoma patients represented 26 cases (32%) and among these patients we selected those who died postoperatively over a period of 16 years.

Demographical data, clinical staging data, tumor extension after Masaoka classification, thymoma type after WHO classification, completeness of surgery, adjuvant treatment and patient follow-up data were obtained from the patients records and from detailed patient or family interviews (tab.I). The end point was mortality of the patients till august 2013.

TABLE I

Clinical and pathological characteristics of the patients.

No. / NAME	SEX	AGE	OSSERMAN	WHO	MASAOKA	OP	MTS	POT	ST	DEATH CAUSE
1. LT	F	33	III	B2	III	CR-R at 10 Y-CR		R/C	16 y	IMA
2.ME	F	45	III	B2	III	CR		R/C	6y	ARF
3.PP	F	45	III	B2	II	CR		R	3m	ARF
4.AM	M	40	III	B2	II	CR		R/C	6y	AHF
5.CT	F	60	IV	B2	II	CR		R/C	9y	ARF
6.AM	F	46	III	B2	II	CR		C	1y	ARF
7.AF	M	27	-	C	IV	BX	lung	R/C	2y	MTS
8.GM	F	31	-	C	III	CR	bone	C/ST	2y	MTS
9.PA	M	41	IIB	B1	II	CR		C	3m	ARF
10.IV	M	54	IIB	B1	II	CR		-	2y	ARF

RESULTS

Survival time ranged between 3 months and 16 years. Mean survival time was 4.45 years and mortality rate was 38.4%.

Sex ratio F/M was 3/2 and mean age at the time of operation was 42.2 years. In 8 patients III and IV Osserman stage myasthenia gravis was associated. 2 patients without myasthenia gravis were diagnosed with a locally advanced stage of thymic carcinoma; in one case resection was feasible while in the youngest one only a biopsy could be performed.

According to WHO pathological classification of thymoma, 2 cases were included in type C, those without myasthenia gravis associated and the rest in type B1 (2 cases) and B2 (6 cases).

According to Masaoka classification which assesses the grade of tumor invasion, all cases presented invasive thymoma which is considered malignant, even in the

absence of cells atypia; 6 cases type II, 3 cases type III and one case with type IV. Apart of one case in stage IV, all cases benefitted of complete surgical resection.

One case presented at 10 years with tumor recurrence that required re-resection with survival time of 6 years, the cause of death being not related to operation or recurrence.

The majority of patients (9 cases) received postoperative adjuvant therapy such as radiotherapy, chemotherapy or both. Radiotherapy was indicated mainly for Masaoka stage III and IV patients.

No perioperative and early postoperative mortality was recorded. At the time of hospital discharge an obvious improvement of symptoms was observed for the patients with thymoma associated with myasthenia gravis.

On long term follow-up the cause of death was related to aggravation of myasthenia gravis (60%) in 6 cases, to cardiac failure in 2 cases (acute myocardial infarction and constrictive pericarditis) and in 2 cases to metastatic disease (thymoma related death - 20%).

DISCUSSION

Thymomas are rare thymic epithelial tumors but represent the most frequent tumor of the mediastinum after the age of 40 without gender preference, unlike lymphoid thymic hyperplasia frequently encountered in young female patients (1, 3).

The presence of myasthenia gravis must be regarded as a favorable factor from the diagnostic point of view because the myasthenia symptoms alert patients to seek medical advice and thymoma in such cases can be discovered in a resectable surgical stage. But in our series the association of severe clinical form of myasthenia gravis seems to be related with fatal outcome. Even after a period of improvement postoperatively, myasthenia gravis has reappeared in aggravated and treatment refractory forms being responsible for acute respiratory failure and death. Aggravation of MG postoperatively might be explained by tumor recurrence or exacerbation of extrathymic autoimmunity.

On the contrary, other studies reported that the presence of myasthenia gravis did not show statistical correlation with survival on a multivariate analysis (1, 2, 4, 9). The reported long-term outcome of thymoma patients is related to tumor stage, WHO histotype, completeness of surgical removal and type of treatment (1, 5, 6).

Morgenthaler et all. found that the prognosis is best predicted by stage of the tumor determined intraoperatively and is poorer in patients with incomplete resection. Irradiation and chemotherapy have important roles in the management of thymomas, particularly in advanced stages (3, 7, 8, 9).

The pathological type encountered in our series after WHO classification was mostly B2 and C, which are recognized as aggressive tumors.

Complete surgical resection is a good prognostic factor and when extended thymectomy is feasible the recurrence of tumor is rare. In one case, recurrence was diagnosed and reoperated at 10 years resulting in a good outcome. The patient survived 6 years after reoperation, the cause of death (acute myocardial infarction) being unrelated to thymoma.

Tumor recurrence is rare in complete resected cases. If over a period of 2 years the disease is well controlled then the chance of prolonged survival is increased. The critical period seems to be 2 years postoperatively in which the patient should be closely follow

up, such as every 3 months.

CONCLUSIONS

The association with myasthenia gravis facilitates the diagnosis of thymomas in an early, resectable stage, but our results suggest that despite an initial improvement, myasthenia gravis might become more aggressive and refractory to immunosuppressive treatment. Another important favorable prognostic factor is the completeness of surgical resection although the influence of pathological type of thymoma remains unclear. In our series, the histopathological type of thymoma with fatal outcome was type B and C according to WHO classification. Aggravation of myasthenia gravis was the first cause of death in spite of complete resection and intensive immunosupressive treatment.

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THE EFFICACY OF THE PEGINTERFERON TREATMENT IN CHRONIC HEPATITIS HDV AND COMPENSATE LIVER CIRRHOSIS

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THE EFFICACY OF THE PEGINTERFERON TREATMENT IN CHRONIC HEPATITIS HDV AND COMPENSATE LIVER CIRRHOSIS (Abstract). **Aim**: to evaluate the efficiency of the treatment with Peginterferon alfa 2a 180 mcg/week, 48 weeks in patients with chronic hepatitis or compensated liver cirrhosis HDV and predictive factors of response to treatment**.** **Material and methods**: prospective study that enrolled 50 patients with chronic hepatitis or compensated cirrhosis HDV between the 1st of January 2011 – 3st of December 2011. The diagnosis of chronic HDV infection was made based on the presence of detectable anti HDV IgG antibodies and HDV-RNA. Patients were evaluated at baseline by CBC, liver function tests, HBV profile, HDV RNA, and by liver biopsy/Fibrotest for evaluating fibrosis and necroinflamatory activity. At 24 weeks CBC (count blood cells), liver function tests, quantitative HBsAg and at 48 and 72 weeks biochemical tests, HDV RNA, HBV DNA, quantitative HBsAg, were performed. Adverse reactions to the treatment were recorded. **Results**: SVR (sustained virologic response) was recorded in 12 patients (24%) and biochemical response in 28 patients (56%). SVR was correlated with low-grade fibrosis, age, the aminotransferase value and the value of HBsAg at the beginning of the treatment. In week 48 HDV RNA was undetectable in 20 patients (40%). The therapy was well tolerated, except two patients for whom the discontinuation of the treatment was decided for severe exacerbation of cytolysis, respectively hepatic decompensation. **Conclusions**: In a representative group of patients, the treatment with Peginterferon once again proves its efficacy in treating chronic HDV. **Keywords**: DELTA HEPATITIS, CIRRHOSIS, PEGINTERFERON TRATMENT.

HDV chronic hepatitis is the most severe of chronic viral hepatitis, representing an important public health problem. The hepatitis delta virus (HDV) is a defective RNA virus that requires the presence of the HBV for replication and transmission. Of the HBsAg carriers, approximately 5% also have the HDV infection, affecting 15 to 20 million people worldwide. The evolution is rapid from chronic hepatitis to liver cirrhosis (1) and hepatocellular carcinoma (2).

HDV infection is difficult to treat;

pegylated interferon seems to be the only efficient molecule naive as well as in pre-treated patients, with a virologic response of ~ 20-47 % (3). HDV activity depends on HBsAg, not on the HBV replication or the HBV DNA level (4). Measurement of HBsAg levels during the treatment may be more useful than HDV RNA negativity in predicting the virologic response. The main concern is the use of interferon is neutropenia, thrombocytopenia, and severe hepatic cytolysis flare that may have dramatic consequences in patients with a deficient hepatic reserve (5). Studies with nucleoside analogues demonstrated that these molecules have no effect on hepatitis delta.

The aim of our study is to evaluate the effectiveness of the treatment with peginterferon α2a 180 mcg/week, 48 weeks in patients with chronic HDV infection in chronic hepatitis or compensated cirrhosis, and the predictive factors of response to treatment. TGP normalization at 48 and 72 weeks, HDV RNA negativity and the decrease of HBsAg levels were followed.

MATERIAL AND METHODS

We performed a prospective study that enrolled patients with chronic hepatitis or compensated cirrhosis VHD, present in the Gastroenterology and Hepatology IC Fundeni Clinic during January 1 2011-31 December 2011. The inclusion criteria were: age over 18 years, HBsAg and anti-HDV antibodies of over 6 months, HDV RNA detectable viremia, hepatic cytolysis with ALT $\geq$ 2xN, and histological evidence of chronic hepatitis or cirrhosis. Exclusion criteria were pregnancy, lactation, other causes of liver damage, hepatocarcinoma or decompensated cirrhosis, thrombocytopenia, neutropenia <1.000/mm^3, Hb <9 g/dl, severe cardiac, lung, neuro-psychiatric co morbidities.

Liver cirrhosis was diagnosed by PBH/FibroMax (F4) or imaging studies: abdominal ultrasound or computer tomography. They were evaluated at baseline with the CBC, liver function tests, profile HBV (HBsAg quantitative HBeAg, HBeAb, AcHBc, DNA-HBV), HDV RNA. At 24 weeks CBC, liver function tests, quantitative HBsAg were performed, and at 48 and 72 weeks CBC, biochemical tests, HDV RNA, HBV DNA, quantitative HBsAg.

Quantitative HBV DNA was detected by using the Real Time PCR (detection limit of 5 IU/ml), anti-HDV antibodies through the method ETI-AB-DELTA-2 (Diasorin), HDV RNA by Real Time- PCR (limit of detection 500 IU/ml). The flare was defined as an increase of aminotransferase levels of 3 times in comparison with the value from the onset of treatment (6).

Sustained virological response (SVR) was defined as undetectable HDV RNA at 48 and 72 weeks. The biochemical response is represented by the normalization ALT at the end of treatment. Non-responders were defined as still having detectable HDV-RNA viral load at the end of treatment.

The fibrosis and necro-inflammatory activity were evaluated by liver biopsy (METAVIR score) or Fibrotest, performed at baseline and at the end of the treatment. An improved histological aspect was considered to be the regression by at least 1 point in the fibrosis score and 2 points in the necro-inflammatory activity for the two determinations. The adverse reactions to treatment were also recorded.

Statistical analysis: the continuous variables such as age, the aminotransferasis, the viremia, platelet counts were expressed as mean value +/- SD (standard deviation).

Categorical variables such as gender, environment, response to treatment, stage of fibrosis were expressed as a percentage (and no. of Patients). Comparisons were made using Fisher's exact t-test, the Pearson correlation coefficient and Anova method (unidirectional analysis of variant), through which the predictive response factors were calculated. Differences were considered statistically significant at a p value <0.05. All data were processed using *SPSS 13.0* statistical analysis program.

RESULTS

50 patients were included in the study, 39 (78%) patients with chronic hepatitis and 11(22%) patients with liver cirrhosis (tab. I).

TABLE I

Baseline characteristics of patients

Characteristics	Chronic hepatitis (39p)	Cirrhosis (11p)	p
Age at onset (years)	41.72+/-12.61	49.36+/-10.03	0.0710
Sex	20F/19M	3F/8M	0.1430
Environment	6R/33U	0R/11U	0.2050
AST at onset (UI/l)	77.44+/-29.45	99.45+/-35.78	0.0420
ALT at onset (UI/l)	97.23+/-47.43	126.55+/-37.07	0.0650
Bil T at onset (mg/dl)	0.77+/-0.22	0.97+/-0.19	0.0110
Albumin (mg/dl)	4.52+/-0.45	3.79+/-0.68	0.0000
INR	0.98+/-0.167(1-1)	1.1+/-0.21 (1-2)	0.0600
Leukocytes (/mm3)	6471.03+/-1735.4	4940+/-2116.7	0.0170
Hb (g/dl)	13.82+/-1.35	13.33+/-1.21	0.2790
Platelets ($X10^{3/mm3}$)	192.667+/-52.226	103.091+/-13.397	0.0000
HBsAg	3598.87+/-2661.25	5227.27+/-2446.26	0.0750
HBsAg ($X10^{6}$)	2.41+/-14.75	0.021+/-0.067	0.5960
HDV RNA ($X10^{6}$)	12.66+/-34.083	8.19+/-9.92	0.6710
HBeAg (+) / HBeAg (-)	2p/37p	0p/11p	0.6050
HBeAb (+) / HBeAb (-)	34p/5p	10p/1p	0.6040
AcHBc (+) / AcHBc (-)	38p/1p	11p/0p	0.7800

SVR was recorded in 12 patients (24%), 11 patients with chronic hepatitis and 1 patient with liver cirrhosis, and biochemical response in 28 patients (56%): 25 patients with chronic hepatitis and 3 patients with liver cirrhosis. Among patients with SVR, 3 were pretreated with standard IFN, the remaining 9 patients were naive.

Alanine amino transferase (ALT) was normal in 28 patients at week 48, and in 29 patients at week 72 (fig.1).

At end of treatment (EOT) 20 patients had undetectable HDV RNA viremia. Only 12 of them maintain the same status of HDV RNA. For 8 EOT patients the viremia becomes detectable at 72 weeks. 30 patients (60%) were considered non-responders, 23 with chronic hepatitis and 7 patients with liver cirrhosis (fig.2).

The clearance of HBsAg at 48 weeks of treatment was observed in 1 patient. At 72 weeks from the start of treatment the HBsAg was negative in 12 patients (tab. II). Patients who presented the negativation of the HBsAg were compared with those who did not show the negativation, and it

was found that HBsAg negativation is not correlated with young age 44.42+/-13.44 (p=0.749), sex (F/M 7p/5p) (p=0.257), progressive form of liver disease – chronic hepatitis/cirrhosis (11 p/1p) (p=0.184). HBsAg clearance was associated with decreased ALT 30.05+/-7.052 (p=0.002) and low-grade fibrosis (p=0.048).

HBs seroconversion, with the appearance of anti HBs antibodies was recorded in 5 patients at 48 weeks of treatment and in 17 patients at week 72.

The reduction of the HBsAg level may be a prognostic factor of response to treatment, because HDV requires the presence of HBsAg as an envelope (7).

At baseline the HBV DNA viral load was undetectable in 20 patients, knowing that HBV replication is inhibited by the presence of HDV virus that is dominant. Reactivation of HBV under treatment was recorded in 2 patients from those with SVR.

A liver biopsy was performed for 32 patients, and 18 were evaluated by Fibrotest. The same evaluation by liver biopsy or Fibrotest was performed at the end of treatment. Histological improvement was recorded in 26 patients (52%). No patient recorded a deterioration of the histological aspect.

Predictors for the onset of the response (tab. III): the degree of fibrosis (the lower the degree of fibrosis, the higher is the probability of response) p=001, age of patients at the beginning of the treatment p=0.007, ALT and AST at the onset of treatment p=0.017 vs p=0.030, total bilirubin p=0.005, albumin p=0.000, INR p=0.031, HBsAg at start p=0.000.

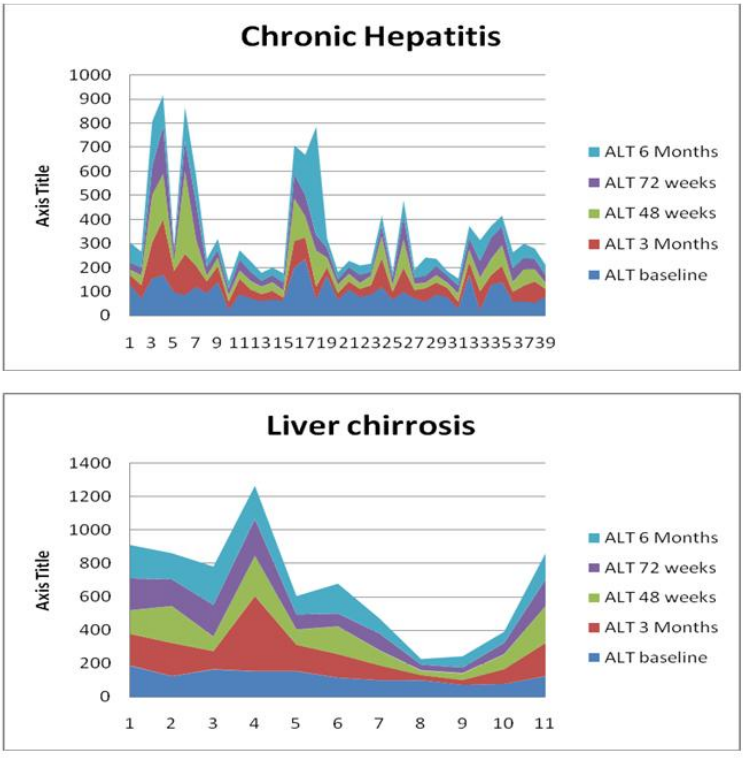

Fig. 1. Evolution of ALT in patients with chronic hepatitis and cirrhosis

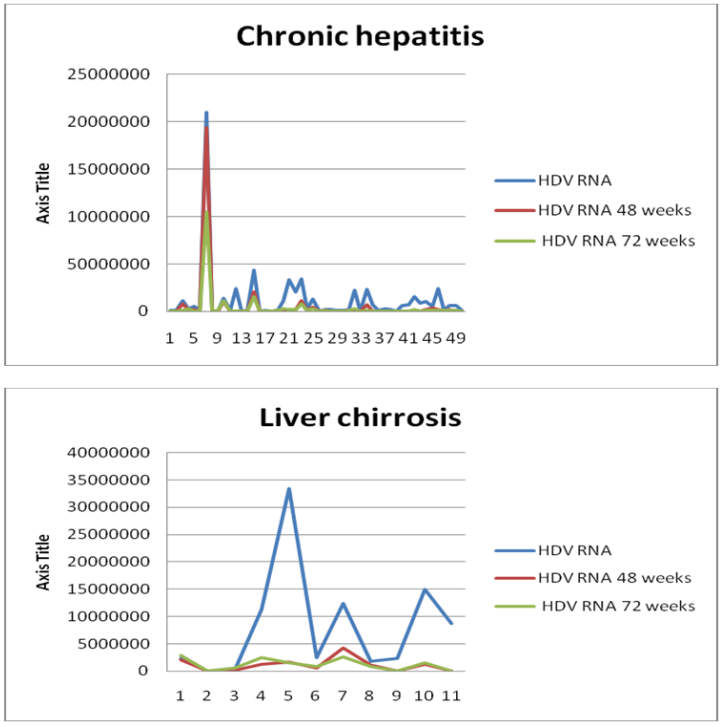

Fig. 2. Evolution of HDV RNA in chronic hepatitis and cirrhosis

TABLE II

Evolution of Ag HBs

	HBsAg at start	**HBsAg at 3 months**	**HBsAg 48 weeks**	**HBsAg 72 weeks**
Mean	3957.12	2251.28	1724.82	1573.16
Std.Deviation	2679.171	2357.786	2072.711	2068.699
Minimum	100	50	0	0
Maximum	9000	8000	7006	7006

The treatment response does not correlate with: sex (p=0.800), HBeAg (p=0.442), baseline viraemia: HBV DNA (p=0.270), HDV RNA (p=0.227).

Although patients with liver cirrhosis were included, tolerability was good (tab. IV).

Thrombocytopenia was more common in patients with cirrhosis: 10% versus 2% in chronic hepatitis (p=0.001); it was not symptomatic and no hemorrhaging manifestations or bleeding was recorded.

Hepatic decompensation occurred in 1 patient (2%): jaundice syndrome (bil T = 3 mg/dl) in week 40 when the treatment was discontinued.

In one of the non-responders, a severe hepatic cytolysis flare was recorded AT x 5 N (2%), the deterioration of the general status caused the discontinuation of treatment at week 26. The only cirrhotic patient who recorded SVR, presented elevated aminotransferases over the entire course of the treatment, but remained asymptomatic.

TABLE III

Evolution of treatment response

Results	Chronic hepatitis	Liver cirrhosis	p
HDV RNA negative at 48 weeks	18p/39p(46.15%)	2p/11p(18.18%)	0.090
HDV RNA negative at 72 weeks	12p/39p(30.8%)	1p/11p(9.1%)	0.144
Normal ALT at 48 weeks	24p/39p(61.5%)	2p/11p(18.18%)	0.013
Normal ALT at 72 weeks	24p/39p(61.5%)	2p/11p(18.18%)	0.013
Normal ALT and HDV RNA negative at 72 weeks	11p/24p(45.8%)	0p/2p(0%)	0.323
HBsAg at 3 months	1954.21+/-2295.19	3304.55+/-2378.49	0.094
AST at 3 months	60.21+/-32.418	119.73+/-101.17	0.002
ALT at 3 months	64.49+/-43.82	153.27+/-115.75	0.000
HBV DNA 48 weeks	$0.032*10^6$+/-$0.200*10^6$	31.36+/-74.36	0.600
HDV RNA 48 weeks ($X10^6$)	6.75+/-30.94	1.11+/-1.25	0.551
HBsAg 48 weeks	1227.82+/-1522.72	3486.91+/-2803.79	0.001
AST 48 weeks	55.23+/-45.49	103.82+/-60.81	0.006
ALT 48 weeks	65.82+/-66.47	127.82+/-74.35	0.011
HBV DNA 72 weeks ($X10^6$)	0.018+/-0.075	0.012+/-0.040	0.806
HDV RNA 72 weeks ($X10^6$)	3.938+/-16.916	1.175+/-1.079	0.593
HBsAg 72 weeks	931.51+/-1440.43	3848.09+/-2405.56	0.000
AST 72 weeks	46.46+/-26.73	90.45+/-46.56	0.000
ALT 72 weeks	56.1+/-39.94	120.09+/-66.09	0.000
AST 6 months	58.67+/-43.965	99.27+/-41.988	0.009
ALT 6 months	69.87+/-74.46	135+/-65.2	0.012

TABLE IV

Treatment side effects

Side effects	Total value	Chronic Hepatitis	Liver cirrhosis	p
Physical asthenia	46% (23/50)	36%(18/50)	10%(5/50)	0.62
Arthralgia	12% (6/50)	10%(5/50)	2%(1/50)	0.604
Weight loss	30% (15/50)	20%(10/50)	10%(5/50)	0.184
Myalgia	16% (8/50)	14%(7/50)	2%(1/50)	0.43
Nervousness	6% (3/50)	4%(2/50)	2%(1/50)	0.534
Thrombocytopenia	12% (6/50)	2%(1/50)	10%(5/50)	0.001
Anemia	2%(1/50)	2%(1/50)	0	0.78
Leukopenia	6%(3/50)	4%(2/50)	2%(1/50)	0.534
Jaundice	2%(1/50)	0	2%(1/50)	0.22
Insomnia	6%(3/50)	6%(3/50)	0	0.466
Depression	8%(4/50)	8%(4/50)	0	0.357
Alopecia	2%(1/50)	2%(1/50)	0	0.78
Loss of appetite	12%(6/50)	8%(4/50)	4%(2/50)	0.396
Fever	12%(6/50)	12%(6/50)	0	0.205
Cytolysis	20%(10/50)	8%(4/50)	12%(6/50)	0.004
Nausea	6%(3/50)	2%(1/50)	4%(2/50)	0.118
No adverse effects	24%(12/50)	20%(10/50)	4%(2/50)	0.472

Peg IFN dose reductions due to leukopenia was necessary in 3 patients.

Under treatment HBsAg, HDV RNA and HBV DNA have the same trend (fig. 3).

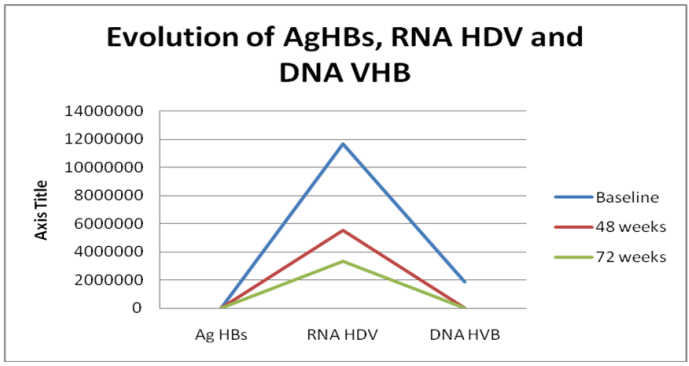

Fig. 3. Evolution of AgHBs, HDV RNA and HBV DNA

DISCUSSION

The Peginterferon treatment resulted in a important reduction of the HDV-RNA at the end of the 48 weeks of treatment, both in patients with hepatitis and with liver cirrhosis, but without statistical significance.

Similar studies have reported values of SVR in chronic HDV infection between 17 and 43%. Niro (37%) (8), Wedemeyer (28%) (9). Erhardt reports a biochemical response in 3 patients (25%) and SVR in 2 patients (17%) (10). Castelnau, in his study conducted on 12 patients, obtains a biochemical response in 8 patients (57%), EOT in 8 patients (57%) and SVR in 6 patients (43%) (11). These results with such different values are probably due to the fact that the studies were conducted on small groups of patients. However, all of them prove the superiority of the Peginterferon treatment versus the standard IFN.

During treatment, ALT levels remain high in many of the patients, especially those with SVR, but there are patients with normal ALT that have not managed to turn their HDV RNA negative. No evidence of a parallel decrease of aminotransferases and HDV RNA was emphasized, so their determination is not of accuracy in the monitoring of the treatment effectiveness.

Although there are studies that show that in the HDV infection as well, HBsAg levels correlate with HDV viremia and can be used as a surrogate marker in monitoring the treatment with PegIFN, in our study we found only the same trend of evolution (12).

In patients with a slow response, which significantly reduce their viremia, but without managing to turn it negative, we wonder if they would benefit from increasing the treatment period beyond 48 weeks. However, there were authors that showed that a 2-year period, is not superior to a 1 year one (13).

As in other studies, the response to treatment was correlated with patient age, low grade fibrosis, the HBsAg and aminotransferases values at the onset of treatment (14, 15). No apparent correlation between the treatment response and HDV viremia was observed, as in other studies (16, 17).

CONCLUSIONS

The treatment with Peg IFNα 2 for a period of 48 weeks is effective. Results, although encouraging, are not maintained over time, at 72 weeks many patients relapse (18).

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A CASE OF SEVERE HYPOALBUMINEMIA ASSOCIATED WITH CHRONIC CONGESTIVE HEART FAILURE: THE ROLE OF THE TRICUSPID REGURGITATION

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A CASE OF SEVERE HYPOALBUMINEMIA ASSOCIATED WITH CHRONIC CONGESTIVE HEART FAILURE: THE ROLE OF THE TRICUSPID REGURGITATION (Abstract): Hypoalbuminemia is considered an independent predictor of mortality, especially in elderly patients. It is common in patients with congestive heart failure, when is due to several mechanisms: increased volume of distribution, significant stasis in the mesenteric circulation and altered protein metabolism in the liver. These alterations are even more pregnant when tricuspid regurgitation is associated or aggravated by different risk factors (recent infections, anemia, hyperthyroidism). We present the case of an elderly patient with severe hypoproteinemia and important hypoalbuminemia associated with congestive heart failure and aggravation of tricuspid regurgitation. The differential diagnosis concluded that hypoalbuminemia was influenced by tricuspid regurgitation as it enhanced liver dysfunction and enteral protein absorption due to increased stasis in mesenteric system. On the other hand, hypoalbuminemia contributed to the progression of heart failure by favoring myocardial edema, volume overload, and diuretic resistance. This is why correct management of this situation should include removal of subclinical excess of fluid and renutrition. A multidisciplinary approach is needed in order to achieve a good control of the symptoms and a significant improvement of quality of life. Keywords: HYPOALBUMINEMIA, CONGESTIVE HEART FAILURE, TRICUSPID REGURGITATION.

Hypoalbuminemia can be caused by various conditions, including acute and chronic inflammation, heart failure, malnutrition, hepatic cirrhosis and nephrotic syndrome (1). Low serum albumin is considered an independent predictor of mortality, especially in elderly patients (2). However, albumin or prealbumin are not considered as defining characteristics of malnutrition in elderly (even if Mini Nutritional Assessment is part of the Comprehensive Geriatric Evaluation) because recent evidence show that serum levels of these proteins do not change in response to changes in nutrient intake (3).

CASE REPORT

We present the case of a 87-year-old

woman with a history of numerous cardiovascular diseases (atrial fibrillation, deep venous thrombosis with repetitive pulmonary embolism, severe pulmonary hypertension and chronic congestive heart failure) and thyroid hyperfunction (with normal function under medication). She was admitted in the Geriatric Clinic for aggravating signs of congestive heart failure. She was referred to our clinic for the first time 6 months previously. Ever since, she is under adequate diet and chronic medication: diuretics, digoxin, aspirin and carvedilol, which she takes regularly.

Clinic examination and exhaustive investigations were performed in order to diagnose the main cause that induced her present symptoms and the comparative results with previous hospitalization revealed the following: recent onset of pruritus; dysphonia for the last 3 months; progression of tricuspid regurgitation (grade II → grade III on echocardiography); aggravation of hypoproteinemia (45 mg/l → 38 mg/l) and hypoalbuminemia (21 mg/l → 18 mg/l).

Based on these elements, we considered that this episode of heart failure could be due to the following causes: 1. onset of a new medical condition: neoplastic (dysphonia, pruritus), parasitic infection (pruritus), new alteration of thyroid function; 2. new episode of pulmonary thromboembolism (D-dimers were negative); 3. natural evolution of her ischemic cardiomyopathy; 4. aggravation of hypoalbuminemia.

Exhaustive investigations did not detect any of these suppositions, so we concentrated on the possible causes of hypoalbuminemia as risk factor for heart failure other then the heart failure itself:

- intake deficit – the MNA (Mini Nutritional Assessment) was normal and the Comprehensive Geriatric Assessment (ADL, IADL, GDS, sarcopenia test) revealed a normal nutritional behavior;

- protein loosing enteropathy, a medical condition difficult to prove in very old patients; anyway, the aggravation of tricuspid regurgitation could have increased stasis in venous circulation and decreased normal absorption of nutrients;

- deficit of protein synthesis due to hepatic failure, but the liver tests were normal and abdominal ultrasound and CT were within normal ranges; however, liver function could have been modified by increased stasis due to tricuspid regurgitation;

- increased catabolism due to acute/ chronic inflammation (in our case we considered cancer, tuberculosis, hyperthyroidism, acute hepatitis) but we found no data to confirm these suppositions;

- increased losses due to an unknown renal problem, but renal function was normal.

We concluded that hypoalbuminemia was an important factor in the aggravation of heart failure but, in the same time, aggravation of tricuspid regurgitation and severe heart failure were key causes for hypoproteinemia and hypoalbuminemia. These conditions were tightly interconnected and we took these aspects into consideration when devising therapeutical management. We combined high doses of diuretics, adequate doses of anticoagulants and digoxin with intense treatment of hypoalbuminemia (recombinant human albumin, intravenous amino acids and parenteral nutrition) and we registered a good evolution, with significant decrease of symptoms and important amelioration of quality of life.

DISCUSSION

Low serum albumin is an important predictor of clinical outcome and mortality

in elderly patients. For every 10 g/L decrease in serum albumin level, mortality increases by 137% and morbidity by 89% (1). This is why diagnosis of hypoalbuminemia and, more importantly, identification of the causes that led to this condition, are very important for practitioners.

Hypoalbuminemia is quite common in elderly population due to several causes closely related to aging itself (4). Dietary intake deficit is frequently encountered due to:

- Undernutrition, particularly in institutionalized patients. This age-associated physiologic reduction in appetite and food intake, which has been termed "the anorexia of aging," contributes to the development of pathologic anorexia, and is associated with substantial adverse effects. (5);

- Depression-associated anorexia. Even if depression is not an inevitable part of aging, it is definitely more frequent in old people due to several causes (retirement, the death of loved ones, increased isolation, and medical problems). Geriatric Depression Scale (GDS) is also an important compound of Geriatric Assessment and should alert the practitioner if psychological therapy is needed;

- Drug-induced anorexia: antiparkinson agents (levo-dopa), antibiotics (co-trimoxazole metronidazole), diuretics (clopamide), chemotherapic agents (cytarabine, dacarbazine), anti-Alzheimer's agents (tacrine), anticonvulsants (zonisamide) are known for their negative effect on appetite (6);

- Age-induced changes of digestive system, including dental and hepatic functions (1).

Other frequent causes of hypoalbuminemia in elderly patients are: chronic heart insufficiency, liver cirrhosis, nephritic/nephrotic syndrome, and acute or chronic inflammatory syndrome. If liver and renal problems are relatively easy to identify, inflammation is quite a challenge for physicians.

Inflammation, either acute (burns, infections) or chronic (arthritis, dementia, Crohn's disease) are more often associated with vague symptoms as malaise, fatigue and lack of appetite than with classic symptoms such as fever, headaches, chills, muscle stiffness, and fatigue. Chronic inflammation is typically detected with lab tests including C-reactive protein, complete blood count (CBC) and complete metabolic panel (CMP). Sodium level should be reviewed to see if it is low, in which case, the albumin level may be diluted due to overhydration. The source of chronic inflammation could take a while to identify but anti-inflammatory treatment should be considered in order to influence hypoalbuminemia.

There are several mechanisms that lead to hypoalbuminemia in congestive heart failure (7). If the synthesis of albumin is normal, hypoalbuminemia results from an increased volume of distribution (which will increase hydrostatic pressure and favor migration of fluids in interstitium). There are many situations when right heart failure is accompanied by significant stasis in the mesenteric circulation, which would affect protein absorption from digestive tube and protein metabolism in the liver; these additional mechanisms for hypoalbuminemia are even more pregnant when tricuspid regurgitation is associated or aggravated by different risk factors (recent infections, anemia, and hyperthyroidism).

Recent studies (8, 9) found that there are independent associations between anemia, hyponatremia, severe tricuspid regurgitation, low serum cholesterol and reduced serum

albumin; interestingly, body mass index was not predictive of albumin levels; hypoalbuminemia was common among patients with acutely decompensated heart failure and was independently associated with increased one year mortality.

The direct relanshionship between tricuspid regurgitation and severe hypoproteinemia is mentioned by Sekine (10); moreover, Lau (11) considers that they are not only related but the severity of tricuspid regurgitation is responsible for the severity of hypoalbuminemia because of the stasis in hepatic venous circulation and impairment of hepatic protein synthesis. In the same time, hypoalbuminemia may also contribute to the progression of heart failure by favoring myocardial edema, volume overload, diuretic resistance (12) and exacerbation of oxidative stress and inflammation. This was the case of our patient, as the specific heart failure treatment didn't work but only when associated with treatment of hypoalbuminemia.

CONCLUSION

Hypoalbuminemia is frequently encountered in patients with heart failure and is mainly related to the malnutrition-inflammation association. Other causal factors include hemodilution, liver dysfunction, renal and enteral loss. Evidence is growing that hypoalbuminemia independently predicts incident heart failure in elderly patients, as well as mortality in patients with heart failure regardless of left ventricular ejection fraction. We report a case with severe congestive heart failure associated with important tricuspid regurgitation and hypoalbuminemia foe which correct management included removal of subclinical excess of fluid and renutrition. A multidisciplinary approach is needed in order to achieve a good control of the symptoms and a significant improvement of quality of life.

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NEWS

IMPORTANT BIOLOGICAL ACTIVITIES OF POMEGRANATE LEAVES

A group of researchers from University of Toulouse conducted a study that evaluated the antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of different extracts (hexane, dichloromethane, ethyl acetate, ethanol and methanol extracts) obtained from *Punica granatum* leaves. The total phenolic content varied between 8.8 ± 0.3 and 127.3 ± 1.6 mg gallic acid equivalent/g dry weight. Ethyl acetate extract contained the highest value of total phenolics (127.3 ± 1.6 mg gallic acid equivalent/g dw), followed by methanol (85.2 ± 2.4 mg gallic acid equivalent/g dw) and ethanol extracts (82.6 ± 1.5 mg gallic acid equivalent/g dw). The total flavonoids content varied between 1.2 ± 0.05 and 76.9 ± 2.45 mg quercetin equivalent/g dw. The ethanol extract contained the highest level of flavonoids. The distribution of total tannins content in the extracts showed that tannins were highly concentrated in the hexane extract of pomegranate leaves (260.8 ± 11.5 mg catechin equivalent/kg dw). The dichloromethane extract was found to contain the lowest concentration of tannins (63.7 ± 1.7 mg catechin equivalent/kg dw). Anthocyanins content in the pomegranate leaves depended on the extracting solvent. The hexane extract contained the highest total anthocyanins content (3.73 ± 0.02 mg cyanidin-3-glucoside equivalent/g dw). The methanol extract showed good DPPH and ABTS scavenging effects (IC_{50}=5.62 and 1.31 mg/L, respectively). The strongest 5-lipoxygenase (5-LOX), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition activities were determined for the ethanol extract (IC_{50} values of 6.20, 14.83 and 2.65 mg/l, respectively); the best cytotoxic activity against MCF-7 cells was determined for the methanol extract (IC_{50} = 31 mg/L). These important biological activities showed that the methanol extract of *P. granatum* leaves is a natural source of antioxidants and might be used as a natural additive in food and pharmaceutical industries. Further *in vivo* studies should be done (Bekir J, Mars M, Souchard JP, Bouajila J. Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (*Punica granatum*) leaves. *Food Chem Toxicol* 2013; 55: 470-475).

Alexandru Vasincu

THE PITFALLS OF DIFFERENTIAL DIAGNOSIS OF LUMBAR SPINE EPIDURAL LESIONS – EXEMPLIFICATION WITH TWO PARTICULAR CASES AND A REVIEW OF THE LITERATURE

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THE PITFALLS OF DIFFERENTIAL DIAGNOSIS OF LUMBAR SPINE EPIDURAL LESIONS–EXEMPLIFICATION WITH TWO PARTICULAR CASES AND A REVIEW OF THE LITERATURE **(**Abstract): **Background and study aims**: Lumbar spinal epidural lesions could belong to a wide spectrum of pathologies. However, some cases present with rare conditions that can cause significant neurological deficit and raise serious diagnostic challenges. We present two such cases where the clinical picture and the imaging findings failed to elucidate the actual diagnosis, which came as an intraoperative and pathological surprise. **Patients**: The first case is that of a 78 years old male that presented for low back pain, bilateral S1 radicular sciatica, paraparesis, and difficulty walking. The neurologic exam showed the absence of the rotulian reflexes bilaterally, and a partial sensory loss in the crural dermatomes. The MRI showed an epidural mass, contrast enhancing at the L1-L2 level, suggestive for an extraxial tumor. The second case is that of a 62 years old woman that presented for a hyperalgic sciatica involving the right S1 root with parestesias in the corresponding dermatome. MRI showed a cystic, contrast enhancing lesion in the L5-S1 recess, mimicking a cystic neuroma. Both patients underwent surgery and histopathological exams were performed from the excised pieces. **Results**: In the first case the surgery resulted in complete removal of the pathological process and histopathological result returned chronic organized hematoma, concordant with the intraoperatory aspect. The evolution was good. In the second case the histopathological result came back as pulpous disk. Again the evolution was good with complete neurological recovery. **Conclusion**: In the presence of an epidural mass with clear clinical symptomatology, rare entities like chronic epidural hematoma or posterior migrated disk material should be always considered as a differential diagnosis in patients with suspicion of extradural chronic compressions. In these cases, only the intraoperatory and histopathological exams can provide a clear diagnosis. **Keywords**: SPINAL, EPIDURAL, DIFFERENTIAL DIAGNOSIS.

Extradural spinal lesions fall within the general classification of traumatic, infectious, neoplastic, developmental, or degenerative pathologies. However, rare cases present with atypical imagistic findings that leave the correct pathological diagnosis to intraoperative and microscopy exam. Significant symptomatology involving the *conus medularis* and the lumbar-sacral roots make these lesions surgical emergen-

cies. For example, acute epidural hematoma is a common complication in spinal surgery (1, 2), but those that result in a neurological deficit are extremely rare (0.1 %) (3). Chronic epidural hematoma is on the other hand very rare (4). The clinical features of a chronic lumbar epidural hematoma may be similar to those of a herniated disk or to those of lumbar spinal stenosis. We present two cases treated in our department recently that we believe strongly illustrate the polymorphism of epidural lesions and the consequent pitfalls of a correct diagnosis in the presence of a lumbar spinal epidural lesion.

CASE 1

The first case is that of a 78 years old male that presented for low back pain, bilateral S1 radicular sciatica, paraparesis, and difficulty walking. Neurological examination at admission showed paraparesis with a sensory loss at the L1 level, absent knee and ankle jerk reflexes. Babinski sign was negative on both sides. No sphincter dysfunction was found.

We performed a MRI exam with contrast enhancement that revealed an epidural mass at L1-L2 level medial-lateral to the left, occupying more de ¾ of the spinal canal at its largest (fig.1). It showed inhomogeneous contrast enhancement, and radicular and *conus medularis* compression.

The patient was operated for prostate adenoma, 2 years before and 8 months before presentation (with normal PSA value 3 months before), both surgeries performed under epidural anesthesia. As co morbidities we learned that the patient also has rheumatoid polyarthritis stage II, operated inguinal herniation, obesity, mild thrombocytopenia.

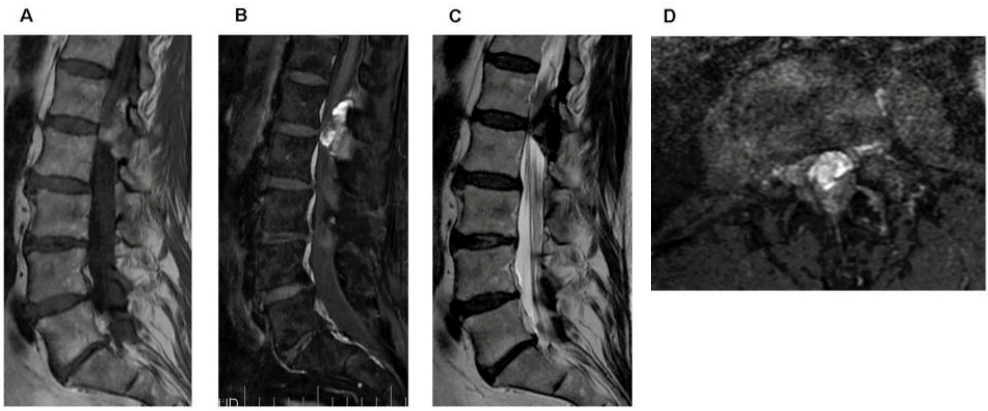

Fig. 1. Preoperative MRI findings in case 1. Sagittal T1 before (A) and after (B) contrast administration showing the cystic lesion at the L5-S1 level with ring-like enhancement. C) the axial enhanced T1 aspect of the lesion.

Postoperatively the neurologic symptomatology remitted except for the sensitive symptoms and the patient was discharged to the rehabilitation clinic for further treatment.

A standard midline posterior approach was chosen and an L1 laminectomy was performed to expose the spinal canal. During the surgery, the dural sac was found to be compressed by a well circumscribed, red-grayish lesion, located in the left poste-

ro-lateral space of the vertebral canal with no adhesions to the nerve roots, but moderately adherent to the dural sac. Under magnification, the lesion was incised, and it contained an isolated well-formed chronic hematoma that appeared to be under pressure (fig. 2). Excision of the thick capsule was performed and the posterior part of the vertebral body was exposed. We observed no disc herniation at the L1–L2 level.

Histopathological examination reported a chronic encapsulated hematoma with reparative fibrous tissue without evidence of malignancy.

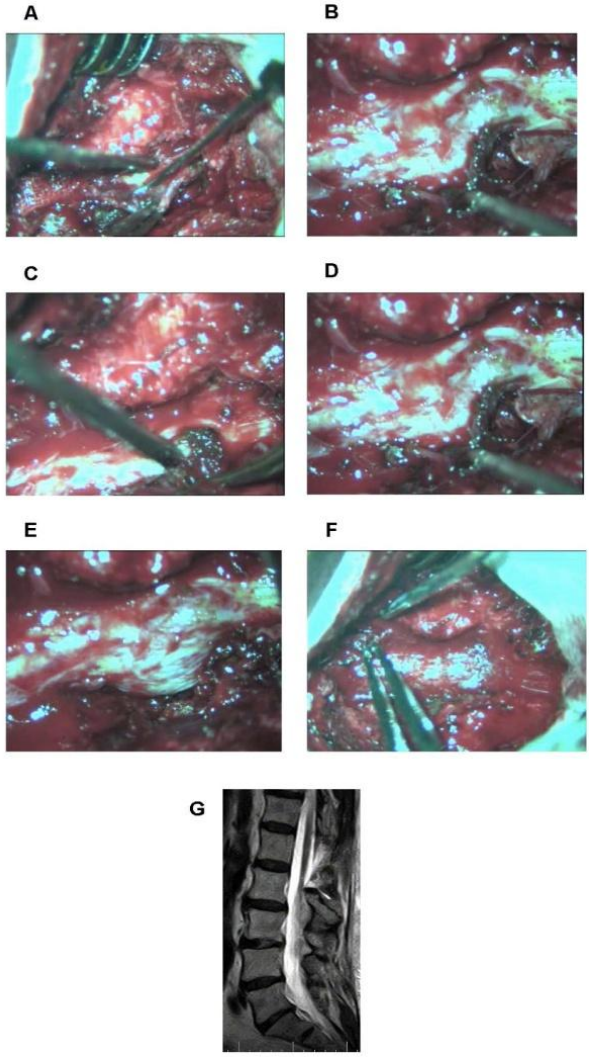

Fig. 2. Intraoperative findings in case 1. A) The pathological mass in the extradural space after cyst content expression (A), during dissection (B) and at the discovery of the dural sac (C). D-E) conjunctive adherences to the dural sac, during dissection. F) final aspect of the dural sac and spinal canal.

CASE 2

The second case is that of a 62 years old female who presented to our department complaining of hyperalgic sciatica of the right S1 root, with paresthesias in the corresponding dermatome, progressive for 1.5 months, after an initial crisis 4 months prior to presentation, self-limited. The neurologic exam showed superficial hypoesthesia on the right S1 dermatome and an achilean reflex of lower amplitude. The conservatory treatment had very little effect on the symptomatology. From the patient's history the only relevant finding was a lumbar trauma 4 months ago (falling down the stairs).

We performed a MRI of the lumbar spine which showed a cystic lesion at the level of L5-S1 foramen, attached to the anterior aspect of the articular process of S1 vertebra (fig. 3A). The finding made us complete the MRI investigation after contrast medium enhancement. The lesion was enhancing circumferentially, keeping the cystic aspect (fig. 3B).

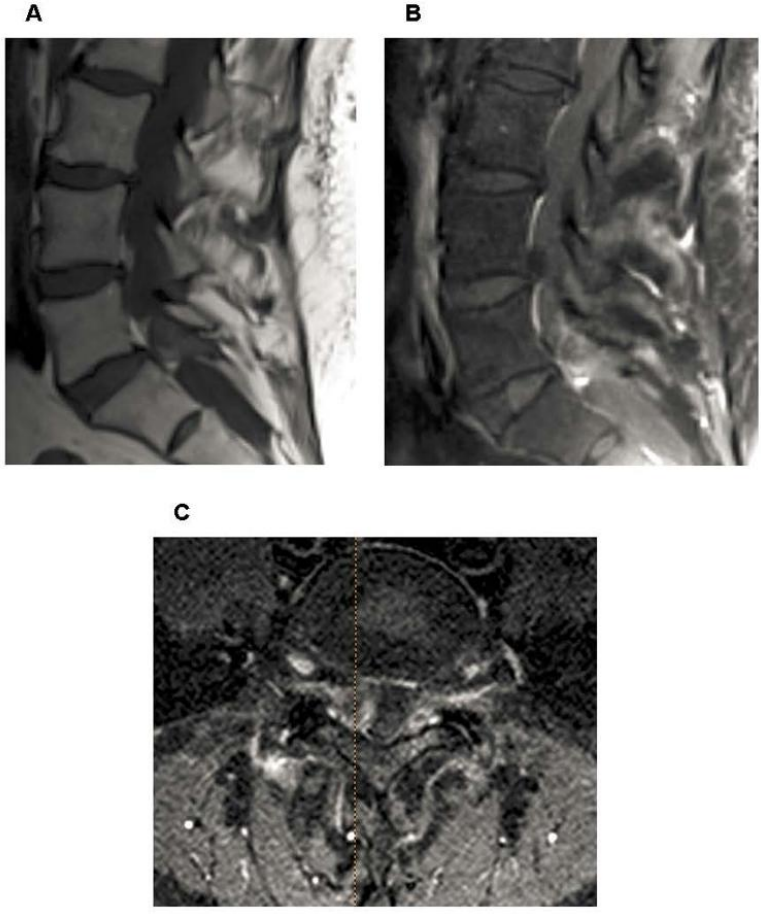

Fig. 3. Preoperative MRI images of case 2. Sagittal planes of native T1 (A), T1 after contrast enhancement (B), and T2 (C) demonstrating the mass occupying lesion at L1-L2 level with heterogeneous contrast enhancement situated in the posterior compartment of the spinal canal. D) axial view of the lesion on enhanced T1-weighted image.

With an unclear clinical and imagistic diagnosis we went on with surgery. We approached the contents of the lumbar canal at L5-S1 level through a standard posterior approach with L5 hemilaminectomy. The bony resection exposed a canal with an unusual aspect. *Ligamentum flavum* was undistinguishable and the canal was filled with a fibrous conjunctive slightly hemorrhagic mass. Under the microscope, at the attempt to find a plan of dissection laterally a brownish fluid was expressed from a false cavity. The dissection continued of what appeared to be a conjunctive cyst till the complete removal of pathologic material and complete exposure of the free dural sac (fig. 4). The pathological exam returned the result of degenerated pulpous disk.

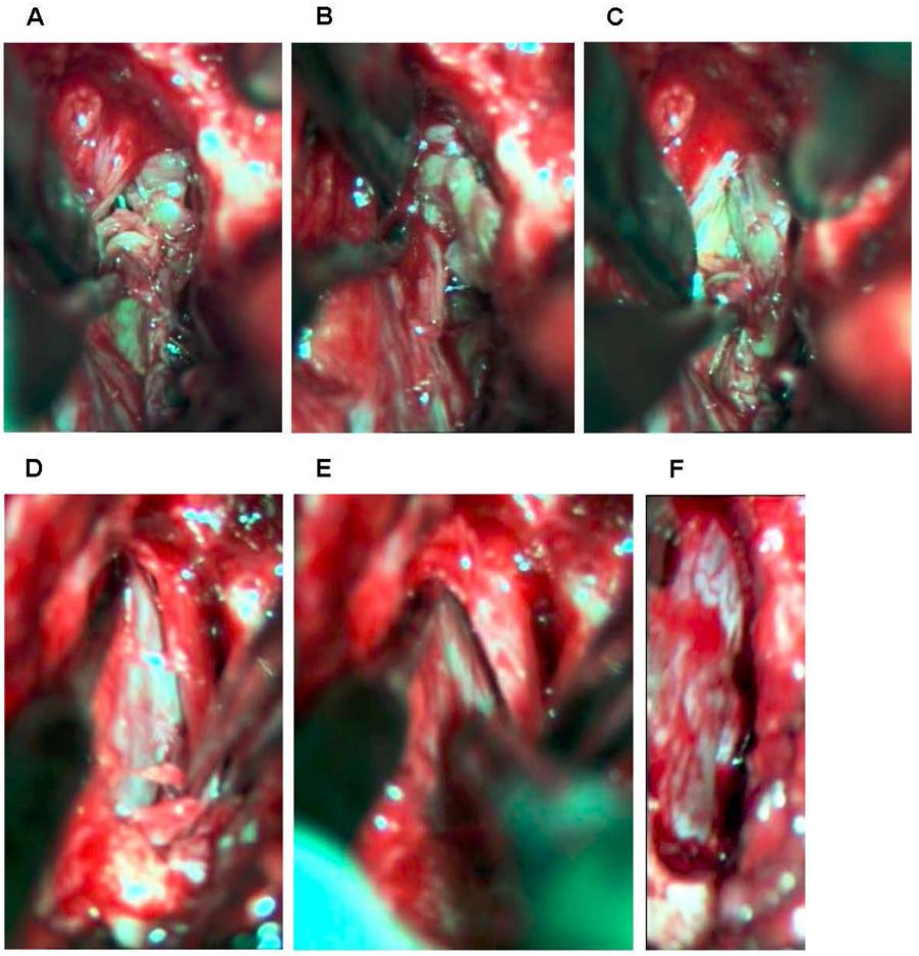

Fig. 4. Intraoperative findings in case 2. A) Initial aspect after canal exposure with capsule dissection. B) Opening of the capsule reveals an organized chronic hematoma. C-E) dissection and excision of the chronic hematoma. F) Final aspect of the spinal canal and dural sac. G) Sagittal T2 weighted MRI image one month postoperatively, documenting the complete removal of the pathologic mass and the normal anatomy of the neural structures).

DISCUSSION

Epidural lesions of the lumbar spine belong to a variety of pathologies and can occur from a variety of situations. Although the tumoral pathology has by far the highest incidence, rare instances can occur and can generate serious difficulties in differential diagnosis. Chronic epidural hematomas in this region in particular seem to have a polymorphic imaging behavior, leading to preoperatory misdiagnosis and, consequently, intraoperatory surprises. They represent extremely rare lesions (0.1 %), being most commonly associated with multilevel surgery, coagulopathies, neoplasia, post lumbar puncture, or the use of high doses of anticoagulant medication (5).

Albeit our first case presented with a recognized risk factor in his history (lumbar puncture for epidural anesthesia) two particularities stand out and prone to discussion. First, the level of the hematoma is 1-2 levels above the level of the puncture. Secondary, the size of the hematoma does not correlate with the symptomatology. It became symptomatic just recently, after more than four months of complete clinical silence, in the case of a clear chronic organization. This divergence might suggest that we may very well be in the presence of a spontaneous chronic lumbar epidural hematoma with a second rebleed that decompensate the initial asymptomatic hemorrhagic collection. It seems to be more likely that the appearance of chronic hematomas occurs in the *conus medullaris* because at this level there are higher pressures in the spinal cord and their arterie (6). The suggested explanation implies that tearing of the epidural veins occurs during intense exercise, which increases the thoracic or abdominal pressure. It is known that the epidural blood plexus volume and pressure depend on the summation of the blood output between intracranial, intrathoracic and intrabdominal blood flow changes (4). Other authors estimate that the hematomas come from an arterial source (7). Graziani et al. (8) proposed as a possible cause for these spontaneous hematomas an epidural cavernous hemangioma.

Posterior epidural disc migration on the other hand is as well a very rare event. Many differential diagnoses are possible in the posterior epidural space other than disc disease. Diagnosis of posterior migrated disc fragment with subtle clinical findings, even though the free fragment has to pass through many anatomical barriers including the nerve roots, poses a clinical challenge. Many differential diagnoses should be kept in mind. MRI findings (especially with contrast enhancement) are useful yet could tell a different story and leave the correct diagnosis to intraoperative tissue sample and microscopy.

Our first case represents a very particular case where an unusual posterior migrated disk and lumbar trauma working together in generating a very particular imagistic and intraoperatory finding. Although known as possible, even if rare, the repeated lumbar trauma was the trigger for subclinical hemorrhages within the yellow ligament and in its vicinity. As a result the disk-ligament-blood complex evolved into a conjunctive mass with liquid content (containing blood byproducts). The clinical picture of clear S1 sciatica was the only indication of a possible herniation; however the MR findings were far from such an instance. The correct diagnosis was possible just after the histopathological investigation of the resection piece.

The clinical symptoms of an epidural hematoma can be similar to those found in

disc herniations, spinal stenosis or spinal tumor (9). A ventrally located hematoma should be differentiated from a posterior longitudinal ligament cyst as described by Toussaint (10). The MRI images of a hematoma can simulate an intracranial tumor or a neurinoma.

CONCLUSION

Although not an unusual location for pathological processes, the lumbar epidural space can be the scene of rare and highly atypical pathologies. In the presence of an epidural mass with clear clinical symptomatology, rare entities like chronic epidural hematoma or posterior migrated disk material should be always considered as a differential diagnosis in patients with suspicion of extradural chronic compressions. In these cases, only the intraoperatory and histopathological exams can provide a clear diagnosis.

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METACHRONOUS LUNG CANCER - CASE PRESENTATION

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METACHRONOUS LUNG CANCER - CASE PRESENTATION (Abstract): Multiple primary tumors can be synchronous when detected simultaneously or metachronous when detected after a variable time interval. We present the case of a 62-year-old female patient with a history of keratinized squamous cell carcinoma of the cervix, stage $T_{3b}N_xM_x$, operated, and treated by chemotherapy and radiation therapy which develops after three years a large cell neuroendocrine carcinoma of the lung detected by imaging. Of major importance in the selection of the optimal therapeutic approach was the accurate determination of the histological type of the metachronous tumor by microscopy and immunohistochemical techniques. The discrete respiratory symptoms and the absence of lung imaging screening accounted for tumor development to an inoperable stage, thus emphasizing the importance of close monitoring of the oncologic patient, thus greatly increasing their chances of survival. **Keywords**: METACHRONOUS CANCER, LUNG CANCER, CERVICAL CANCER.

Multiple primary tumors in a patient are characterized as synchronous, when detected symultanously, or metachronous, when there is a variable time interval between the detection of the first tumor and the detection of a second or even a third tumor. These are the more common as those surviving a form of malignancy live longer (1). The exact causes of the development of other primitive tumors in some oncologic patients are unknown, but radiotherapy and chemotherapy used to treat the first cancer are believed to be predisposing factors.

A recent study (1), using data from the Rhode Island Cancer Registry covering a period of about 20 years, showed a steady upward trend of cancers diagnosed as a second primary tumor from 11.5% to 20.3% in men and 12.8% to 20.7% in women. Of all metachronous cancers, the lung, colorectal, breast and prostate ones account for 54.3% of cases, the average time to diagnosis being 6.5 years in men and 4.8 years in women.

In the literature, multiple primary lung tumors are often described (2-5), but lung cancers metachronous to other organ cancer are less frequent (6).

CASE PRESENTATION

We present the case of a 62-year-old female patient who presented at the Pulmonary Rehabilitation Clinic of the Iasi Rehabilitation Hospital for episodes of inspira-

tory dyspnea, mucus coughing and fatigue, with onset 3 months earlier. The patient was not a smoker, did not drink alcohol, and was not exposed to respiratory hazards.

In her medical history there was a cervical cancer diagnosed in stage $T_{3b}N_xM_x$ three years earlier, treated multisequentially: preoperative radiotherapy and cisplatin chemotherapy, surgery (radical hysterectomy type C_1 and pelvic lymphadenectomy level 2) with favorable postoperative course, followed by postoperative radiation therapy. Pathological examination of the surgically removed specimen established the histopathological type of the cervical tumor - keratinized squamous cell carcinoma.

Physical examination at the time of presentation revealed no significant changes except for some wheezes over the anterior left hemithorax and grade I obesity (BMI = 31 kg/m^2). Laboratory tests revealed a nonspecific inflammatory syndrome (ESR = 54 mm/1h, CRP = 2.82 mg/dl), mixed dyslipidemia (total cholesterol = 225 mg/dl, HDL-cholesterol = 33.43 mg/dl), and impaired fasting glucose (114.1 mg / dl), while ventilatory function tests were within normal range.

Given the patient's symptoms and history, thyroid and abdominal-pelvic ultrasounds were recommended; these showed normal issues without nodular organ lesions or lymphadenopathy. Instead, pulmonary X-ray, the first one performed after genital cancer was surgically removed, showed an ovalary opacity of 7/5 cm, relatively well-defined, located in the left para- and suprahilar areas (fig. 1), which required a chest computed tomography (native and with intravenous contrast).

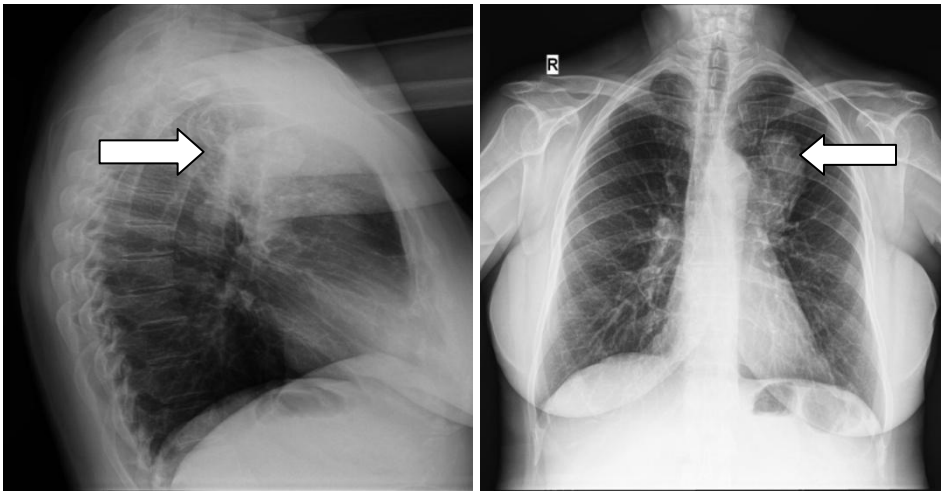

Fig. 1. Chest X-ray
(left – lateral view, right – anterior-posterior view; white arrow – tumoral mass)

The resulting images helped to characterize more precisely the expansive mass as solid, inhomogenous, with areas of necrosis, located in the left upper lobe, with no cleavage plane with the aortic arch and left pulmonary artery (fig. 2). Also, there were no mediastinal lymphadenopathies and pleuropericardial effusion.

In this clinical and imaging context, a late secondary lesion of cervical cancer

was suspected, and the determination of serum squamous cell carcinoma antigen (SCCA) was recommended to guide the diagnosis but the result was within normal range (SCC = 0.2 μg /L). The patient was referred to the Iasi Oncology Clinic and Thoracic Surgery Clinic, with a diagnosis of probable left upper lobe lung primary cancer.

Surgery with curative intent was limited only to taking multiple biopsies from different tumor areas as intraoperatively the lung tumor proved to be adjacent to the large vessels of the left pulmonary hilum, surrounding and discretely compressing them.

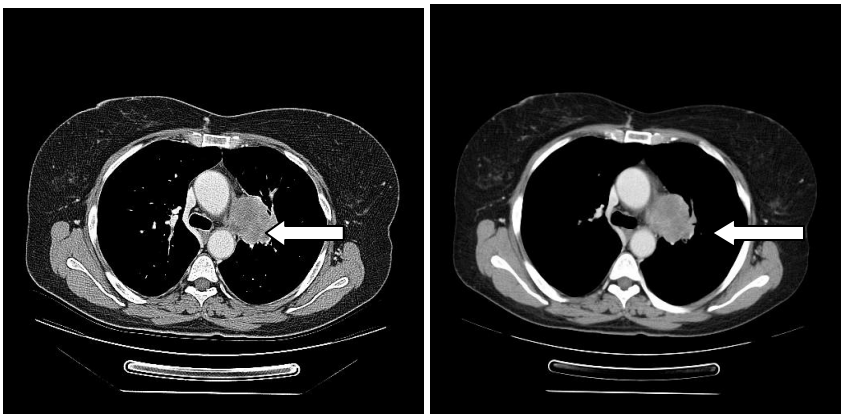

Fig. 2. Chest computed tomography
(left – native, right – intravenous contrast; white arrow – tumoral mass)

Microscopic examination (hematoxylin-eosin, van Gieson) of the collected tumor fragments showed an undifferentiated carcinoma. For an accurate characterization of the tumor multiple immunohistochemical markers were tested (tab.I), the final diagnosis being pulmonary large cell neuroendocrine carcinoma.

TABLE I
Immunohistochemical tumor markers

Immunohistochemical markers	Result
CK7	Zonal positive distribution
CK 5	Positive in very rare cells
TTF-1	Poorly positive zonal distribution
p63	Negative
Synaptophysin	Focally positive
CD56	Zonal positive distribution

The oncologist initiated primary chemotherapy with carboplatin 600 mg and paclitaxel 300 mg, with good immediate clinical tolerance.

DISCUSSION

This case brings up a relatively rare

condition of the oncologic patient who is monitored for locoregional recurrence or metastatic spread, but after clinical recovery, the likelihood of new primary tumors is overlooked. However, statistics show that approximately 20% of cancer survivors develop one or more metachronous cancers (1), requiring a careful, lifelong follow up of these patients.

The patient was diagnosed with lung cancer late and the prognosis was worsened mainly due to the discrete alarm symptoms and also by the absence, for a long time (three years) of a screening chest X-ray.

Of major importance after the discovery of the metachronous lung cancer process was the accurate histological typing of the new tumor in view of optimal selection of therapeutic approach. Initially, serum SCCA was determined, this tumoral antigen being well related with the clinical course of squamous cell cervical cancer (the first tumor in our patient) in terms of recurrent or progressive disease or presence of residual tumor after treatment (7). The very small seric levels suggested that a diagnosis of late pulmonary metastasis of the cervical carcinoma was unlikely. Immunohistochemical techniques, supported by data in the literature (8), were useful in determining the neuroendocrine nature of the second primary lung cancer, poorly differentiated and large cell - type, unfortunately associated with a poor response to chemotherapy (9); also, its perivascular location was contraindication to surgical resection of the tumoral mass.

In conclusion, given the fact that the time interval for the development of metachronous primary tumors in an oncologic patient is unpredictable, but usually takes years, the opportunity for screening and early diagnosis, mainly by imaging monitoring, has not to be neglected, the chances of survival of these patients being thus improved.

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ORTHOSTATIC INTOLERANCE AND CHRONIC FATIGUE SYNDROME - POSSIBLE RELATED CONDITIONS

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ORTHOSTATIC INTOLERANCE AND CHRONIC FATIGUE SYNDROME - POSSIBLE RELATED CONDITIONS (Abstract): The connection between orthostatic intolerance and chronic fatigue syndrome was first introduced in 1995. It was demonstrated that many patients with chronic fatigue syndrome also had some form of orthostatic intolerance. Some studies suggested that dysautonomia may be the common problem in patients with these syndromes. Although these conditions affect an important number of people, especially younger adults, orthostatic intolerance and chronic fatigue syndrome are among the least understood of the autonomic disorders and sustained research is focused particularly on elucidating their pathogenesis and identifying the most effective methods of treatment. **Keywords:** ORTHOSTATIC INTOLERANCE, CHRONIC FATIGUE SYNDROME, AUTONOMIC NERVOUS SYSTEM, DYSAUTONOMIA.

Although it is a condition that affects an important number of people, especially younger adults, orthostatic intolerance (OI) is one of the least understood of the autonomic disorders and it is the subject of a sustained research particularly focused on elucidating the pathogenesis and identifying the most effective methods of treatment. It is known by various names: ,,irritable heart", ,,neurocirculatory asthenia", ,,partial dysautonomia", ,,hyperadrenergic orthostatic hypotension", ,,vasoregulatory asthenia"or ,,soldier's heart". This syndrome has described by Da Costa in 1864, during the American Civil War, affecting young soldiers under physical and mental stress and characterized predominantly by cardiovascular symptoms in the absence of organic suffering (1).

In 1996, following a multidisciplinary conference under the auspices of the American Academy of Neurology and the Autonomic American Society, a brief definition of orthostatic hypotension (OH) was published (2). In Europe, the first guidelines for the management of syncope that included orthostatic intolerance syndromes (OIS) were published in 2001 and reviewed in 2004 by the European Society of Cardiology. To highlight the importance of this problem and because of the increasing number of studies regarding this issue an

update was necessary, the latest version of the guidelines, published in 2009 in the European Heart Journal, was developed in collaboration with the European Heart Rhythm Association, Heart Failure and Heart Rhythm Society Association (1). A few years later, progress in understanding OH and OIS has made it necessary to clarify and expand the original definition, so that in 2011, by a consensus statement endorsed by the American Autonomic Society, the European Federation of Autonomic Societies, the Autonomic Research Group of the World Federation of Neurology and the Autonomic Disorders Section of the American Academy of Neurology the definition, path physiology and clinical forms of the OH were updated and published. Also added were the definitions of two highly prevalent disorders of OI: neutrally mediated (reflex) syncope and postural orthostatic tachycardia syndrome (POTS) (2).

The connection between OI and CFS was first made public in 1995 by Peter Rowe, MD and associates at Johns Hopkins University, who identified a type of OI called neurally mediated hypotension in CFS patients. Since that year, scientists have learned much more about the kinship between OI and CFS.

ORTHOSTATIC INTOLERANCE– CLINICAL, EPIDEMIOLOGICAL AND PATH PHYSIOLOGICAL ASPECTS

Orthostatic intolerance is the development of a set of characteristic symptoms while standing or sitting upright such as dizziness/lightheadedness, pre-syncope, syncope; weakness, fatigue, lethargy, palpitations, sweating, visual disturbances, hearing disturbances (including impaired hearing and tinnitus) and pain in the neck (occipital/paracervical and shoulder region), low back pain, or precordial pain (3, 4). These conditions are a result of inappropriate reflexive bodily responses, regulated by the autonomic nervous system, to compensate the effect of gravity upon the distribution of blood. Predominately in younger individuals, often under the age of thirty five, these syndromes affect more women than men, both children and adults (3, 4, 5, 6, 7, 8, 9, 10).

According to the 2009 syncope management guidelines, there are three clinical forms of OI, and, of all, POTS is frequently associated with chronic fatigue syndrome (CFS). This condition affects mostly young women (11) in a 5:1 ratio over males and most patients are between 20 to 40 years of age (12). They present with severe complaints of OI, but not syncope, symptoms of cerebral hypo perfusion and autonomic over activity that are relieved by recumbence (2), marked increase in heart rate, greater than 30 beats/min, usually to 120 beats/min or higher, and transient systolic blood pressure decrease of more than 20 mm Hg, with recovery within the first minute of tilt (13), criteria that may not be applicable for individuals with low resting heart rates. For individuals aged 12–19 years the required increment is at least 40 beats/minute.

The etiology and pathophysiology of POTS are unknown, but are likely to be heterogeneous. According to Braunwald, major mechanisms are denervation (neuropathic postural orthostatic tachycardia syndrome), deconditioning, and a hyper adrenergic state, each of these three major mechanisms being exacerbated by hypovolemia (12, 13). An important aspect is that the syndrome is also associated with recent viral illness, a limited or restricted autonomic neuropathy, and CFS (2).

CHRONIC FATIGUE SYNDROME– CLINICAL, EPIDEMIOLOGICAL AND PATH PHYSIOLOGICAL ASPECTS

Chronic fatigue syndrome is the common name for a group of significantly debilitating medical conditions. It is characterized by new, unexplained fatigue that lasts for at least 6 months, is unrelieved by rest, is not due to exertion, is not caused by other medical conditions (14) and may be worsened by physical or mental activity. It is also known as chronic fatigue immune dysfunction syndrome, myalgic encephalomyelitis, post-viral fatigue syndrome or by several other terms.

Although biological, genetic, infectious and psychological mechanisms have been proposed, the etiology of CFS is not clearly understood and it may have multiple causes.

We know that symptoms may vary in number, type, and severity from person to person and, unfortunately, there is no specific diagnostic laboratory test or biomarker for chronic fatigue syndrome (15). Although it's name does not express the importance of this illness, CFS brings with it a number of debilitating symptoms: bone-deep fatigue, which is accompanied by other symptoms like sleep difficulties (falling asleep, staying asleep and waking up feeling unrefreshed), concentration and short-term memory problems, joint pain (without swelling), muscle pain, tender lymph nodes, sore throat, and headache. A distinctive symptom is post-exertional malaise, a worsening of symptoms following even very modest physical or mental exertion that can persist for days or weeks. About 25% of people with this syndrome are fully disabled by the illness (16).

Because these symptoms are common with many other conditions and in the absence of any specific laboratory test or biomarker, it is frequently misdiagnosed. Even so, at least one million people in the United States are affected and many others worldwide (17). Another research has shown that CFS is about four times as common in women as men, but it does not make any differences when it comes to age, racial, ethnic and socioeconomic group (18, 19).

We know now that CFS is not a form of depression, and many patients have no diagnosable psychiatric disorder. Also, some abnormalities of the brain have been found in CFS patients using magnetic resonance imaging scans, single photon emission computed tomography and positron emission tomography, predominantly affect the temporal lobes of the brain. Cognitive impairment is common in these patients, the most frequently documented abnormalities being the difficulty in information processing, memory, and/or attention.

CFS patients have disordered expression of genes that are important in energy metabolism.

They have a state of chronic low-grade immune upregulation and substantial evidence of poorly functioning natural killer cells. There is a growing body of evidence of more frequent latent active infection with various herpesviruses and enteroviruses in CFS patients, *Epstein Barr* virus and cytomegalovirus. Other infectious agents can also trigger CFS, including the bacterium that causes Lyme disease, *Giardia duodenalis,West Nile* virus and human parvovirus B19.

Abnormalities of the autonomic nervous system have been found by numerous independent researchers. These include a failure of the body to maintain blood pressure several minutes after a person stands up,

abnormal responses of the heart rate to standing, and unusual pooling of blood in the veins of the legs. Some have also found low levels of blood volume (20, 21).

DISCUSSION

It was demonstrated that many CFS patients (up to 97% in some studies) have some form of OI and it seems to be a particular problem in young people (3, 10, 22, 23, 24). Some studies have suggested that dysautonomia may be the common problem in patients with this syndrome. During tilt-table testing, more than 60% of patients with CFS show abnormal blood pressure or heart rate responses (sudden hypotension or severe bradycardia or tachycardia), along with decreased consciousness (12). OH can result in fainting very quickly after standing and can be diagnosed with a simple in-office test (measuring the blood pressure first while lying down and again upon standing) (3). Unlike patients with OH, in which case the drop of the blood pressure occurs within the first three minutes of standing, chronic fatigue syndrome patients with OI often have a delayed form of OI (25), meaning that the changes do not develop for many minutes after standing, the standard test for acute OH being ineffective in diagnosis. A tilt table test in CFS is considered to be positive if a patient experiences orthostatic symptoms and blood pressure and/or heart rate changes, whether or not he or she faints (3, 5).

There are several hypothesized causes of OI syndrome relevant to CFS, all leading to inadequate blood circulation that may reduce the amount of blood getting back to the heart and brain. Researchers have also identified several physiological abnormalities in CFS patients that are related with autonomic nervous system problems such as POTS. In five studies, adults and adolescents with CFS had elevated heart rates at rest compared to healthy and sedentary controls (8, 10, 23, 26, 27), although two studies found no difference (5). Heart rate further increased when patients underwent a tilt test, a common finding in POTS (3, 8, 10, 27, 28, 29). In addition, three studies - one in adults (27) and two in adolescents (9, 30) found that heart rate variability is significantly reduced in CFS compared to controls. This means that instead of having a heart rate that change appropriately when faced with orthostatic stress, many CFS patients have reduced modulation of their heart rate, suggesting impairment of the autonomic nervous system (9). In contrast, one study of adults with CFS found that heart rate variability is similar to that in controls (3, 30). An important issue is that the effective treatment for patients with OI and CFS must be individualized. Since no cause or cure has been identified, treatment programs should be directed at relieving symptoms, which can allow patients to live almost a normal life (21).

The first line of treatment should be non-medical interventions, such as increasing fluids and salt intake, tilting the head of the bed up a few degrees, wearing compression garments and learning to avoid the situations that can make OI worse (standing in long lines, being in warm environments, and eating large, heavy meals)(4). Also, the therapeutic importance of exercise training and improved physical conditioning is becoming an important strategy for the deconditioned patient (12). Because they often experience guilt, anger, anxiety and depression, it is important to include counseling for emotional and mental health. Educating patients, cognitive behavioral therapy, a form of psychological therapy

often used to treat chronically ill patients are moderately effective treatments (31, 32) that, sometimes, can be useful in treating some patients (30). If these are not effective, doctors may initiate pharmaceutical treatments (4).

Whether relief of any dysautonomic symptoms in these patients may relieve the symptoms of fatigue is unclear. In general, treatment for POTS helps greatly to alleviate some symptoms, but rarely fully resolves the CFS (3).

CONCLUSIONS

Further research is required to determine how orthostatic intolerance is involved in chronic fatigue syndrome. It is clear from past studies that orthostatic intolerance is associated with chronic fatigue syndrome, but the degree and meaning of that association is still a focus of vigorous research (3).

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NEURO-PROPRIOCEPTIVE FACILITATION IN THE RE-EDUCATION OF FUNCTIONAL PROBLEMS IN FACIAL PARALYSIS. A PRACTICAL APPROACH

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NEURO-PROPRIOCEPTIVE FACILITATION IN THE FUNCTIONAL RE-EDUCATION OF IN FACIAL PARALYSIS. A PRACTICAL APPROACH (Abstract): Facial paralysis, in the form of Bell's syndrome, is an acute paralysis of idiopathic origin. Disability in patients with this medical condition is the result of impairment or loss of complex and multidimensional functions of the face like emotion expression through facial mimics, facial identity and communication. **Aim:** This study aimed to present new and improved practical manual techniques in the area of facial neuromuscular facilitations and to review the literature for disability indexes and facial nerve grading. **Material and methods:** We present the practical modality of using neuro-proprioceptive facilitation techniques, such as rhythmic initiation, repeated stretch (repeated contractions), combination of isotonics and percussion, and also report the effects of these techniques in three Bell's syndrome patients which were previously evaluated. **Conclusions:** Recovery from facial paralysis can be a difficult and long lasting process and the utilization of a grading system may help the physical therapist. The effects of this type of therapy may help benefit the patient if the therapist is well trained and familiar with the neurophysiological background. **Keywords:** FACIAL PARALYSIS, PROPRIOCEPTIVE FACILITATION, BELL'S SYNDROM, HAUSE-BRAUCKMANN SCORE

Facial nerve paralysis, because of the dysfunctional problems that occur at the level of a very personal part of patient's body, who gives their personality, needs special consideration. Because speech, mastication, and expression of moods and emotions are based on the ability to move the facial musculature, successful treatment of facial nerve paralysis and especially functional reeducation are a vital concern for the physical therapists (1).

Bell's palsy has an incidence of approximately 20 cases of 100.000 persons and it's considered to be of idiopathic etiology. The onset is sudden, often proceeded by facial dysesthesia, epiphora, pain, hyperacusis, dysgeusia, and decreased function of the lacrimal gland (2, 3).

We found that very few patients with Bell's palsy are referred from the neurology services to physiotherapy centers. Very often, the patients are told to do nothing and that the muscle function and facial expressions will return without any intervention. Patients that are referred to physical therapy are typically treated with elec-

trical stimulation of the facial muscles and exercises with maximal effort. The results of such interventions were less then optimal resulting in the development of mass action or synkinesis (4).

Facial neuromuscular reeducation with tailored exercises for patients had been demonstrated to be effective in impairments related to facial paralysis (5).

MATERIAL AND METHODS

We are presenting a modality of manual stimulation for the reeducation of functional disorders in facial paralysis inspired from the proprioceptive neuromuscular facilitation concept of H. Kabat. This approach was used in clinical settings on Bell's palsy syndrome patients with positive effects on increasing muscle control and overall functionality; we also report the effects of this technique in three Bell's syndrome patients which were previously evaluated.

The techniques utilized in the rehabilitation of facial paralysis are tailored and executed specifically for every patient separately taking into account the following lines in the therapeutic conduct: degree of muscle function, functional disability and pain.

The techniques that we have used in managing facial paralysis conditions are: rhythmic initiation, repeated stretch (repeated contractions), combination of isotonics and percussion of tendons or marginal fascia of the muscle.

Rhythmic initiation was utilized in patients who could not perform any movement or when the therapist wanted to teach a motion or improve the coordination of that motion; this is achieved starting from passive range of motion and progressively passing to active range of motion with or without manual resistance. Description: the therapist starts by moving the patient passively through the range of motion using verbal commands to set the rhythm, and at a point in the treatment when the patient can promote movement he will first accompany the therapist and then will move against opposed force from the therapist (6, 7).

Repeated stretch was used at the beginning of the range of motion but also through range in case of muscle weakness and also when required for the initiation of motion, increasing strength and range of motion. Description: the therapist elongates the muscle just at the level when he feels a slight tension of the muscle (especially in flaccid situations) and then gives a "tap" to lengthen the muscle and evoke the stretch reflex; at that point the patient receives a verbal command to move and the therapist is resisting the resulted reflex and voluntary muscle contraction.

Combination of isotonics was performed in the program of rehabilitation at the time when the patient would have voluntary muscle contraction and movement for the following goals: achieving active control of motion, coordination and muscle strengthening. Description: the therapist is resisting the movement of the patient through the active range of motion desired, at the end of which the therapist tells the patient to stay in that position for the purpose of stabilization of the contraction. When the stabilization is achieved therapist tells he patient to allow the part to be moved slowly back (eccentric contraction).

In the functional rehabilitation of facial nerve paralysis is important to know the movement of the principal muscles and how to appropriate stimulate them in such a manner as to obtain the maximum muscular response. In the following photos we are presenting the specificity of manual facili-

tation for different muscles and the afferent explanation.

Stimulation of the frontalis muscle is made by the therapist pushing downwards with a diagonal component of inwards movement. The verbal command is: "Lift your eyebrow up and wrinkle your forehead, be surprised" (fig. 1).

Fig. 1. Stimulation of frontalis muscle

Stimulation of the corugator muscle is made by the therapist pulling upward with a diagonal component of outward movement. The verbal command is: "Pull your eyebrows down, look angry" (fig. 2).

Stimulation of orbicularis oculi muscles is made by pulling up the inferior and superior parts of the muscle at the same time with a diagonal component and resisting at the movement of the muscle from the contralateral side to reinforce the weak muscle. The verbal command is: "Close your eyes" (fig. 3).

Stimulation of levator labii superioris and procerus muscles is made by pulling downward superior lip. The verbal command is: "Show your upper teeth and wrinkle your nose" (fig. 4).

Stimulation of zygomaticus major and minor muscles is made by pushing the upper corner of the lip downwards and with a diagonal component of inward movement.

Stimulation of buccinator and risorius muscles is made by pulling the corners of the mouth medially; if the patient will push more interior towards your fingers it will stimulate more the buccinators muscle. The verbal command is: "Pull the corner of your mouth" (fig. 5).

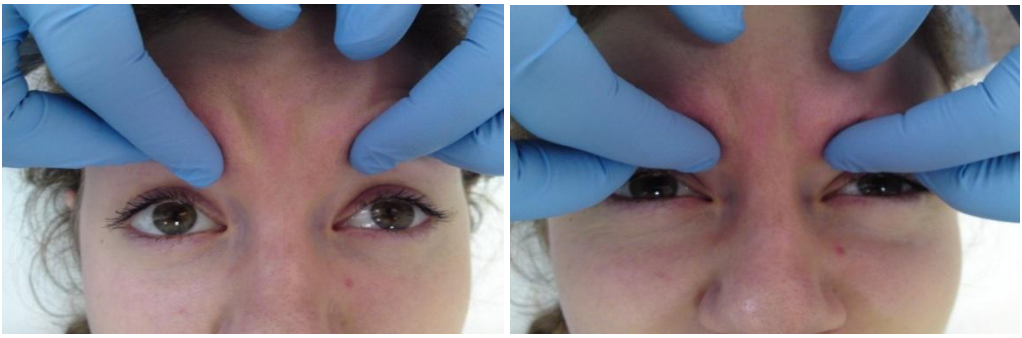

Fig. 2. Stimulation of corugator muscle

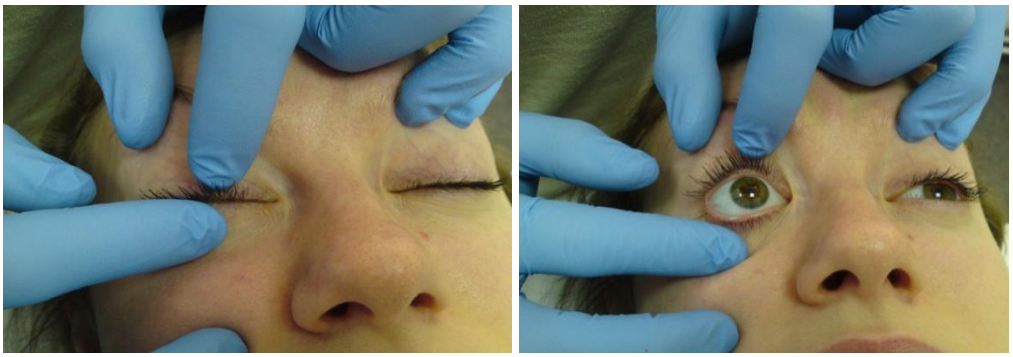

Fig. 3. Stimulation of orbicularis oculi muscle

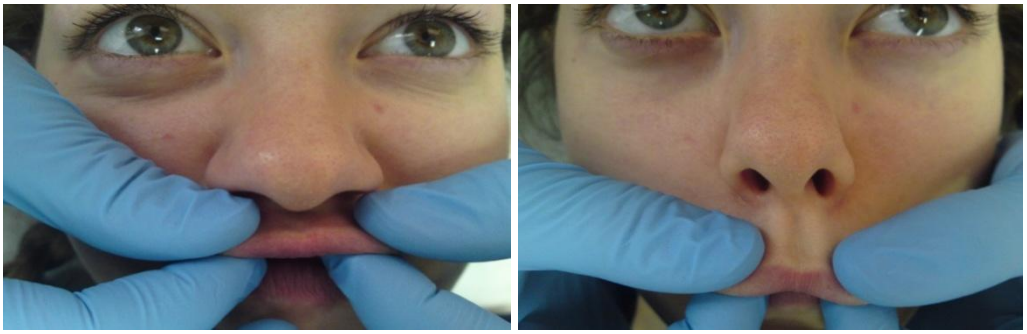

Fig. 4. Stimulation of levator labii superioris

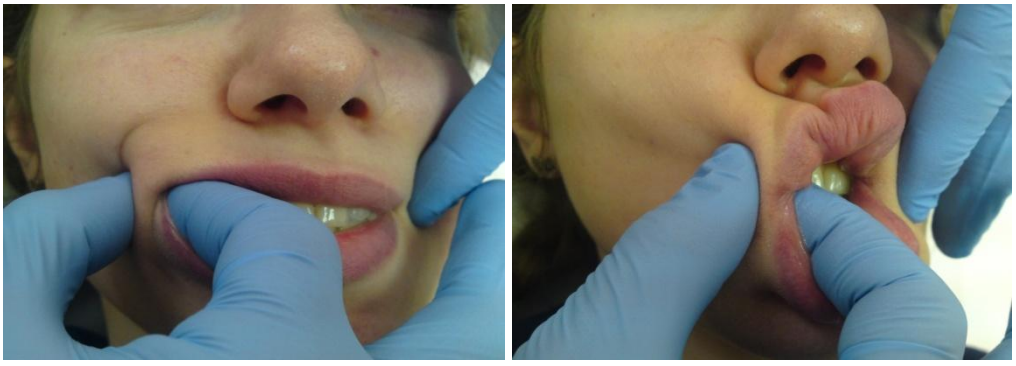

Fig. 5. Stimulation of buccinator and risorius

Stimulation of depressor anguli oris is made by pulling up the corners of the mouth. The verbal command is: "Push down the corners of your mouth. Look like you are sad". Stimulation of oribularis oris muscle is made by pulling outwards the corner of the mouth. The verbal command will be: "Kiss, whistle". This muscle has a great importance in closing the mouth.

CASE PRESENTATIONS

We are presenting three cases of facial paralysis (Bell's syndrom) that were treated at the Physio-kinesitherapy and Rehabilita-

tion Center, Iasi. All the patients that we are presenting were treated with the proprioceptive neuromuscular facilitation (P.N.F.) techniques that we have previously discussed for the duration of 30 sessions of treatment. For the clinical examination of the functional disorders we have chosen, after a review of articles, the Hause-Brauckmann facial nerve grading system which gives a qualitative and a quantitative assessment (8, 9).

S.U. has presented to our clinic on 9.04.2012 with a right facial paralysis caused by the inflammation of the seventh facial nerve. After the clinical examination the qualitative score on Hause-Brauckmann facial grading was V and the quantitative score was 1/8 – 2/8, that meant severe dysfunction with only barely perceptible motion and at rest with facial asymmetry. Characteristics: no movement at the level of the forehead, incomplete eye closure, excessive function of lachrymal glands and slight movement at the level of the mouth.

A.V. has presented to our clinic on 6.07.2012 with a right facial nerve paralysis caused by a car accident with head trauma. After the clinical examination the qualitative score was V and the qualitative score was 1/8 – 2/8. Characteristics: no movement at the level of the forehead, incomplete eye closure with no lachrymal gland action, and slight movement at the level of the mouth.

S.A. has presented to our clinic on 10.09.2012 with a right facial nerve paralysis caused by facial nerve inflammation. After the clinical examination the qualitative score on Hause-Brauckamann scale was graded IV and the quantitative score was 3/8, that meant moderately sever dysfunction with obvious weakness and normal symmetry of the face in rest position. Characteristics: no movement at the level of the forehead, incomplete closure of the eye and asymmetrical closure of the mouth with maximal effort.

RESULTS

After 30 sessions of P.N.F. manual therapy the patients' time of recovery was different from one another and came in different stages.

Patient **S.U.** had a good outcome of its treatment improving his qualitative score from grade V to grade I and the quantitative score from 1/8 to 8/8. The lacrimal gland ceased to work excessively and the blinking of the eyelash came through. The asymmetry of the face at stand position was no more visible and the overall facial expressions were reinstated.

Patient **A. V.** improved his qualitative Hause-Brauckmann score from grade V to grade I and the quantitative score from 1/8 to 8/8. The eyelash started to blink and lachrymal glands resumed their action.

Patient **S.A.** has improved her qualitative score from grade IV to grade I and the quantitative score from 3/8 to 8/8. (tab. I, fig. 6)

TABLE I

Analysis of the Hause-Brauckamann score

Patient name	**Before treatment**		**After treatment**	
	Qualitative score	Quantitative score	Qualitative score	Quantitative score
S. U.	V	1/8	I	8/8
A. V	V	1/8	I	8/8
S. A.	IV	3/8	I	8.8

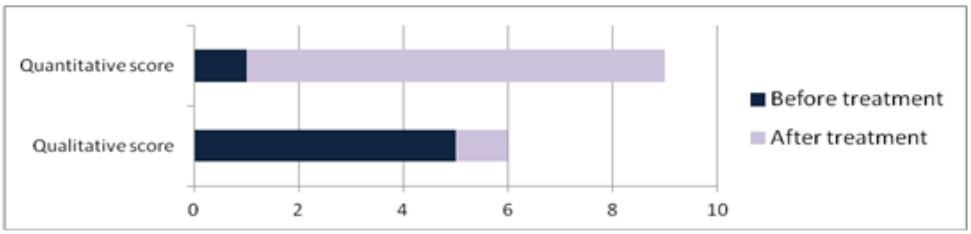

Fig. 6. Evolution of the Hause-Brackmann score

Moreover, because of the negative impact that it has on social integration of the facial paralysis subjects, the patients were more inclined to socialize after the treatment because of the regained conscience over their facial mimics and expressions.

CONCLUSIONS

Recovery from facial paralysis can be a difficult and long lasting process and the utilization of a grading system may help the physical therapist. The effects of this type of therapy may help benefit the patient if the therapist is well trained and knows the neurophysiological background.

Facial paralysis is one of the dysfunctions that actively affect the desire of the patients to communicate in social environments and from this idea it is of most importance for them to be treated their functional disabilities at the level of one part of the body that gives them the identity and their personality.

Furthermore, the success of the program depends on the relation established between the physical therapist and the patient, the perseverance and the motivation that lies behind.

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MACRO-CREATINE KINASE SYNDROME AS AN UNDERDIAGNOSED CAUSE OF CK-MB INCREASE IN THE ABSENCE OF MYOCARDIAL INFARCTION: TWO CASE REPORTS

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MACRO-CREATINE KINASE SYNDROME AS AN UNDERDIAGNOSED CAUSE OF CK-MB INCREASE IN THE ABSENCE OF MYOCARDIAL INFARCTION. CASE REPORTS (Abstract): When assessing an acute coronary syndrome (ACS) by means of high serum levels of creatine kinase (CK) and its MB fraction (CK-MB), one must keep in mind that there are several other causes for an increase of these markers, such as myocarditis, pericarditis, heart failure, severe aortic stenosis, stroke, renal failure, malignant hyperthermia, Reye syndrome, polymyositis, and borreliosis (1). Also, there are cases when CK-MB is falsely increased due to certain abnormalities that occur in the CK isoenzymes. One such example is the formation of the so-called macro-creatine kinase complexes (macro-CK) that give a false increase of the CK-MB fraction. We report two clinical cases where macro-CK was the cause of apparent increase in serum CK and CK-MB: in a 79-year old male with a history of coronary disease and a 82-year old female with permanent atrial fibrillation. **Keywords**: MACRO-CK SYNDROME, CK-MB FRACTION, ACUTE CORONARY SYNDROME.

Acute coronary syndrome (ACS) includes unstable angina and myocardial infarction. The only way to differentiate between unstable angina and myocardial infarction without ST segment elevation (NSTEMI) reveal myocardial necrosis by dosing biomarkers of myocardial cytolysis. Introduction of cardiac enzymes in clinical practice increased the rate of diagnosis of myocardial infarction by 30%.Cardiac markers are extremely important diagnostic tools especially in the elderly because of atypical clinical forms (insidious onset painless forms, atypical pain) and poor electrocardiographic changes through the predominance of NSTEMI forms.

CK-MB may increase in other diseases than acute myocardial infarction, such as myocarditis, cardiomyopathy, pericarditis, congestive heart failure, close aortic stenosis, stroke, renal failure, malignant hyperthermia, Reye's syndrome, strenuous efforts, trauma muscle, polymyositis, dermatomyositis, Duchenne muscular dystrophy, myoglobinaemia, borreliosis (1).

CK-MB is the most widely used marker for evaluating patients with acute coronary syndromes. Cardiac troponins (I and T) have greater sensitivity and specificity because they are contractile proteins that

are found only in the cardiac myocytes (2). Serial measurements of total CK and CK-MB, associated with troponin measurements may better indicate temporal evolution and infarct size (3, 4).

Creatine kinase (CK) consists of 3 fractions (CK-MM, CK-MB, and CK-BB). A report of total CK / CK-MB> 50% is suggestive for the presence of macro CK complexes (type 1 or 2), resulting in a reaction type Ag-Ac. The occurrence of these immune complexes produces a false increase in CK-MB which can 't be differentiated except by electrophoresis (5).

CASE REPORT I

Patient 1 is known with significant coronary and hypertensive damage ; at the age of 76 years he was performed angioplasty with active pharmacologically on circumflex artery and at the age of 78 years a coronary angiography is carried out again because of prolonged chest pain and occurrence of electrocardiographic changes in dynamics. The coronary angiography revealed the following changes: left coronary artery - LAD stenosis 80% II, 80% ostial stenosis, stent re-stenosis minimum CX I, stenosis 80% GM I, GM II 90% ostial stenosis, right coronary artery - 80% stenosis CD I CD III 80% stenosis, 80% stenosis IVP with surgical myocardial revascularization indication that the patient has timed it.

In April 2012, he was admitted in Geriatric Hospital Clinic "Dr. C.I. Parhon " for precordial pain having angina character and worsening in the last 4 hours before addressing and which partially wakened after Nitroglycerin administration.

Aim at internment: BP = 140/80 mm Hg, FC = 76 b / min, rhythmic, without signs of cardiac decompensation.

Electrocardiogram: sinus rhythm, FC = 70/min, AQRS = -30 °, concentric left ventricular hypertrophy type, ISL = 40 mm

Holter ECG: sinus rhythm throughout the recording

Echocardiography: significant left ventricular wall hypertrophy, SIV = 16 mm, EF = 60%, without hypo-kinetic areas.

Of laboratory results note: total CK = 245 U / l, CK-MB = 226 U / L, troponin T hs = 11 ng / ml (normal: 0-14 ng / ml), SGPT = 30 U / L, SGOT = 31 U / l, LDH = 175 U / l, glucose = 98 mg%.

Because of increased CK-MB, troponin T hs normal, ratio CK / CK-MB> 50%, without significant electrocardiographic abnormalities dynamic and echocardiographic appearance's CK electrophoresis was performed (tab. I). Suspected a false increase in CK-MB. Macro CK Complex has been identified type 1 (1.56 micromol / SL), total CK = 2.5 (normal: 2.85 micromol / SL), CK-MB = 0 micromol / sL (normal: <0.1 micromol / SL), CK-MM = 0.94 micromol / sL (normal: <2.85 micromol / SL), CK-BB = 0 micromol / sL (normal: 0) (tab I, fig. 1, 2).

TABLE I

Results of CK izoenzyme electrophoresis in patient 1

	Patient values	**Normal values**
CK	2,5 µmol/sl	< 2,85 µmol/sl
CK-MM	0,94 µmol/sl	< 2,5 µmol/sl
CK-BB	0 µmol/sl	0,00 µmol/sl
CK-MB	0 µmol/sl	< 0,1 µmol/sl
Macro CK	1,56 µmol/sl (62% of CK)	0 µmol/sl

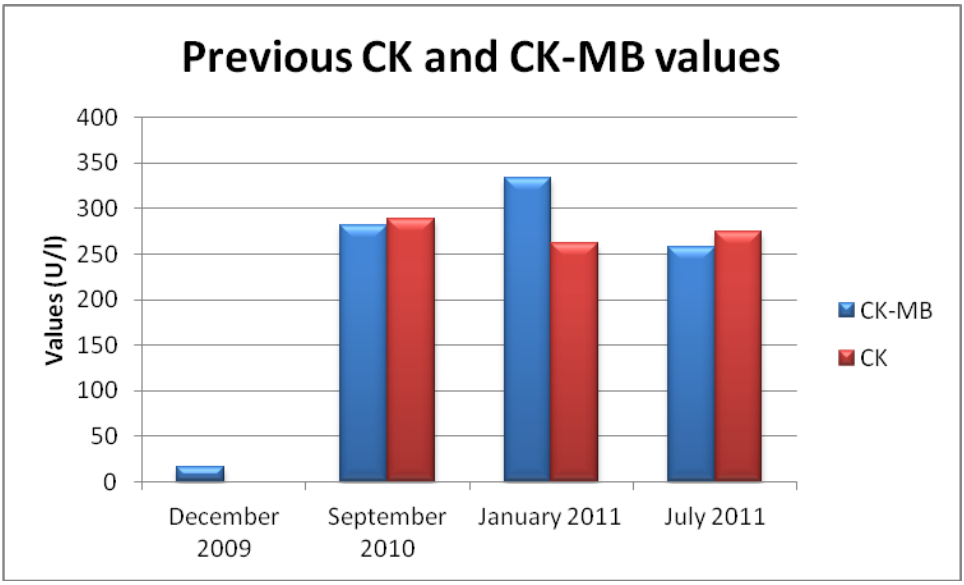

Fig. 1. CK and CK-MB values during previous hospital stay.
Values in 2009 are previously recorded PTCA

CASE REPORT II

Patient 2, well known hypertensive with permanent atrial fibrillation and heart failure NYHA class II left side is admitted for dyspnea at minor efforts occurred two days ago due to psycho-emotional stress. Associates prior atypical chest pain, with emphasis on compression onset 24 hours ago.

Aim at internment: BP = 150/90 mm Hg, FC = 110 / min.

Electrocardiography: Atrial fibrillation, FCM = 104/min, AQRS = 30°, negative T waves in V1, V2.

Echocardiography: EF = 50%, SIV = 11 mm, without hypo-kinetic areas without pericardial fluid, VCI completely inspiratory collapse.

From the laboratory results its is noted: total CK = 248 U / l, CK-MB = 274 U / L, troponin T hs = 17 ng / ml, SGPT = 36 U / l, ALT = 28 U /l.

CK s electrophoresis was performed as in the case of the first patient. It has been identified macro CK complex type 1 (4.33 micromol / sL) = 4.79 total CK, CK-MB = 0 micromol / SL, CK-MM = 0.46 micromol / SL, CK-BB = 0 micromol / SL (tab II, fig. 2).

TABLE II

Results of CK izoenzyme electrophoresis in patient 2

	Patient values	**Normal values**
CK	4,79 µmol/sl	< 2,85 µmol/sl
CK-MM	0,46 µmol/sl	< 2,5 µmol/sl
CK-BB	0 µmol/sl	0,00 µmol/sl
CK-MB	0 µmol/sl	< 0,1 µmol/sl
Macro CK	4,33 µmol/sl (90% of CK)	0 µmol/sl

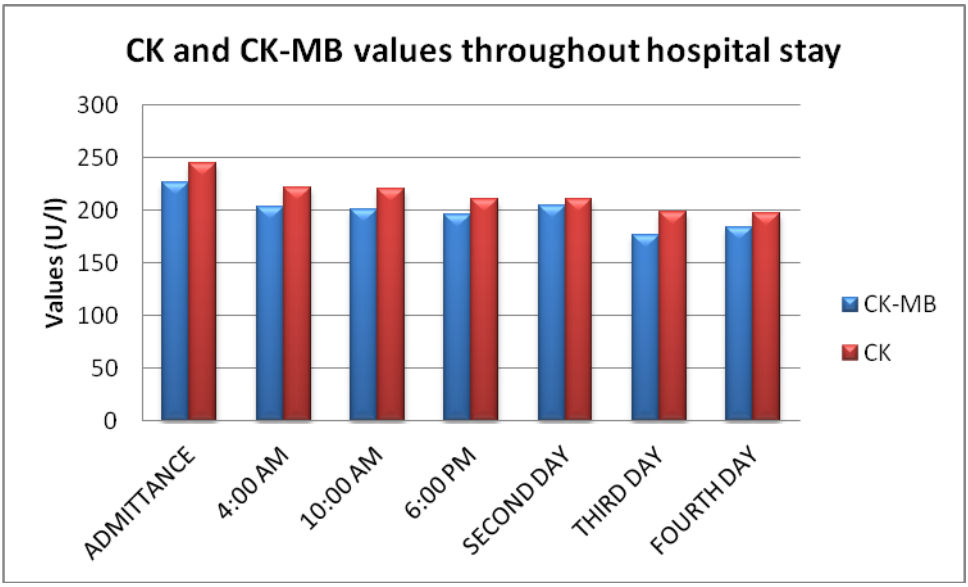

Fig. 2. CK and CK-MB values throughout hospital stay

DISCUSSION

In both cases one can notice a large increase in CK-MB values that could not be explained by acute myocardial ischemia or other common causes of this enzyme increase. Analyzing the previous documents of patient 1 reveals a constant value increase of CK-MB. The only normal value was recorded only in 2009 after stent angioplasty. On the internment in January 2011 and August 2011 the lowest CK-MB value was 125 U / l despite vigorous treatment, properly administered.

A report of total CK / CK-MB> 50% is suggestive for the presence of macro CK complexes. The occurrence of these immune complexes produces a false increase in CK-MB can t be differentiated except with electrophoresis. The presence of macro CK complex still doesn't have a precise use being possible to increase in many other diseases (6).

There are two types of macro CK: type 1 and type 2. Macro CK type 1 occurs via antigen-antibody reaction type between CK-BB and IgG or, less commonly, between CK-MM and IgA (7, 8). Macro CK type 1 (incidence = 0.45% - 1.2%) occurs in hypothyroidism, autoimmune diseases, cancer, myositis, cardiovascular disease and even apparently healthy individuals. It is more common in women and in people over 70 years (9). Macro CK type 2 (incidence = 0.5 - 3.7%) is close to home and migrate mitochondrial CK-MM band. In more severe disease than type 1 appears: malignancy (most commonly colon or prostate cancer) and severe liver disease with a poor prognosis (10).

Macro CK complex appearance does not determine great increase value of total CK (<500 U /l). It should be suspected a false increase in CK-MB in the presence of atypical symptoms of acute ischemia, lack of electrocardiographic changes, echocardiographic and persistent increases regardless of the therapeutic scheme administered (11).

Growth of these enzymes, with a total CK report /CK-MB under 5% is suggestive for non cardiac muscle damage. Troponin specificity and sensitivity is higher than CK-MB determination. Troponin T hs shows frequently increased values than CK-MB in the absence of acute coronary ischemia therefore it is important to determine its dynamics. Any significant increase in intraventricular pressure can lead to abnormal Troponin T hs without myocardial infarction. In patient 2, the slightest increase of Troponin T hs at interment with normalization in the next determination was interpreted in the context of heart failure and atrial fibrillation with fast frequency.

There are few cases of this type reported in literature. Among these, some associate the presence of this syndrome with digestive disorders such as Crohn's disease or ulcerative colitis (12) or persistent diarrhea (13). In other reports it has been noticed associations with cancer (14) or myopathies (15). Rosa Jimenez & et al (16) presents a series of 7 cases with macro-CK type 1 syndrome, of which 3 cases had a history of cardiovascular disease and the rest were not known. Patients reported in this article are the first reported in Romania with macro-CK syndrome.

CONCLUSIONS

Highlighting cases of macro CK is useful for avoiding invasive investigations and unnecessary treatments. The particularity of the first patient case consists in a false increase of CK-MB at a patient with three-vessel lesions and indication for revascularization surgery. The systolic function is maintained within normal limits and poses no arrhythmia at ECG Holter monitoring, in significant myocardial ischemia. The particularity of the second case is the combination of a significant increase in CK-MB in the presence of a chest pain caused by musculoskeletal pain (possible intricate pain) and without electrocardiographic changes in dynamics and without echocardiographic hypo-kinetic areas. Electrophoresis Creatine is currently quite expansive investigation and can be suspected the presence of complex macro CK emergency when the total report CK / CK-MB> 50%.

Discrepancy between the values of CK-MB and troponin must raise a question mark for the diagnosis of acute myocardial infarction and the values of these biomarkers must be correlated with clinical examination, electrocardiographic and echocardiographic aspect.

Guidelines for acute coronary syndrome should mention this false increase in CK-MB as a variant of differential diagnosis.

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NEWS

A NEW METHOD FOR DETECTION OF BACTERIAL PROTEIN SECRETION

Bacteria interact with host cells through secretion systems which carry proteins through bacterial membrane. Developing methods to monitor bacterial protein secretion can bring new information for diagnostic of bacterial infections. Enzymatic or fluorescent marking of proteins to reveal genes involved in transmembrane export of bacterial proteins can used to study the interaction of bacteria with host cell. A recent study conducted by M. M. Champion and collaborators describe a modified form of bacterial proteomics using a MALDI-TOF mass spectrometer, which directly detects the proteins from whole colonies. This method is able to specifically monitor a special protein secretion system, which is a major virulence determinant in *Mycobacterium tuberculosis, Bacillus* and *Staphylococcus* species. Also this method can be applicable to study other bacterial protein export systems and for the identification of compounds that inhibit bacterial protein secretion (M. M. Champion, E. A. Williams, G. M. Kennedy, P. A. DiGiuseppe Champion**.** Direct Detection of Bacterial Protein Secretion Using Whole Colony Proteomics**.** *Molecular & Cellular Proteomics*, 2012; 11 (9): 596-604).

Cătălina Luncă

BIOCHEMICAL AND ULTRASOUND ASPECTS IN CHRONIC VIRAL HEPATITIS C PATIENTS

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BIOCHEMICAL AND ULTRASOUND ASPECTS IN CHRONIC VIRAL HEPATITIS C PATIENTS (Abstract): **Aim:** Biochemical and ultrasound investigations in patients with chronic hepatitis C on a 48-week anti-viral regimen. **Material and methods:** A group of 6 patients, 4 women and 2 men, age range 24-61, were evaluated at the beginning of therapy and compared after 48 weeks of peginterferon alfa-2a and peginterferon alpha-2b regimen at doses established by the National Treatment Program approved by the Romanian National Health Insurance House. **Results**: ALT and AST levels have decreased in all patients. The viral load was undetectable in two patients, both females, after 48 weeks of therapy. Ultrasound images showed a decrease in size of the right and left hepatic lobes and portal vein diameter after 48 weeks. **Conclusions**: Inhibition of virus by interferon and ribavirin resulted in the reduction of inflammatory phenomena and regenerative fibrosis noticed after a very long period of time (at least 48 weeks). Further studies performed on larger patient groups are needed in order to better describe all these changes. **Key words**: CHRONIC VIRAL HEPATITIS C, INTERFERON, RIBAVIRIN, ULTRASOUND

In Romania, the average prevalence of hepatitis C in general population is approximately 5%, but it appears to be underestimated. As to mortality rate from chronic liver disease, Romania ranks fourth in Europe with 44.5%ooo deaths, as compared with the European average of 15%ooo.

70 to 80% of hepatitis C infection cases become chronic. Currently, no vaccine is available for hepatitis C (1). The diagnosis is made on elevated aminotransferase levels, antibodies to hepatitis C and hepatitis C virus RNA in the blood and liver biopsy confirming the diagnosis (2).

Careful screening is necessary before starting the treatment to determine the most appropriate approach for the patient. Combination anti-viral therapy with interferon and ribavirin has been the mainstay of hepatitis C treatment. Unfortunately, interferon is not widely available globally, it is not always well tolerated, some virus genotypes respond better to interferon than others, and many people who take interferon do not finish their treatment. This means that while hepatitis C is generally considered to be a curable disease, for many people this is not a reality (3).

Evaluation of treatment possibilities and contraindications, testing of hepatitis C virus genotype, and estimation of side effects are important therapeutic algorithm steps in patients with chronic hepatitis C. The use of subcutaneous peginterferon once a week and oral ribavirin 1000-1200 mg daily is the treatment of choice in these patients (4).

MATERIAL AND METHODS

Our study was conducted on a series of 6 patients with chronic viral hepatitis C, 4 women and 2 men, age range 24-61, evaluated at the beginning of therapy and compared after 48 weeks of treatment with peginterferon alfa-2a and peginterferon alpha-2b at doses recommended by the National Treatment Program approved by the Medical Committee of the Romanian National Health Insurance House.

Patients were assessed biochemically – alanine (ALT) and aspartate (AST) aminotransferase, serologically, and ultrasonographically. Viral markers and viral load, degree of necroinflammatory activity and level of fibrosis, development of complications (necrosis and liver fibrosis), size and other changes in the portal vein, morphological and clinical changes were some benchmarks. Patients were reassessed on completion of treatment (at 48 weeks of starting regimen).

Liver fibrosis was assessed by approved methods (PBH and FibroScan), in limits for chronic hepatitis (F1 to F3 Metavir score): of the six patients one had F1; four F2; and one patient F3 score.

RESULTS

Biochemical investigations. Comparatively, ALT and AST levels, baseline and after 48 weeks of anti-viral treatment, decreased in all study patients, but the most important decrease in ALT level was from 43 to 24 UI/ml (case 5, female, age 61) and in AST from 30 to 18 (the same patient); the smallest decrease was from 86 to 79 for ALT (case 2, male, age 51) and from 71 to 66 for AST (the same patient) (fig. 1, 2).

Viral load also decreased in all hepatitis C patients, but was undetectable in two cases (both females, aged 61 and 24, respectively) at the end of the 48 week- antiviral therapy.

Ultrasound investigations. We followed the size of right and left hepatic lobes (RHL, LHL), which decreased after the anti-viral regimen. The most significant decrease in RHL size was also noticed in case 5, from 124 to 101 mm and the less significant in case1 (female, age 41) – from 138 to 130 mm (fig. 3). Case 3 (male, age 47) showed the most significant decrease in LHL size, from 65 to 50 mm, while in case 1 the decrease was of only 3 mm, from 71 to 68 (fig. 4).

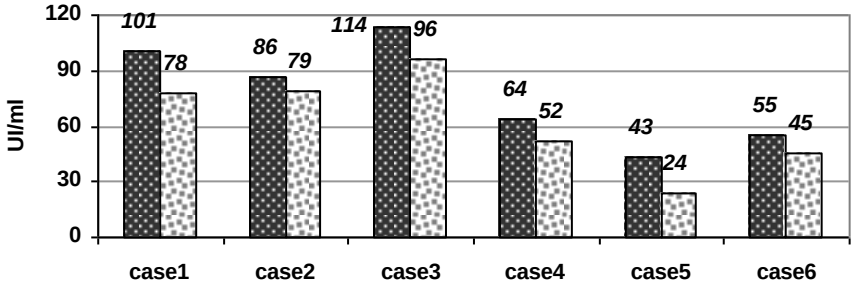


Fig. 1. Decrease in ALT levels in treated patients

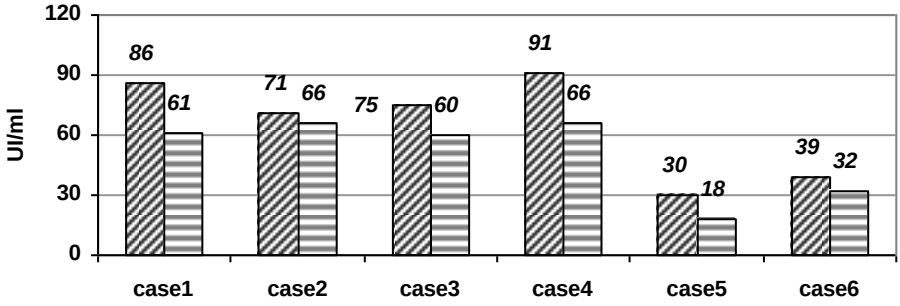
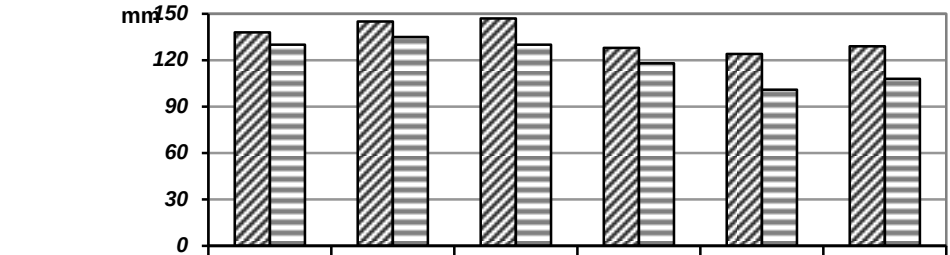
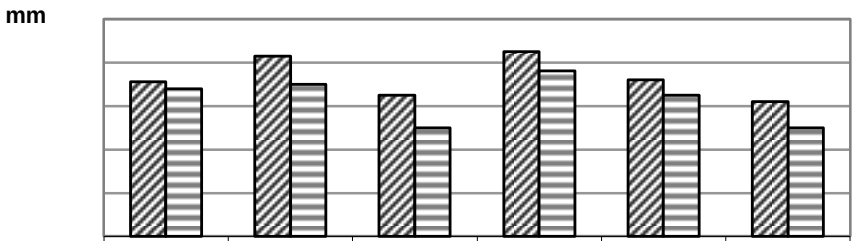


Fig. 2. Decrease in AST levels in treated patients



	case1	case2	case3	case4	case5	case6
before treatment	138	145	147	128	124	129
after 48 weeks	130	135	130	118	101	108

Fig. 3. Decrease in RHL size



	case1	case2	case3	case4	case5	case6
before treatment	71	83	65	85	72	62
after 48 weeks	68	70	50	76	65	50

Fig. 4. Decrease in LHL size

Significant changes in portal vein diameter after 48 weeks of anti-viral therapy were also recorded. A reduction of 1.5 mm was noticed in one case, 1 mm in two; 0.5 mm in one; and no reduction in the remaining two patients (fig. 5).

In all patients, the above-mentioned biochemical concentration levels and diameter values were induced by hepatic distress in the context of liver regenerative fibrosis, the clinical and ultrasound outcomes being moderate (fig. 6).

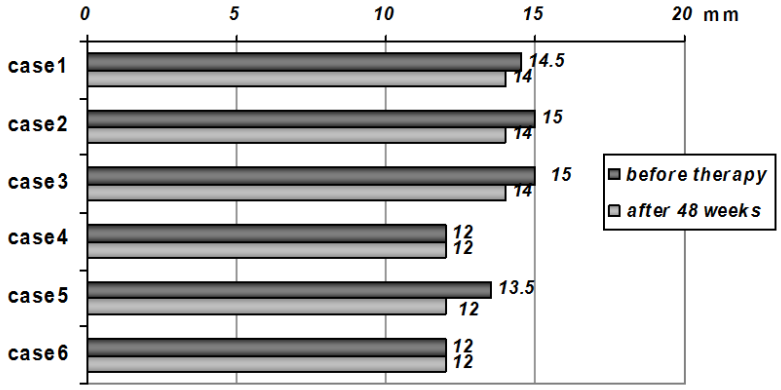


Fig. 5. Decrease in portal vein diameter

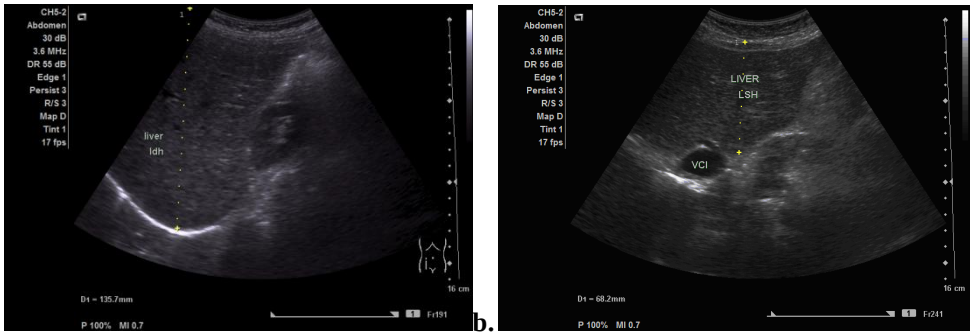


Fig. 6. Ultrasound images at the beginning (a) and after 48 weeks (b) of antiviral treatment (case 1, female, age 41)

DISCUSSION

In a systematic review, Shepherd et al. (5) mentioned that in the two trials testing pegylated interferon plus ribavirin against non-pegylated interferon plus ribavirin the combined percentage of sustained virological response (SVR) was 55%. In the four trials that evaluated pegylated interferon mono-therapy against non-pegylated interferon the combined SVR rates were 31% for pegylated interferon and 14% for non-pegylated interferon. Adverse events have been reported, but they did not differ substantially from levels of adverse events in regimens involving non-pegylated interferon.

Another meta-analysis on chronic hepatitis C therapy by Ford et al. (6) in middle-income countries highlighted that the treatment with pegylated interferon and ribavirin was associated with better outcomes than other regimens, and only 4% of patients included in the meta-analysis discontinued treatment because of adverse events.

Awad et al. (7), in a meta-analysis conducted in 2010, stated that peginterferon alpha-2a significantly increased the number of patients who achieved a sustained virological response (SVR) *vs.* peginterferon alfa-2b. The metaanalysis of adverse events leading to treatment discontinuation in
cluded 11 trials and revealed no significant differences between the two peginterferons. Authors suggested that peginterferon alpha-2a was associated with higher SVR than peginterferon alfa-2b.

Hézode et al. (8) in a study performed on a series of 91 patients with chronic hepatitis C and significant fibrosis (>7.0 kPa) who received therapy with pegylated interferon-α and ribavirin, mentioned that a significant liver stiffness decrease was observed during therapy, which continued after treatment only in patients who achieved SVR.

In a study carried out in South-East Asia, Chavalitdhamrong and Tanwandee (9) showed that during a follow-up interval of about 3 years in 171 chronic hepatitis C patients with SVR after effective anti-viral therapy there were no evident signs of either biochemical or clinical relapse of liver injury in all individuals.

In a multivariate analysis, Vergniol et al. (10) asserted that, whatever the virological response, treatment for hepatitis C was associated with an improvement of FibroScan and Fibrotest values. These non-invasive methods, and especially FibroScan, should be useful for assessing treatment efficacy in clinical trials of new regimens.

CONCLUSIONS

The comparative analysis of the study patients highlighted the homogeneity of the group: all individuals presented criteria for inclusion in a standard therapeutic protocol: elevated ALT and AST levels; liver fibrosis determined by approved methods (PBH and FibroScan), in limits for chronic hepatitis (between F1 and F3 Metavir score); an expected decreasing trend of the above-mentioned parameters during the therapy.

Virus inhibition by interferon and ribavirin led to a reduction of inflammatory phenomena and regeneration fibrosis after 48 weeks of therapy.

Along with treatment, "helper" factors, such as diet and lifestyle - very restricted in some cases, hepatic protective drugs, administration of various plant products, and the so-called sparing social and environmental factors, also contributed to the improvement of follow-up treatment parameters.

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NEWS

DENTIN REGENERATION USING DECIDUOUS PULP STEM/PROGENITOR CELLS

During the last decade, tissue engineering has been extensively used in the regenerative medicine. Mesenchymal dental stem derived from teeth are easily accessible multipotent cells with the capacity to differentiate into distinct cell types. Reparative dentin formation is essential for maintaining the integrity of dentin structure during caries or trauma. In this study, it was investigated the stem/progenitor cell-based tissue engineering for dentin regeneration in a large animal model. Porcine deciduous pulp stem/progenitor cells (PDPSCs) were mixed with a beta-tricalcium phosphate (β-TCP) scaffold for dentin regeneration. Different concentrations of PDPSCs were tested to determine the optimal density for dentin regeneration. Aliquots of 5×10(5) PDPSCs in 1 mL resulted in the highest number of cells attached to the scaffold and the greatest alkaline phosphatase activity. PDPSCs were labeled with green fluorescent protein (GFP) and the optimal cell numbers mixed with β-TCP were used to repair pulpchamber roof defects in the premolars of swine. Four weeks after transplantation, GFP-positive PDPSCs were observed in PDPSC-embedded scaffold constructs. At 16 weeks after transplantation, the PDPSCs mixed with β-TCP significantly regenerated the dentin-like structures and nearly completely restored the pulp chamber roof defects. This study demonstrated that the PDPSC/scaffold construct was useful in direct pulp-capping and provides pre-clinical evidence for stem/progenitor cell-based dentin regeneration. Stem cells could be of benefit in development of techiques for regenerative dentistry (Zheng Y, Wang XY, Wang YM, Liu XY, Zhang CM, Hou BX, Wanq SL. Dentin regeneration using deciduous pulp stem/progenitor cells. J Dent Res 2012; 91(7):676-682).

Gianina Iovan

UNELE ASPECTE PRIVIND HEPATITELE VIRALE B ŞI C. OBSERVAȚII PE UN LOT DE PACIENȚI TRATAȚI ÎN JUDEȚUL BACĂU, ÎN PERIOADA 2007-2010

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SOME ASPECTS REGARDING VIRAL HEPATITIDES B AND C. OBSERVATIONS ON PATIENTS TREATED IN THE BACAU COUNTY, BETWEEN 2007 AND 2010 (Abstract): The study aim was to assess some features of samples of patients assisted for viral hepatitis B and C, as well as cirrhosis, and included in a national antiviral treatment protocol. Material and methods. The study considered patients assisted in the medical assurance system in the Bacau County, Romania, during 2007 and 2010. Results. A sample of 1127 patients with chronic viral hepatitides B and C was assisted, from which 154 cases with B virus (13.7%) with a male predominance and 973 with C virus (86.3%) and a female predominance. An increase trend for both hepatitides prevalence was highlighted and a number of non-responders every year (18.1% of total cases). Conclusions. A correct diagnosis and treatment of\cases with chronic viral hepatitis B and C and to have a smaller number of non-responders represent major purposes of specialists in the Bacau County also. **Key words**: VIRAL HEPATITIS B, VIRAL HEPATITIS C, ANTIVIRAL TREATMENT, INTERFERON

*Hepatita virală B (*HVB) continuă să constituie o importantă problemă de sănătate publică pe plan mondial, cu toate că actualmente există mijloace eficiente de prevenție activă. Manifestările care decurg din evoluția autolimitantă sau persistentă a infecției cu virus B, îmbracă un spectru clinic foarte variat, de la hepatita acută cu evoluție rezolutivă la formele fulminante, de la starea de purtător sănătos, la ciroză şi cancer hepatic primar (CHP), respectiv la boli cronice extrahepatice (renale şi cardiovasculare) (1, 2, 3).

Hepatita virală C (HVC) este considerată inițial ca o hepatită transmisă aproape exclusiv (85-90%) prin sânge contaminat, astăzi s-a dovedit faptul că poate să apară şi sporadic la persoane fără antecedente transfuzionale, achiziționată comunitar (2, 4). Dominanta caracteristică a infecției cu VHC este evoluția preponderent persistentă, silențioasă din punct de vedere clinic, cu o tendință marcată de cronicizare şi malignizare. Se aproximează că 50% din infecțiile persistente evoluează spre hepatită cronică, 10-20% din hepatitele cronice trecând în ciroze. Incidența anuală a decompensării cirozelor, decompensări care pot conduce la deces în 2-5% din cazuri (1, 4, 5).

În prezent, există o multitudine de mijloace terapeutice, cu mecanisme variate de acțiune şi eficiență inegală, unele aflându-

se încă în faza de evaluare. În individualizarea tratamentului trebuie să se țină seama de o multitudine de parametri, printre care şi răspunsul imun endogen față de virusurile hepatitice (6, 7, 8, 9).

MATERIAL ŞI METODE

Studiul a luat în considerație un lot de pacienți cu HVB şi HVC, asistați în sistemul de asigurare medicală din județul Bacău, în perioada 2007-2010.

Criteriile de includere au cuprins cele legate de pozitivitatea diagnosticului referitor la prezența hepatitei virale B, C şi / sau D, prezența hepatitei cronice şi / sau a cirozei hepatice în contextul infecției virale, eligibilitatea pentru a intra într-o schemă terapeutică cu interferon şi antivirale, precum şi calitatea de asigurat la CAS Bacău. *Criteriile de excludere* au cuprins infirmarea diagnosticului de hepatită virală cronică.

Culegerea datelor a fost efectuată pe baza rapoartelor statistice existente la Casa de Asigurări pentru Sănătate Bacău, iar prelucrarea şi interpretarea s-au realizat cu ajutorul softului MS Excel şi SPSS 16.0.

REZULTATE ŞI DISCUȚII

1. Unele caracteristici demografice ale lotului studiat. În perioada 2007-2010, în județul Bacău, au fost asistați terapeutic 1127 pacienți cu hepatite virale cronice cu virus B şi C. Dintre aceştia, 154 au fost cazuri cu virus B (13,7%), iar 973 pacienți au prezentat hepatită C (86,3%).

Prevalența cea mai ridicată a fost întâlnită în anul 2010, prin cumularea cazurilor din anii precedenți (fig. 1), incluse în schemele terapeutice comune, la care se adaugă un număr, din ce în ce mai ridicat, de cazuri noi.

Distribuția pe sex arată o predominanță a bărbaților în lotul de cazuri cu HVB şi predominanța netă a femeilor în HVC (fig. 2, 3). De asemenea, tendința este crescătoare, pe seama cazurilor incluse în schemele de tratament, la care s-au adăugat unele îmbolnăviri.

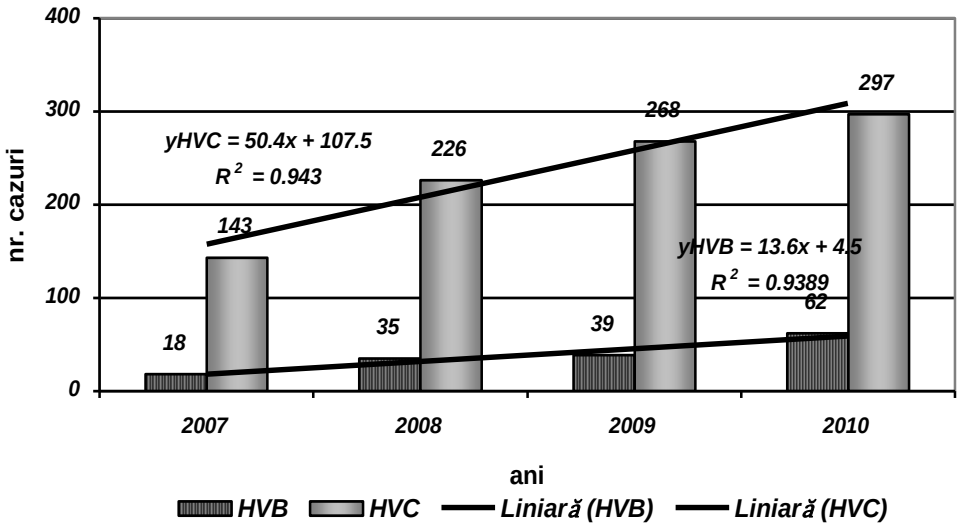

Fig. 1. Distribuția anuală a pacienților cu hepatite virale B şi C, luați în evidență în vederea tratamentului antiviral, în județul Bacău, în perioada 2007-2010

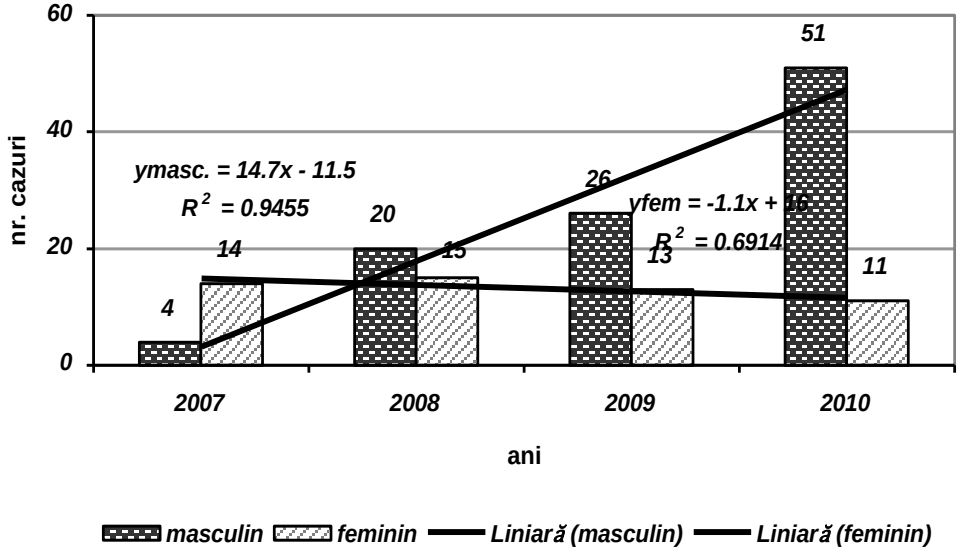

Fig. 2. Distribuția anuală şi pe sex a pacienților cu hepatită virală B cronică

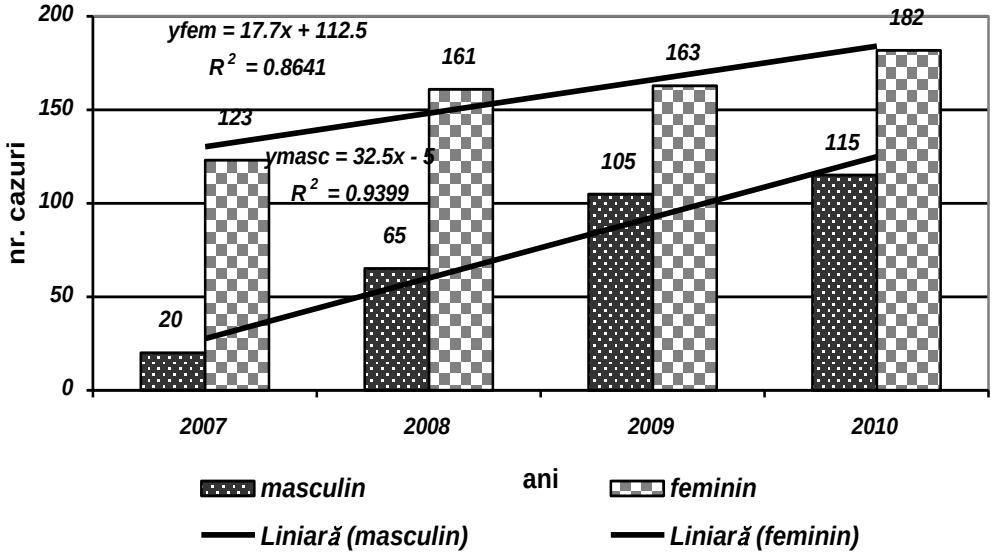

Fig. 3. Distribuția anuală şi pe sex a pacienților cu hepatită virală C cronică

Dacă pentru HVB, s-a observat o uşoară tendință de scădere a incidenței bolii la sexul feminin, pentru HVC, atât la bărbați, cât şi la femei, tendințele au fost de creştere semnificativă. Situația înregistrată se poate explica prin existența în populația

judeţului Bacău a unui număr în creştere de surse de virus C, ceea ce presupune un risc crescut de apariţia unor noi cazuri în anii următori.

2. Descrierea lotului de studiu, pe ani calendaristici. În ***anul 2007***, au fost incluşi în lot 18 cazuri de HVB, dintre care 4 bărbaţi şi 14 femei (raportul M/F = 0,28), iar cu HVC, 143 pacienţi, dintre care 20 de sex masculin şi 123 feminin (raportul M/F = 0,16). S-a observat predominanţa femeilor, în special pentru HVC, fapt care poate fi explicat printr-o frecvenţă mai mare a modalităţilor de transmitere (investigaţii paraclinice invazive) a virusului hepatitic C, în populaţia feminină. Datele noastre concordă cu cele de la nivel mondial, referitoare la distribuţia pe sex a HVC (1, 2, 4).

La lotul de 161 cazuri s-au adăugat 12 pacienţi cu ciroză hepatică, dintre care 5 bărbaţi şi 7 femei (tab. I). Vârsta medie a lotului studiat a fost de 50 ani şi 65,2% peste.

TABELUL I

Distribuţia pe sex a cazurilor de hepatite virale şi ciroze, incluse în tratament, în anul 2007

Categorii	Nr. cazuri	Bărbaţi		Femei	
		Nr.	%	Nr.	%*)
HVB	18	4	22,2	14	77,8
HVC	143	20	13,9	123	86,1
Ciroză hepatică	12	5	41,6	7	58,4

*) pentru valorile mai mici de 10, proporţiile sunt orientative

Au fost incluşi în tratament încă 84 pacienţi cu HVC, dintre care 75% în stadiul F2, 15% - F3 şi 5% - F4. Asocierile virale s-au evidenţiat numai cu Ag HBs – 14 cazuri din care 2 nonresponderi; B + C + D – niciun caz; B + D – 9 cazuri din care 1 nonresponder.

În ***anul 2008***, a fost adăugat, la tratamentul standard, şi Entecavir. Pentru HVB, s-a utilizat doar interferonul, iar pentru pacienţii cu HVC, asocierea interferon cu ribavirină. Din lotul de 35 cu HVB, 20 (57,1%) au primit tratament cu Entecavir (raport M/F = 17/3), iar ceilalţi 15 pacienţi, au fost trataţi cu interferon standard (3 x 10 M UI / săptămână) (10, 11, 12).

În studiu au fost incluşi, de asemenea, 226 pacienţi cu HVC, 65 bărbaţi şi 161 femei. Astfel că cele 71 cazuri noi de boală au determinat o rată de atac (incidenţă specifică) pentru HVC, în judeţul Bacău, pentru anul 2008, de 31,1%.

Evaluarea asocierilor virale a evidenţiat: pacienţi cu AgHBs prezent – 20 dintre care 5 au finalizat tratamentul; B + D – 10 pacienţi, cu 6 cazuri cu tratament finalizat; B + C – 5 cazuri din care toate rămase în schema terapeutică; B + C + D – niciun caz (tab. II). În acest an, 31 (11,8%) au fost pacienţi nonresponderi.

TABELUL II

Asocieri virale înregistrate la pacienţii cu hepatită cronică, în anul 2008

Virusul	Nr. total cazuri	Din care, cu tratament finalizat
Ag HBs prezent	20	5
B+D	10	6
C	226	0
B+C	5	0
B+C+D	0	0

În ***anul 2009***, au fost urmărite 39 cazuri de HVB, 26 bărbaţi şi 13 femei (raport M/F = 2). Cu HVC cronică, au fost 268 pacienţi, 105 bărbaţi şi 163 femei (raport M/F = 0,64). Din acest lot, un număr de 103 (38,4%) cazuri au fost scoase din schema terapeutică, fiind considerate *nonrespon-*

deri, astfel: la 4 săptămâni – 24 (23,3%); la 12 săptămâni – 76 (73,7%); la 24 săptămâni – 3 (4%).

Asocierile virale au fost: cu Ag HBs – 29 pacienți dintre care 4 ieșiți din tratament; B + C – 4 cu 0 nonresponderi; B + D – 5 cu 2 nonresponderi; B + C + D – niciun caz.

În ***anul 2010***, au fost înregistrate 62 cazuri de HVB, 51 pacienți de sex masculin şi 11 feminin (raportul M/F = 4,63).

În acelaşi an, au fost asistate 297 cazuri, 115 bărbați şi 182 femei (raportul M/F = 0,63), 121 (40,7%) au ieşit din tratament, iar dintre aceştia, 69 (23,2%) au fost consideraţi nonresponderi. Pentru un pacient, schema terapeutică a inclus şi Adefovir.

Pentru întreaga perioadă studiată, 204 (18,1%) au fost pacienți nonresponderi.

CONCLUZII

În perioada 2007-2010, în județul Bacău, au fost asistați terapeutic 1127 pacienți cu hepatite virale cronice cu virus B şi C. Dintre aceştia, 154 (13,7%) au fost cu virus B, iar 973 (86,3%) cu HVC, cu predominanța bărbaților pentru HVB şi cu diferență puternic semnificativă statistic ($p< 0,005$) pentru femeile cu HVC.

Morbiditatea a evidențiat o tendință crescătoare pentru ambele hepatite (B şi C), pentru întreaga perioadă de studiu, fapt explicat prin menținerea în tratament a pacienților în supraveghere, la care s-au adăugat cazurile noi. Acest fapt va conduce la creşterea costurilor asociate cu scăderea calității vieții.

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12. *** *Protocolul therapeutic în hepatita cronică şi ciroza hepatica cu virus B şi C* (Anexa 9). Monitorul Oficial al României, partea I, nr. 386 bis/10.VI.2010.

SECȚIUNE **TIP ARTICOL**

DIAGNOSTIC APPROACHES IN INFECTIVE ENDOCARDITIS

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DIAGNOSTIC APPROACHES IN INFECTIVE ENDOCARDITIS (Abstract): Infective endocarditis is a true systemic infection and a life-threatening disease associated with high mortality. **Aim**: To evaluate the problems that occur during making the diagnosis of infective endocarditis, in order to highlight the need of other diagnostic prospects. **Material and methods**: Retrospective study using clinical, microbiological, and echocardiographic findings from 45 patients admitted to the Iasi Infectious Diseases Hospital in the interval January 2007 - January 2011. **Results**: A positive diagnosis of infective endocarditis was made based on Duke Criteria. Inflammatory syndrome revealed leukocytosis with neutrophilia in 42% of the patients. In 91% of the cases fever syndrome was present. Blood cultures were positive in almost 45% of the cases, and the identified etiologic agents were *Staphylococcus spp.*, *Streptococcus spp.*, *Achromobacter spp.*, *Klebsiella spp.*, *Enterococcus spp.*, *E.coli*. In 95% of the patients, the echocardiographic appearance was a major criterion for diagnosis. Associated diseases were most often present with rebound on the course. Cardiac complications occurred despite treatment and re-evaluations. Ten percent of our cases required transfer to cardiology and cardiac surgery units. **Conclusions**: Microbiologic diagnosis was mainly based on cultured-dependent methods that often fail because of previous antibiotic therapy or the involvement of fastidious microorganism. In this case, advances in molecular diagnostics have yielded new tools (polymerase chain reaction - PCR techniques) to diagnose this disease. **Key words**: ENDOCARDITIS, DUKE CRITERIA, BLOOD CULTURE, ECHOCARDIOGRAPHY, PCR.

Infective endocarditis is a life threatening disease associated with high mortality, its sudden or insidious onset causing heart damages, or aggravates a preexisting one which as the disease progresses may affect other organs (1). Although the primary focus of the infection is confined to the endocardium, microbial shedding by continuous bacteremia and embolization of vegetation fragments makes infective endocarditis a true systemic infection. This particular form of systemic infection with unlimited development still has a great impact on the adult population, but not only (2).

While developments in cardiac imaging, therapeutics and surgical techniques have led to improved outcomes in individual patients, the overall incidence has remained relatively stable from the 1950`s through 2000, about 3.7 to 10 cases per 100,000 patient/year (3). The predisposing risk factors have changed over time with intravenous drug abuse, intravascular prosthe-

ses, nosocomial exposure, hemodialysis, and age-related valvular sclerosis being now more prevalent than rheumatic heart disease, especially in the developed countries (4). In addition to these risk factors, studies show that the causative flora implicated in infective endocarditis has also evolved, with *Staphylococcus aureus* emerging as the predominant pathogen, while a recent population-based study disputes this (5).

According to current guidelines, infective endocarditis is diagnosed if microbiological infection of the endocardium is demonstrated. The standard procedure includes clinical and echocardiographic findings plus identification of the causative pathogen (6). In unclear cases, diagnosis may be based on Duke criteria (7). Identification of the causative microorganism is crucial, not only for a proper diagnosis, but also for appropriate antibiotic treatment and management decision, e.g. surgery during active infective endocarditis. Blood cultures are accepted as the gold standard for pathogen identification and quantitative susceptibility testing.

However, in up to 31% of suspected infective endocarditis cases (8, 9), blood cultures remain negative due to previous antibiotic treatment, slow-growing, fastidious, or difficult-to-culture organisms (10), or small amounts of microorganisms per volume. According to the most recent studies, the patients with culture-negative endocarditis, often associated with more complicated courses, may benefit from pathogen identification by polymerase chain reaction (PCR) techniques (6, 11).

MATERIAL AND METHODS

We performed a retrospective study using clinical, microbiological, and echocardiographic findings from 45 patients admitted to the Iasi Infectious Diseases Hospital Iasi between January 2007 and January 2011.

A positive diagnosis of infective endocarditis was based on the clinical picture and laboratory findings according to Durack/Duke diagnosis and classification criteria of infective endocarditis (two major criteria, one major criterion and 3 minor criteria, or 5 minor criteria).

The clinical diagnosis relied on the concurrent presence of major criteria (positive blood cultures, damaged valve at echocardiography, a new heart murmur, worsening or changing the character of existing murmur, though not a sufficient criterion) and minor criteria (predisposing factors: cardiac, intravenous addiction, fever; vascular events: emboli, aneurysm, fungal, intracranial hemorrhages, septic pulmonary infarction, Janeway lesions; immunological manifestations of glomerulonephritis, Osler nodes, Roth spots, presence of rheumatoid factor; suggestive but not conclusive echocardiographic images to be considered a major criterion; also microbiological data: positive blood culture, serological evidence of acute infection with a germ possibly implicated in the etiology of infective endocarditis, splenomegaly, elevated ESR, increased CRP, presence of venous catheters, microscopic hematuria).

The bacteriological diagnosis revealed the causative agent in blood cultures (three samples), and transthoracic and/or transesophageal echo-cardiography in dynamics allowed the identification of certain valvular changes.

RESULTS AND DISCUSSION

Between January 2007 and January 2011, 45 patients with the diagnosis of infective endocarditis were hospitalized at the Iasi Infectious Diseases Hospital.

The yearly distribution of our infective endocarditis cases during the study interval was: most cases were recorded in 2010 (15 cases), followed by 2009, with 11 cases, 2007 and 2008 with 9 cases each, and 1 case in January 2011.

The patients were aged 9 to 76 years, most cases belonging to 40-66 years age group (36 cases); the mean age was 49 years. The patients were prevalently males (35 cases), and residing in urban areas (38 cases).

A positive diagnosis of infective endocarditis was made according to Duke Criteria. Inflammatory syndrome revealed leukocytosis with neutrophilia in 42% of patients. All clinical signs and symptoms specific for infective endocarditis were present in our patients. All patients (45 cases) presented cardiac murmurs, and the most severe symptom was physical and mental fatigue. In 41 (91%) of them fever syndrome was present (fig. 1).

Blood cultures were positive in 20 (44.5%) of the cases. Table I lists the identified etiologic agents: *Staphylococcus spp.*, *Streptococcus spp.*, *E.coli*, *Enterococcus spp.*, *Achromobacter spp.*, *Klebsiella spp.* Blood cultures remained negative in 25 (55.5%) of the cases.

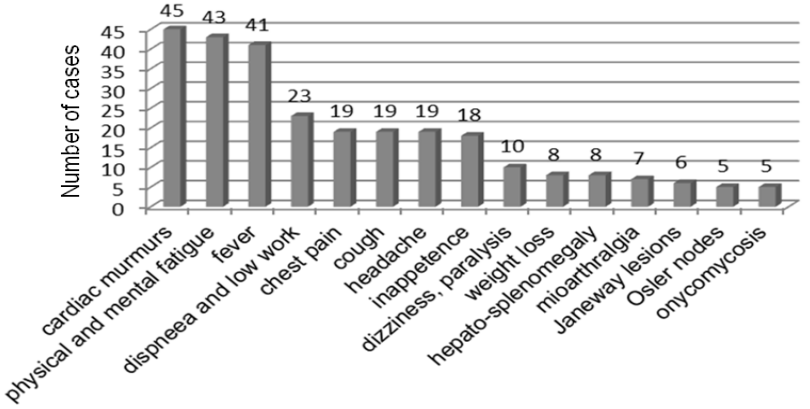

Fig. 1. Clinical signs and symptoms

TABLE I

Distribution of positive blood cultures with the identified etiologic agents, and negative blood cultures

Blood culture	Cases	
	Number	Percentage
Positive blood cultures	20	44.5
Staphylococcus aureus	7	15.5
Streptococcus spp.	4	8.8
Escherichia coli	3	6.6
Enterococcus faecalis	3	6.6
Achromobacter specialis	2	4.4
Klebsiella ozaenae	1	2.2
Negative blood cultures	25	55.5

For a complete diagnosis, transthoracic echocardiography was performed and the echocardiographic appearance was a major criterion for diagnosis. Vegetations were present in 95% of cases on different valves, while a normal appearance could be seen in few cases (4,2%), (fig. 2).

Echocardiographically, the cardiac vegetations were different in size and character (fig. 3).

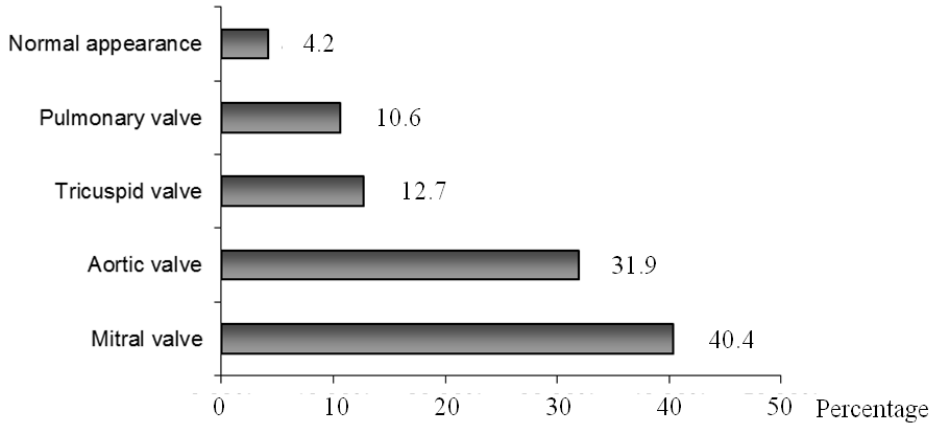

Fig. 2. Echocardiographic distribution of vegetations

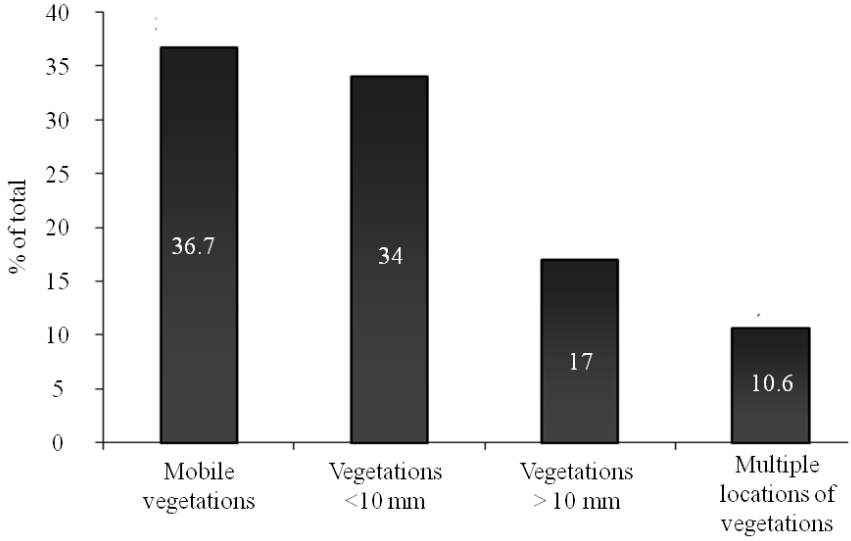

Fig. 3. Echocardiographic character of vegetations

Associated diseases were most often present and had rebound on the course. In most cases, anemia was the severe associated pathology (Table II). Cardiac complications, especially heart failure, occurred despite treatment and re-evaluations (Table III). Ten percent of cases required transfer to cardiology and cardiac surgery units.

TABLE II
Distribution of associated diseases

Associated pathology	Cases	
	Number	Percentage
Anemia	37	82.2
Global chronic heart failure	24	53.3
Mitral insufficiency	17	37.7
Aortic insufficiency	15	33.3
Chronic active hepatitis virus B +D, C	9	20
Arterial hypertension	8	17.7
Tricuspid insufficiency	7	15.5
Aortic stenosis	5	11.1
Severe pulmonary hypertension	5	11.1
Functional acute renal failure	5	11.1
Chronic renal failure	4	8.8

TABLE III
Distribution of complications

Complications	Cases	
	Number	Percentage
Cardiac failure	24	53.3
Pericarditis	6	13.3
Thromboembolism	5	11.1

CONCLUSIONS

Infective endocarditis being a very serious condition it is important to make a quick and appropriate diagnostic, so that the specific treatment to be initiated in time for patients.

A problem appears when blood cultures remain negative, and for finding the etiologic agent a better method than blood culture is needed. The pathogens identified in this study are in the spectrum of the literature reports. The predominant etiologic agent found in the present study was *Staphylococcus aureus*.

Of particular importance was diagnostic imaging by echocardiographic appearance which was a major diagnostic criterion.

Cardiac symptoms dominated the clinical picture. The complexity of associated pathology caused insidious developments.

The microbiologic diagnosis was mainly based on cultured-dependent methods that often fail because of previous antibiotic therapy or the involvement of fastidious microorganism. In this case, advances in molecular diagnostics have yielded new tools to diagnose this disease. Patients with culture-negative endocarditis may benefit today from identification of pathogens by polymerase chain reaction (PCR) techniques.

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NOUTĂȚI

DETECȚIA ADN BACTERIAN FOLOSIND NANOPARTICULE DE AUR

Metodele PCR utilizate în diagnosticul bolilor infecțioase necesită personal special instruit şi reactivi foarte costisitori. Padmavathy B şi colab. au explorat posibilitatea folosirii nanoparticulelor de aur (AuNPs) pentru detecția ADN bacterian din probe, printr-un procedeu mai ieftin şi mai uşor de realizat. Au fost elaborate protocoale pentru detecțiia sensibilă şi specifică a tulpinilor de *E. coli* folosind sonde oligonucleotidice cuplate cu nanoparticule de aur (AuNP- oligo probe) care hibridizează cu ADN-ul țintă din probele analizate. Detecția rapidă şi directă a genomului bacterian este posibilă printr-un viraj de culoare, observabil cu ochiul liber, datorită agregării sondelor. Metoda nu necesită amplificarea ADN, are o durată mai mică de 30 de minute după extracția ADN şi o limită de detecție de ~54 ng pentru ADN-ul neamplificat. (Padmavathy B, Vinoth Kumar R, Jaffar Ali BM. A direct detection of *Escherichia coli* genomic DNA using gold nanoprobes. *J Nanobiotechnol* 2012;10(1):8).

Teodora Vremera

CARDIOVASCULAR AND METABOLIC COMORBIDITIES - THERAPEUTIC DIFFICULTIES. CASE REPORT

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CARDIOVASCULAR AND METABOLIC COMORBIDITIES - THERAPEUTIC DIFFICULTIES. CASE REPORT (Abstract): Cardiorespiratory arrest is still a major emergency with a disappointing success rate despite the experts' efforts and the advanced equipment available nowadays. Most cases occur outside the hospital, the experience of resuscitation teams being essential in minimizing long-term neurological sequelae. We report the case of a 67-years-old female patient, old hypertensive, with coronary disease, diabetic, with multiple macro and microvascular complications of diabetes, who was hospitalized for the investigation of a home resurrected cardiorespiratory arrest, with multifactorial etiology. **Keywords:** DIABETES MELLITUS, CARDIORESPIRATORY ARREST, STROKE, ARTERIAL HYPERTENSION.

There are several factors that lead to the cardiorespiratory arrest. The chances of survival of a patient in the cardiopulmonary arrest decrease with time (1). It is extremely important for patients in the cardiorespiratory arrest to receive treatment as quickly as possible regardless of the circumstances in which the arrest occurred and the available resources (2, 3).

Atherosclerotic cardiovascular disease is the leading cause of death in industrialized countries (4). Longevity and number of co-morbidities increased (5). The rate of stroke increases with age, doubling for both men and women after the age of 65 (6, 7). Studies have shown that atrial fibrillation is associated with a 4 to 5-fold increase in ischemic stroke risk, independent of the presence of valvular heart disease. The choice of therapy for the prevention of stroke in patients with atrial fibrillation depends on several factors, including the risk of stroke, the risk of bleeding during anticoagulation therapy and the preference or availability of the patient (8, 9).

Judicious anamnesis and a rigorous, objective, complex and complete clinical examination are very valuable. Proper risk stratification allows prompt and efficient management with significant predictive implications. During the diagnosis, the degree of risk must be constantly re-evaluated.

The therapeutic scheme is guided by patient's co-morbidities. Guidance notes must be followed but apply individually (10).

CASE REPORT

We present the case of a 67-year-old female patient, from rural area, old hypertensive **(**BP max= 170/90 mmHg)**,** diabetic since age of 49 (about 6 months insulin treated), with medical history of acute lower myocardial infarction at age of 65 treated by percutaneous transluminal coronary angioplasty with stent on the anterior descending artery (coronarography at that time revealing tricoronary lesions), dyslipidemia, paroxysmal atrial fibrillation episode at the age of 66 converted with Amiodarone with the initiation of oral anticoagulant treatment with Acenocumarol, an episode of upper digestive hemorrhage manifested by melena 2 weeks before admission (by oral anticoagulation overdose), digestive endoscopy performed after hemodynamic stabilization showing aspect of gastritis and duodenitis, but with the absence of freshly digested blood or lesions with hemorrhagic potential), with acute post-hemorrhagic anemia, who is urgently hospitalized after resuscitation at home, with prompt response to resuscitation maneuvers.

Post-resuscitation, during hospital transportation, the patient develops bradypsychia, bradylalia, right hemiparesis (MRC = 4/5) and generalized contractions. The native cranio-cerebral computed tomography exam performed approximately 40 minutes after the cardiorespiratory arrest occurred showed signs of chronic ischemic stroke in left front-parietal lobe and the right caudate nucleus without the detection of acute ischemic lesions. Also described is diffuse cortical/subcortical atrophy, right putamen calcification and bilateral carotid calcifications.

The objective examination reveals an influenced general state, afebrile, conscious, bradypsychia, difficult cooperation through patents of systemic atherosclerosis, norm ponderal (BMI = 22.4 kg/m^2), stigmata of atherosclerosis – gerontoxon (fig. 1a.) mucocutaneous pallor, dehydrated skin, malar telangiectasias, trophic limb disorders, inferior limb onychodystrophies, left foot ulceration with hematic crust localized in the calcaneal region, imprecisely delimitated with irregular contours and loss of substance, approximately 4/3cm long, and painful edges (fig. 1b.); in the left elbow region of the there is a 5/6 cm nodular swelling area with medium to large hardness, slightly mobile on the deep plane with the presence of Celsius signs (fig. 1c.); SpO_2 = 98% in ambient air, quasi-continuous dyspnea, apexian shock in VIIth intercostal left ventricle on the anterior axillary line, TA = 130/70 mmHg, AV = 72 bpm, rhythmic cardiac noises, III/VI aortic systolic with irradiation on both carotid arteries, mitral systolic murmur grade III/VI with axial irradiation, liver at 2 cm under the rib cage, low pulsatile arteries (left <right), quasi-immobilization in bed (motions only in the bed plan).

An ECG examination was performed at admission with the following findings: sinus rhythm, QRS = 90°, AV = 60 / min, T negative in DI, aVL, QS DIII, T negative V_5-V_6, ST elevation sketch in V1-V4.

The biological investigations revealed mild leukocytosis, normocytic hypochromic anemia, hyperglycemia, nitrate retention syndrome and nonspecific inflammatory syndrome.

Chest radiography reveals cardiomegaly –cardiothoracic index> 0,5, a long elongated left inferior arch, calcification of aortic knob; lung aspect without evolutionary lesions (fig. 2).

a. **b.** **c.**

Fig. 1. Patient assessment highlights

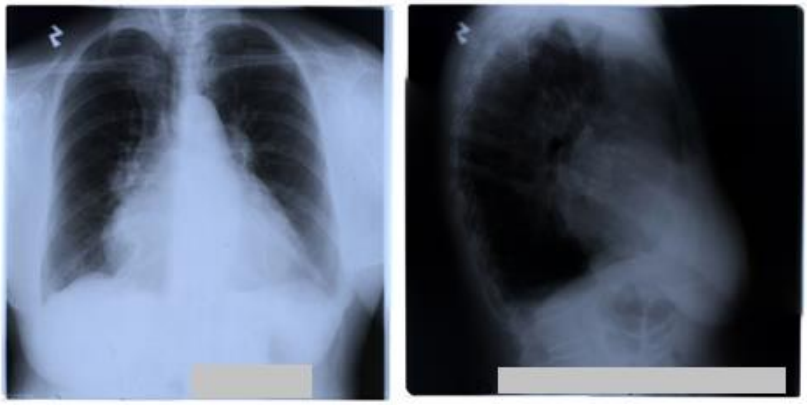

Fig. 2. Chest X-ray

Abdominal ultrasound described liver steatosis, folded gallbladder with calculus (several stones of 5-6 mm), hypoechogenic lipomatosis pancreas, small dimensions of the kidneys (right kidney = 104 mm, parenchymatous index = 7-14mm, 2 mm mean calcification in a dilute calyx, left kidney = 92 mm, parenchymatous index= 11 mm); uterus surgically extirpated; otherwise, abdominal ultrasound does not describe other changes.

Echocardiography reveals cardiomegaly, biatrial dilatation and degenerative mitral valvular changes (mitral ring fibrosis) and aortic (aortic valve sclerosis) with delayed relaxation diastolic dysfunction, EF = 40%, FS = 20% (fig. 3).

Due to the presence of the nodular formation in the left elbow region, a soft ultrasound was performed which found in the subcutaneous tissue a non-homogeneous, imprecisely defined collection, with maximum dimensions 8/17/33 mm, with no Doppler signal inside and in the periphery; and a diffuse infiltrate appearance of adjacent subcutaneous tissue without signs of thrombosis of neighboring vessels.

Vascular surgery consult established the diagnostic of superficial phlebitis located in the inferior third of the upper left limb. Also, the presence of the ulcerative lesion at the left calcaneal region required an eco-Doppler investigation that revealed medio calcinosis in the left femoral artery and in the bilateral popliteal arteries, severe stenosis in the left popliteal artery (90%), with the recommendation of establishing a conservative treatment.

Fig. 3. Echocardiographic changes

It was also determined that the ankle-brachial index that showed the presence of middle obstruction on the right (0.61) and severe on the left (0.4).

We established a general treatment with antibiotic treatment associated with coronary vasodilators, antihypertensive treatment, hypolipidemic, monotherapy antiplatelet drugs, gastro protector, antiarrhythmic, diuretic, martial and topical therapy has been instituted.

Clinical evolution was slowly favorable with progressive improvement in dyspnea, tendency to epithelialization of the plantar ulcer, and remission of inflammatory phenomena from the left elbow, but eluded by neurological complications and diabetes by plurivascular affection, with increased bradylalia and bradypsychia, and evolution to mixed aphasia.

DISCUSSION

Major cardiovascular events at hypertensive patients are preceded by the development of structural and functional cardiovascular and renal abnormalities, most of which are recognized as significant independent predictors of subsequent cardiovascular complications (11).

People with type 2 diabetes have a 1.54 to 4 times higher risk of developing a coronary disease, with a younger onset with a higher severity of the lesion and a lower prognosis, compared to individuals without diabetes. Studies have also shown that cerebrovascular disease morbidity is 2-5 times higher in type 2 diabetic patients than in non-diabetic individuals (4). In the general population, women show a delay of about 10 years compared to men, regarding the occurrence of subclinical atherosclerosis and cardiovascular events (12).

The recommendations of the American College of Chest Physicians (ACCP) guidelines for atrial fibrillation are as follows:

- Warfarin is routed to all high-risk patients and to all patients over 75 years of age, regardless of their risk;
- Low-risk patients and patients under 65 years of age should be treated with aspirin;
- Patients aged 65-75 without risk factors will receive oral anticoagulant treatment based on associated comorbidities (9).

The prognosis of this case is reserved both in the short term for the possibility of occurrence of immediate thromboembolic complications with vital and long-term risk

through coronary complications (patient with tricoronary lesions), rhythm and/or conduction disorders, hemorrhagic transformation of chronic stroke from the territory front-parietal left, complications secondary to diabetes. Also, the prolonged decubitus caused by the right hemiparesis causes trophic and tissue complications.

CONCLUSIONS

Caring for patients with multiple comorbidities requires knowledge of both the manifestations caused by the pathology involved, and especially the difficult issues within the therapeutic plan. Individualized therapeutic regimens, flexibility and empathy towards the wishes of each patient, providing psychological and emotional comfort that motivates them to constantly care for their health, are just a few principles that underlie therapeutic monitoring. In this context, a multidisciplinary therapeutic approach, medical teamwork, collaboration with the family doctor and other specialties is needed that manages the evolution of the patient's general pathology.

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EFECTELE ETANOLULUI ASUPRA EVOLUȚIEI INTOXICAȚIILOR ACUTE CU BENZODIAZEPINE

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THE EFFECTS OF ETHANOL ON THE EVOLUTION OF THE ACUTE BENZODIAZEPINE POISONING (Abstract): The depressing effects on the nervous central system (NCS) induced by benzodiazepines and ethanol are similar. The complications are rare in the benzodiazepine poisoning, but are a lot more frequent in association with other depressing drugs for the NCS (especially alcohol). **Material and methods:** We analyzed retrospectively patients with benzodiazepine poisoning admitted in the Internal Medicine Clinic – Toxicology during 2003 – 2009.The study attempted a complex evaluation of the consequences of acute and chronic alcoholism on the evolution of acute benzodiazepinepoisoning and the description of the clinic evolution and paraclinical particularities of the patients under investigation. **Results:** 343 patients with benzodiazepine poisoning were admitted, 150 were tested through measurement of alcohol level, leading to values between 1 – 415 mg/dl. Chronic alcoholism in personal pathological antecedents of the patients determined a relative risk of intoxication 1.46 times higher. The hospitalization period varied from 1 to 8 days for patients with chronic alcoholism and from 1 to 14 days for patients with acute alcoholism, a statistically important difference. **Conclusions:** During the period under investigation, from the total of patients admitted for acute benzodiazepine poisoning, 2 deaths were registered. Of the two deaths, one patient showed ethanol coingestion. **Key words:** BENZODIAZEPINE POISONING, CHRONIC ALCOHOLISM, ACUTE ALCOHOLISM.

Intoxicaţiile acute cu benzodiazepine (BZD) constituie o cauză importantă de morbiditate şi mortalitate în întreaga lume. Cel mai adesea, intoxicaţiile survin la pacienţi etilici cronici sau pe fond de etilism acut,ceea ce face ca managementul lor să devină extrem de dificil.

BZD administrate în doze peste limita terapeutică produc doar semne minime şi moderate de toxicitate, incluzând ataxie, dizartrie şi letargie, dar dozele foarte mari pot cauza comă, hipotensiune, hipotermie şi depresie respiratorie, necesitând intubaţie oro-traheală şi ventilaţie asistată (1, 2).

În intoxicaţiile cu benzodiazepine complicatiile sunt rareori semnalate, dar sunt mult mai frecvente în asociere cu alte droguri deprimante ale sistemului nervos central (SNC), în special alcoolul.

Depresia SNC este mai accentuată la vârstnici, la pacienţi cu boli cronice (ciroză, hepatopatii) şi la pacienţi în tratament cu medicaţie ce interferă cu metabolismul hepatic al BZD: macrolide (claritromicina,

eritromicina), blocante calcice (verapamil, diltiazem), antifungice (fluconazol, ketoconazol), antidepresive (fluoxetin), inhibitori de pompă protonică (omeprazol). De asemenea, coingestia de suc de grapefruit şi de alcool poate creşte concentraţia serică de BZD (3, 4, 5, 6).

Efectele deprimante asupra SNC induse de BZD şi etanol sunt similare. Alcoolul acţionează asupra complexului funcţional format de acidul gamma-aminobutiric (GABA), receptorul pentru BZD şi canale de clor, crescând afinitatea de legare a BZD la situsul BZD, având efect aditiv şi de potenţare a efectului deprimant SNC şi la nivelul aparatului respirator (2,8,9,10).

Lucrarea urmăreşte evaluarea complexă a consecinţelor etilismului acut şi cronic asupra evoluţiei intoxicaţiilor acute cu BZD si descrierea particularităţilor clinico-evolutive şi paraclinice ale pacienţilor incluşi in studiu.

MATERIAL ŞI METODE

Cercetările au inclus 343 pacienţi intoxicaţi cu BZD, asistaţi în Clinica Medicală –Toxicologie şi ATI a Spitalul Clinic de Urgenţe Iaşi, în perioada 2003-2009. Pe baza investigaţiilor clinice şi paraclinice, s-a încercat evidenţierea influenţei etilismului cronic sau acut, ca factor de risc, asupra stării de sănătate a populaţiei. Culegerea datelor s-a realizat prin investigaţii clinice şi paraclinice, analiza foilor de observaţie şi ancheta clinico-epidemiologica. Pentru prelucrarea şi interpretarea datelor obţinute s-au folosit metode epidemiologice, de laborator şi statistico-matematice.

REZULTATE SI DISCUTII

1. Evaluarea nivelului alcoolemiei la pacienţii intoxicaţi cu benzodiazepine.

Din numărul total de pacienţi intoxicaţi cu BZD, 43,7% dintre pacienţi au fost testaţi prin măsurarea alcoolemiei, obţinându-se valori de la 1 la 415 mg/dl. (fig. 1).

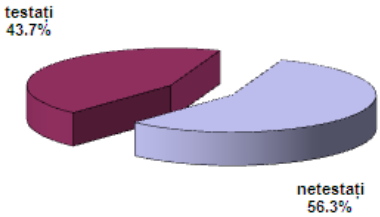

Fig. 1. Distribuţia pacienţilor intoxicaţi cu benzodiazepine în relaţie cu investigarea alcoolemiei

Valorile normale ale alcoolemiei, indicate de Laboratorul de Biochimie a Spitalului de Urgenţă „Sf. Ioan" Iaşi, se află în intervalul 1-10 mg/dl, iar cele înregistrate peste această limită la pacienţii testaţi la internare au variat de la 11 la peste 300mg/dl (tab. I, fig. 2).

2. Analiza în relaţie cu sexul pacienţilor cu etilism peste limita normală,a remarcat căponderea pacienţilor de sex masculin (29/56) cu valori peste limita admisă nu a prezentat diferenţe semnificative faţă de cea a pacienţilor de sex feminin (51/94) (χ^2=0,02; GL=1, p=0,901) (fig. 3).

Valorile medii ale alcoolemiei, înregistrate la pacienţii de sex feminin au fost semnificativ mai scăzute (74,16±9,84 $g^o/_{oo}$) decât la cei de sex masculin (87,70±11,54 $g^o/_{oo}$) (t=7,33; GL=148; p<0,001).

TABELUL I

Nivelul alcoolemiei la pacienţii intoxicaţi cu benzodiazepine

Alcoolemie (mg/dl)	% din totalul pacienţilor testaţi
11-100	14,5
101-200	17,7
201-300	12,9
>300	9,7

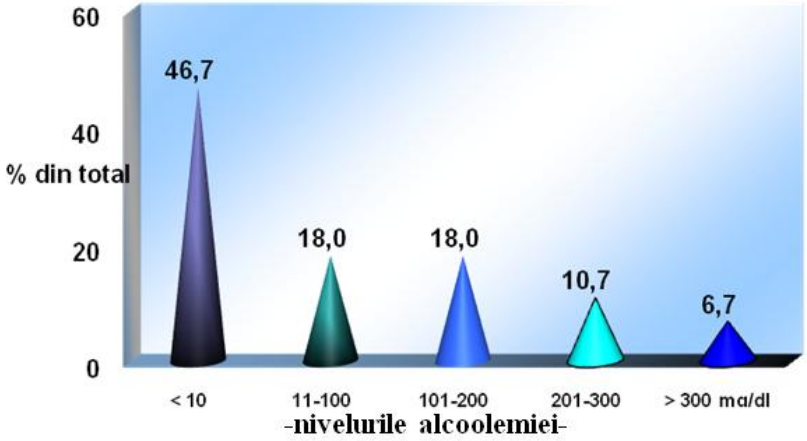

Fig. 2. Distribuția pacienților intoxicați cu benzodiazepine în relație cu nivelul alcoolemiei înregistrat la internare

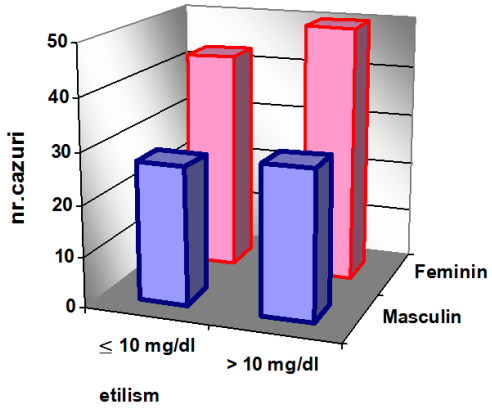

Fig. 3. Distribuția pe sexe a pacienților intoxicați cu benzodiazepine în relație cu nivelul alcoolemiei înregistrat la internare

3. Analiza cazurilor în relație cu mediul de proveniență.

A rezultat că ponderea cazurilor provenite din mediul urban cu alcoolemie peste limita normală din total testați (60/98) a fost semnificativ mai crescută comparativ cu cele din mediul rural (20/52) (χ^2=6,19; GL=1; p=0,013); riscul relativ de intoxicație cu benzodiazepine a fost de 1,59 ori mai mare la pacienții din urban cu coingestie de alcool (RR=1,59; IC95%: 1,09÷2,32) (fig. 4).

Valorile medii ale alcoolemiei, înregistrate la pacienții proveniți din mediul urban (106,45±90,61 $g^o/_{oo}$) nu au fost semnificativ mai crescute decât la cei din mediul rural (93,39±57,33 $g^o/_{oo}$) (t=1,08; GL=148; p>0,05).

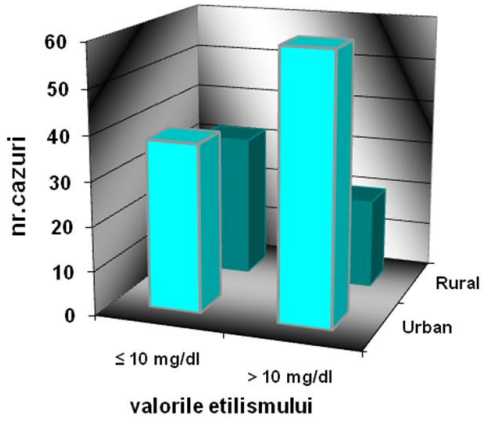

Fig. 4. Distribuția pacienților testați în relație cu nivelul alcoolemiei înregistrat la internare și mediul de proveniență

4. Evaluări privind prevalența cazurilor de etilism şi durata de spitalizare

Anamneza pacienților cu coingestie de alcool din lotul studiat a identificat existența a 46,7% cazuri cu etilism acut, iar ponderea celor cu etilism cronic, cu valori la internare ale concentrației de alcool peste limita normală (32/47) a fost semnificativ mai mare față de cazurile cu etilism acut (48/103) (p=0,023). Etilismul cronic în antecedentele patologice personale ale pacienților a determinat un risc relativ de 1,46 ori mai mare de intoxicație (RR=1,46; IC95%: 1,10÷1,94) (fig. 5, 6).

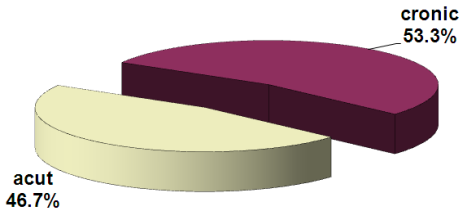

Fig. 5. Distribuția pacienților cu coingestie de alcool în relație cu tipul de etilism

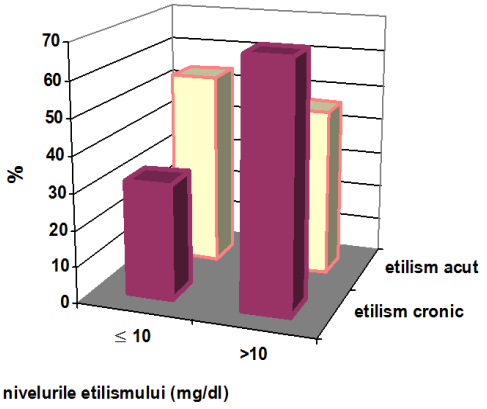

Fig. 6. Distribuția pacienților intoxicați cu benzodiazepine în relație cu nivelul alcoolemiei înregistrat la internare şi antecedentele patologice personale

Pacienții cu intoxicații cu benzodiazepine şi coingestie de alcool au înregistrat o perioadă de spitalizare care a variat de la 1 la 8 zile pentru cei cu etilism cronic şi de la 1 la 14 zile în cazul etilismului acut.

În relație cu prezența etilismului acut la pacienții intoxicați cu BZD, valorile medii ale perioadei de spitalizare au fost semnificativ mai crescute (t=3,13; GL=148; p<0,002) (fig. 7).

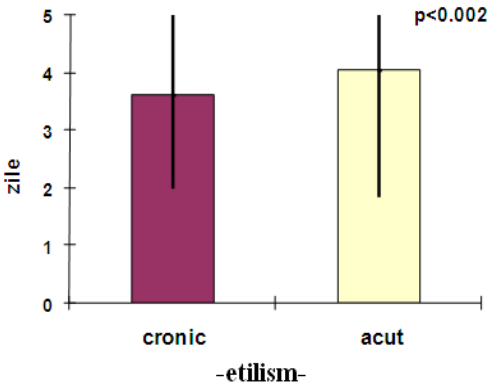

Fig. 7. Valorile medii ale perioadei de spitalizare la pacienții intoxicați cu benzodiazepine în relație cu tipurile de etilism

În perioada studiată,din totalul pacienților spitalizați pentru intoxicație acută cu BZD s-au înregistrat 2 decese, din care numai un pacient a fost cu etilism acut, diferență nesemnificativă din punct de vedere statistic (p=0,936).

CONCLUZII

Ponderea pacienților de sex masculin (29/56) cu valori peste limita admisă a alcoolemiei nu a prezentat diferențe semnificative comparativ cu cei de sex feminin (51/94) (χ^2=0,02; GL=1, p=0,901).

Etilismul cronic în antecedentele pato-

logice personale ale pacienților a determinat un risc relativ de intoxicație de 1,46 ori mai mare.

Perioada de spitalizare a variat de la 1 la 8 zile la pacienții cu etilism cronic şi de la 1 la 14 zile la cei cu etilism acut, diferența fiind semnificativă statistic.

Din totalul pacienților spitalizați pentru intoxicație acută cu BZD s-au înregistrat 2 decese, dintre care unul la un pacient spitalizat cu etilism acut, cu o diferență statistică nesemnificativă (p=0,936).

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CARACTERISTICILE INFECŢIEI CU VIRUS HEPATITIC C (VHC) ÎN COLECTIVITĂŢI ÎNCHISE

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CHARACTERISTICS OF HEPATITIS C VIRUS (HCV) INFECTION IN CLOSED COMMUNITIES (Abstract). **Aim:** To determine the prevalence of HCV in closed communities and the relations between risk behavior and HCV infection. **Material and methods**: The persons deprived of personal freedom selected for this study have filled out an epidemiological questionnaire including identity and epidemiological data (detention history, evaluation of the risk factors), as well as a written consent. All the subjects were tested for the presence of anti-HCV antibodies. **Results**: This study included 326 of the total of 500 persons deprived of personal freedoms at the Bacau Correctional Facility The prevalence of hepatitis B and C was 15.34 %, and that of HCV 5.21%, higher than in the general population. Risk factors associated with HCV in closed communities were injected drugs, homosexuality, and tattooing. **Conclusions**: The results confirm that the population in correctional facilities is at high risk for infection with hepatitis viruses (both B and C), the modes of disease transmission being already known. **Key words**: HEPATITIS C VIRUS, PREVALENCE OF HCV INFECTION, RISK BEHAVIOR, CORRECTIONAL FACILITIES

Prevalenţa VHC la nivel mondial este de 3%, ceea ce corespunde unui număr de aproximativ 170 milioane persoane (1). Această estimare este realizată cel mai frecvent pe baza ratei prevalenţei în rândul donatorilor de sânge, ceea ce face ca numărul real să fie subestimat.

Până în prezent s-au identificat şase genotipuri (notate de la 1 la 6) şi mai multe subtipuri ale VHC, a căror răspândire şi răspuns la tratament sunt diferite (2). Pentru România valorile prevalenţei sunt cuprinse între 1,8-9,6% (3).

OMS estimează că la nivel mondial se înregistrează mai mult de 1,1 milioane decese legate de infecţia cu VHC (care, ca număr de persoane, este de patru ori mai frecventă ca HIV) (4).

Principala cale de transmitere a infecţiei cu VHC este cea sanguină, putându-se realiza în două moduri: direct (de ex. prin transfuzii de sânge) sau indirect (de ex. prin administrarea intravenoasă de droguri, urmare a partajării echipamentului de injectare, această cale fiind considerată principalul factor de risc în ţările dezvoltate). Au fost identificate următoarele căi posibile: utilizarea drogurilor intravenos; transplantul de organe; contactul sexual (cu un IDU – injection drug user); detenţia mai mare de trei zile; scarificarea religioasă; heteroagresiuni/agresiuni cu un obiect contaminat cu

sânge sau alte fluide biologice infectate; piercing, tatuaj, acupunctură; infecţii în instituţii sanitare (ocupaţionale şi nosocomiale) (1,2,5-7).

Utilizarea drogurilor injectabile a devenit cea mai frecventă formă de transmitere a VHC. Majoritatea infecţiilor nou dobândite apare la indivizii care îşi injectează sau şi-au injectat droguri ilicite. Seroprevalenţa anticorpilor anti VHC în grupul IDU poate ajunge la 80-90% (8), având variaţii ce ţin de regiune, comportament la risc, status socio-economic, etc. Infecţia VHC este asociată şi cu antecedentele de utilizare intranazală (prizare) a cocainei (alcalină şi corozivă), datorită prezenţei sângelui pe dispozitivele de prizare şi partajării acestora (9,10). Statusul de VHC în rândul IDU este asociat cu: partajarea seringilor şi a echipamentului de injectare; numărul de ani de injectare; frecvenţa injectării; vârsta; nivelul consumului de drog; coinfecţia cu VHB; consumul excesiv de alcool; detenţia; sexul masculin (11,12).

Literatura de specialitate recunoaşte că utilizarea ilicită a drogurilor reprezintă factorul de risc principal şi major în transmiterea VHC (13-18). Consumul de droguri şi detenţia cresc riscul de infecţie VHC. Rata încarcerărilor printre IDU, ca şi proporţia IDU în rândul deţinuţilor sunt mai mari decât în populaţia generală (12). Intrarea într-un sistem penitenciar scade (măcar teoretic) consumul de droguri, dar reprezintă foarte des un prim pas în iniţierea consumului acestor substanţe. Studii citate de K. Dolan (19) arată că deţinuţii din penitenciare cu regim de securitate scăzut au risc mai mare de infecţie datorită posibilităţii injectării de droguri (48%) decât cei din penitenciare de securitate medie (27%) sau maximă (18%). Ca şi în cazul infecţiei cu VHB, la consumul de substanţe ilicite se adaugă şi alţi factori de risc (20): comportament şi activitate sexuală la risc în penitenciare (14,16), istoricul detenţiei (numărul sentinţelor, durata totală a timpului petrecut în închisoare, frecvenţa reîncarcerărilor, tatuarea şi piercing-ul corporal realizate în penitenciar în condiţii improprii şi cu echipamente improvizate (21), leziuni (autorăniri, heteroagresiuni), afecţiuni mentale (22), suprapopularea/supraaglomerarea. Prevalenţa infecţiei virale C în rândurile p.p.l. diferă foarte mult în funcţie de regiune, tipul penitenciarului (gradul de securitate), structura populaţieie penitenciare, prezenţa şi importanţa unor grupuri de risc (19).

Scopul acestui studiu a fost stabilirea prevalenţei infecţiei virale C în colectivităţi închise (penitenciar) şi stabilirea factorilor de risc ai transmiterii VHC în aceste comunităţi.

MATERIAL ŞI METODĂ

În studiu au fost incluşi rezidenţii Penitenciarului Bacău pe o perioadă de 3 ani (2007-2010). După semnarea consimţământului informat, subiecţii au completat un chestionar epidemiologic şi au fost supuşi unui examen clinic general. Chestionarul epidemiologic a cuprins date de identificare şi date epidemiologice (istoricul detenţiei/detenţiilor, evaluarea factorilor de risc: administrare de droguri injectabile, tatuare, hetero/autoagresiuni, partajul obiectelor de toaletă, intervenţii chirurgicale sau stomatologice, călătoria în străinătate şi eventual în zone cu endemicitate crescută). Toţi subiecţii au fost testaţi pentru prezenţa Ag HBs prin metoda ELISA.

Datele statistice au fost prelucrate cu ajutorul programului SPSS 16.0. Am utilizat pentru compararea valorilor testul Chi pătrat, iar valorile neparametrice au fost interpretate cu ajutorul testului Mann-

Whitney de comparare a rangurilor.

REZULTATE

Din totalul de 500 deţinuţi din Penitenciarul Bacău, 326 au semnat consimţământul informat şi doar 141 au completat chestionarul epidemiologic.

Vârsta medie a lotului a fost de 30,94 ± 9,67 ani (femei 32,84 ± 8,92 ani, bărbaţi 30,65 ± 9,77 ani), cu o vârsta medie a subiecţilor cu VHC de 28 ani (95% IC 24,14-31,81). Au fost completate 141 de chestionare (43,25% dintre subiecţii incluşi în studiu). Doar la 197 (60,43%) dintre deţinuţii incluşi în studiu s-au efectuat analize medicale complete. Au existat 31 persoane (22%) pentru care au fost realizate analize medicale complete şi care au acceptat să completeze şi chestionarul, neexistând situaţii de p.p.l. incluse în studiu şi care să nu fi răspuns măcar la unul din cele două forme de investigare folosite. Întregul lot a fost însă testat pentru prezenţa Ac anti VHC (anticorpi anti VHC).

În urma analizelor efectuate s-a obţinut o prevalenţă a Ac anti VHC de 5,21%. Datorită structurii penitenciare din perioada desfăşurării studiului, numărul de cazuri cu Ac anti VHC prezenţi a fost mai mare la p.p.l. de sex masculin de 4,66 ori.

Majoritatea celor incluşi în studiu a făcut parte din grupa de vârstă 20-45 ani, prezenţa Ac anti VHC fiind preponderentă la aceeaşi grupă de vârstă. Pentru a vedea dacă s-au înregistrat diferenţe semnificativ statistice între valorile vârstei pe diferitele categorii de subiecţi incluşi în lotul de studiu, am folosit testele de semnificaţie statistică. Testul de normalitate Kolmogorov – Smirnov a aratat că valorile vârstei nu respectă legea de distribuţie normală (Z = 1,805, p = 0,003), deci pentru a compara aceste valori am folosit doar teste neparametrice. Am folosit testul Mann-Whitney de comparare a rangurilor, care a aratat că nu există diferenţe semnificative statistic între valorile vârstei pe sexe (U = 5200.500, p = 0.084) şi nici între subiecţii pozitiv / negativi pentru anticorpi anti VHC (U = 2172.000, p = 0.229).

Pentru ambele sexe mediul de proveniență a fost preponderent urban. Pentru infecţia virală C mediul rural acţionează ca factor de protecţie, riscul asociat fiind de 0,655, însă nu au existat diferenţe semnificativ statistice între frecvenţa infecţieie virale C în mediul urban faţă de mediul rural (OR 2,15, p=0,457).

Majoritatea subiecţilor era de etnie română (82,45%), dintre aceştia 92,3% având Ac anti VHC prezenţi (OR 1,96, p=0,522).

Nivelul de educaţie nu a influenţat probabilitatea infecţiei VHC. Cei mai mulţi aveau terminate 12 clase (liceul) ca nivel de studii, 7,9% dintre aceştia prezentau Ac anti VHC (p=0,603).

În lotul studiat nivelul socio-economic era mediu spre scăzut, 14,7% dintre şomeri prezentau Ac anti VHC, spre deosebire de doar 3,2% dintre subiecţii angajaţi (p= 0,028).

Cea mai mare frecvenţă a infecţiei virale C a fost înregistrată la persoanele necăsătorite (12,5%) spre deosebire de cele căsătorite, unde frecvenţa a fost de 8,5% (p=0,364). În urma completării chestionarului au fost identificaţi mai mulţi factori de risc asociaţi cu infecţia virală C (tab. I).

În rândul subiecţilor cu relaţii sexuale cu parteneri de acelaşi sex riscul de infecţie virală C este de 9,5 ori mai mare. Utilizarea de droguri intravenoase creşte riscul de infecţie virală C de 8,1 ori.

Prezenţa tatuajelor creşte riscul de infecţie virală C de 2,6 ori, majoritatea tatuajelor la cei infectaţi fiind realizate în penitenciar.

TABEL I

Factori de risc asociaţi cu VHC în colectivităţi închise

Caracteristici	Prevalenţa VHC (%)	OR (95% IC)	RR	p
Călătorii în străinătate	7,4	0,693 (0,203-2,321)	0,716	0.343
Alte condamnări	11,1	1,500 (0,477-4,714)	1,444	0.485
Homosexualitate	33,3	13,750 (3,827-49,398)	9,500	<0.001
Tatuaje	12,7	2,850 (0,749-10,844)	2,161	0.111
Leziuni autoprovocate	13,3	1,744 (0,498-6,110)	1,644	0.380
Consum de droguri intravenoase	60,0	18,900 (2,823-126,558)	8,160	<0.001
Tratamente injectabile la domiciliu	12,2	1,694 (0,536-5,354)	1,609	0.365

DISCUŢII

Infecţia cu VHC recunoaşte drept cale majoră de transmitere calea sanguină. Alte căi importante sunt cea sexuală şi cea verticală (materno-fetală). A doua cale de transmitere nu a făcut obiectul studiului.

Prevalenţa infecţiei virale C în lotul studiat a fost de 5,21%, mai mare faţă de prevalenţa VHC din populaţia generală a României - 3,52% (23).

Structura populaţiei penitenciare a influenţat unele rezultate. Faptul că în perioada desfăşurării studiului, populaţia de sex feminin a penitenciarului era de aproximativ 5% din total a determinat o prevalenţă mai mare a infecţiei VHC în rândul persoanelor de sex masculin. Aceste rezultate sunt în contradicţie cu un studiu (23) efectuat la nivel naţional, în care persoanele de sex feminin aveau o proporţie a infecţiei cu VHC mai mare decât cele de sex masculin (care acţionează ca factor protectiv), infecţia fiind de asemenea mai frecventă în mediul rural (din cauza sanitaţiei, educaţiei şi nivelului socio-economic mai scăzute aici). În studiul nostru însă, dimpotrivă, mediul rural acţionează ca factor de protecţie, pentru cei care provin din acest mediu, existând de 0,655 ori mai puţine şanse de a se infecta cu VHC. Acest fapt este explicat prin mediul de proveniență a deţinuţilor, cei mai mulţi provenind din mediul urban, unde grupurile ce se angajează în activităţi la risc (de ex. consumul de droguri ilicite) sunt mai numeroase, la nivelul penitenciarului aceste grupuri agregându-se şi continuând acelaşi comportament la risc (mediul penitenciar este recunoscut ca fiind un loc propice pentru astfel de activităţi). Atât în studiul sus-amintit (23), cât şi în studiul nostru, nivelul ridicat de educaţie a constituit un factor protector faţă de achiziţionarea infecţiei cu VHC, la fel ca şi statusul marital (căsătoria este un factor protector) şi statusul socio-economic (cei neangajaţi sau neimplicaţi într-o activitate productivă legală au şanse mai mari de a se infecta cu VHC).

Un aspect aparte şi foarte obişnuit printre deţinuţi, indiferent din care sistem penitenciar provin, îl reprezintă tatuarea. Tatuajul este recunoscut ca factor de risc în transmiterea infecţiei cu VHC (20,24). În studiul nostru 82,3% din tatuaje au fost realizate în penitenciar, tatuarea fiind un factor de risc pronunţat pentru infecţia VHC (de 2,616 ori mai multe şanse de infecţie). Deşi nu constituie calea majoră

de transmitere, relaţiile sexuale cu parteneri de acelaşi sex (25) (homosexualitatea temporară - pe perioada detenţiei) reprezintă un factor de risc foarte important, existând de 9,5 ori mai multe şanse de infecţie, leziunile autoprovocate având un risc mediu de infecţie cu VHC.

Corespunzător datelor din literatura de specialitate (11,26) utilizarea substanţelor ilicite reprezintă un risc foarte important pentru infecţia VHC. Dacă se adaugă încă un factor de risc (de ex. tatuarea), riscul creşte exponenţial (9). Importanţa acestei căi de transmitere, încercarea de a înţelege modalităţile de transmitere a VHC la IDU s-au concretizat în numeroase studii (10,27), inclusiv în realizarea de modele matematice ale transmiterii şi ale influenţei utilizării de substanţe legale în achiziţionarea VHC de către IDU (12,28). Numărul de condamnări şi anii petrecuţi în detenţie determină un risc, deşi slab, de a achiziţiona infecţia cu virusurile hepatitice. Aceste argumente sunt în favoarea aserţiunii potrivit căreia detenţia în general (prin modul de viaţă şi comportament pe perioada executării pedepsei) are un rol de seamă în transmiterea infecţiei cu virusuri hepatitice şi în perpetuarea unui important izvor de virus. Rezultatele obţinute de noi contrazic un studiul citat mai înainte (23) potrivit căruia detenţia nu se corelează din punct de vedere epidemiologic cu infecţia VHC.

În studiul efectuat nu am găsit ca fiind factori de risc pentru infecţia cu VHC administrarea de tratamente injectabile la domiciliu, împărţirea obiectelor personale de igienă, accidentele de circulaţie, acupunctura, frecventarea saloanelor de cosmetică/manichiură/pedichiură, intervenţiile chirurgicale sau spitalizările.

Inexistenţa unui vaccin (în momentul de faţă) împotriva VHC face imperios necesară realizarea unei strategii de screening, prevenţie şi tratament (12,27) prin adoptarea unor programe bine elaborate, care să aibă drept ţintă grupurile cu risc major, un astfel de grup constituindu-l deţinuţii.

Datele obţinute susţin necesitatea imperioasă a elaborării unor programe de screening, prevenţie şi tratament în rândul persoanelor instituţionalizate (mai ales din perspectiva absenţei, în momentul actual, al unui vaccin anti VHC), dar şi întreprinderea unor iniţiative legislative care să sprijine astfel de programe.

CONCLUZII

Datele obţinute concordă cu cele din literatură, ele demonstrează că infecţia cu VHC este mai frecventă decât în populaţia generală. Valorile mai mici sunt probabil rezultatul structurii populaţiei carcerale din Penitenciarul Bacău, în momentul realizării studiului. Este posibil ca realizarea unui astfel de studiu în facilităţi corecţionale cu o altă structură a populaţiei să conducă la date mai apropiate de cele din alte ţări.

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ECTOPIC ADRENOCORTICOTROPIC HORMONE SYNDROME CAUSED BY A CARCINOID TUMOR OF THE THYMUS. A CASE REPORT

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ECTOPIC ADRENOCORTICOTROPIC HORMONE SYNDROME CAUSED BY A THYMIC CARCINOID. CASE REPORT. (Abstract). Ectopic ACTH secretion accounts for less that 10% of all causes of endogenous Cushing's syndrome. Carcinoids are rare thymic tumors, and when associated with ACTH hypersecretion display local or distant aggressive behavior. A 32-year-old woman was admitted to the Endocrinology Unit for obesity, moon face, facial hirsutism, hyperpigmentation, and secondary amenorrhea. Laboratory test confirmed the hypercortisolism and excess ACTH, while dexamethasone suppressive test was negative. Thorax computed tomography (CT) showed an antero-superior mediastinal tumor invading the pericardium and left mediastinal pleura. A complete resection through median sternotomy of the tumor, pericardium and left mediastinal pleura was performed. After a one-year symptom-free period, hypercortisolism recurred, confirmed by laboratory findings. Although no signs of local recurrence were seen on thorax CT, left internal mammary lymph nodes involvement and vertebral body metastases at C7 and L1 were found. Refractory electrolyte disturbances could not be corrected resulting in severe cardiac arrhythmia and death from cardiac arrest. The reported case draws attention on the aggressiveness of ACTH-secreting thymic carcinoid both oncologically and also due to the refractory electrolyte disturbances with fatal outcome. **Key words:** CARCINOID, THYMUS, ECTOPIC ACTH SECRETION, CUSHING'S SYNDROME

Ectopic ACTH secretion accounts for less than 10% of all causes of endogenous Cushing's syndrome (1,2). Carcinoid tumours are derived from enterochromaffin cells and are classically described as originating from the embryological foregut, midgut or hindgut. Thymic carcinoids are rare thymic tumours, divided into three forms: nonsecretory tumours, hormonal secretory tumours and tumours associated with multiple endocrine neoplasia type 1 (3-5).

CASE REPORT

A 32-year old female patient was admitted in the Endocrinology Unit with typical clinical aspect of hypercortisolism (moon

face, obesity, facial hirsutism, hyperpigmentation, secondary amenorrhea), the onset being noticed three months ago.

The endocrinological work up confirmed the hypercortisolism, as follows:

- plasmatic cortisol was high: 582 ng/mg at 8 am (normal: 50-230 ng/mg) and 590 ng/mg at 11 pm (normal: 30-150 ng/mg);
- urinary cortisol was, also, high: 532 ug/24 h (n 9-180);
- low plasmatic potassium, metabolic alkalosis and hyperglycemia were considered secondary to hypercortisolism;
- ACTH was also elevated (292 pg/ml, n < 46) and, given the negative dexamethasone suppressive test, the diagnosis of Cushing's syndrome was endorsed.

In order to investigate the source of ACTH excess, a radiography of the skull and a magnetic resonance image (MRI) of the head were performed, but no abnormalities were shown. Except polycystic ovaries, ultrasound and computer tomography of the abdomen were normal.

Thorax CT revealed an anterior mediastinal mass of 32/42/49 mm in contact with pericardium and left mediastinal pleura, consistent with a tumor of the thymus (fig. 1).

Fig. 1. Pre-operative thorax CT image. An anterior mediastinal mass of 32/42/49 mm in contact with pericardium and left mediastinal pleura was evident.

The preoperative diagnosis was of an ectopic ACTH secreting thymic tumor. The patient was scheduled for surgery and a medical preoperative treatment consisting of beta-blockers, angiotensin converting enzyme inhibitor, ketoconasole, spironolactone, and oral antidiabetics was commenced.

On July 2008 the patient underwent an extended thymomectomy through a longitudinal complete sternotomy. The tumour was located in the antero-superior mediastinum, invading the pericardium and left mediastinal pleura which were resected en block with the thymic tumour.

Pathology assessment

Macroscopically it was described a 5/4/2 cm tumour, of hard consistency and fatty aspect on cross section. The microscopical examination showed, besides thymic tissue with interstitial sclerosis and calcifications, a malignant proliferation with rare mitoses and foci of necrosis. The tumor exhibited a compact architecture and cellular pleomorphism, with invasion of the extracapsular space and vascular emboli (fig. 2, a) and b). No malignant cells were found in the mediastinal fatty tissue, pericardium and pleura portion removed with the tumor.

The immunohistochemistry (IHC) assessment showed - enolase neuron specific (NSE) was intense positive (fig. 3), chromogranin was positive (fig. 4), synaptophisin was intense positive (fig. 5), - citokeratine cocktail (MNF 116) was moderate positive (fig. 6) and Ki 67 was 10% (fig. 7).

Given the IHC, the final diagnosis was of a carcinoid carcinoma of the thymus with ACTH secretion. The immediate postoperative course was uneventful and the patient was discharged 12 days following surgery. The clinical examination and hormonal assessment at follow-up examination after one month showed the disappearance of the hypercortisolism were: plasma cortisol - 97,4 ng/ml at 8 am and 3,9 ng/ml at 11 pm; urinary cortisol - 178,9 μg/24 h; ACTH 37 pg/ml.

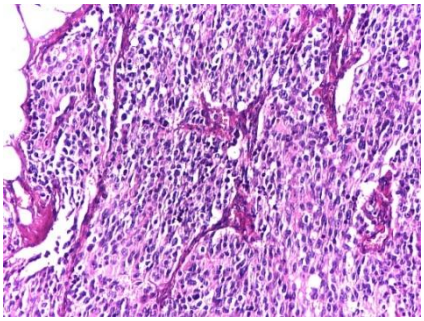
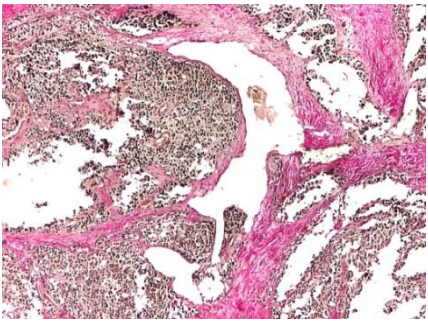

Fig. 2. Optical tumor microscopy images:
a) compact architecture and cellular pleomorphism; b) vascular emboli.

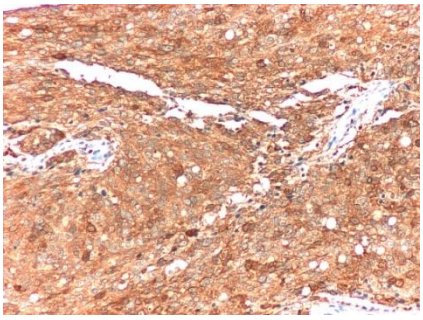

Fig. 3. IHC - NSE intense positive

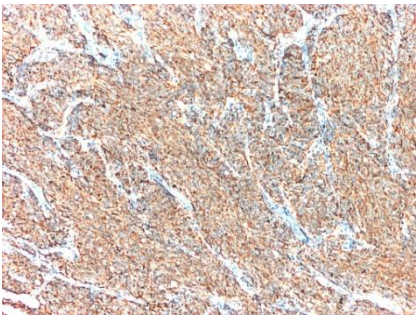

Fig. 4. IHC – chromogranin positive

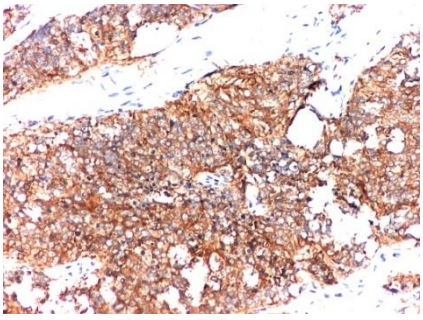

Fig. 5. IHC - synaptophisin intense positive

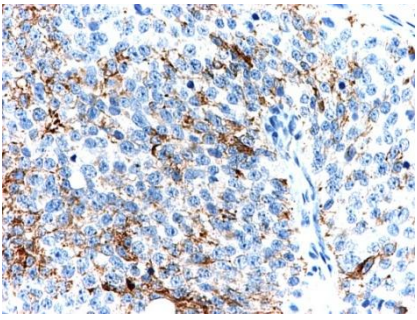

Fig. 6. IHC – MNF16 moderate positive

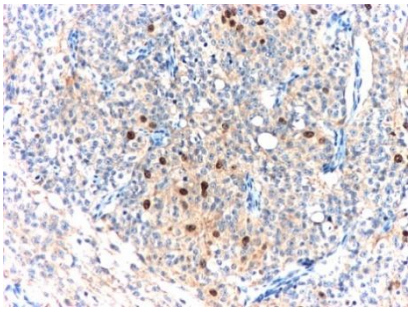

Fig. 7. IHC – Ki 67 10 %

The patient was disease-free for one year following surgery she, but in July 2009 the symptoms of Cushing's syndrome reoccurred, confirmed by the lab tests. The thorax CT scan revealed left internal mammary lymph nodes enlargement and metastases of the body of C7 and L1 vertebrae, confirmed by bone scintigraphy. Under these circumstances the patient was referred to oncology department where two

sessions of chemotherapy with carboplatin and etoposid were followed by 2 shots of octreotide acetate. The thorax CT and the scintigraphy were repeated three months later and revealed an increase of the vertebral metastases. Due to electrolyte disturbances the patient was readmitted in the endocrinology department with severe low serum K (2.1 mmol/l), metabolic alkalosis and hyperglicemia.

The persistence of electrolyte disturbances led to the indication of bilateral adrenalectomy but in February 2010 the patient was admitted in the Intensive Care Unit for ventricular fibrillation due to refractory hypokaliemia. Despite intensive monitoring and therapy an acute myocardial infarction occurred and the patient died from cardiogenic shock and unresponsive cardiac arrest.

DISCUSSION

The carcinoid tumors of the thymus associated to Cushing's syndrome are aggressive tumors and usually produce local and distant metastases (3, 5). ^{18}F-FDG PET/CT, that integrates both functional and structural imaging in one device, can improve the accuracy of tumor localization in patients with ectopic Cushing's syndrome and further improve the prognosis of the patients by adequate treatment (6). The diagnosis represents a challenge to the endocrinologist as well as the therapy against life-threatening hypercortisolemia. Ectopic ACTH hormone secretion is more commonly associated with small cell carcinoma of the lung followed by islet cell cancer, medullary thyroid cancer, thymic carcinoid, pheochromocytoma and gastro-intestinal carcinoid. In 16% of patients, the tumor cannot be found, in this circumstance the patients are likely to benefit from a bilateral adrenalectomy (2). Clinicians should be aware of the patho-physiological changes of the thymus gland such as reactive thymic lymphoid hyperplasia, resulting from rapidly dropping cortisol levels occurring about six months after remission of hypercortisolism by medical treatment, in cases of still unknown primary tumor (7). The high dose dexamethasone suppression test showing lack of suppression along with CT scanning are the mainstay of the diagnosis (1, 4). ^{111}In and, more recently, ^{99m}Tc somatostatin analoques scintigraphy is a potentially useful imaging exploration in diagnosis and location of the carcinoid tumours, making evidence of intense uptake on the lesion (8, 9). Patients with ectopic ACTH secretion more likely present with persistent hypertension and hypokalemic metabolic alkalosis, which is resistant to huge doses of potassium supplementation. Profound hypokalemia and hypertensive crises from mineralocorticoid action of cortisol may lead to cardiac and vascular complications including ventricular arrhythmias. Medical therapy involves use of steroid synthesis inhibitors, such as Ketoconazole, Mitotane, Metyrapone, Octreotide, Amino-gluthethemide. Ketoconazole, which acts by blocking corticosteroid production by inhibiting 17-hydroxylase and 11- hydroxylase, is the therapy of choice because of its low incidence of side effects. Octreotide acts by binding to the sst2 somatostatin receptor, suppressing ACTH release. It is effective both on its own and in combination with Ketoconazole. Spironolactone, an aldosterone receptor antagonist, is used to decrease urinary potassium wasting secondary to activation of mineralocorticoid receptors. Surgery combined with medical therapy improves morbidity and quality of life. An important

feature of carcinoid tumors is their expression of somatostatin (SST) receptors. Somatostatin analogues are the best therapy for controlling the symptoms of malignant carcinoid syndrome. Octreotide has a half-life of approximately 1.5 hours and can be given as a subcutaneous or intravenous injection. SST analogues have been shown to be the best therapy for controlling carcinoid symptoms. In some studies, it has been shown that SST analogues inhibit tumour growth as monotherapy in up to 8% of treated carcinoid patients or even greater anti-tumour effect in combination with other agents, for example, interferon-α (10).

CONCLUSIONS

The reported case underlines the aggressiveness of the thymic carcinoids with ectopic ACTH secretion from the oncological point of view, but also regarding the refractory electrolyte disturbances with fatal outcome.

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ARTERIAL AND VENOUS THROMBOSIS–TWO FACES OF THE SAME DISEASE. CASE REPORT

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ARTERIAL AND VENOUS THROMBOSIS-TWO FACES OF THE SAME DISEASE. CASE REPORT (Abstract): Abnormal activation of the coagulation cascade in cancer patients leads to arterial and venous thrombotic events. If the link between venous thromboembolism and malignancy has long been known, cancer-related stroke is a newly recognized entity. We present the case of a 90-year-old woman who developed two concurrent ischemic strokes in the right posterior cerebral artery territory and deep vein thrombosis of the left lower limb, cascade of events that led to the diagnosis of a cancer. We highlight some of the particularities of cancer-related stroke to raise awareness upon this complication that leads to increased morbidity and mortality in cancer patients. We suggest that special attention should be paid to patients with concomitant thrombotic events in different vascular areas because a systemic underlying substrate may be involved. **Keywords:** CANCER, VENOUS THROMBOSIS, ARTERIAL THROMBOSIS, CANCER-RELATED STROKE.

Malignancy disturbs the hemostatic system and, consequently, the cancer patients are at high risk of both thrombosis and hemorrhage. Cancer cells activate the coagulation cascade by multiple mechanisms: tissue factor, inflammatory cytokines, cancer procoagulants, tumor mucins (1). The coagulation abnormalities may result in both venous and arterial thrombosis. Venous manifestations of cancer-associated thrombosis have been studied extensively. But in addition to venous thromboembolism (VTE), cancer-related stroke is emerging as a new severe complication of a malignancy.

CASE REPORT

We present the case of a 90-year-old woman who presented to our clinic for swelling of the left lower limb, with onset 7 days earlier and progressive worsening. The patient declared that she was hypertensive and a month earlier she suddenly developed complete loss of function in the left leg, being bedridden since then. At that time the patient did not seek medical advice. After 3 weeks of bed rest the left leg started to swell from calf to thigh. Due to the progressive worsening of swelling, the patient had been convinced by her family

to go to the hospital.

On admission the patient had high blood pressure (180/80 mmHg). She had sinus rhythm on ECG and minor ST segment abnormalities interpreted as expression of ischemic heart disease. The transthoracic echocardiography of the left heart revealed ventricular hypertrophy with normal systolic function and diastolic dysfunction (abnormal relaxation pattern), mild atrial dilatation. The mitral and aortic degenerative valvular changes were without hemodynamic significance. The evaluation of the right heart was normal. There were no discontinuities at the level of interatrial and interventricular septum. No cardiac sources of embolism were detected by transthoracic echocardiography. The left lower limb was plegic, with severe edema. The clinical signs were highly suggestive of deep vein thrombosis (DVT) of the left leg. Ultrasound examination confirmed the thrombosis of the left common and superficial femoral veins and of the popliteal vein (fig. 1). The patient needed to receive anticoagulant treatment. Prior to the initiation of anticoagulation, a craniocerebral computed tomography scan was performed. The presence of any expansive intracerebral processes and intracranial hemorrhages was excluded. Two areas of hypodensity, one of 14/18/15 mm located parasagittal frontal-parietal right and the other of 12/10/15 mm located on the topography of right semi oval center, were suggestive of subacute-chronic ischemic strokes in the territory of the terminal branches of the right posterior cerebral artery (fig. 2). The anticoagulant treatment was started with low molecular weight heparin (enoxaparin). After 5 days of anticoagulant treatment, a minor genital bleeding occurred. Abdominopelvic ultrasound was immediately performed. On the uterus topography, a nonhomogeneous hyperechogenic structure, with the largest diameter of 26 mm, highly suggestive for endometrial malignancy was found. The cancer antigen 125 (CA 125) serum level was 55.1 IU/mL, 2.6 times higher than the upper limit of the normal values. Invasive procedures were proposed to the patient, but she refused and asked her family to take her home. Only palliative recommendations were formulated at discharge.

Fig. 1. Ultrasound examination of the left leg. Recent, partially occlusive thrombosis of the left common femoral vein.

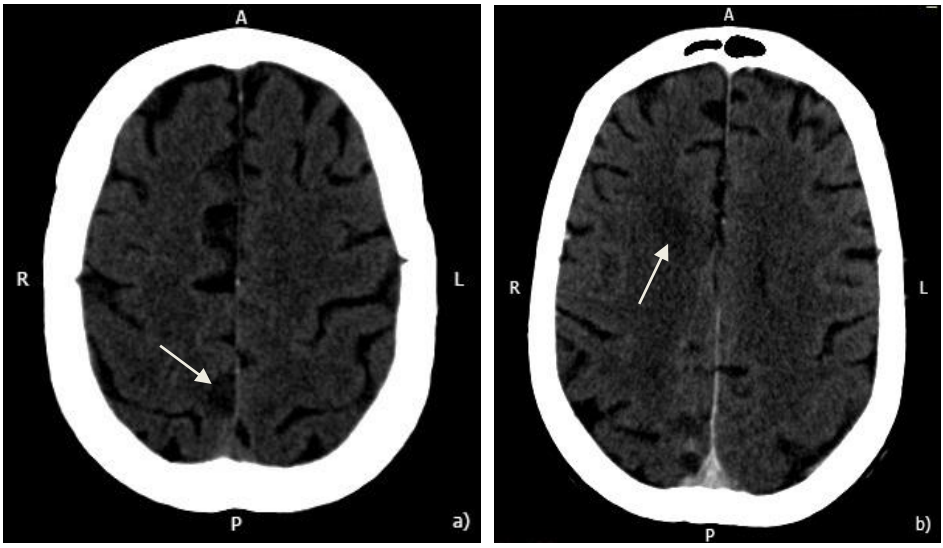

Fig. 2. Craniocerebral computed tomography with contrast enhancement. a) Area of hypodensity of 14/18/15mm located parasagittal frontal-parietal right; b) Area of hypodensity of 12/10/15 mm located on the topography of the right semi oval center.

DISCUSSION

Advanced age is associated with an increased risk for stroke. Atrial fibrillation, hypertension, atherosclerosis of extracranial and intracranial blood vessels, hyperlipidemia and diabetes mellitus are all highly prevalent in the elderly and they are also major risk factors for stroke. Due to immobility, stroke patients are at high risk for VTE, the pulmonary embolism ranking third among the most common causes of case fatality in stroke (2). Depending on the diagnostic methods, studies reported that up to 80% of patients with ischemic stroke who do not receive prophylactic therapy could developer DVT (3). In our case, we initially presumed that the patient suffered an ischemic stroke in the right posterior cerebral artery territory, with crural monoplegia, and as she was bedridden the venous stasis in the plegic leg favored the development of DVT. Dehydration, commonly found in the geriatric population and stroke patients, could also have contributed to the development of venous thrombosis.

The presence of the uterine tumor required reconsideration of the pathophysiological mechanism of these two vascular events. Thus, besides the venous stasis determined by immobilization and hemoconcentration due to dehydration, it was necessary to consider the imbalances that cancer brings upon hemostasis. It is well known that a malignancy increases the risk of DVT due to the alteration of one or more factors of the Virchow's triad. The tumor invades and compresses the surrounding structures, produces proinflammatory and prothrombotic mediators, thereby causing endothelial dysfunction, hypercoagulability and stasis. Patients with active cancer have a 4- to 8-fold increase in VTE incidence compared with the general population (4). The overall risk of VTE in patients with uterine cancer is relatively small, of 2.7%

in a 2-year incidence, but it can vary from 1.5% among women with local stage disease to 10.5% among women with advanced disease (5). It is very important to bear in mind that the presence of VTE in a patient with malignancy is a negative prognostic factor because in these patients the survival rate decreases up to 6-fold compared with patients without VTE (6). In patients with endometrial cancer, the elevated serum CA 125 concentrations are associated with deep myometrial invasion, extrauterine spread and reduced survival.

Although in the last decade there have been major changes in antithrombotic therapy due to the emergence of non-vitamin K antagonist oral anticoagulants (NOAC), low molecular weight heparin (LMWH) is still the guideline endorsed treatment of patients with cancer associated VTE (7). Old age (age > 75 years) is a bleeding risk factor in the anticoagulated general population whether the anticoagulant is warfarin, LMWH, or a NOAC (4). The anticoagulant therapy raises 2-6 folds the bleeding risk in cancer patients (8) and moreover patients who have the highest risk for VTE also have the highest risk for bleeding (4).

More recently, it has been studied the link between cancer and stroke. A malignancy may directly or indirectly lead to stroke. Several mechanisms have been proposed: direct tumor compression of blood vessels, hypercoagulability, non-bacterial thrombotic endocarditis and treatment-related effects (9).

As DVT may be the first manifestation of a malignancy (10), an ischemic stroke can also be the first manifestation of a cancer (1). Case reports linking stroke to cancer started to be published more than 10 years ago. In one case, stroke was the first manifestation of a concealed pancreatic neoplasia (11) and in another case a recurrent cerebral embolism led to the diagnosis of an ovarian cancer (12). One small study analyzed the clinical and imagistic particularities of stroke patients, whose cancers were diagnosed at stroke presentation (13) and it was emphasized that multiple cerebral infarctions of an unknown etiology must trigger the search of a concealed cancer. A study conducted on 348 consecutive patients with acute ischemic cryptogenic stroke, with and without cancer diagnosis, concluded that multiple infarcts involving multiple vascular territories are associated with the presence of malignancies (1414). One study reported that 67% of strokes in cancer patients appear on imaging as multiple concurrent embolic events, suggesting that clot formation and embolization are often responsible (15). This hypothesis is confirmed in our patient's case, by identifying two concurrent recent strokes, one located parasagittal frontal-parietal right and the other located on the topography of the right semi oval center. Moreover, gynecologic neoplasms are reported to have the highest potential for developing of ischemic stroke.

The most important mechanism linking cancer and stroke is the abnormal activation of the coagulation cascade. Tissue factor (TF) and cancer procoagulant (CP) are the most important procoagulant molecules that are release directly from the tumor cells (16). TF promotes thrombosis by activating the coagulation cascade after binding to Factor VII, while CP exerts its prothrombotic effect through the activation of Factor X. In one study, TF was identified in the atherosclerotic plaques of symptomatic patients with high-grade carotid artery stenosis, so it has been hypothesized that TF may destabilize the atherosclerotic plaques (17). A highly significant association of TF expression with the occurrence

of cerebral micro embolism was also found (17). Furthermore, tumor cells release cytokines such as tumor necrosis factor-alpha, interleukin-1 and interleukin-6 which are able to inhibit the natural anticoagulant pathway of C protein and to induce endothelial cells, monocytes, and cancer cells to express TF, thereby enhancing the thrombosis (9). So, the available data suggest that hypercoagulability-induced stroke is a real entity.

The anticoagulation with LMWH is the treatment of choice for VTE in cancer patients, but information regarding cancer-related stroke are lacking. The effect of anticoagulation on micro embolism was assessed in one study (18) and the results showed that anticoagulation has the potential to attenuate cancer-induced hypercoagulability leading to stroke. The recently published results of the OASIS-Cancer Study have demonstrated that the effective correction of hypercoagulability in patients with cancer-related stroke may play a protective role in survival of these patients (1). A risk score to estimate the need for anticoagulation for primary prevention of VTE in cancer patients already exist (19), but how to choose high-risk cancer patients to receive anticoagulation for stroke prevention is still a matter of debate and the role for antiplatelet in the secondary prevention of cancer-related stroke also remains to be investigated.

CONCLUSIONS

We present a case of concomitant arterial and venous thrombotic event to raise awareness upon the importance of identifying its underlying mechanism. Generally, the thrombotic events in the elderly people have a complex and multifactorial etiology. Through multiple mechanisms a neoplasia can disrupt the coagulation-fibrinolysis balance and it may trigger thrombotic events. Cancer must not be considered only a trigger for *in situ* vascular thrombosis or venous thromboembolism. We want to highlight that an active malignancy is associated with the activation of the coagulation cascade and of the inflammatory pathways in both the arterial and venous system and in patients with concomitant arterial and venous thrombotic events the presence of a systemic substrate should be investigated.

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NEWS

AN INNOVATIVE SUTURING BIOMATERIAL WILL REINVENT THE SURGERY RECOVERY

A group of researchers from Donghua University, Shanghai, China conducted a very interesting study about synthesizing an "intelligent" biomaterial which is prepared successfully from bacterial cellulose and chitin and using it to obtain novel filament which can meet the demands of surgical sutures. The bacterial cellulose was used as a nanocrystals (BCNCs) for improving the mechanical performance of regenerated chitin (RC) fibers. These two materials have excellent biocompatibility and biodegradability with low toxicity when implanted in vivo. A detailed characterization comprising morphological observations, infrared spectroscopy, mechanical properties assessment, enzymatic degradability determination and in vitro biocompatibility and in vivo evaluations (murine skin wound closure) indicated that the BCNC/RC yarns meet the requirements for use as surgical sutures and promote wound healing without adverse effects (Huanling Wu, Gareth R. Williams, Junzi Wu, Jianrong Wu, Shiwei Niu, Heyu Li, Haijun Wang, Limin Zhu, Regenerated chitin fibers reinforced with bacterial cellulose nanocrystals as suture biomaterials, *Carbohydrate Polymers* 2017; 180: 304-313).

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UN „FALD" CAPCANĂ ÎNTR-O BOALĂ TAKAYASU

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A TRAP „FOLD" IN A TAKAYASU ARTERITIS (Abstract): Sometimes, acute aortic dissection diagnosis, a significant cardiological and surgical emergency, is particularly difficult. Therefore, Takayasu arteritis associated dissection fold may be a real diagnostic „trap", in spite of all modern exploratory techniques. **Key words:** AORTIC DISSECTION, TAKAYASU ARTERITIS, DIAGNOSIS

Disecţia acută de aortă este o mare urgenţă cardiologică şi chirurgicală, mortalitatea în cazul bolii netratate fiind de 1% pe oră, în primele 24 de ore, ajungând în urgenţă, în cazul bolii netratate chirurgical până la 90% la 3 luni de la diagnostic. Cu toate mijloacele moderne de diagnostic şi progresele din chirurgia cardiovasculară, mortalitatea în disecţia de aortă rămâne mare (rata de supravieţuire la un an într-un studiu multicentric european a fost de 52% în tipul I, 69% în tipul II şi 55% în tipul III) (1).

Evidenţierea prin diferite metode a unui fald de disecţie poate fi uneori extrem de dificilă. Această situaţie se regăseşte şi în următoarea prezentare de caz clinic, în care prezenţa unei comorbidităţi – boala Takayasu (o posibilă cauză de disecţie de aortă) – a indus importante dificultăţi în confirmarea sau infirmarea ipotezei de disecţie acută a aortei.

Boala Takayasu este o afecţiune idiopatică rară, caracterizată printr-o inflamaţie vasculară cronică care afectează aorta şi ramurile sale mari, inclusiv arterele coronare, carotide, pulmonare şi renale (2, 3, 4). În evoluţie se produce fibroza pereţilor arterelor afectate, cu apariţia manifestărilor clinice specifice stenozelor sau ocluziilor arterelor dintr-un anumit teritoriu, formării de anevrisme care pot evolua spre disecţie (2, 5, 6).

PREZENTAREA CAZULUI

Pacienta S.A., în vârstă de 43 ani, se adresează în urgenţă clinicii noastre cu ipoteza de diagnostic – Disecţie acută de aortă, ipoteză susţinută de examenul CT toracic. Pacienta este internată în Unitatea de Terapie Intensivă Coronarieni, motivele fiind: durere retrosternală de repaus cu iradiere în braţe, interscapulară şi la baza gâtului, accentuată de mişcările corpului, cu durata de aproximativ 3 ore şi fără răspuns la nitroglicerină sublingual, survenită cu 24 ore anterior internării, dispnee la eforturi mici; actual asimptomatică. Antecedentele personale patologice ale pacientei sunt importante. Astfel, de la vârsta de 30 ani pacienta este diagnosticată cu boală Takayasu (inclusiv prin angiografie), ocluzie de arteră subclaviculară stângă, insuficienţă aortică gradul III şi hipertensiune arterială esenţială. Este nefumătoare, neagă consumul cronic de alcool şi desfăşoară o activitate intelectuală. Din istoricul bolii reţinem şi apariţia de aproximativ un an a dispneei la eforturi mici şi a unor dureri retrostenale de efort tipic anginoase (spre deosebire de durerea

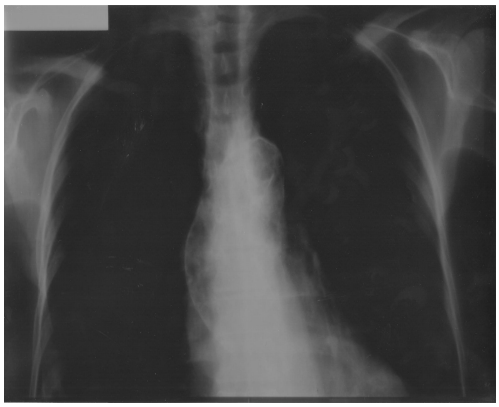

Fig. 1. Radiografia toracică

Fig. 2. Ecocardiografia transtoracică

toracică care a determinat internarea actuală). Ultimul tratament urmat a fost cu perindopril 2,5mg/zi şi pentoxifilin 800 mg/zi.

La examenul obiectiv, datele patologice înregistrate au fost : puls regulat, tahicardic (100/min.) ; TA braţ drept – 70/40 mmHg ; TA braţ stâng – nemăsurabilă ; TA coapse – 200/60 mmHg ; suflu diastolic de regurgitare aortică ; suflu sistolic carotidian stâng ; puls absent la artera radială şi humerală stângă şi diminuat pe dreapta, artere femurale pulsatile bilateral, fără semne de ischemie acută periferică.

În acest moment ne-am întrebat dacă avem de-a face sau nu cu o urgenţă majoră, ipotezele de diagnostic clinic fiind :

- Disecţie acută de aortă.
- Sindrom coronarian acut.
- Durere pericardică.
- Durere de perete toracic.

Biologic s-au evidenţiat : sindrom inflamator nespecific moderat : VSH 24 mm/1 oră, fibrinogen 587 mg%, GA 14000/mm^3, CRP 8,5 mg/l ; metabolism lipidic şi glucidic normal ; markeri de necroză miocardică fără creştere patologică : CK-MB 17 u/l, TGO 20 u/l, LDH 443 u/l, TnT negativă ; funcţie hepatică şi renală normală ; D-dimeri absenţi.

Electrocardiograma relevă : tahicardie sinusală 95/min ; HVS ; unde T ample, pozitive simetrice în V1-V4.

Radiografia toracică evidenţiază aorta intens calcificată (Fig. 1).

Ecocardiografia transtoracică efectuată în urgenţă a relevat (Fig. 2, Fig. 3) : VS dilatat, cu disfuncţie sistolică şi diastolică (FE 35%, FS 18%, DTDVS 58 mm, DTSVS 48 mm, SIV 10 mm, PP 9 mm, DSM 18 mm, VTDVS 141 ml, VTSVS 84 ml, E/A > 1) ; insuficienţă aortică gradul IV ; aortă intens calcificată, de dimensiuni normale ; posibil „fald" de disecţie de la nivelul inelului aortic şi până la jumătatea crosei, paralel cu peretele anterior aortic ; absenţa lichidului pericardic.

Ecografia abdominală : aortă abdominală intens calcificată, de dimensiuni normale, dar cu posibil fald de disecţie sub emergenţa arterei renale stângi.

Tomografia computerizată efectuată anterior internării : aortă de dimensiuni normale, intens calcificată ; disecţia aortei ascendente toracice pe o lungime de 17 mm, cu punct de plecare supravalvular (Fig.4) ; posibilă disecţie a aortei abdominale – 11 mm, caudal de emergenţa arterei renale stângi (Fig. 5).

În evoluţie, pacienta prezintă o durere retrosternală, cu răspuns prompt la nitroglicerină sublingual şi accentuarea discretă a modificărilor de fază terminală în teritoriul inferolateral.

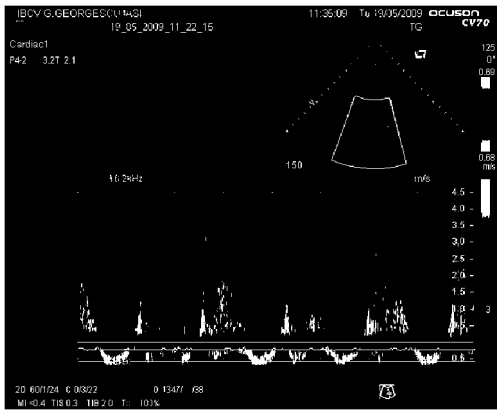

Fig. 3. Ecocardiografia transtoracică

În continuare, principala problemă a fost diagnosticul de urgenţă cardiovasculară, în această etapă ipotezele de diagnostic rămânând :

1. Disecţie acută de aortă :

- argumente : tomografia computerizată, ecocardiografia, ecografia abdominală ;
- „ciudat” : disecţie concomitentă a aortei în 2 teritorii strict limitate aflate la distanţă.

2. Angină pectorală instabilă :

- argument : modificări de fază terminală în dinamică ;
- contraargument : caracterul atipic al durerii anginoase prelungite.

Diagnosticul de certitudine a fost tranşat prin angiografie care a evidenţiat :

- aorta ascendentă, crosa şi aorta descendentă sever calcificate ;
- stenoză 95% a arterei subclaviculare drepte ;
- ocluzia arterei subclaviculare stângi, stenoză 40% a arterei carotide comune stângi ;
- subocluzie de arteră vertebrală stângă ;
- coronare cu plăci ateromatoase difuze, fără stenoze semnificative ;
- insuficienţă aortică gradul IV, fără elemente de disecţie a aortei, artere renale permeabile.

În acest context, diagnosticul final a fost :

Fig. 4. Tomografia computerizată toracică

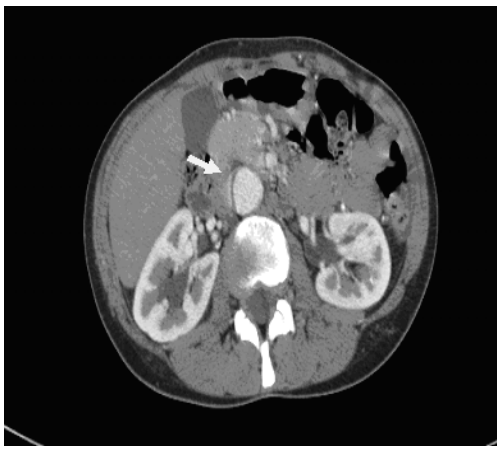

Fig. 5. Tomografia computerizată abdominală

1. Angină pectorală de efort
2. Boală Takayasu
3. Ocluzie arteră subclaviculară stângă
4. Stenoză arteră subclaviculară dreaptă
5. Stenoză arteră carotidă comună stângă
6. Insuficienţă aortică gradul IV
7. Cardiopatie hipertensivă
8. Insuficienţă cardiacă cronică clasa III NYHA

Probleme terapeutice au fost :

- scăderea TA (măsurată la nivelul coapsei) până la valori care să nu determine

fenomene ischemice cerebrale sau la nivelul membrelor superioare ;
- tratamentul insuficienţei aortice, cu indicaţie de protezare valvulară, dar cu risc intraoperator extrem de mare, rezultat din calcificarea intensă a aortei ascendente ;
- tratamentul leziunilor stenotice/ocluzive arteriale prin angioplastie cu stent sau *by-pass* când se impune, proceduri de asemenea cu risc crescut.

DISCUŢII

Principala problemă în cazul prezentat a fost diagnosticul diferenţial între faldul de disecţie şi peretele aortic intens calcificat în contextul bolii Takayasu. Arterita Takayasu este o vasculită granulomatoasă de etiologie neprecizată, mai frecventă la femei, care afectează aorta toracică, abdominală şi ramurile mari ale acestora. A fost descoperită în 1908 de oftalmologul Mikito Takayasu, care a descris modificările specifice de la nivelul vaselor oculare la o femeie japoneză în vârstă de 21 ani. Boala are un prognostic rezervat, mortalitatea fiind de 75% fără tratament şi de 10% cu tratament. Complicaţiile arteritei Takayasu sunt multiple, o bună parte regăsindu-se sau punând probleme de diagnostic diferenţial în cazul prezentat : HTA (prin reducerea fluxului sanguin renal), insuficienţa aortică, insuficienţă cardiacă, angină pectorală, infarct miocardic acut, anevrisme aortice care pot diseca, etc.

CONCLUZII

Cazul este particular prin dificultatea şi complexitatea diagnosticului diferenţial dintre „faldul" de disecţie şi peretele aortic intens calcificat în contextul bolii Takayasu, o afecţiune vasculară rară, severă, cu multiple complicaţii arteriale. De asemenea, în acelaşi context, subliniem limitările terapeutice importante : protezare aortică dificilă tehnic datorită aortei ascendente intens calcificate ; controlul tensiunii arteriale în contextul leziunilor de pe arterele carotide şi subclaviculare.

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THE MANAGEMENT OF SKIN TUMORS EXTENDED TO THE ORBIT

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THE MANAGEMENT OF SKIN TUMORS EXTENDED TO THE ORBIT (Abstract) **Aim:** This paper focuses on presenting the experience of the authors on the management of orbital invasion from periocular skin malignancies. **Material and methods:** We reviewed 18 cases of skin tumors extended to the orbit regarding the initial location, clinical signs, diagnostic methods, the surgical treatment and reconstruction procedure, the tumor histology, as well as the postoperative outcome and complications. **Results:** The most encountered location of the malignancy was at the level of the inferior eyelid. The predominant histological type was squamous cell carcinoma, followed by basal cell carcinoma and glandular carcinoma. Thirteen of the included patients underwent orbital exenteration, with five necessitating an extended orbital exenteration to the neighboring structures. Various reconstructive procedures were used for closure considering the extent of the defect, ranging from split thickness skin graft, local and regional flaps, to microvascular free flaps. The overall postoperative results were favorable with accurate defect coverage and few complications. **Conclusions:** Orbital invasion by skin malignancies can be adequately managed by orbital exenteration and a closure method suitable to the extent of the postoperative defect, the patient's age and comorbidities. **Keywords:** ORBITAL EXENTERATION, ORBITAL INVASION, SKIN CANCER, RECONSTRUCTION.

Periorbital non-melanoma cutaneous malignancies are common encounters, making up for approximately 5 to 10 % of all skin cancers (1). Basal cell carcinoma is the main type of malignancy involving the periorbital skin, representing approximately 90% of lesions. It is more commonly found on the inferior eyelid and medial canthal area, while sebaceous gland carcinoma is encountered mainly in the superior eyelid region. Squamous cell carcinoma and sebaceous gland carcinomas each account for less than 10% of periocular skin malignancies. Orbital invasion is reported in less than 4% of cases and it is a more likely occurrence in older patients with neglected large lesions located in the medial canthal area, in patients with a history of previously excised skin malignancies and the presence of local recurrence, as well as in association with aggressive histologic subtypes and the ones expressing perineural invasion (2, 3).

The infiltration of intra-orbital structures can be clinically silent in one third of cases (1). If not accurately diagnosed, it can lead to residual tumor and the onset of local recurrence, with a worse overall prognosis. Clinically apparent orbital invasion is generally associated with large ag-

gressive tumors and the surgical treatment can therefore lead to significant morbidity and deformity. The indications for orbital exenteration must be carefully considered for ensuring oncologic safety with minimal morbidity. The accurate diagnosis of the extent of tissue invasion and proper surgical planning can help decide the best option.

The purpose of this paper is to present our experience regarding the surgical management of skin tumors with orbital extension, focusing on the main challenges encountered regarding the extent of tissue removal, the options for reconstruction and the associated complications.

MATERIAL AND METHODS

We performed a review of patients diagnosed with periocular skin malignancies in between January 2013 and April 2018. We included only the patients who had clinical or imaging evidence of orbital invasion and were diagnosed with non-melanoma skin cancer. Patients with unresectable tumors were excluded from the study, as well as patients who did not undergo tumor removal due to contraindication or due to the patient's refusal. Patients diagnosed with skin invasion from malignancies arising in the adjacent orbital or sino-nasal structures, according to the history of the disease and the final histology, were also excluded from the study. The medical charts of all 18 patients included in the study were analyzed for information regarding the clinical signs, the tumor location and size, the diagnosis of orbital invasion, the type of tumor removal procedure and reconstruction, the postoperative complications and outcomes.

RESULTS

A total of 18 patients with non-melanoma skin cancer extended to the orbit were identified. There were 11 men (61.1%) and seven women (38.9%), aged between 57 and 83 years old. Three (16.6%) patients had recurrent tumors from previously operated carcinomas in the same region. Four (22.2%) of them had performed preoperative radiotherapy. Three (16.6%) patients had a history of skin cancer surgery in other head and neck regions, and two (11.1%) patients had previous surgery for cutaneous malignancies in other regions of the body. There were 11 (61.1%) cases of SCC (squamous cell carcinoma), six (33.3%) BCC (basal cell carcinoma) and one (5.6%) glandular carcinoma. The initial location of the tumor was at the level of the inferior eyelid in eight cases (44.4%), the superior eyelid in five patients (27.7%), the medial canthus in two (11.1%), lateral canthus in one patient (5.6%), the nasal region in one case (5.6%), the genian region in one patient (5.6%). Upon presentation, 15 (83.4%) patients had clinical signs suggestive for orbital invasion, consisting of exophthalmos in six patients (33.3%), globe displacement in five patients (27.7%), limited eye movements in four cases (22.2%), diplopia in six patients (33.3%), infraorbital hypoesthesia in two cases (11.1%), visual impairment in seven patients (38.9%) and tumor adherent to the subjacent bone on palpation in nine patients (50%). In the other three (16.6%) cases, the patients had no obvious clinical signs of orbital invasion. Paraclinical investigations CT in 12 (66.6%) cases and MRI in 9 (50%) patients were necessary for determining the tumor spread and diagnosing orbital invasion. Associated clinical findings and symptoms were the presence of a palpable mass in 18 cases (100%), pain in seven patients (38.9%), bleeding in 14 (77.7%), epiphora in six cases (33.3%). The greatest external tumor diameter was 6

cm, while the smallest tumor was 3 cm. None of the patients with squamous cell carcinoma had lymph node metastasis upon presentation.

Tumor removal was performed in all patients after histological confirmation by incisional biopsy. Exenteration was performed in 13 (72.3 %) cases and an extended orbital exenteration was necessary in five (27.7%) patients. Partial preservation of one or both eyelids was performed in eight (44.4%) cases. The exenteration defect was covered by a split thickness skin graft in three patients (16.6%), by an association of local and regional flaps in two cases (11.1%), a temporalis muscle flap in five patients (27.7%), a lateral pedicled frontal flap in association with titanium mesh in three patients (16.6%), a lateral pedicled frontal flap in two cases (11.1%), one medial pedicled frontal flap (5.6%), one major pectoralis muscle flap (5.6%) and one latissimus dorsi flap (5.6%).

The postoperative results were favorable regarding the complete tumor removal and obtaining an accurate defect closure with a short healing time. The postoperative scars and resulting deformity due to the absence of the orbital tissues could be easily camouflaged by wearing an eye patch. During the postoperative time there were few local complications that resolved by local and systemic treatment methods. They included partial flap necrosis in three cases (16.6%), wound dehiscence and infection in two patients (11.1%), bone exposure and delayed secondary healing of the exenteration socket in two patients (11.1%). There was one (5.6%) case of intraoperative cerebrospinal fluid fistula that was managed using a latissimus dorsi flap. Five (27.7%) patients underwent postoperative radiotherapy. Follow-up at one month, three months and six months did not show any signs of tumor recurrence.

The good postoperative outcomes are exemplified by the help of a clinical case (fig. 1-5).

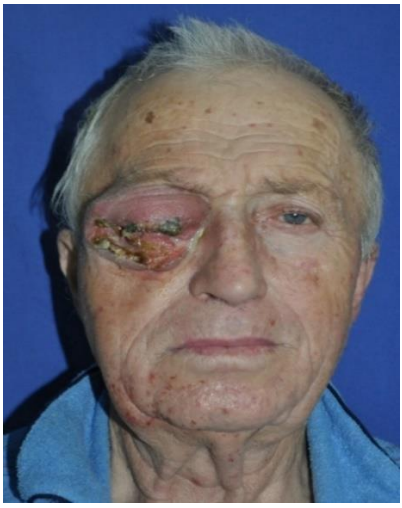

Fig. 1. Patient with a squamous cell carcinoma originating in the inferior eyelid with orbital extension.

Fig. 2. The surgical treatment consisted of an extended orbital exenteration with resection of the lateral orbital wall and of the skin in the inferior third of the right temporal region.

Fig. 3. The plasty of the defect was achieved by rotation of the right temporalis muscle flap into the orbital socket and covering of the muscle with a split thickness skin graft.

Fig. 4. Clinical view of the reconstruction one week postoperative.

Fig. 5. Final aspect of the reconstruction one year after surgery.

DISCUSSION

The management of cutaneous tumors with orbital invasion differs regarding the extent of tissue removal in the presence of various patterns of orbital invasion and histologic types. In our study, clinical or radiological evidence of orbital invasion, consisting of tumor tissue extending beyond the orbital septum, into the orbital fat, was an indication for orbital exenteration in all cases to ensure complete tumor removal and decrease the recurrence rate. This is consistent with most of the existing literature on the subject, but there are also authors that suggest a more conservative approach, opting for globe-sparing procedures in anterior orbital invasion from basal cell carcinoma (4, 5, 6, 7).

The slow local invasion and the absence of lymphatic spread, characteristic for most subtypes of basal cell carcinomas, may allow a less aggressive tissue removal. However, basal cell carcinoma in the periocular region is generally associated with more aggressive types considering the growth pattern, such as infiltrative, morpheic and basosquamous (4, 8, 6). Perineural spread is also exhibited by some histological types and it is a major cause of local recurrence. Additionally, the location of malignancy development in the periocular skin, particularly the medial canthal region and superior nasal-genian fold region favor the deep spread of tumor at the junction of various anatomical elements. Residual tumor may grow extensively before a recurrence diagnosis is made. In consideration of all these factors, we consider advisable to perform an initial orbital exenteration for malignancies eroding through the orbital septum into the orbital fat, regardless of the histology. Complete or partial preservation of an eyelid and conjunctiva can be performed without compromising oncologic safety in most cases, considering the location of the cutaneous malignancy. This aspect is important for obtaining better aesthetic results of the orbital socket reconstruction (9).

Although less common than basal cell carcinoma in the periocular region, squamous cell carcinoma is responsible for most cases of orbital invasion that require orbital exenteration procedures (10). We found similar results in our case series, consisting of 61.1% squamous cell carcinoma cases out of the total.

Due to its aggressive rapid growth pattern and possibility of lymphatic and hematogenous metastasis, cutaneous periorbital squamous cell carcinoma requires an initial radical resection, involving an orbital exenteration for all malignancies invading beyond the orbital septum. The controversy is regarding lymph node dissection in the presence of a high-risk situation and absence of macroscopic nodes. Lymph node metastases from periocular squamous cell carcinoma may be encountered in up to 25% of cases. Laterally located orbital malignancies drain mostly in the intraparotid and peri parotid lymph nodes, while medial tumors drain via the angular and facial vein into the facial and submandibular lymph nodes (11). The same tumor characteristics associated with a higher risk of orbital invasion, are also related to a higher rate of lymph node metastasis for squamous cell carcinoma lesions: increased tumor size, recurrence of previously operated tumors, the depth of invasion, less differentiated histologic subtypes, perineural spread, patient over 70 years of age (12, 13). In our study we did not encounter any patient with lymph node involvement at the time of the initial presentation, despite the

presence of some of the mentioned features associated with a higher risk for lymphatic metastasis. In the absence of clinically or imaging lymph node involvement, we did not consider necessary to perform a prophylactic lymph node dissection. This is the general management preferred by most surgeons, although some advocate for sentinel node biopsy in high-risk cases (11, 14). With absence of lymph node removal, a careful oncologic surveillance is crucial for the early detection of possible lymphatic metastasis.

Considering the extent of the postoperative defect, several techniques may be used for closure. The simple orbital exenteration socket heals by secondary intention through granulation tissue formation that takes place over three to four months (15). Covering the socket with a split thickness skin graft can shorten the healing interval. If radiotherapy is planned postoperatively, a good, rapid healing of the socket is desired, thus a local or regional flap may be used. For defects involving the bony walls of the orbit and the neighboring anatomical structures, opening the adjacent sinus cavities (6, 16), a regional flap or a free flap is often necessary. Due to the advanced age of the patients and comorbidities, we preferred the covering of the orbital socket using a split thickness skin graft when the orbital walls were intact, without communication with the adjacent sinus cavities. This method allowed shorter surgery time, good results, decreased complications, good oncologic surveillance by direct inspection of the orbital socket, and the possibility to start postoperative radiotherapy. In defects involving the bony walls, we favored the use of local and regional flaps for closure, with good overall results and few complications. Extended defects involving a wide skin surface and a wide opening of adjacent cavities, or associating a cerebrospinal fluid fistula, were closed using a free flap.

Almost one quarter of orbital exenteration procedures are followed by the onset of complications (15). Studies have found similar complications to the ones encountered in our case series, including exposed bone with prolonged healing of the residual socket, fistula formation and chronic drainage, tissue necrosis and infection, pain (15, 17). Most complications were managed conservatively, without the need for invasive procedures. We encountered one case of intraoperative cerebrospinal fluid fistula consecutive to the removal of the extended tumor. The closure of the fistula was achieved using a latissimus dorsi flap that was also necessary for covering the remaining skin defect and the exenteration socket.

The main purpose of surgery is achieving local control of the disease and adequate closure of the defect for a rapid postoperative healing, in detriment to the aesthetic result. Secondary procedures can be performed for improving cosmesis. An orbital epithesis can be performed to recreate the lost facial features, to decrease the psychological impact of the resulting deformity and increase social integration of the patients. Since the manufacturing of a natural looking epithesis with a good adaptation and comfortable wear is challenging, most patients prefer wearing an eye patch for camouflaging the reconstructed region.

CONCLUSIONS

We found that cutaneous malignancies invading the orbit can be adequately managed surgically by orbital exenteration and extended orbital exenteration with good results and few complications. A variety of

techniques can be employed for the closure of defects with various extents. The challenges encountered were choosing the most appropriate method for individual defect reconstruction in the presence of comorbidities and advanced age, as well as establishing the opportunity of performing a prophylactic lymph node dissection for orbital squamous cell carcinoma in the absence of clinically or imagistic nodal involvement. Postoperative surveillance is crucial for patients with orbital invasion for the early detection of local recurrence and lymph node metastasis.

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NEFRITA HENOCH-SCHONLEIN: PROBLEME DE DIAGNOSTIC ŞI PROGNOSTIC LA COPIL

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HENOCH-SCHONLEIN NEPHRITIS: DIAGNOSIS AND PROGNOSIS PROBLEMS IN CHILDHOOD (Abstract): Henoch-Schonlein purpura (HSP) is the most common vasculitis in childhood and is characterized by sistemic leukocytoclastic angiitis, mainly affecting small vessels of the skin, joints, gastro-intestinal tract and kidneys. Renal involvement is the most important cause of morbidity and mortality in patients with HSP. The aim of this study was to evaluate renal involvement in children with HPS and the prognoses of short- and long-term outcome of patients diagnosed with HS nephritis. **Material and method**: We conducted a retrospective study based on observation files of hospitalized children with Henoch-Schonlein purpura admitted in the Nephrology Department" Sf. Maria" Hospital, during 20 years. **Results**: The results showed that the prognosis of Henoch-Schonlein nephritis is relatively good and long-time morbidity is predominantly associeted with initial presentation and renal involvement. **Key words**: HENOCH-SCHONLEIN PURPURA, CHILDHOOD, OUTCOME, HENOCH-SCHONLEIN NEPHRITIS

Purpura Henoch-Schonlein (PHS) este cea mai comună vasculită întâlnită în copilărie. Este o afecţiune multisistemică ce afectează în general pielea, articulaţiile, tractul gastro-intestinal şi rinichii, dar pot fi interesate şi alte organe. Leziunea vaselor mici este mediată de imunglobulinele A. Afectarea renală este importantă deoarece fixează prognosticul bolii.

Ne-am propus, printr-un studiu retrospectiv, evaluarea manifestării renale şi a prognosticului pe termen scurt (sub 6 luni) sau lung (peste 6 luni) a pacienţilor diagnosticaţi cu PHS. Cazurile au fost internate şi urmărite în Clinica a IV-a Pediatrie, UMF „Gr.T. Popa" Iaşi.

MATERIAL ŞI METODĂ

Au fost urmăriţi 39 de pacienţi, internaţi într-un interval de 20 de ani în Clinica a IV-a Pediatrie, care au îndeplinit cel puţin 3 din cele 6 criterii ACR (American College of Rheumatology) modificate (1). S-au evaluat datele clinice iniţiale, datele epidemiologice şi principalele modificări urinare. Am încercat aprecierea evoluţiei pe termen scurt şi lung a nefritei Henoch-Schonlein (NHS) sub diferite scheme de tratament.

Am urmărit durata şi persistenţa modificărilor renale la toţi pacienţii cu NHS.

Parametrii utilizaţi au fost:

a) Clinici: hematuria macroscopică; edemele; oliguria; hipertensiunea arterială (HTA).
b) Biologici: creatinina serică şi rata de filtrare glomerulară - RFG (calculată după formula Schwartz), proteine totale, albumina serică, lipide totale, colesterol.
c) Urinari: hematuria microscopică (testul Addis), proteinuria pe 24 ore.

d) Histologici : puncţia biopsie renală (PBR) a fost efectuată în 10 cazuri. Criteriile de PBR au fost : persistenţa proteinuriei (peste 1,5 g pe 24 ore), prezenţa sindromului nefrotic sau nefritic şi scăderea RFG. La pacienţii la care s-a practicat PBR s-a urmărit corelaţia dintre substratul histologic şi evoluţia pe termen scurt sau lung.

Pacienţii cu NHS au fost împărţiţi în 5 grade în funcţie de manifestările clinice iniţiale conform clasificării Meadow (2) modificate :

Gradul I - hematurie microscopică : 12 cazuri (38,5%)

Gradul II - proteinurie medie persistentă (<1,5 g pe 24 ore) ± hematurie : 11 cazuri (28,2%)

Gradul III - sindrom nefritic parţial : hematurie, scăderea RFG, oligurie, HTA, edeme : 11 cazuri (28,2%)

Gradul IV - sindrom nefrotic : proteinurie >50 mg/kgc/zi, hipoalbuminemie, hiperlipidemie, edeme : 2 cazuri (5,1%)

Gradul V - nefrită/nefroză : 3 cazuri (7,7%).

Pentru aprecierea evoluţiei pacienţii au fost încadraţi în 2 grupe :

Grupul I a cuprins gradele I şi II (23 cazuri).

Grupul II a cuprins gradele III, IV, V (16 cazuri).

g) Tipul de tratament utilizat : anticoagulant (tip I), corticoterapie orală (tip II) şi alte imunosupresive : pulsaţii de Metilprednisolon, Ciclofosfamidă (tip II).

h) Evoluţia a fost clasificată conform criteriilor Meadow (2) :

- A - normală : fără HTA, modificări urinare, creatinina serică normală
- B - modificări urinare minime : proteinurie minimă ± hematurie macroscopică sau microscopică recurentă
- C - boală renală activă : proteinurie >50 mg/kgc/zi, creşterea creatininei serice
- D - insuficienţă renală cu scăderea RFG <60 mL/min/1,73m^3

Evoluţia pe termen - scurt : până în 6 luni şi - lung : mai mult de 6 luni

Datele au fost încărcate şi prelucrate cu ajutorul funcţiilor statistice din EXCEL, EPIINFO şi SPSS.

REZULTATE

Parametri clinici

Manifestarea renală exprimată clinic prin sindrom nefritic a fost întâlnită la 71,8% dintre pacienţii cu PHS. Remarcăm faptul că sexul feminin asociază mai frecvent afectare renală comparativ cu cel masculin, diferenţa fiind statistic seminificativă între sexe ($p=0,03$), dar nu şi raportat la vârsta pacientului ($p>0,05$).

Parametri biologici

a) Retenţia azotată ca manifestare a NHS a fost întâlnită la debutul afecţiunii la 30,8% dintre cazuri, 53,8% aveau o RFG normală în momentul diagnosticului, iar 15,4% din cazuri nu aveau creatinina lucrată la debut. Remarcăm normalizarea valorilor creatininei (de la 1,44 la 1,17 mg%) şi creşterea RFG (de la 87 la 93,9 mL/min/1,73m^3) la pacienţii cu retenţie azotată la debut, pe parcursul derulării studiului, ceea ce sugerează o evoluţie favorabilă.

b) Hipoproteinemia, ca expresie a sindromului nefrotic asociat nefritei HS s-a întâlnit în 20,5% dintre cazuri. Se remarcă o corelaţie indirectă, slabă ($r = -0,14$) între valorile scăzute ale proteinelor totale şi valoarea crescută a creatininei, precum şi o corelaţie directă ($r = +0,44$) între normalizarea RFG şi valoa-

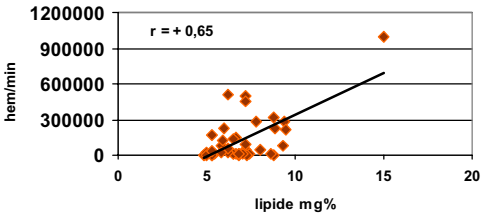

Fig. 1. Corelaţia dintre lipide şi hematurie la pacienţii cu NHS

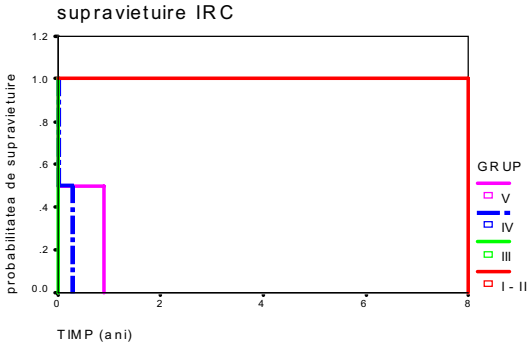

Fig. 2. Curba de supravieţuire Kaplan-Meier în funcţie de perioada de monitorizare, valorile hematuriei şi evoluţia spre IRC

rea normală a proteinelor totale. Se poate spune că hipoproteinemia influenţează negativ RFG.

c) Hipercolesterolemia a fost întâlnită la debut doar la 12,8% dintre cazuri. Aparent se remarcă faptul că valorile colesterolului cresc odată cu creşterea creatininei serice, dar dislipidemia (valori crescute ale lipidelor totale şi ale colesterolului) nu reprezintă un factor de progresie a leziunilor de NHS .

Parametri urinari

a) Hematuria microscopică a fost prezentă la 66,7% dintre pacienţi la debut. Remarcăm o corelaţie directă slabă între hematuria microscopică şi valorile mari ale creatininei serice (r = +0,27) şi o corelaţie indirectă (r = -0,32) între hematuria masivă şi scăderea RFG. Există însă o puternică corelaţie directă (r = +0,68) între hematurie şi hiperlipidemie (fig. 1).

b) Proteinuria de rang nefritic a fost întâlnită la 38,5% dintre cazuri şi cea de rang nefrotic la 17,9% la debutul studiului. Proteinuria a diminuat pe parcursul monitorizării lotului, ca efect al terapiei instituite, fără ca aceasta să fie statistic semnificativă (p > 0,05).

c) Pentru aprecierea evoluţiei NHS pe parcursul desfăşurării studiului, cazurile au fost încadrate în 5 grade conform clasificării Meadow (2).

Cele mai multe cazuri de NHS (87,2%) sunt incluse în gradele I, II şi III conform clasificării Meadow. Cea mai lungă perioadă de supravieţuire de organ se întâlneşte la cei cu afectare renală gradul I (manifestată doar prin hematurie microscopică), iar supravieţuirea este scăzută la cei incluşi în gradul V (cu manifestări de nefrită/nefroză), ajungând la maxim 12 luni.

Pacienţii cu gradele I şi II de afectare renală au o evoluţie favorabilă pe termen scurt, care diferă semnificativ statistic (p = 0,0009) comparativ cu cea de la pacienţii cu gradele III, IV, V.

În 6 cazuri (15,4%), pacienţii au evoluat spre IRC terminală şi au necesitat supleerea funcţiei renale prin dializă. În ceea ce priveşte evoluţia pe termen lung, aceasta este de asemenea favorabilă la pacienţii cu gradele I şi II de afectare renală, conform clasificării Meadow , comparativ cu cei care se încadrează în gradele III, IV şi V, diferenţele dintre ele fiind semnificative statistic (fig. 2).

În funcţie de clasificarea Meadow am subîmpărţit lotul de studiu în două grupuri, pentru a studia evoluţia nefritei Henoch-Schönlein către IRC şi dializă :

Grup I : în care intră gradele I şi II din clasificarea Meadow - 23 pacienţi (59%)

Grup II : în care intră gradele III, IV şi V din clasificarea Meadow - 16 pacienţi (41%).

Evoluţia, clasificată după criteriile Meadow, se poate observa în tabelul I.

Se remarcă din acest tabel că un singur pacient clasat în grupul II a prezentat evoluţie încadrată în grupul D. În acest caz, afectarea renală nu a fost investigată la debut, nefiind posibilă încadrarea corectă.

Astfel, prezenţa sindromului nefritic sau nefrotic, precum şi manifestările tip nefrită/nefroză la debul PHS se asociază cu evoluţie nefavorabilă atât pe termen scurt cât şi pe termen lung, influenţând net supravie-

TABELUL I

Evoluţia purpurei Henoch-Schönlein pe grupuri de studiu

GRUP	n	%	Evoluţia pe termen scurt				Evoluţia pe termen lung			
			A	B	C	D	A	B	C	D
Grup I										
Grad I	12	30,8	9	3	-	-	3	2	-	-
Grad II	11	28,2	7	3	1	-	8	-	-	1
Grup II										
Grad III	11	28,2	-	11	-	-	1	7	1	-
Grad IV	2	5,1	-	-	1	1	-	-	-	2
Grad V	3	7,7	1	1	-	1	1	1	-	1

TABELUL II

Evoluţia sub tratament a pacienţilor împărţiţi pe grupuri de studiu.

	Tratament	n	Evoluţia pe termen scurt				Evoluţia pe termen lung			
Grup I			A	B	C	D	A	B	C	D
Grad I	I	4	3	1	-	-	-	1	-	-
	II	1	1	-	-	-	-	1	-	-
	I/II	2	1	1	-	-	1	-	-	-
Grad II	I	1	1	-	-	-	1	-	-	-
	I/II	7	4	2	1	-	5	-	-	1
	I/II/III	1	1	-	-	-	1	-	-	-
Grup II										
Grad III	I	1	-	1	-	-	-	1	-	-
	I/II	5	-	5	-	-	1	2	1	-
	I/II/III	4	-	4	-	-	-	4	-	-
Grad IV	I/II	1	-	-	-	1	-	-	-	1
	I/II/III	1	-	-	1	-	-	-	-	1
Grad V	I/II/III	3	1	1	-	1	1	1	-	1

TABELUL III

Evoluţia NHS pe termen scurt, respectiv pe termen lung funcţie de încadrarea histologică

Clasa de NHS	Nr.	Evoluţia pe termen scurt (< 6 luni)	Evoluţia pe termen lung (> 6 luni)
Clasa II	1	A	A
Clasa III	1	A	A
	6	B	B
Clasa IV	1	D	D
Clasa IV/V	1	C	D

ţuirea renală.

Evoluţia sub tratament a fost favorabilă de asemenea la pacienţii din grupul I (gradele I şi II Meadow de afectare renală). Din cele 16 cazuri incluse în grupul I, la 10 s-a obţinut remisiunea completă la 6 luni de urmărire şi doar la 8 s-a menţinut această remisiune (tab. II). Majoritatea acestor pacienţi au primit corticoterapie oral.

Pacienţii cu sindrom nefritic au avut o evoluţie pe termen lung relativ favorabilă : din cele 10 cazuri, 9 au continuat să prezinte doar hematurie microscopică şi/sau proteinurie.

Cazurile cu sindrom nefrotic au avut evoluţie nefavorabilă pe termen lung, cu scăderea RFG sub 60 mL/min/1,73 m^3. Se poate afirma că proteinuria de rang nefrotic este factor de prognostic nefavorabil pentru NHS.

Puncţia biopsie renală a fost efectuată în 10 cazuri, iar în 2 din cazuri a fost necesară repetarea ei, datorită evoluţiei nefavorabile. Se poate remarca evoluţie favo-

rabilă în cazul NHS clasă II şi doar într-un singur caz de NHS clasă III, celelalte cazuri de NHS au evoluat cu persistenţa modificărilor urinare minime (proteinurie < 1 g%o ± hematurie).

Clasa IV de NHS a avut o evoluţie nefavorabilă atât pe termen scurt cât şi lung.

DISCUŢII

În lotul studiat majoritatea pacienţilor (71,8%) diagnosticaţi cu PHS au asociat la debut manifestări renale: HTA (43,6%), edeme, oligurie. Evoluţia lor a fost relativ favorabilă, dar aceşti pacienţi au fost urmăriţi pe o perioadă relativ scurtă de timp: media a fost de 34,22 luni, cu limite între câteva zile şi 124 de luni. Unii pacienţi nu s-au mai prezentat la control şi astfel evoluţia lor nu a putut fi apreciată. În literatură se citează necesitatea urmăririi evoluţiei pe o perioadă de cel puţin 10 ani (supravieţuirea renală la 10 ani a fost de 73%). Scharer et al. au efectuat un studiu cu privire la evoluţia la 20 de ani a unor copiii cu PHS şi s-a raportat că aceasta a fost nefavorabilă la unii dintre pacienţi cu boală moderată iniţial şi cu recuperare completă. Aceşti pacienţi, după 20 de ani de evoluţie, se aflau în diferite stadii de IRC (3,4). Acest model de evoluţie se regăseşte doar într-un singur caz din lotul nostru.

Un alt studiu realizat de un grup de finlandezi, pe un grup de 52 de pacienţi diagnosticaţi cu PHS şi investigaţi 24 de ani mai târziu, relevă că acei pacienţi fără modificări urinare în faza acută au avut o evoluţie, în general, benignă. De asemenea ei relatează că nu există o corelaţie între histologia iniţială şi evoluţia la distanţă. Acei pacienţi cu manifestare iniţială severă au avut un risc şi mai crescut de evoluţie nefavorabilă (5,6,7), ceea ce s-a confirmat şi pe lotul nostru.

Aceste rezultate au fost explicate prin continuarea depunerii în mezangiul glomerular de IgA, chiar dacă faza acută a fost depăşită. O altă explicaţie ar fi legată de susceptibilitatea lor genetică (8).

Din cei 23 de pacienţi (gradele I, II Meadow) care la debut au prezentat doar hematurie microscopică, respectiv protienurie moderată ± hematurie, la 16 dintre ei s-a notat o evoluţie favorabilă pe termen scurt şi la 11 şi o evoluţie favorabilă pe termen lung. Pacienţii doar cu hematurie au prognostic benign, iar cei care au asociat şi proteinurie pot prezenta persistenţa anomaliilor urinare.

În ceea ce priveşte histologia renală , numeroasele studii efectuate relevă faptul că aceasta nu poate fi un factor predictiv pentru persistenţa sechelelor renale şi nici pentru viitoarele recăderi renale. Prezenţa semilunelor în procentaj crescut, a fibrozei interstiţiale şi extinderea depozitelor mezangiale sunt corelate cu risc crescut de IRC (9,10,11). În studiul nostru, prezenţa acestor modificări histologice a determinat evoluţie spre IRC stadiul IV.

Nu există tratament specific pentru NHS. Tipul de tratament aplicat în lotul nostru s-a demonstrat a nu avea un efect semnificativ asupra prognosticului de supravieţuire renală, dar corticoterapia s-a dovedit a avea efect benefic în unele cazuri.

CONCLUZII

1. Pacienţii încadraţi în gradele I şi II de afectare renală, conform clasificării Meadow, au avut o evoluţie favorabilă atât pe termen scurt cât şi pe termen lung, care diferă semnificativ statistic ($p=0,0009$) comparativ cu cea de la pacienţii cu gradele III, IV, V.
2. Histologia renală clasa IV a determinat evoluţia spre IRC, iar majoritatea cazurilor încadrate în clasa III au prezentat în timp doar modificări urinare minime.
3. Pacienţii cu PHS necesită o perioadă de urmărire de cel puţin 10 ani, iar cei cu manifestări de nefrită vor fi evaluaţi periodic pe tot parcursul vieţii.

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NOUTĂŢI

Clopidogrelul Şi inhibitorii de pompĂ de protoni

Clopidogrelul este un inhibitor al agregării plachetare utilizat frecvent în asociere cu aspirina la pacienţii cu infarct miocardic, însă are ca efecte adverse creşterea riscului de sângerare gastrointestinală ; în vederea reducerii acestui risc se pot asocia inhibitori de pompă de protoni (IPP). Există mai multe studii care au demonstrat că această asociere poate determina diminuarea efectului antiplachetar al Clopidogrelului, crescând riscul de evenimente trombotice. FDA (Food and Drug Administration) şi EMEA (European Medicine Agency) au tras un semnal de alarmă în privinţa acestei asocieri medicamentoase, recomandând utilizarea de IPP doar la pacienţii cu risc de sângerare gastrointestinală foarte crescut. Un studiu recent pare însă să contrazică aceste temeri. Studiul a inclus 56.406 pacienţi danezi, cu vârste peste 30 ani, internaţi pentru infarct miocardic acut. S-a urmărit rata de apariţie a unui eveniment trombotic (infarct de miocard, atac vascular cerebral), la 30 zile de la infarctul de miocard, pacienţii fiind apoi urmăriţi timp de 1 an. Din totalul pacienţilor 9137 (16,2%) au prezentat evenimente trombotice, 43,8% au primit tratament cu Clopidogrel şi la 27,3% s-a asociat IPP. Studiul a demonstrat că nu au existat diferenţe semnificativ statistice între cele doua loturi de pacienţi, rata de evenimemte trombotice fiind similară (p=0,72). Astfel rezultatele acestui studiu de cohortă demostrează că asocierea dintre Clopidogrel şi un IPP nu creşte suplimentar riscul de evenimente trombotice. (Charlot M, Ahlehoff O et al. Proton pump inhibitors after MI linked to increased risk for adverse cardiovascular outcomes. *Ann Intern Med* 2010 ; 153 (6) : 378-386).

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LICHEN PLANUS PIGMENTOSUS INVERSUS IN A 67-YEAR-OLD WOMAN. CASE REPORT

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LICHEN PLANUS PIGMENTOSUS INVERSUS IN A 67-YEAR-OLD WOMAN. CASE REPORT (Abstract): Lichen planus pigmentosus inversus (LPPI) is a chronic inflammatory disorder, presenting with brown patches and macules over flexural areas. Due to its histological appearance, LPPI is believed to be a pauci-inflammatory variant of classical lichen planus. Although it is a rare disorder, its epidemiology is variable, case series being reported all over the world. Herein, we report the case of a 67-year-old woman with asymptomatic, brown to purple, macules and patches, distributed over multiple intertriginous areas. Even though current evidence suggests that LPPI is generally resistant to treatment, our patient showed improvement following topical treatment with high-potency corticosteroids. LPPI may be an under-reported disease due to misdiagnosis and it should be considered in the differential diagnosis of pigmentation over flexural areas. **Keywords:** LICHEN PLANUS PIGMENTOSUS INVERSUS, HYPERPIGMENTATION, DERMOSCOPY.

Lichen planus pigmentosus inversus (LPPI) is a rare, chronic inflammatory skin condition, characterized by the appearance of brown patches and macules in intertriginous areas (1). Herein, we report the case of a 67-year-old woman without any relevant personal or family history presenting with LPPI with multiple flexural involvement.

CASE REPORT

A 67-year-old woman presented with a 3-week history of asymptomatic, brown to purple macules and patches, localized over multiple intertriginous areas including axillae, neck, abdominal, and inframammary folds. She did not remember having come into contact with any plants, animals or chemicals. A causal relationship with drugs, sun exposure, or trauma could not be established. Her history was negative for fever, weight loss, and hepatitis B or C virus infection. Patient informed consent was obtained.

Clinical examination revealed multiple brown to purple macules and patches, distributed over the neck, axillary, inframammary, and abdominal folds (fig. 1A-D). The inguinal folds were spared. The macules and patches varied in diameter, from as small as 2-3 mm to several centimeters, some of them coalesced into linear structures (fig. 1A, 1B). The mucosae, hair, and nails were not involved. The patient was otherwise healthy and general clinical examination revealed no abnormalities.

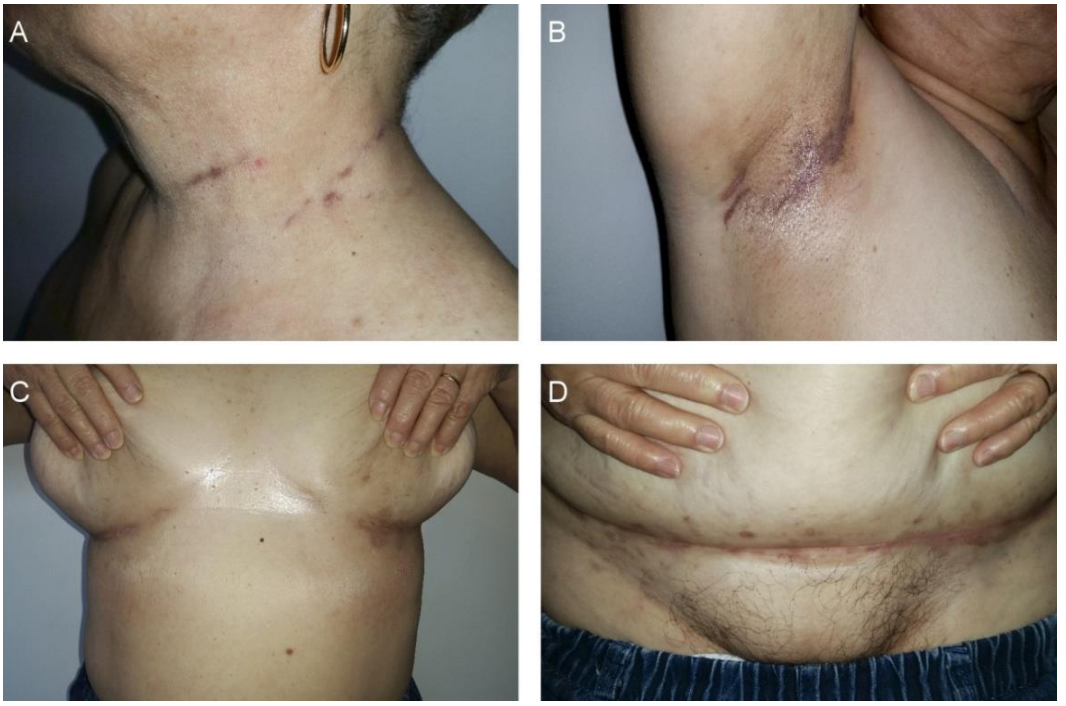

Fig. 1. Clinical images. A-D
Hyperpigmented patches over the neck, axillary, inframammary, and abdominal folds.

Dermoscopic examination of the lesions revealed a mottled white-brown-purple pattern in the absence of a pigmented reticulum (fig. 2B). Homogeneous whitish-pink areas (black arrows) and brown-grey or blue-grey globules and dots (white arrows) could be observed at the periphery of some lesions (fig. 2A). Dermoscopic differential diagnosis included pigmented basal cell carcinoma, pigmented Bowen's disease, pigmented actinic keratosis, and melanoma.

Laboratory evaluation including complete blood cell count, fasting blood sugar levels, liver function tests, serum electrophoresis, serum electrolyte levels, and urinalysis were all within normal limits.

The histopathological examination of a punch biopsy taken from a lesion in the right axilla showed a slightly hyperplastic epidermis with compact hyperkeratosis, hypergranulosis, and vacuolar alteration of the basal layer. The inflammatory infiltrate of lymphocytes, histiocytes, and prominent melanophages in the upper dermis was moderate, and had a characteristic lichenoid disposition (fig. 3A, 3B).

Based on all these clinical, dermoscopic, and histological findings, the patient was diagnosed with LPPI and started on topical high-potency corticosteroids.

Follow-up at one month showed moderate improvement of the neck, axillary and abdominal lesions, and slight improvement of the inframammary ones. At this time, tacrolimus 0.1% ointment was added to the treatment regimen, and at 2-months follow-up all lesion sites showed further improvement (fig. 4A-D).

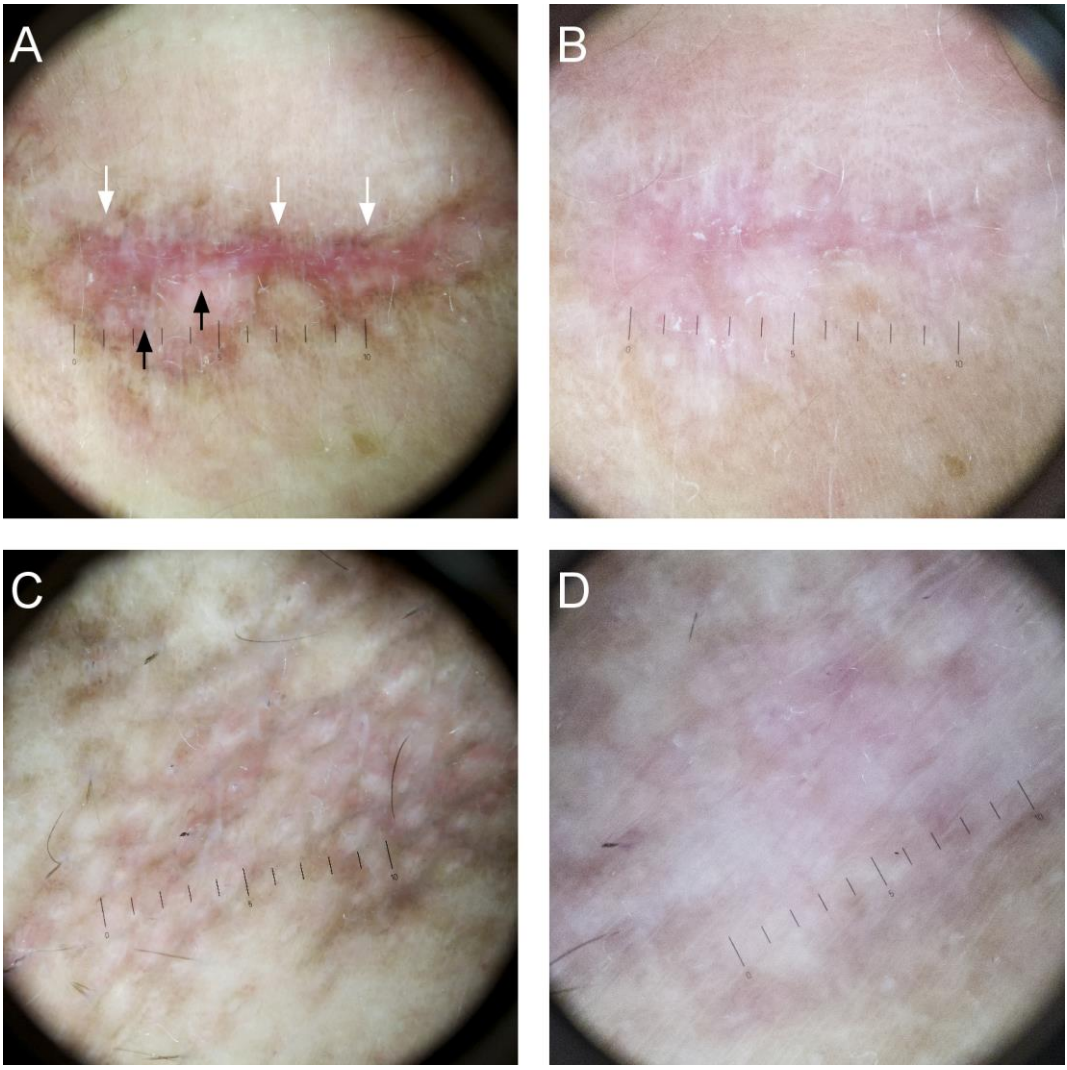

Fig. 2. Dermoscopic features of LPPI.
A - Neck lesion showing homogeneous whitish-pink areas (black arrows) and brown-grey or blue-grey globules and dots (white arrows) towards the periphery;
B - Neck lesion at 2 months follow-up: overall pigmentation is significantly reduced, and globules and dots are absent;
C - Mottled white-brown-purple pattern in the absence of a pigmented reticulum (right axilla);
D - Right axilla lesion at 2 months follow-up shows improvement of the mottled pattern.

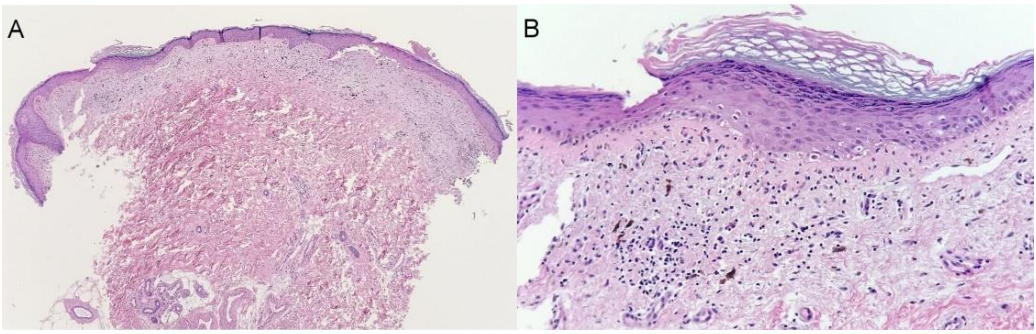

Fig. 3. Histological aspects of LPPI.
A, B Compact hyperkeratosis, hypergranulosis, vacuolar degeneration of the basal layer, moderate upper dermal inflammatory infiltrate showing a characteristic lichenoid disposition (Hematoxylin-Eosin stain, 40x and 400x, respectively).

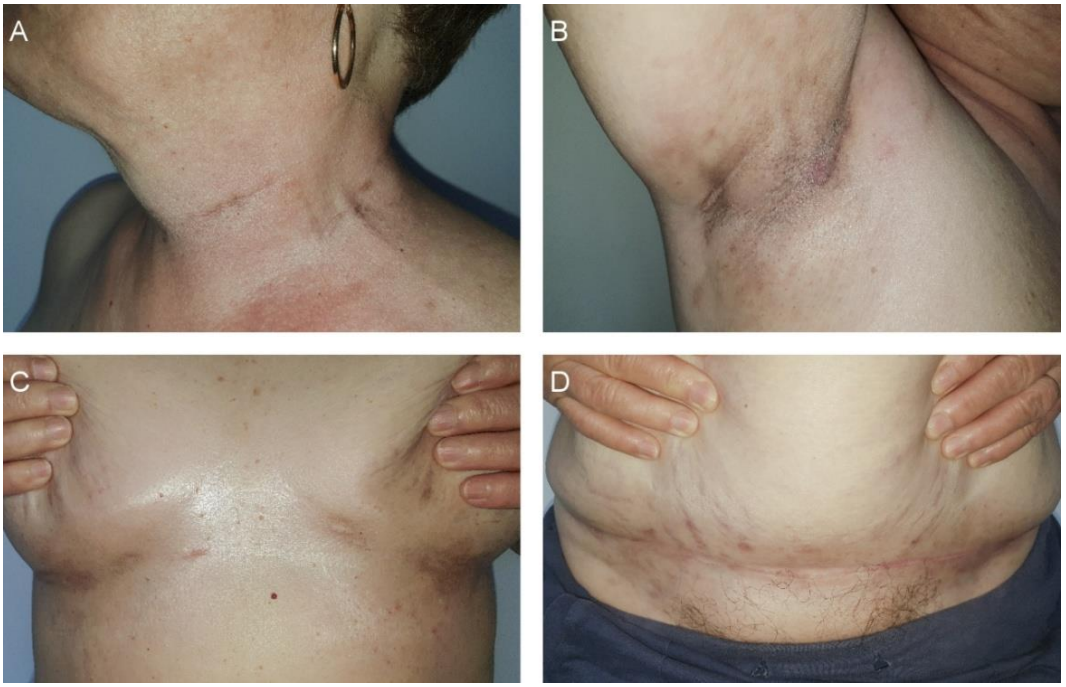

Fig. 4. Clinical images at 2 months follow-up.
A-D - Improvement across all affected areas.

DISCUSSION

The term of lichen planus pigmentosus inversus was coined by Pock *et al* (1) in 2001 after the condition was observed in seven patients with lesions similar to LPP, but distributed in flexural areas. To our knowledge, only 80 cases of LPPI, including the present case, have been reported in the literature until now. LPPI's etiology and physiopathology are poorly under-

stood, but due to histological similarities, many authors have suggested it arises through epidermal flattening of LP lesions in flexural areas (2). The literature confirms the absence of nail, hair and mucosal involvement (3, 4), also observed in our case.

LPPI lesions begin as oval to round macules ultimately coalescing into confluent areas of pigmentation with variable color ranging from slate-blue to steel grey, brown (1, 4), or even purple. Lesion morphology includes linear, reticular, blotchy, blaschkoid or perifollicular patterns (5). The disorder most often affects the axillae, groin, and inframammary folds (5-8), two of these three areas also being affected in our patient. The dermoscopic aspects of LPPI are poorly covered by literature (9-11), and include diffuse brown patches containing grey-brown or grey-blue dots and globules, features similar to those observed in our patient. Histologically, reported cases have mainly described marked pigment incontinence, hydropic degeneration of the basal layer, and a scanty inflammatory infiltrate (1, 7), the regressive lichenoid reaction being in accordance with our findings. The differential diagnosis of LPPI includes LPP, LP actinicus, acanthosis nigricans, pigmented contact dermatitis, and erythema dyschromicum perstans (EDP). A negative history for contact with common allergens rules out pigmented contact dermatitis in this case. Acanthosis nigricans has a more verrucous surface and lacks a lichenoid reaction on histology. EDP affects darker skinned individuals and prefers non-flexural areas of the trunk and limbs, its active lesions showing erythematous borders (1, 7, 12). Reports of diseases associated with LPPI include a single a case of lichen planopilaris (13), one of hepatitis C (14), a drug-induced case by a propolis containing topical agent (15), and an occupational systemic metal sensitization (16). Due to LPPI's reported resistance to treatment including topical (7, 8, 17) and systemic (9) corticosteroids, and topical calcineurin inhibitors (17) some only recommend symptomatic treatment (9, 18), and it has been suggested that a "wait and watch" approach may be the best strategy in LPPI cases (4). Contrary to this, topical treatment with high-potency steroids (clobetasol propionate 0.05%) for only one month led to lesion improvement, our patient continuing to improve constantly after the addition of topical tacrolimus 0.1% ointment.

CONCLUSIONS

Lichen planus pigmentosus inversus seems to be under-reported, probably due to the fact that it is often misdiagnosed. LPPI should, therefore, be considered in the differential diagnosis of cutaneous pigmentation located in flexural areas. More cases are required in order to validate the isolated reports of LPPI's dermoscopic pattern. Even though many authors deem the disorder as "easy to diagnose but hard to treat", our patient showed constant improvement with conventional topical treatments, thus suggesting that some cases of LPPI may be quicker to respond to therapy than others, inevitably leading to questions regarding as to why this may happen.

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OBSTRUCTIVE SLEEP APNEA PATIENTS VOICE ANALYSIS

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OBSTRUCTIVE SLEEP APNEA PATIENTS VOICE ANALYSIS (Abstract). It is generally considered that obstructive sleep apnea may associate larynx abnormalities – either anatomic or functional in nature ; considering the vocal tract as a series of uniform loss cylindrical pipes the idea of non-invasively exploring this by means of spectral analysis emerged. The aim of our study is to evaluate potential changes in acoustic features of voice in sleep apnea patients as compared to matched controls in order to develop a potential screening test. **Material and method** : Sleep apnea subjects and controls were asked to produce sustained vowels such as "a" and "i" which were recorded and analyzed. Some parameters were estimated – average of fundamental frequency, peak amplitude variation and compared and others are still to be developed. **Key words** : OBSTRUCTIVE SLEEP APNEA, VOICE, FOURIER ANALYSIS

Sleep apnea syndrome is a relatively recently recognized disorder of respiration during sleep. Although a number of factors contribute to its pathogenesis, sleep related changes in ventilatory control, specifically control of upper airway muscles may play a key role in its pathogenesis.

Affected patients have repetitive periods of apnea (i.e., cessation of breathing) that occur during sleep. A period of more than 10 seconds without airflow is generally considered to constitute an apneic episode, and patients with this syndrome often have hundreds of such episodes during the course of a night's sleep. The term *hypopnea* is used to describe a reduction in airflow of 50% or more but without the complete cessation of airflow implied by the term *apnea*. Because episodes of apnea and hypopnea commonly coexist, the broader term *sleep apnea–hypopnea syndrome* is sometimes used. Sleep apnea syndrome is surprisingly common ; estimates suggest a prevalence of 2% to 4% in middle-aged adults. Men are affected more commonly than women, and affected women are typically postmenopausal.

The aim of our study is to evaluate the changes in acoustic features of voice in sleep apnea patients as compared to matched controls in order to develop a potential screening test.

METHOD

Study type : pilot, prospective, monocentric

Subjects

Our study included 40 male subjects divided in two groups : the obstructive sleep apnea group - 20 subjects and the control group - 20 subjects. The groups were matched for age – mean age control 54.6 +/- 5.8 mean ages OSA 52.6+/-10.

Procedure

The subjects were asked to emit two phonemes : [a:] and [e:] for as long as they considered possible with their mouth open and to focus on obtaining a rear resonance. To achieve this purpose a visual incentive

was used. Furthermore subjects were encouraged to perform "at comfortable loudness and pitch" – a situation which according to previous research is linked to a 63-75 dB interval. All recordings were performed in a quite room as a soundproof booth was not available on site.

The subjects were recorded using an electric microphone (frequency range 50--15000 Hz, sensitivity -72dB) at 20-30 cm away from mouth coupled to a Realtek soundcard to a .wav file (48,000 kHz sampling rate). The files were edited and the first and last seconds removed.

Signal analysis was performed using EFx Hist software package and the following parameters were considered for further statistical analysis: close quotient mean (%), mean jitter (%), mean shimmer (%), harmonics to noise ratio (dB), percentile values of the frequency distribution (Hz) and fundamental frequency. For ease mean shimmer and mean jitter values were multiplied by 100. Statistical analysis was performed using SPSS 13.0.

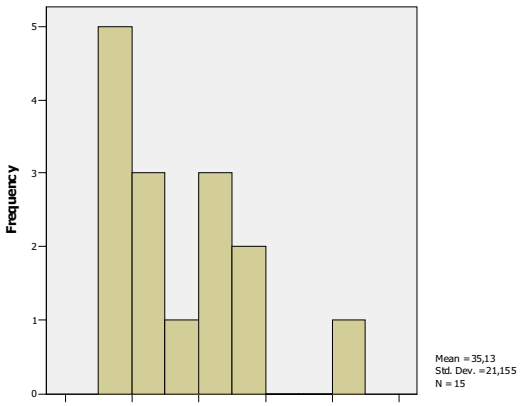

Fig. 1. AHI distribution for OSA group

RESULTS

The OSA subjects had a mean apnea--hipopnea index (AHI) of 35.1+/-21.1 events/hour with a minimum of 14 and a maximum of 86. The distribution of AHI values among OSA group subjects is presented in fig. 1.

Mean voice time was 3.3+/-1.9 seconds with a maximum of 7.5 and a minimum of 0.8 seconds (after editing) for vowel "a" and 3.4+/-2.2 for vowel "i".

As far as fundamental frequency for the two phonemes was concerned - for "a" there was a mean of 131+/-25 Hz in the OSA group and 133+/-25 in the control group; for "i" there was a mean value of 140+/-29 Hz in the OSA group and 148+/-31 Hz in the control group. There were 3 and respectively 4 cases in the OSA and control group for which the fundamental frequency was lower for "i" as compared to "a" – these cases were excluded from further analysis.

There was a statistically significant mean difference as far as the ratio between the two fundamental frequencies was concerned – mean difference 6.5% (95% confidence interval 1.2-11.7%) for p=0.02 (independent samples t-test)

TABLE I
Significant differences for vowel "a" parameters

	Group	N	Mean	Std. Deviation	95% Confidence interval of the difference
Jitter Mean	OSA	15	45.1	13.3	20.0
	Control	17	14.0	16.9	42.2
Shimmer Mean	OSA	15	21.0	10.5	9.3
	Control	17	5.4	6.5	21.9
HNR Mean	OSA	15	17.1	4.4	-5.4
	Control	17	20.1	1.9	-0.5
40% Percentile	OSA	15	204.8	72.0	8.9
					96.3

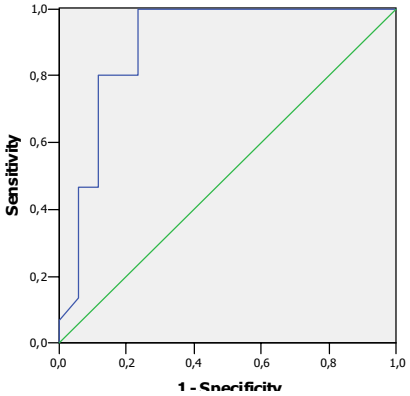

Fig. 2. ROC curve for jitter mean vowel "a"

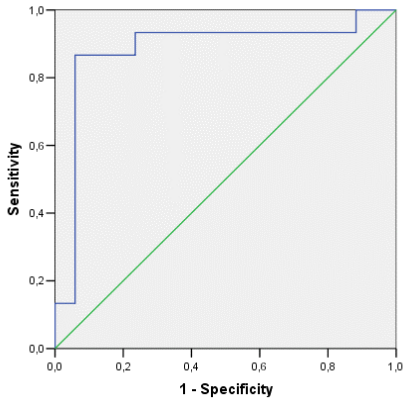

Fig. 3. ROC curve for shimmer mean vowel "a"

As far as the rest of aforementioned parameters are concerned statistically significant between group differences for vowel "a" is presented in table I.

For vowel "i" there were statistically significant differences for jitter mean (mean difference 32.1% ; 95% confidence interval 18.6 – 45.5%) and shimmer mean (mean difference 12.8% ; 95% confidence interval 17.5 – 18.1%).

Using the value of mean jitter for vowel "a" in order to discriminate between groups the following ROC curve (fig. 2) was generated (Area under curve 0.89, asymptotic significance 0.001) ; the cut-off value of 20.2 implies a sensitivity of 86.7% and a specificity of 76.5% but the presence of ties suggests potential bias.

The ROC curve for shimmer mean for vowel "a" is presented in fig. 3 (Area under curve 0.882, asymptotic significance 0.001) and the cutoff value of 10.65 associates a sensitivity of 86.7% and a specificity of 94%.

DISCUSSION

During the last 2 decades, a great deal has been learned about the pathogenesis and the risk factors leading to obstructive sleep apnea syndrome. Normally, inspiration is characterized not only by contraction of the diaphragm, resulting in negative airway pressure, but also by increased activity of a number of upper airway muscles acting to keep the pharynx patent. The genioglossus muscle is particularly important in this regard because it prevents the tongue from falling against the posterior pharyngeal wall and occluding the pharynx.

In patients with obstructive sleep apnea, structural and functional factors often work together to allow the upper airway to close during inspiration. In most patients, an excess of soft tissue in the upper airway, often as a consequence of obesity, compromises the size of the pharyngeal opening. During sleep, particularly rapid eye movement sleep, loss of activity of the upper airway muscles allows inspiratory collapse of the soft tissues and obstruction of the upper airway. Airflow eventually resumes after each episode of obstruction as the patient arouses (although these "micro arousals" often are not evident to the patient), when activity of the upper airway muscles is restored, and when the airway temporarily becomes patent. However, as the patient falls asleep again, inspiratory muscle activity is again lost, and the cycle repeats itself. Because of the importance of structural factors contributing to a small upper airway, patients who are obese (with short, fat necks) or who have a small jaw (micrognathia), a large tongue, or large tonsils are at particular risk for obstructive sleep apnea.

Obstructive sleep apnea commonly occurs

in children of 2-6 years and subsides later as tonsils and adenoid tissue involutes – this fact suggests that larynx and pharynx conformation may play a pathogenic role. Furthermore there are case reports on newly set sleep apnea following vocal cord paralysis (1,2).

Voice analysis has been referred to as a non-invasive and easily implemented tool able to provide useful information complementing standard ENT examinations. To some degree vocal shimmer and jitter may be considered normal but there is a large body of evidence suggesting that increased voice perturbation may be detected in the presence of various larynx conditions (3). These and other vocal determinations are currently used to document and monitor treatment outcomes (4,5).

Since upper airway anatomy seems to be inextricably related to both sleep apnea pathogenesis and vocal characteristics we may infer that some vocal parameters may potentially be used as predictors.

Study limits: The results need to be interpreted considering the group size – rather small given the pilot stage of the study.

Furthermore there was some variability as far as patient performance was involved – the large variations in total voice time may partially explain some of the differences. One explanation for this situation may consist in some associated conditions such restrictive ventilatory disorders (such as obesity-hypoventilation syndrome) which are prone to limit the ability to phonate for longer periods of time; on the other hand some patients reported that they felt awkward performing the requested maneuvers.

There was also some variability concerning the timing of recordings –usually early morning for OSA subjects and late afternoon for control subjects – this may have produced noisier recordings for the first group. Furthermore there is no data on voice parameters variations during the day and we may assume that OSA patients may have experienced a higher stress level under given conditions (night spent in sleep laboratory).

However despite these drawbacks the present results suggest that some vocal changes may occur in sleep apnea patients – either sharing a common cause such as some form of neuropathy and subsequent vocal chord dysfunction either as an effect of this condition. At least in theory there are clinical conditions which predispose to sleep apnea - we may mention hypothyroidism, acromegaly, tonsillitis, adenoiditis, various neurological conditions (focal and systemic), various drugs, tobacco and alcohol all of which usually associate hoarseness.

It is hard to estimate the utility of such parameters as predictors and as screening tests – our OSA subjects had mostly advanced forms of the disease and this situation is usually identified by a skilled physician without further need for screening tests. Given this future study on lower OSA stages subjects is necessary to asses the significance of this approach.

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NEW FRONTIERS IN THE HISTOLOGICAL DIAGNOSIS OF DEGENERATIVE AORTOPATHIES MANIFESTED AS AORTIC ANEURYSMS AND DISSECTIONS

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NEW FRONTIERS IN THE HISTOLOGICAL DIAGNOSIS OF DEGENERATIVE AORTOPATHIES MANIFESTED AS AORTIC ANEURYSMS AND DISSECTIONS (Abstract): Nowadays, the histopathological study of surgical specimens is an essential part of the diagnosis of aortic diseases. Despite the increasing clinical-therapeutic complexity of aortic pathology, the criteria for histopathological diagnosis have not been updated for years. In this respect, we applied a consensus document established in 2016 by a staff of pathologists, who improved our knowledge about noninflammatory degenerative aortic lesions by revising, unifying the nomenclature on this topic and bringing terminology and diagnostic criteria up to date. Older terms such as cystic medial necrosis and medionecrosis were replaced by more accurate terms such as mucoid extracellular matrix accumulation, elastic fiber fragmentation and/or loss, and smooth muscle cell nuclei loss. The main aim of the paper is to draw attention on noninflammatory aortopathies, including standard terminology and new grading system, and to define/present histopathological diagnostic criteria applying to these degenerative aortic diseases. The use of this accessible and standardized nomenclature by all general pathologists is important for future diagnosis and research papers as it will allow drawing comparisons between future findings. **Keywords:** AORTIC ANEURYSM, AORTIC DISSECTION, MEDIAL DEGENERATION.

Thoracic aortic aneurysm (TAA) and aortic dissection (AD) are most frequently characterized by degenerative medial lesions**.** Aortic degenerative disease is a common aortic disorder caused by genetic or acquired conditions and manifests as dilated (aneurysm) or dissecting aortic condition. Nowadays, the histopathological (HP) study of surgical specimens is an essential part of the diagnosis of aortic diseases. Medial degeneration (MD) is characterized by smooth muscle cell loss, alteration of elastic fibers and accumulation of the basophilic mucoid pools within the media of the aortic wall. This degenerative process also extends to the elastic components of the adventitial layer. The weakened aortic wall tends to aneurysm formation and dissection (1). Because the meaning of the histopathological terms used to describe MD has become confusing, there is a need for unified nomenclature for the histopathological lesions of the noninflammatory degenerative ascending aorta diseases (2). The application of the consensus document enabled us to assess the extent of lesions by using a new grading scale in order to better and more consistently classify aortic lesions and to

analyze the connection between HP patterns and etiopathogenetic factors.

Histology of the normal ascending aorta

The aorta is a large elastic artery composed of three layers, the intima, media and adventitia, containing some cellular and extracellular components: endothelial cells overlying the intimal layer, smooth muscle cells (SMCs) within the muscular-elastic lamellar unit, elastic fibers, fibrillary extracellular matrix and collagen fibers. The intima is made up of a single layer of endothelial cells overlying loose connective tissue with scarce SMCs and is separated from the media by the internal elastic lamina. Tunica media is organized in morpho functional muscle-elastic lamellar units consisting of bands of elastin filaments with interposed SMCs and separated by collagen fibers and non-fibrillary matrix. The adventitia is composed of loose connective tissue and contains the vasa vasorum, which constitutes the blood supply to the aortic wall, and is separated from the media by the external elastic lamina. This complex organization provides structural substrate elasticity (2).

Histopathological substrate of degenerative aortopathies

Definition and Terminology of Degenerative Aortopathies. The term "degenerative aortic diseases" refers to aortic disorders characterized by cellular and extracellular matrix lesions that are not caused by inflammation, representing the most common clinical expression of thoracic aortic aneurysm (usually located in the ascending aorta) and aortic dissection. The degenerative medial lesions are characterized by fragmentation of elastic fibers, loss of smooth muscle cells, and accumulation of mucoid material (3).

The earliest terminology of degenerative aortic lesions was the classic description of medionecrosis by Erdheim in 1930. For years the concept of "degenerative lesions" replaced "Erdheim's cystic medial necrosis", term used by Erdheim to describe fragmentation of elastic fibers, loss of smooth muscle cells, and accumulation of basophilic ground substance. In 1986, the term medial degeneration replaced the old terminology such as cystic medial necrosis (cyst-like structures containing proteoglycans) and medionecrosis (loss of SMC nuclei), because the observed lesions were neither cystic nor necrotic. Since then, no specific terms have gained full acceptance in describing degenerative medial lesions until 2014. The new terms and definitions were the result of a constant consensus process (of the membership of the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology) to create a single unified and consistent set of terms to describe noninflammatory degenerative aortic pathologies (2).

Histopathological Lesions in Aortic Degeneration. From a histological viewpoint, noninflammatory aortic diseases are caused by a great number of disorders involving many histopathological (HP) lesions. These medial degenerative lesions result in medial wall weakening that progresses to aneurysm, dissection, or both (4).

Aortic degenerative lesions include various lesions affecting the medial structural aortic components, either cellular, or extracellular (ECM- extracellular matrix) (1): (a) elastic fibers: thinning with widening of interlamellar space and / or fragmentation and/or loss of elastic fibers; (b) non-fibrillary ECM (mucopolysaccharides or glycosaminoglycans): mucoid material pooling within pseudo cystic spaces variable in size; (c) smooth muscle cells: decrease or loss of SMCs; severe lesions may be accompanied by "laminar medial necrosis" characterized by band–like SMC loss with subsequent elastic fiber collapse; (d) colla-

gen fibers: increase of collagen fibers resulting in substitutive fibrosis.

Eponyms for Selected Histopathological Terms Related to Aortic Medial Degeneration. The Cardiovascular Pathology study group noted that the individual main components of medial degeneration are: MEMA - mucoid extracellular matrix accumulation, EFF/EFL - elastic fiber fragmentation and/or loss, SMCNL - smooth muscle cell nuclei loss, LMC - laminar medial collapse, and MF - medial fibrosis (2).

The New Grading System of Degenerative Lesions. Medial lesions were subclassified and graded (3) according to their distribution and severity, depending on whether they cross multiple lamellar units. The degree of medial degenerative lesions may vary from very little to severe destructive aortic medial alterations (fig.1). Therefore, the grading should be reported as mild/moderate/severe and the distribution should be reported as focal, multifocal, or extensive.

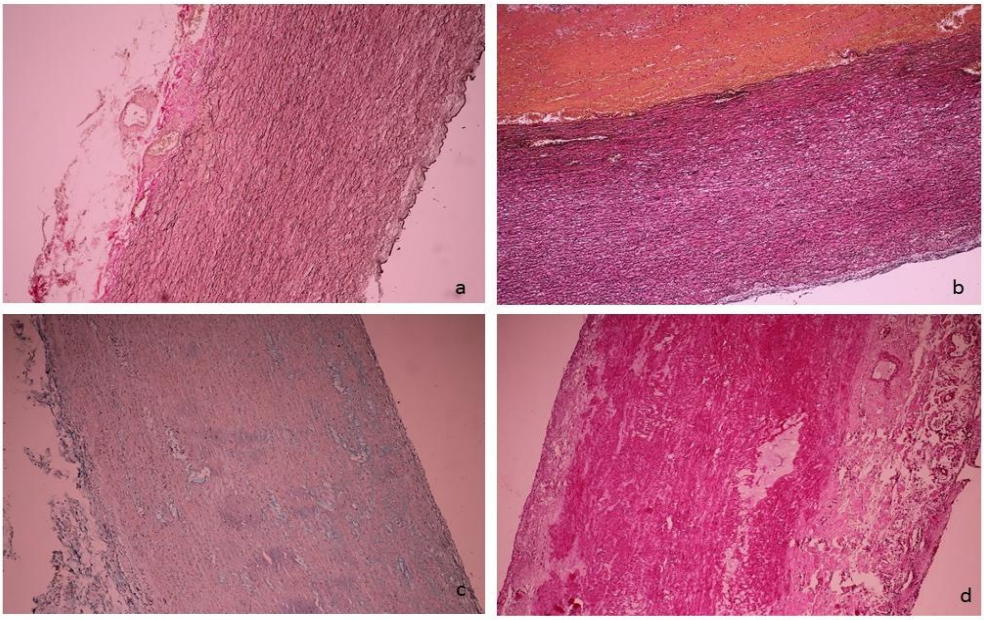

Fig. 1. a) TAA-mild MD and b) AD-mild MD, EVG x10: media with patchy smooth muscle cell loss and mild elastic fiber fragmentation; c) TAA-moderate MD, AB x10: rare smooth muscle cell nuclei loss, multifocal elastic fiber fragmentation and MEMA; d) TAA- severe MD, HE x10: band-like smooth muscle cell loss, severe extensive elastic fiber fragmentation/loss and multifocal trans lamellar MEMA**.**
Abbreviations: AB-Alcian Blue; EVG-Elastic von-Gieson; HE-Hematoxylin-Eosin.

The degree of severity is rated as follows: Mild: lesion involves up to 3 lamellar units; Moderate: lesion involves 4-10 lamellar units; Severe: lesion involves >10 lamellar units. The extent of individual lesions is graded as follows: Absent: the lesion type is not present; Focal: involvement of up to 10% of the media area; Multifocal: multiple areas of involvement of 10–30% of the media area; Extensive: involvement of the media area >30% (4). The application of this grading system in the assessment of all ele-

mentary aortic wall lesions is essential to find new correlations between HP patterns and etiopathogenetic factors.

Etiology

Aortic medial degenerative disease is currently subdivided into two main categories: sporadic and familial degenerative aortopathies (syndromic and non-syndromic) (5).

Degenerative HP lesions may be the consequence of: (a) the normal aging process, in which aortic wall alteration and repair are physiological events; (b) an early and accelerated pathological process in individuals with altered hemodynamic forces (e.g. hypertension, etc.) or in patients with syndromic or nonsyndromic heredofamilial diseases of connective tissue (e.g. Marfan syndrome).

Generally, the causes of thoracic aortic aneurysm (TAA) include inherited syndromes, congenital, atherosclerosis, noninfectious aortitis and infections, and unknown causes (6).

A major cause of noninflammatory thoracic aneurysms is represented by inherited connective tissue disease syndromes, specifically Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), Ehlers-Danlos syndrome (EDS) and familial thoracic aortic aneurysm and dissection (FTAAD) (11).

Marfan Syndrome. MFS is an autosomal dominant disease, with 25% of patients having no family history. Cardiovascular manifestations of MFS include aortic root dilatation, ascending aortic dilatation, aortic dissections, other sites of aortic aneurysm, and mitral valve prolapsed. The histological feature of aortic aneurysms in MFS is loss of elastic laminas with pooling of proteoglycans, so-called medial degeneration. Histological lesions are also seen to lesser extents in familial non-Marfan aortic dissection and those associated with bicuspid aortic valve (7).

In the absence of any surgical treatment, patients with MFS have a 50% risk of developing aortic dissection during their lifetime. The aortic dilatation observed in MFS is the result of defects in a specific component of the elastic fiber, i.e. fibrillin-1, which is encoded by the *FBN1* gene on chromosome 15 (8).

The clinical diagnosis of Marfan syndrome can be set based on the revised Ghent nosology criteria and confirmed molecularly by analysis of the FBN1 gene. MFS treatment involves the use of beta-blocker and angiotensin-converting enzyme inhibition therapy to achieve reduction of hemodynamic stress and delay the progression of arterial dilatation. When critical dilatation of the aorta occurs, with significant risk for aortic dissection or rupture, aortic root replacement surgery is performed (9).

Loeys-Dietz syndrome. LDS is an autosomal dominant Marfan-like connective tissue disorder that is an increasingly recognized cause of thoracic aortic aneurysm. It results from genetic mutations in the transforming growth factor beta receptors 1 and 2 (TGFBR1 and TGFBR2). From the histological point of view, there is diffuse medial degeneration, which may be subtle (10). The cardiovascular manifestations in LDS are arterial tortuosity with aneurysms and dissections. LDS has an earlier onset than MFS, with recommendations for prophylactic aortic root replacement at younger ages and with smaller aortic dimensions (11).

Ehlers-Danlos syndrome. Type IV EDS (vascular type) is due to defects in type III procollagen (COL3A1) (19). It causes vascular fragility with aneurysm formation, rupture, and dissections, with possible life-threatening consequences. When the aorta is involved, it may lead to multiple rupture, usually affecting normal-caliber artery. Patients with type IV EDS may also have TAAs (12).

The diagnosis of type IV EDS is based on physical examination, biochemical analysis of the collagens on cultured skin fibroblasts and molecular analysis of the

COL3A1 gene. No current etiological treatment is available, and management focuses on symptomatic control.

Familial thoracic aortic aneurysm and dissection. FTAAD is a rare condition that affects a subgroup of patients with HP medial degenerative disease. In people affected by this condition, the thoracic aorta may become weakened, stretched and/or enlarged. This can lead to a sudden tear of the inner wall of the aorta that allows blood to dissect the aortic wall. These aortic abnormalities can be life-threatening due to aortic rupture (13).

Idiopathic, noninflammatory aneurysms are also associated with congenital conditions (bicuspid aortic valve) and acquired conditions (hypertension).

Bicuspid Aortic Valve with Ascending Aortic Aneurysm. From a genetic viewpoint, little is known about bicuspid aortic valve (BAV). BAV is a common congenital cardiac defect that affects 1% to 2% of the general population, who run a risk of proximal aortic aneurysms and dissections. Unicuspidal aortic valve is associated with TAA at an even higher rate.

The cardiac manifestations of the BAV disease, which becomes manifest in most patients during the course of their lifetimes, are isolated aortic regurgitation, isolated ascending aortic aneurysm, aortic regurgitation associated with aortic aneurysm, isolated aortic stenosis, and aortic stenosis with aneurysm (14).

Approximately 25% of patients with TAAs have no known cause or association. The major association is systemic hypertension.

Hypertension. Varying degrees of degeneration can be seen in patients without familial syndromes, as an acquired form, usually associated with hypertension (HTA).

Differences between these degenerative HP lesions in various primary and secondary conditions, including aging process, are quantitative rather than qualitative. The involvement of cell cytoskeleton and extracellular matrix components is at the basis of the main degenerative histopathological elementary lesions (15).

Other Rare Causes. *Aortic atherosclerosis* (a) appears to play a major role in aortic arch, descending thoracic and abdominal aorta disease. Atherosclerosis can result in aortic wall weakening, making it prone to aneurysm formation or dissection. *Inflammatory aortic disorders* (b) represent a third broad category of predisposing factors causing aortic diseases. These occur in the context of isolated or systemic disorders such as inflammatory vasculitis. They include infectious and non-infectious causes (8). *Traumatic aortic dissection*(c) often occurs at the level of the left subclavian artery with formation of a chronic pseudoaneurysm (16).

Clinical manifestations of degenerative aortopathy

Degenerative changes of the aortic media and consequent weakening of the aortic wall lead to aneurysm formation and dissection. Aneurysms are less common in the thoracic aorta, where they are more prone to dissection.

Thoracic Aortic Normal Anatomy. Anatomically, the thoracic aorta is divided into subcomponents. The thoracic aorta consists of the aortic root, the ascending aorta (with an average diameter of 3 cm), the arch, and the descending thoracic aorta, with an average diameter of 2.5 cm.

Aortic Aneurysm. An aneurysm is defined as a dilatation at least 50% above the normal diameter of an artery. In the thoracic aorta, a diameter of 3 cm or greater is generally considered aneurysmal. Usually, the term aortic aneurysm refers to an aortic dilatation of over 5cm in diameter. The growth of aneurysm may be indolent. Serial imaging studies can show a slow growth rate (about 1 mm yearly) until the diameter is

about 5 cm, at which time it may be more rapid (17).

TAAs can involve the aortic root, ascending aorta, arch, descending aorta, or a combination of these locations. The combination of aortic root dilatation and ascending aneurysm is termed "annuloaortic ectasia".

Clinically, a large percentage of patients with noninflammatory aortic disorders come in with asymptomatic thoracic aortic aneurysms. These thoracic aneurysms are usually found incidentally by imaging studies. Sometimes, after unexpected rupture, the pathologist may find them during the autopsy. Usually, symptoms are related to aortic insufficiency (aneurysms involving the aortic root or ascending aorta), dissection, or rupture (18).

Aortic Dissection. Aortic dissections are frequently superimposed on noninflammatory TAAs; these aneurysms are named "dissecting aneurysms". Anyway, aortic dissections may occur with medial degeneration in the absence of a pre-existing aneurysm.

AD is defined as separation of the layers within the aortic wall. It is caused by splitting of the aortic wall within the external media, which results in the formation of an aortic false lumen that runs along a true lumen. The hallmark of classic aortic dissection is an intimal tear, with blood entering the aortic wall and formation of an intimal flap protruding within the false lumen of the aortic wall (19).

Aortic dissection, representing the most threatening acute aortic syndrome, includes other dissection variants such as intramural hematomas, penetrating aortic ulcers (especially in abdominal aorta) and symptomatic aneurysms (18). Intramural hematoma (IH) and penetrating aortic ulcer (PAU) differ from classic dissection by the absence of an intimal flap. IH is a non-communicating blood collection in the aortic wall with blood lumen. IH can result in primary rupture of the vasa vasorum. Unlike a true dissection, there is no loss of intimal continuity, no entry tear, and thus no intimal flap. Recent advances in diagnostic imaging can reveal these entities (19). PAU develops when an atheromatous plaque erodes into the aortic media. Depending on how far into the aortic wall the plaque erosion occurs, there can result an intramural hematoma or even complete aortic rupture. This lesion is more commonly encountered within the abdominal aorta (20). Dissections occurring in younger patients (younger than 40 years) typically occur in the context of connective tissue disorders such as Marfan syndrome, Loeys-Dietz, congenital bicuspid aortic valve, and previous aortic surgery (14, 17).

In patients with chronic aortic dissection (present for >2 weeks) surviving the period of increased mortality, the aortas often dilate and are at higher risk for aneurysm formation due to reduced elasticity of the thinner aortic wall remaining after dissection. A rare type of AD is iatrogenic aortic dissections. Aortic cross-clamping or cannulation during cardiac surgery and angiographic catheters can disrupt the aortic intima resulting in iatrogenic dissections anywhere along the aorta's course. These dissections are usually diagnosed and treated at the time of surgery (21).

CONCLUSIONS

The goal of this paper is to draw attention on several noninflammatory aortic disorders which can be related to TAA and AD in young and adult patients. While some are more prevalent than others, it is clear that the severity of the clinical spectrum of many of these disorders is highly variable. This would be the cause of underdiagnosing this aortopathy.

In this respect, we would like to point out the importance of a good familial history, HP examination and genetic screening. Usually, features of acquired MD in the aging aorta bear close resemblance to medi-

al changes in the genetic syndrome, while in the case of genetic syndromes, these degenerative changes are more extensive, and they occur at an earlier age.

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THE INFLUENCE OF DIABETES ON GLAUCOMA PROGRESSION – PROSPECTIVE STUDY

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THE INFLUENCE OF DIABETES ON GLAUCOMA PROGRESSION - PROSPECTIVE STUDY (Abstract): Purpose of the study: To outline the influence of diabetes on the progression of glaucoma. **Material and methods:** The study (prospective, case-control) analyzed the data from 102 patients with glaucoma admitted to the Clinic I Ophthalmology of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, between October 2011 and November 2015, divided into two study groups, depending on the presence of diabetes, as follows: First group 51 patients with Diabetes and Glaucoma (study group) and Group II 51 patients with glaucoma, without Diabetes (control group). In all patients, the following parameters were recorded: VA (visual acuity), IOP (intraocular pressure), visual field progression, corneal thickness, and fundus appearance, where the presence of DR (diabetic retinopathy), its progression and form when it existed, as well as optic disc appearance. Also, it was noted: the duration of glaucoma, diabetes and the type and quantity of antiglaucoma and antidiabetic medications used and the number of VF examinations (visual field) and OCT (ocular coherence tomography), glycemic and HbA1c values. **Results:** At the end of the study, for the patients in group I there was a significantly decrease visual acuity in patients with severe glaucoma ($p = 0.001$). The mean IOP values did not show statistically significant differences compared to the evolution of glaucoma. At the end of the study, the C/D ratio (cup / disc) was significantly higher in patients in group II ($p = 0.017$). The presence of Diabetes had a negative impact on RNFL (retinal nerve fiber layer) among patients with an advanced form of glaucoma. For the patients in the study group, who had a high blood glucose level, it was noted: a significant decrease in the mean IOP, a significant decrease PSD (pattern deviation) and significant decrease mean MD (mean deviation). **Conclusions:** In our study, the data from the analysis of VF, OCT and clinical examination parameters indicate an unfavorable progression of glaucoma among diabetic patients compared to those without. **Keywords:** POAG, NTG, OCT, MD, PSD, DIABETES.

Glaucoma and diabetes are two chronic conditions that have an increased prevalence among the population over 40 years old. Both diabetes and glaucoma lead to irreversible vision loss after a period of evolution. Numerous studies have attempted to find possible associations between diabetes and primitive open-angle glaucoma (POAG) with conflicting results.

MATERIAL AND METHODS

The study (prospective, case-control)

analyzed data from 102 glaucoma patients admitted to Clinic I of Ophthalmology at "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, between October 2011 and November 2015, divided into two study groups, depending on the presence of diabetes, as follows:

- Group I - 51 patients with diabetes and glaucoma (study group);
- Group II - 51 patients with glaucoma without diabetes (control group).

Inclusion and exclusion criteria. The inclusion criteria were: POAG or NTG (normal tension glaucoma) for the control group and the study group with diabetic patients. Patients with other types of glaucoma (pseudo-exfoliative, pigmentary, cortisonic, neovascular, and narrow-angle primitives) were excluded from the study. Patients with age-related cataracts were also excluded, whose visual acuity (VA) was very low.

In all patients, the following parameters were recorded: VA, decrease in IOP (with Goldmann Aplanotonometer), perimetric evolution (MD and PSD recording by *Humphrey Field Analyzer II Zeiss Automatic Perimeter*), Corneal Thickness and fundus examination with the *Volk 78D* lens. The presence of DR, its progression and its form was noted, but also the aspect of the optic disc. It was also noted: duration of glaucoma and diabetes, the type of diabetes (1, 2 and mixed), number and type of antiglaucoma and antidiabetics medication, the number of examinations VF and OCT. Inconclusive OCT and VF examinations were excluded from the analysis. For all patients, glycemic and glycosylated hemoglobin (HbA1c) values were recorded to confirm or discontinue the diabetic status of the patients in both groups. At the same time, in the clinical examination, the anterior chamber angle was analyzed by gonioscopy for the correct fit of the glaucoma. OCT examinations were performed at the *OFTAPROF Clinic* with Cirrus HD-OCT Zeiss using the Optic Disc Cube 200 x 200 protocol. The following were recorded: mean retinal nerve layer thickness (RNFL), RNFL symmetry, Neuroretinal rim, optic disc area, mean C/D ratio, vertical C/D ratio, volume of the optic disc cup.

Another necessary condition for participation in the study was to have VF and OCT examinations at intervals of at least one year between the initial and final assessment. The study only analyzed the data from the most affected eye of the two.

All patients enrolled in the study agreed to participate, signing an informed consent in accordance with the Helsinki Declaration (June 1964).

The criteria for the diagnosis of NTG and POAG were age greater than 35 years, normal intraocular pressure (IOP) or more than 21 mmHg without treatment, open anterior chamber angle, damage to the optic nerve characteristic of glaucoma (ratio C/D> 0.5) , VF defects.

Evaluation of DR changes was performed with the *Volk* lens and retinal photography at the *Fundus Camera Zeiss*, monitoring all quadrants. Progression was defined by any newly developed clinical element that led to a more severe form.

In the case of the study group, only 21 of the 51 patients performed at least one second OCT exam. For the study group, when analyzing the OCT parameters, only the data from the 21 patients was included.

Statistical analysis. With a sampling error of 9.5%, the study represents a representative sample. The data was systematized and centralized into a *SPSS version 18.0* database and processed with the fol-

lowing statistical functions: *ANOVA* test, *t-Student* test, *F (ANOVA)* test, correlation coefficient *Pearson* (r), χ^2 test, *Kruskal-Wallis* correlation, linear regression, multiple regression, *ROC* curve.

RESULTS

Visual acuity: At the beginning of the study, visual acuity was significantly higher in patients from Group II (p = 0.015). At the end of the study, visual acuity significantly decreased in patients from Group I in those with severe glaucoma (p = 0.001).

The mean values of the IOP: did not show statistically significant differences depending on the evolution of glaucoma.

C/D. At the onset of the study, the C/D ratio was significantly higher in patients in group II (p = 0.017). At the end of the study, the C/D ratio was slightly lower in patients in group I (p = 0.680).

After analyzing the initial and final C/D report in both batches, we conclude that the tendency was equalization that occurred mainly through "recovery" (which translates into growth) of the C/D in the study group. In other words, in the diabetic group, the increase in C/D ratio is more pronounced**.**

RNFL. At the start of the study, RNFL was significantly lower in group II patients with moderate and severe forms of glaucoma (p = 0.043). At the end of the study, in Group I RNFL significantly decreased in patients with severe glaucoma (p = 0.001). This development indicates that the presence of diabetes had a negative impact on RNFL among patients with advanced forms of glaucoma (fig.1).

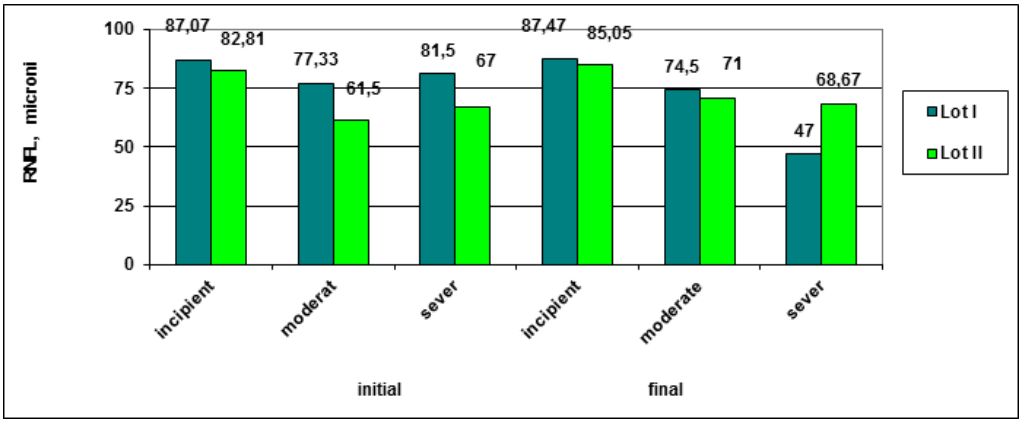

Fig. 1. Average values of RNFL by study group according to MD classification

RNFL Symmetry. At the start of the study, RNFL symmetry was significantly lower in Group I patients with moderate glaucoma and in Group II patients with severe glaucoma (p = 0.001). At the end of the study, there were no statistically significant differences, according to glaucoma development, of the mean values between the analyzed groups (p = 0.546).

Neuroretinal rim. At the start of the study, mean values of the neuroretinal rim were significantly lower in patients from Group II with moderate and severe glaucoma (p = 0.025).

The size of the disc was not statistically significant in the evolution of glaucoma.

Mean C/D. At the start of the study, the mean C/D was significantly higher in pa-

tients with moderate and severe glaucoma in Group II (p = 0.045). At the end of the study, the mean C/D was lower in patients from the first group with severe glaucoma (p = 0.042).

Cup-Disc Volume. At the start of the study, the cup-disc volume was significantly higher in patients with moderate and severe glaucoma from Group II (p= 0.031). At the end of the study, the cup volume was lower in patients from Group I with incipient glaucoma (p = 0.05).

HbA1c. At the start of the study, patients from Group I had a significantly higher level of IOP (19.90 *vs.* 17.30 mmHg; p = 0.029), a difference that was maintained until the end of the study in patients with HbA1c ≥ 7% (16.38 *vs.* 15.30 mmHg, p = 0.001). This would suggest that elevated levels of HbA1c would maintain elevated IOP values, although no correlation between the mean values of the two was found across the study group (tab.1).

TABLE I

Evolution of markers by HbA1c level at patients in the study group

Marker	Initial			Final			$p_{i\ vs\ f}$ HbA1c > 7%
	HbA1c ≥7%	HbA1c <7%	p	HbA1c ≥7%	HbA1c <7%	p	
Visual field							
VA	0.75±0.26	0.84±0.18	0.158	0.75±0.18	0.81±0.24	0.352	0.958
IOP	19.90±5.12	17.30±3.15	**0.029**	16.38±3.61	15.30±2.35	0.201	**0.001**
C/D	0.56±0.19	0.58±0.19	0.668	0.58±0.17	0.57±0.18	0.848	0.495
PSD	2.65±2.27	3.12±2.86	0.537	4.45±3.58	3.64±3.40	0.421	**0.002**
MD	-1.48±3.92	-5.48±3.81	**0.005**	-6.20±7.75	-3.88±6.70	0.258	**0.008**
OCT							
RNFL	83.71±15.40	86.73±13.13	0.455	85.60±14.73	84.45±15.19	0.863	0.199
Symmetry RNFL	69.43±29.22	80.36±8.51	**0.046**	82.50±7.77	77.36±9.65	0.198	**0.040**
Neuroretinian area	1.15±0.23	1.17±0.28	0.762	1.15±0.24	1.10±0.32	0.745	0.885
Disc area	2.04±0.39	2.08±0.41	0.727	1.95±0.22	2.26±0.54	0.105	0.054
C/D average	0.64±0.11	0.64±0.12	0.962	0.63±0.12	0.70±0.10	0.145	0.940
Vertical C/D	0.63±0.12	0.63±0.11	0.959	0.60±0.12	0.67±0.08	0.109	0.891
C/D volume	0.30±0.15	0.38±0.29	0.254	0.26±0.19	0.52±0.37	0.061	0.795

At the end of the study, there was a significant increase in the mean PSD (4.45 *vs.* 3.64; p = 0.002). In patients with an HbA1c level ≥7%, a significant decrease in MD was noted at the end of the study (-6.20 *vs.*-3.88, p = 0.008). As above, elevated levels of glycosylated hemoglobin would induce more severe impairment of visual function monitored by VF examination (tab. I).

RNFL symmetry was significantly lower at the beginning of the study in patients with HbA1c levels ≥ 7% (69.43 *vs.* 80.36; p

= 0.046), increasing significantly by the end of the study (82.50 *vs.* 77.36; p = 0.04).

Blood sugar. At the start of the study, patients in the study group (with diabetes and glaucoma) showed a significantly lower VA level (75% *vs.* 91%, p = 0.019), which is maintained until the end of the study (72% *vs.* 85 %, p = 0.03), and RNFL was significantly lower in the beginning (83.08 *vs.* 91.27; p = 0.05), with the difference decreasing by the end of the study (82.62 *vs.* 88.88, p = 0.177). In patients from the study group who had a high blood sugar levels and remained above the baseline until the end of the study, the following evolution of the biological markers was noted:

- mean IOP values significantly decrease from 18.28 ± 4.76 mmHg to 15.44 ± 3.12 mmHg (p = 0.001);
- PSD significantly increases from 2.91 ± 2.03 to 3.86 ± 3.14 (p = 0.042);
- mean MD significantly decreases from -3.83 ± 7.52 to -4.99 ± 7.87 (p = 0.05).

DISCUSSION

The two batches were comparable in terms of glaucoma stage and time since diagnosis.

Analyzing the number of VFs and OCTs performed by patients, there was noted a more in-depth examination for those diagnosed with NTG and a slightly larger number of examinations of patients with diabetes. These aspects are due to the increased need for the clinician to obtain diagnostic data in the absence abnormal IOP values for patients with NTG, but also due to patients increased ocular comorbidities in diabetic patients, which is also reflected in multiple studies in the literature (1-6).

The presence of diabetes did not correlate with the thickness of the cornea or with the IOP values, both of which lead to the conclusion that if diabetes influences in some way the evolution of glaucoma, this does not happen through a biomechanical mechanism.

There was an indirect correlation of low intensity between C/D values with glycemic status (HbA1c and glucose values). Over the course of the study high values of diabetic parameters (blood sugar or HbA1c) can be correlated with favorable prognostic factors of glaucoma such as low C/D ratio. Looking at the dynamics and comparing the tendency of this correlation between the final and the initial moment, it is noticed that over time, this "favorable" effect of diabetes on glaucoma is no longer as pronounced. Furthermore, it should be stated that higher blood sugar levels have been recorded at baseline, compared to the end of the study and the fact that natural evolution of glaucoma results in increasing the C/D ratio value. In fact, this parameter had a similar evolution in both groups.

Analyzing each type of glaucoma (POAG and NTG) in the evolution of the C/D ratio it is observed that NTG recorded the highest values. Besides the fact that the assessment of NTG is subjective, related to the experience and behavior of each clinician, there is a tendency (at least at the time of diagnosis) to underestimate this ratio under normal IOP values as defined by this pathology. Because of that the diagnosis is decided later, in more advanced stages, where we encounter higher C/D values. This is most likely to be found in the “favorable” improvement in the assessment in people with NTG in the study group (C/D: 0.6 initially versus 0.56 final). In these patients, the presence of comorbidity may influence the examiner's decision. All this information was not sta-

tistically significant. A longer follow-up could provide new clues in this evaluation.

The different correlation of the C/D and VA ratio in the two groups (positive for the glaucoma and diabetes group and negative for the glaucoma group only) is based on low AV rates in patients with diabetes. Most studies assess AV in diabetes in advanced forms with retinal changes. In the early stages (on patients where DR is not significant, similar to the present study), VA values have different behaviors across other studies. In a study from 2000 of two similar demographic groups (age and gender) of 385 patients, there was a low VA value among diabetes patients compared to the control group: 40% *vs.* 31% (7).

On the other hand, in a Swedish study published in 2007, there is no difference in VA between two groups: the study group made up of people in a well-defined population area diagnosed with type 2 diabetes compared to a similar control group, without diabetes. One person blinded because of DR, however, the main cause of decreased vision was age-related macular degeneration in both groups (8).

Discussion based on visual field data

Analyzing the data obtained from the VF examinations, there is a decrease in the MD values in both groups (study and control) for patients diagnosed with NTG and a trend of PSD increase (highlighted by an important statistical correlation: $p = 0.004$) for patients in the study group. This last aspect, even if the values have not reached the pathological area, raises questions about what would have happened if the study period would have been longer.

The absence of correlation between intraocular pressure values and MD and PSD values in both groups may have two causes: Progression prevention by effective antiglaucoma treatment and / or reduce follow up period for the study (2.38 ± 1.20 years study group, respectively 2.26 ± 1.0 years the control group). In addition, this synchronic behavior of the two VF parameters is also reinforced by the statistically strong indirect (normal) correlation obtained between these values throughout the study and within each group ($p = 0.001$).

Analyzing the evolution of the two VF functional parameters MD and PSD, we observe a lower number of VF examinations among the patients in the study group compared to those in the control group:

- Mean differences of the final MD - initially recorded a more significant decrease among people with diabetes compared to those without -1 dB and -0.54 dB ($p = 0.701$);
- Mean PSD differences - initially recorded a more significant increase among people with diabetes compared to those without: + 1dB *vs.* +0.22 dB ($p = 0.041$).

It would also be interesting to divide each group according to the MD classification and then to study the evolution of this parameter over time and to compare similar subgroups on each side. For example: Advanced subgroup (<-12 dB) in study group (diabetes and glaucoma) versus advanced subgroup of control group (glaucoma only). However, in order to have statistical relevance, each subgroup formed within the two groups should have been numerically important, which is, unfortunately, impossible in the present study.

Discussion based on OCT data

The results following the analysis of the OCT data (structural assessment) were conflicting. There was noted an increase in RNFL thickness in both groups, although

the natural tendency is thinning even in healthy subjects. However, comparing the two groups against each other, the increase in RNFL was less in patients with Diabetes.

RNFL Symmetry registered a decline among the study group. This finding, however, was not statistically significant.

The mean C/D value increased by the end of the study in both groups. However, this increase was more important in the study group and had statistically significance ($p = 0.044$). This would suggest the fact that the presence of diabetes has an unfavorable effect on the development of glaucoma disease. The same conclusion can be drawn if we look at the more pronounced increase of the vertical C/D in the group of patients with diabetes and glaucoma. The statistical correlation is, however, weaker.

The volume of the optic disc had increased significantly more among NTG patients in both groups.

On the other hand, diabetic parameters (HbA1c and glycemia) correlated negatively with almost all OCT values: RNFL symmetry, mean C/D, vertical C/D, cup volume. In conclusion, elevated levels of HbA1c and glucose were associated with "more favorable values" of OCT examinations.

From the obtained results, we can observe a significant indications suggesting the negative impact of diabetes on the development of glaucoma. This comes from the analysis of functional data obtained from clinical examinations and VF examination but also partly from the structural analysis, which was revealed by OCT examinations. These findings are in line with the meta-analysis published in 2004 by the team led by Bonovas S., which states that diabetes is a risk factor for POAG (4).

Even though it wasn't statistically significant, a generally positive correlation between IOP and diabetic parameters (HbA1c and glycemia) was observed. This is similar to the results published by Zhao D et al. in 2015. They found that for each increase in blood glucose of 10 mg/dl there was an increase in IOP by a mean of 0.09 mmHg6. However, the data from the study may have been influenced by the introduction of antiglaucoma medication, which was meant to control or even decrease the IOP.

In contrast, the data indicating a protective role that diabetes would have on glaucoma comes mainly from the analysis of intragroup information obtained in the study group (with diabetes and glaucoma), specifically that elevated levels of diabetic parameters were associated with more favorable aspects of glaucoma.

Analyzing the OCT data, an improvement of the RNFL thickness was generally observed in the two groups. This can be explained by small batches and the short period of the study, which could leave room for possible errors and limitations of this type of investigation (OCT). At the same time, as Primus S. and colleagues suggest, this OCT structural objective improvement may be due to the improvement of retinal vascular flow in the early stages of diabetes (9), but also due to the decrease in the mean values of IOP.

Existence of diabetes did not correlate with corneal thickness or IOP values, both of which lead to the conclusion that if diabetes influences in some way the evolution of glaucomatous disease this does not happen through a biomechanical mechanism. This is in contrast to the data published by Zhao D *et al.* which reveals that hyperglycemia could induce an osmotic gradient that would attract an excess of aqueous

humor in the anterior chamber, which could affect trabecular meshwork function and that diabetes may increase the stiffness and central thickness of the cornea (6).

Diabetes negative role. Among the results that suggested a negative influence of diabetes on glaucomatous disease progression, we mention:

- The mean reduction in MD was more significant in patients with diabetes compared to those in the control group: -1 dB *vs.* -0.54 dB (p = 0.701).

- PSD values increased more among patients with diabetes than those with glaucoma alone: +1dB *vs.* +0.22 dB (p= 0.041).

- RNFL symmetry also recorded deterioration among the group: diabetes and glaucoma (p = 0.758).

- increase in mean C/D was more important in the presence of diabetes (p = 0.044).

- The same behavior was also encountered in the evolution of vertical C/D values (p = 0.164). In the literature, Mwanza JC *et al.* emphasizes that for the diagnosis of glaucomatous disease, data are related to the neuroretinal rim area and the vertical cup/disc ratio (10).

The presence of diabetes had a negative impact on RNFL in patients with advanced forms of glaucoma (p = 0.001).

Diabetes protective role. Among the results suggesting a protective role of diabetes on the development of glaucoma were all correlations between blood glucose levels and HbA1c and structural measurements determined by OCT examination: RNFL symmetry, mean C/D, vertical C/D, cup volume. Almost without exception, elevated values of these parameters correlated with less affected structural measurements on the OCT examination.

The study also has limits. The follow-up period was short: 2.38 years of follow-up through VF examinations in the study group and 2.26 in the control group, respectively 1.86 years between final and initial OCT examination in the study group and 1.82 in the control group. Consequently, the number of VF and OCT examinations was rather low, especially the OCTs. The number of patients was low, especially those who underwent OCT examinations in the study group. The majority of patients came from the urban area, showing that at least in rural areas these two diseases remain undiagnosed and unexamined.

It would have been interesting to divide the groups either according, to the stage of glaucoma evolution (early, moderate and severe) in order to assess the impact of diabetes at each of these stages or to the degree of diabetic control (normal, controlled, moderately controlled or uncontrolled). It is expected that, in order to have a statistically significant data with subgroups of this kind, a larger number of patients is required. This is more likely with multicenter studies.

Also, considering the vascular physio pathological mechanisms that appear to be involved in the pathology of the two diseases (diabetes and POAG), it would have been interesting to analyze the ocular perfusion pressure in this study as well.

In order to achieve more accurate results, a longer study period is needed, which considers the chronic nature of the pathologies involved.

CONCLUSIONS

In our study, data from the VF, OCT, and Clinical Examination Parameters analysis indicate an unfavorable progression of glaucoma among diabetic patients compared to those without. This is based

on a more significant degradation of these parameters within the study group compared to the control group. In contrast, data suggesting a favorable impact of diabetes was obtained only inside the study group and showed negative correlation between diabetic parameters (glycemic and glycosylated hemoglobin) and the clinical and paraclinical parameters of glaucoma. However, this information was not supported by statistically significant value.

Patients in the study mostly came from urban areas. This highlights the low addressability and difficult access of people from rural areas to health services. Consequently, both glaucoma and diabetes remain underdiagnosed and underestimated if not among the entire population, at least among those in rural areas.

Longer follow-up studies, including a larger number of patients, with the possibility of dividing into subgroups based on the stage in the two glaucoma disease groups and subgroups with different degrees of therapeutic control of diabetes within the study. This would be much easier if the study were multicenter with a common data recording protocol. Additionally, the study would benefit from analyzing a new parameter represented by ocular perfusion pressure, in order to analyze the vascular mechanism that could be involved in the pathophysiology of the two diseases.

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THE USEFULNESS OF GRONINGEN FRAILTY INDICATOR IN THE UROLOGIC PATIENTS AGED OVER 60 YEARS OLD

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THE USEFULNESS OF GRONINGEN FRAILTY INDICATOR IN THE UROLOGIC PATIENTS AGED OVER 60 YEARS OLD (Abstract): The presence of frailty in an older patient influences his mental status, physical status, his response in stress conditions, including surgery and choosing the proper treatment. The surgical community has recently adopted frailty evaluation as an instrument to estimate the post-surgical risk. Our objective is to evaluate the usefulness of frailty evaluation in the urological patient in terms of post-surgical evolution and post-surgical complications. **Material and methods**: This is a prospective study which included 600 consecutive patients aged 60 and over admitted in Urological Clinic in one year. Demographic data and data regarding comorbidities, urological intervention, hospitalization days and complications were collected. Using Groningen Frailty Index (GFI) patients were divided into two groups: non-frail (GFI 0-4) and frail (GFI 5-15). **Results**: 343 patients with a mean age of 70.24 years were in non-frail group and 257 patients with a mean age of 73.28 years in frail group. Cardiovascular comorbidities predominated in both groups 82.02% (281 patients) in the "non-frail" group and in 84.27% (217 patients) in the "frail" group ($p = 0.4833$). Surgical intervention were performed on 80.39% (276 patients) in the "non-frail" group and on 75.40% (194 patients) in the "frail" group. The rate of post-surgical complications was with 13.7% (34 patients) higher in the frail group ($p = 0.00053$). Frailty is correlated with age ($r = 0.252$, $p<0.0001$), neurological comorbidities ($r = 0.171$, $p<0.0001$) and non-urological malignancies ($r = 0.096$, $p = 0.018$). **Conclusions**: GFI can be a useful tool in the post-surgical evaluation of the elder patients' evolution.
Keywords: FRAILTY, UROLOGICAL OLDER PATIENTS, COMPLICATIONS.

Once with the increase of the average life expectancy, the addressability for conditions that require urological surgery also increases, while an accurate estimation of the surgical risk becomes even more necessary. Elder patients have an increased risk of developing post-surgical complications and major urological interventions (radical nephrectomy, radical cystectomy, prostatectomy etc.) add even more risks. In case these complications occur, they can lead to a multitude of events resulting in a low-quality life, possible loss of independence, high costs of medical care and increased mortality (1). When dealing with an elder patient, taking

the right decision regarding the surgery, becomes difficult and even a real challenge, due to the complexity of elements which play a role in a healthy condition and the lack of instruments which may appreciate/predict the surgical risk (1).

Elements predicting the post-surgical complications commonly used have substantial limitations-most of them are based on a single organ system or are subjective and do not evaluate the physiological supply of the patients (e.g. Lee and Eagle criteria appreciate only the heart function, while the well-known ASA indicator is determined by a subjective estimation of the level of damage in the organ systems and probability of survival) (1, 2). Hence, some elder patients could be subjected to surgical procedures with an unacceptable high risk of post-surgical complications (2, 3). Both the patient and his relatives must be correctly informed about the surgical risk (4, 5) and the possible short-term and long-term complications which may arise, and for now, the urologist cannot offer answers to match the expectations. For the first time in literature, we have tried to evaluate the usefulness of frailty evaluation in the urological patient by using the Groningen frailty indicator in the view of the post-surgical evolution.

The geriatric community acknowledged and developed the concept of "frailty" for a more accurate objectification of the patients' condition, defined as an individual syndrome of physiological supply reduction, which restricts the ability of a patient to respond to stress factors and exposes the patients to adverse reactions/results (1, 6).

The surgical community has recently adopted frailty evaluation as an instrument to estimate the post-surgical risk (2).

The aim of this study is to establish the role that the frailty of the patient-evaluated with the help of the Groningen frailty indicator-can play in appreciating the risks and complications in the pre- and post-surgery periods. It is the first of several studies that explores frailty and its impact on the post-surgical evolution, including oral and maxillofacial surgery, and orthopedic surgery.

The assumed objectives of this study, were: to detect the patients with increased frailty, who could not be identified through other methods (clinical/non-clinical), with the help of the Groningen frailty indicator; establishing the implications of the frailty rate in choosing the urological therapeutic strategy (conservatory/surgical-endoscopic/opened); evaluating the influence of the frailty rate over the surgical risk and subsequent complications.

MATERIAL AND METHODS

In the ongoing study, we have evaluated patients aged over 60 years, hospitalized in the Urology clinic of the "Dr. C. I. Parhon" Hospital in the last 18 months, by filling in a *Groningen Frailty Indicator* (GFI) form at admission. Based on the obtained indicator, these were divided into 2 study groups: 0-4 GFI indicator (considered as "non-frail") and 5-15 GFI indicator (considered as "frail"). The 2 groups were evaluated comparatively in terms of cardiovascular, neurological, pulmonary, metabolic conditions, but also in what the number of hospitalization days and post-surgical complications are concerned.

600 patients aged over 60 years were included in the study. They were hospitalized in the Urology clinic of the "Dr. C.I. Parhon" Hospital between the 1st June 2017 and 1st May 2018. The GFI form was filled in for all the patients, after the informed consent was obtained.

The Groningen Indicator is a tool of screening the level of frailty in patients and includes 15 questions. The questionnaire is divided into 4 geriatric fields: physical function (mobility, daily activities, sight, hearing, weight loss); cognitive function; social function (solitude); psychological function (anxiety, depression).

The categories of answers are positively or negatively formulated variables (0 or 1 point). The indicator may vary from 0 to 15 points. High indicators reveal high levels of frailty: patients with a GFI≥4, were considered as frail. The patients were also asked about the following socio-demographical data: age, gender, civil status, origin environment (urban/rural). The studied age groups were young elders (60-74 years old), adult elders (75-84 years old) and old elders (≥ 85 years old).

Data concerning the following were collected: patient comorbidities (cardiac, neurological, respiratory, metabolic pathologies or other malignancies), urological pathology-malignant, lithiasis. Infectious (urosepsis, orchiepididymitis, prostatitis), others (periurethral adenoma, hydrocele, trauma); surgical intervention (invasive/ opened or minimally invasive/endoscopic); number of hospitalization days; with/ without post-surgical complications.

The statistical analysis was carried out by using *t-Student* and *Chi-Square* and the statistical significance was defined for $p \leq 0.05$.

RESULTS

Between the 1st June 2017 and 1st May 2018, 600 patients hospitalized in the Urology clinic Iasi for a surgical intervention, have filled in the Groningen questionnaire. The patients were aged between 60 and 95 years. Out of these, 33% were women (198 patients) and 67% were men (402 patients). After filling in the questionnaire, 343 patients with a mean age of 70.24 years fell into the Ist study group ("non-frail"), while 257 patients with a mean age of 73.28 years ($p = 0.00001$), fell into the study group of "frail" patients. The origin environment was not significant for the statistics ($p = 0.1034$): in the "non-frail" group, 50.14% (172 patients) were from urban environment and 49.86% (171 patients) from rural environment, while in the "frail" group, 44.35% (114 patients) were from urban environment and 55.65% (143 patients) from rural environment.

Among the associated comorbidities, cardiovascular disease had the highest incidence in both study groups, present in 82.02% (281 patients) of the "non-frail" patients and in 84.27% (217 patients) of the "frail" patients, without being relevant in the statistics between the two categories ($p = 0.4833$).

Neurological conditions (19.35%, $p = 0.000315$), metabolic conditions (33.87%, $p = 0.0196$) and post-surgical complications (13.70%, $p = 0.00053$) had a higher incidence in the "frail" study group, with an impact on the number of hospitalization days, representing an average of 5.97 days (tab. I).

Surgical intervention was carried out on 80.39% (276 patients) of the "non-frail" group and on 75.40% (194 patients) of the "frail" group. In the latter one, the rate of post-surgical complications was with 13.7% (34 patients) higher compared to the "non-frail" group of patients ($p = 0.00053$) (tab. II).

There are no substantial statistical differences regarding the gender of the study group ($p = 0.81$), yet a predominance of male patients can be noticed (tab. III).

TABLE I

Characteristics of the study group according to frailty status

		Non-frail	Frail	p
Number of patients included		343	257	
Age		60-88	60-95	0.000010
Mean age		70.24	73.28	
Gender		F- 95 (26.14%) M-248 (73.86%)	F- 103 (32.66%) M- 154 (67.34%)	0.092954
Environment		U- 172 (50.14%) R-171 (49.86%)	U- 114 (44.35%) R- 143 (55.65%)	0.103400
Comorbidities	Cardiovascular	281 (82.02%)	217 (84.27%)	0.483300
	Neurological	30 (8.82%)	50 (19.35%)	0.000315
	Pulmonary	44 (12.74%)	35 (13.70%)	0.7385
	Metabolic	85 (24.83%)	87 (33.87%)	0.0196
	Other malignancies	25 (7.18%)	32 (12.5%)	0.0345
Urological pathology				0.202
Neoplasia		127	98	
Lithiasis		80	49	
Infectious		41	41	
Others		95	69	
Operated		276 (80.39%)	194 (75.40%)	0.15750
Days of hospitalization		1-64 (5.85)	1-33 (5.97)	0.38400
Complications		16 (5.22%)	34(13.70%)	0.00053

F-female, M-male, U- urban area, R-rural area.

There is a significant statistical difference between the origin environment of the adult elder patients and young elders or old elder ones 116 (60.4%) *vs.* 178 (46.4%) and 12 (50%). Taking comorbidities into consideration, the cardiovascular ones are more frequently encountered in the age group of 75-84 years old (90.1%), compared to the age group of 60-74 years old (79.4%) and the group over 85 years old (83.3%), the difference proving significant in terms of statistics ($p = 0.0006$). The neurological pathology is encountered on a larger scale in the study group of old elders (37.5%) than in the group of young elders (12.2%) and adult elders (14.1%), the difference between these also proving statistically relevant ($p = 0.002$). There are no statistically significant differences in what the pulmonary or neoplasia pathologies are concerned, other than the urological one ($p = 0.299$).

When analyzing the urological pathology according to age groups, statistically significant differences ($p = 0.001$) can be noticed, with a prevalence of the neoplasia pathology, regardless of age, especially in the old elders group (48.5% *vs.* 41.7% and 33.3%). Urological infectious pathology prevails in the age group ≥ 85 years old (25% *vs.* 12.8% and 11.5%), while the lithiasis pathology prevails in young elders (24%) compared to adult elders (17.2%) and old elders (4.2%).

Surgical intervention was carried out with no difference in age in the studied group ($p = 0.219$), encountered in approximately 308 (80.2%) patients aged < 75 years old, in 142 (74%) patients aged be-

tween 75 and 84 years old and in 18 (75%) patients aged over 85 years old. The emergence of complications was similar between the studied groups (35 (9.1%) *vs.* 20 (10.4%) *vs.* 5 (20.8%), p = 0.183).

Elders ≥ 85 years old are frailer compared to the ones from other age groups, so that the GFI has an average of 7 (8-3) in old elders, *vs.* 3 (5-1) in young elders, *vs.* 4 (6-2) in adult elders p<0.0001 (tab. II).

TABLE II

Characteristics of the study group according to age

		Age < 75 years old No.=384	Age 75-84 years old No.=192	Age ≥ 85 years old No.=24	p
Gender	Male	264 (68.8%)	146 (76%)	20 (83.3%)	0.81
	Female	120 (31.3%)	46 (24%)	4 (16.7%)	
Rural		178 (46.4%)	116 (60.4%)	12 (50%)	0.006
Comorbidities	Cardiovascular	305 (79.4%)	173 (90.1%)	20 (83.3%)	0.006
	Neurological	47 (12.2%)	27 (14.1%)	9 (37.5%)	0.002
	Pulmonary	46 (12%)	29 (15.1%)	3 (12.5%)	0.575
	Malignancies	37 (9.6%)	18 (9.4%)	0	0.299
Urological pathology	Neoplasia	160 (41.7%)	64 (33.3%)	11 (48.5%)	0.001
	Lithiasis	92 (24%)	33 (17.2%)	1 (4.2%)	
	Infectious	49 (12.8%)	22 (11.5%)	6 (25%)	
	Others	83 (21.6%)	73 (38%)	6 (25%)	
Operated		308 (80.2%)	142 (74%)	18 (75%)	0.219
Days of hospitalization		5.90±4.90	5.81±3.92	5.75±3.43	0.967
Complications		35 (9.1%)	20 (10.4%)	5 (20.8%)	0.183
GFI		3 (5-1)	4 (6-2)	7 (8-3)	<0.0001

Table note: GFI-Groningen frailty index

TABLE III

Correlations of frailty with the studied parameters

		r	p
Age		0.252	<0.0001
Gender		-0.067	0.100
Rural		-0.862	0.045
Comorbidities	Cardiovascular	0.006	0.855
	Neurological	0.171	0.000025
	Pulmonary	0.012	0.763
	Malignancies	0.096	0.018
Urological pathology	Neoplasia	-0.002	0.969
	Lithiasis		
	Infectious		
	Others		
Operated		0.055	0.180
Days of hospitalization		0.036	0.379
Complications		-0.173	0.00002

The frailty in the older patients hospitalized for urological conditions, is correlated to age ($r = 0.252$, $p<0.0001$), presence of neurological disease ($r = 0.171$, $p<0.0001$) and non-urological malignancies ($r = 0.096$, $p = 0.018$). Frailty is also correlated to the rural origin environment ($r = 0.862$, $p = 0.045$), GFI proving to be higher in those. The presence of complications was associated to frailty in the elders from the studied group ($p<0.0001$). The days of hospitalization ($p = 0.379$), type of urological pathology ($p = 0.969$), presence of cardiovascular ($p = 855$), pulmonary ($p = 0.763$) conditions and days of hospitalization ($p = 0.379$) were not associated to frailty (tab. III).

DISCUSSION

There are numerous studies searching for the right tools to quantify the actual health condition regardless of age (7). Among these, the Groningen indicator seems to be a useful element in establishing the frailty. Meulendijks *et al.* believe that "frailty" with a reduced level of psychological supply is caused by the accumulation of multiple deficits of the organs, resulting in an extremely low endurance to stress (9). In a study carried out on 1549 patients, Irene Drubbel *et al.* certify the usefulness of the Groningen questionnaire, compared to the Frailty Index (FI).

Currently, approximately half of the surgical interventions from USA are carried on patients older than 65 years old. Based on recent population projections, it is estimated that the average workload of surgeons shall increase from 14% up to 47% between 2000 and 2020, because of the old patients. (1). There is no doubt concerning the fact that these patients require a specific approach and the presence of frailty must be taken into consideration in the treatment scheme (8). Due to similar trends in our population, we perform this study to identify particularities and significance of frailty. In our study group the frailty population as older than the non-frail group with no significant differences between men and women. The prevalence of frailty of 42,83% in our group was higher comparing with other data (21,8% in a study perform by Moro *et al.*) despite the fact that our group included patients over 60 years, compared with 70 years in the other study (29). This may be a characteristic of our population, or an indicator for "the biological age" of our study population considering the high prevalence of comorbidities, over 80 % had concomitant cardiovascular comorbidities, but also metabolic and neurological ones.

Once the patient was identified as frail, the physician can consider the risks and benefits of a potential surgical intervention. In the post-surgery period, it is possible to reduce the risk of complications in the frail ones, through a more careful monitoring, a better hydration, nutrition and mobilization. (1) In a study carried on 594 patients aged ≥65 years old, who needed an elective surgical intervention, Martin A. *et al* shows that frailty is an independent factor which predicts post-surgical complications and the number of hospitalization days. (1) These data comply with the results of our study, where frail elders had greater complications compared to the non-frail since the frailty indicator is associated with the emergence of complications. This can be the context for body homeostasis condition, affecting the adaptive ability and association of sarcopenia. All the things mentioned above are body modifications in the context of the emergence of a frail state

and negatively influence the response of the body to stress, causing an increased risk of morbi-mortality (14, 15). Post-surgery complications are frequent in frail-group, but also depends of patients age. In our study, the very old group, aged over 85 years, 20.8% had complications, compared with those below 75 years of age; and the older the patient, the higher the GFI. Similar findings were described in a previous study which included 95,108 urologic cases representing 21 urologic procedures. They found that frailty is strongly associated with both major and minor complications among patients undergoing urologic surgery. Moreover, frailty had a significant association with complications for most urologic procedures examined. (30) Louis M Revenig et. al carries out a similar study on 80 patients and finds the same negative impact on the post-surgical evolution in patients categorized as "frail" after filling in the questionnaire, compared to those considered as "non-frail" and even reported an increased incidence of post-surgical complications in case of open, extensive, elaborate surgical interventions, compared to the endoscopic, urological and minimally invasive ones (6). Further data emphasis the fact that the presence of frailty is a strong predictor of high-grade post-operative complications and mortality following radical cystectomy in bladder cancer patients. Thus, frailty has the potential to be a very clinical aspect for risk stratification and perioperative counseling of bladder cancer patients (31).

Frailty and the emergence of post-surgical complications bring about the increase of the hospitalization period and mortality (16, 17, 18). In our study group, the hospitalization period was similar in frail and non-frail patients and also with no differences regarding patients age (considering the three groups). The explanation results from the multi-disciplinary collaboration in the hospital, which facilitated the post-surgical identification of frail elder patients. Hence, corrective actions could be carried out and a customized tracking of these could be established, while post-surgery, patients were transferred in the Geriatric Clinic, if the case.

The geriatrist R. E. Hubbard and anesthesiologist D. A. Story defined frailty and its quantification as "an elephant in the operating room: easy to spot, yet often ignored". They define frailty as that increased vulnerability of the geriatric patient to intra and post-surgical stress, seen as the mirror of the normal process of aging, but which manifests differently; although there are validated algorithms and questionnaires, it is difficult to quantify the elements leading to an increased risk up to being unacceptable, when talking about an elder patient going into the operating room (12, 32).

Only one study, carried out in Romania by Marinela Olaroiu (13) validates the Groningen indicator as useful in evaluating elder patients' frailty and finds a much greater prevalence in Romanian patients compared to the Dutch ones. Thus, this study provides significant data regarding frailty in elders hospitalized for urological conditions. The great number of patients included in the study increase the importance of the results, since they can be extrapolated to other similar populations. Also, this study is one of the few studies which stress out the necessity of post-surgical evaluation of frailty in the urological patient, even in the surgical one, and of collaborating with the geriatric team in order to reduce the complications and hos-

pitalization period.

There are several limitations to our study including a limited follow-up period, leading to an underestimation of complications. The anesthesia type is not recorded, and the literature data described a relationship between depth of anesthesia and impaired postoperative also suggested a more pronounced impact on the elderly and especially in pre-frail or frail patients (33). Also, data about the cognitive status, sarcopenia and other geriatric syndromes is lacking (34, 35). These data emphases the need for further studies.

CONCLUSIONS

In order to make a correct decision regarding the surgical treatment, it is useful to establish whether the patient is frail or not, by taking into consideration the tight connection between frailty and the increased risk of developing post-surgical complications.

A large part of the urological patients is over 60 years old and anticipating the emergence of post-surgical complications within the context of their age, surgical intervention and comorbidities, is still a challenge. The experience accumulated from those 600 patients indicates the fact that the Groningen indicator can be a useful tool in the post-surgical evaluation of the elder patients' evolution.

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NEWS

EVALUATION OF THE EFFECTS OF BIOCOMPATIBLE MATERIALS ON CELL DEVELOPMENT ON PULP STEM CELLS IN DIRECT PULP CAPPING

A group of researchers have studied the effects of widely used biomaterials in direct capping, using the in vitro method on pulp stem cells. The osteogenetic, odontogenetic and angiogenetic effects of ProRoot MTA (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA), Dycal (DENTSPLY Caulk, USA), Biodentine (Septodont, USA) or Emdogain Gel 30 mg/ml (BIORA AB/Straumann, Switzerland) were studied on stem cell cultures. The analyzes of the genetic markers were performed during a time span of 7 to 14 days following the beginning of the experiment and the goal was to preserve the pulp vitality in the cases group compared to the control group. Statistical analysis was done by one-way ANOVA. The study revealed that Emdogain has a lower cytotoxicity than the other materials. In the case of osteogenesis and odontogenesis it was concluded that all 4 materials stimulate these processes, but Dycal exhibited lower results in angiogenesis, compared to the other materials. Therefore, Emdogain can be a successful material in direct capping due to its biological properties, particularly involved in pulp regeneration (Abdel-Rahman Y., Ramy E., Mohiuddin M. T., Faisal A. Al-Alla, Majed A., Mazen A. A, Shahid S. S. Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. *BMC Oral Health* 2019; 19: 133).

HYDATID CYST WITH MULTIPLE LOCALIZATION. CASE REPORT

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HYDATID CYST WITH MULTIPLE LOCALIZATION. CASE REPORT (Abstract): We present the case of a 21-year-old male, admitted in our Clinic with pain in the left upper quadrant, without any other complains. The biochemical tests were normal, except the Ig E for the hydatid cyst was 668 U/mL with the reference value of 0-87 U/mL. The ultrasound and the computer tomography revealed the presence of cystic tumors in the liver, right lung and in the spleen, with imagistic appearance specific for hydatid cyst. A multidisciplinary surgery team was formed from General Surgery and Thoracic Surgery to perform, in one surgical session, the resection of the multiple cysts, first time through thoracotomy followed by laparotomy. The histopathological examination confirmed the diagnosis of multiple hydatid cyst. **Keywords:** HYDATID HEPATIC CYST, *ECHINOCOCCUS GRANULOSUS*, PERICYSTECTOMY, OPEN SURGICAL APPROACH.

The human hydatidosis is a potentially serious, sometimes fatal, condition caused by cysts containing the larval stages of the *Echinococcus granulosus* (*E. granulosus)* tapeworm that infect dogs and other canines . The tapeworm eggs are excreted in feces of these infected animals and people become infected by ingesting them. The contamination can occur via hand-to-mouth transfer after handling dogs or objects contaminated with the eggs, or from consuming contaminated food or water (1). The uncomplicated hydatid cyst, in most of the cases, is asymptomatic. The diagnostic is established by random abdominal echography or during the surgery performed for another pathology. There are cases were the hydatid cyst was first diagnosed in surgical intervention performed for another pathology, or even during necropsy. The hydatid cyst can be asymptomatic for many years, the clinical manifestations appears in case of large dimensions, depending of the location of the cyst and his complications.

CASE REPORT

We present the case of a 21-year-old male, admitted in our clinic with pain in the left upper quadrant, without any other complains. The biochemical tests were normal, except the Ig E for the hydatid cyst was 668 U/mL with the reference value of 0-87 U/mL. The ultrasound and the computed tomography revealed the presence of

cystic low-density mass in the right lobe of the liver, without signs of calcification of the pericyst of 94/70/88 mm, 207cm^3 in the segment V-VI of the liver, 112/90/99 mm, 560 cm^3 in the spleen (fig. 1), and 60/40 mm in the right lung (fig. 2), with imagistic appearance specific for hydatid cyst.

The patient was on Albendazole 800 mg/day for 22 days with a pause from the treatment for 14 days before the surgical procedure.

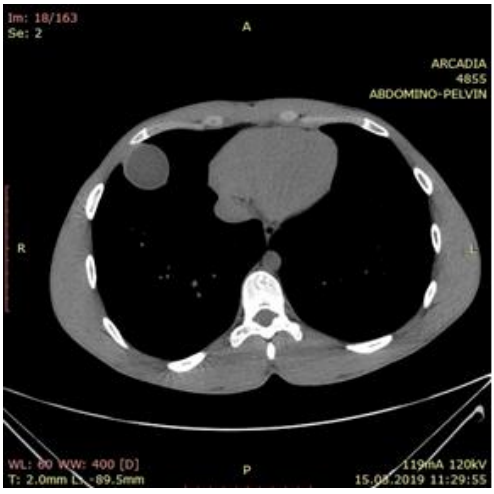

Fig. 1. Cystic tumor in the lung**.**

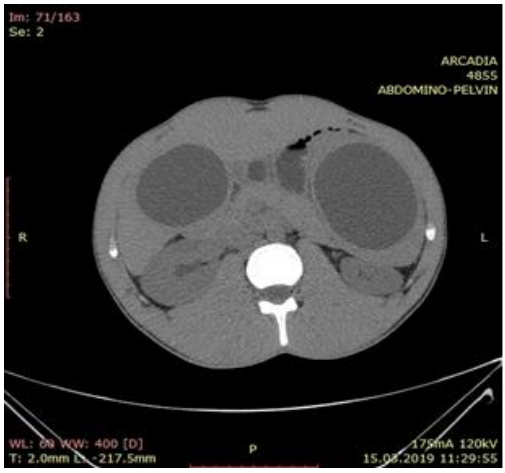

Fig. 2. Cystic tumor in the spleen and liver.

A multidisciplinary surgery team was formed from General Surgery and Thoracic Surgery to perform in one surgical session the resection of the multiple cysts, first time by thoracotomy (fig. 3) followed by laparotomy (fig. 4), with the total cystectomy (fig. 5).

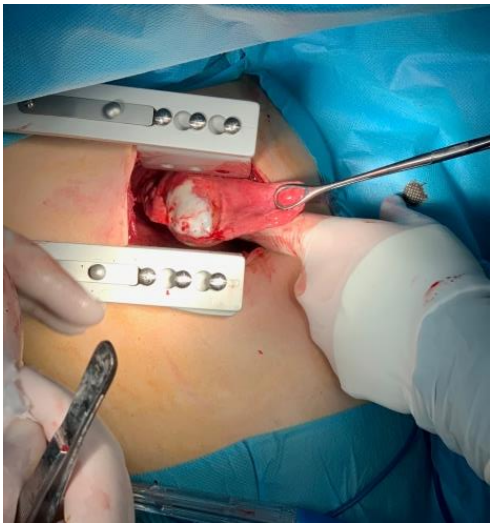

Fig. 3. Removal by thoracotomy

Fig. 4. Removal by laparotomy

Fig. 5. The total cystectomy

The anesthesiologist did the induction in the supine position. A selective bronchial intubation was applied, the endotracheal tube was advanced into the left main bronchus. After that the patient was positioned in the lateral decubitus with the operated side up. He is immobilized with sandbags or a beanbag that is used to support the back and abdomen. Strips of adhesive tape and/or a belt are passed over the hips and secured to the table. The lower arm is on an arm board at a right angle with the table, while the upper arm is rotated forward and upward, and fixed at the elbow. The legs are separated with a pillow. The lower leg is flexed at the knee, while the upper leg lies straight on top of the pillow. To increase the spread of the intercostal space and, thus, facilitate entry into the pleural space, the table can be angulated, or a roll can be inserted under the patient's chest. During positioning, it is important to support the arm and shoulder to prevent brachial plexus or shoulder injuries. These injuries are not uncommon, and they can be the source of pain or immobility of the shoulder postoperatively.

The thoracic team did a postero-lateral thoracotomy, with the reveling of the hydatid cyst in the inferior right lobe and performed a total cystectomy (fig. 3). The pulmonary tissue was sealed with a linear white stapler. A pleural drainage was put in the pleural cavity and the thoracic wall was closed in anatomical plans.

After the thoracic procedure we turn the patient in a supine position and a median laparotomy was performed. We performed a splenectomy and total hepatic cystectomy without incidents. A peritoneal irrigation with hypertonic saline was not necessary because of the removal of the intact cysts (fig. 5). No biliary fistula were revealed. We did the drainage of the splenic lodge, hepatic resection site and Douglas space.

After the surgery the patient evolution was favorable, and the drainage tube were removed consecutively after thoracic x-ray and ultrasound was performed.

DISCUSSION

The liver is the most common location of the hydatid cyst, followed by the lung, with the approximate occurrence rates of 70% and 12%, respectively. In the general population the occurrence of synchronous multiple localization is about 4-25%, in a smaller rate in the spleen. The classic surgical attitude in multiple localization is to perform the thoracic cystectomy and after a time the abdominal intervention to remove the cysts.

The hydatid disease has many complications, such as rupture, suppuration or calcification, but the most common problem is the surgical one represented by the multiplicity of cysts. The approach is difficult in the up mentioned situation requiring sound

operative experience, preferably with a one-stage procedure following appropriate preoperative preparations (2, 3, 4). We performed in one surgical session the resection of the multiple cysts, first time by thoracotomy followed by laparotomy, because the patient was young, in perfect health.

As of now, the surgical management is still the treatment of choice for most cases of hydatid hepatic cysts. It remains the single method that provides complete eradication of the parasite, treatment or prevention of complications and evasion of relapse.

In the table below are presented the indications for surgical management (tab. I) (5).

TABLE I

Indications for surgical treatment of hydatid echinococcal hepatic cysts.

Indications	Contraindications
• Superficial cysts with a high rupture risk • Large cysts, containing multiple daughter cysts • Hepatobiliary communication • Compression of neighboring organs (symptomatic) • Pus containing, infected echinococcal cyst • Nonhepatic echinococcal cysts (lungs, kidneys) • Cysts exerting pressure on adjacent vital organs	• Multiple cysts • Inactive, difficult to access cysts • Dead cysts • Inactive cysts that are partially or totally calcified • Very small cysts • unsuitable patient's condition

To decide the treatment in case of multiple hydatid cyst it is very important to know the physical condition of the patient and the characteristics of the cysts.

When the surgical treatment is not indicated the options are drug therapy along with systematic monitoring of the patient with or without the nonsurgical techniques (*wait & watch approach*).

The desired results of the surgical treatment are the entire removal of the cyst, the prevention of intraoperative contamination of the intraperitoneal cavity, the treatment of the remaining hepatic parenchyma and the identification and treatment of possible hepatobiliary communication.

Generally, the surgical treatment is reserved for complicated cysts, such as the ones that develop a biliary fistula, the perforated cysts or the ones that have daughter cysts inside. If the cysts are superficial ones, smaller than 10 cm, and the patients are not suitable for the percutaneous treatment then the surgical treatment is the election one.

There are three treatments options: a conservative open approach, a radical open approach and a laparoscopic approach (6). Before surgery, no matter the chosen approach for each and every patient, the medical treatment with benzimidazole must take place in order to obtain sterilization of the cyst content and to prevent dissemination and anaphylactic shock.

In all cases, the precautions taken to eradicate even the protoscolioces of the parasite within the content of the cyst, has to be drastic. Isolating the operating field has to be done scrupulously and also using solutions capable to eliminate all cells of the parasite (7).

CONCLUSIONS

The hydatid disease is still a major

problem in the public health system and the main goal, no matter the treatment chosen, has to be parasite elimination along with no recurrence and with minimal morbidity and mortality. Even though the surgical procedures are the best therapeutic option the right technique has to be chosen considering the uniqueness of each patient.

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NEWS

MERIDIAN DIAGNOSTIC ACCURACY STUDY: CLINICAL AND FINANCIAL BENEFITS OF iuMRI

Fetal brains anomalies are found in 1% of pregnancy and 10% of perinatal autopsies, posing a huge burden to the healthcare system. The main technique of screening and diagnosis at the moment is represented by ultrasonography, but this method is associated with an important number of cases of incorrect diagnoses and inaccurate counselling. A group of researchers from the UK performed a prospective multicenter cohort study together with a health economics analysis in order to assess the diagnostic impact, acceptability and cost consequence of intrauterine Magnetic Resonance Imaging (iuMRI) among fetuses with a suspected fetal brain abnormality. After evaluating 570 pregnancies, they reported that iuMRI significantly improves the diagnostic accuracy and confidence for the detection of fetal brain abnormalities, with a small increase in early examination costs but a decrease in cost per management decision. In conclusion, iuMRI can be an excellent diagnostic tool for fetal brain anomalies with a good cost efficiency for the healthcare system. (Griffiths PD, Bradburn M, Campbell MJ, Cooper CL, Embleton N, Graham R, *et al*. MRI in the diagnosis of fetal developmental brain abnormalities: the MERIDIAN diagnostic accuracy study. *Health Technol Assess.* 2019; 23(49): 1-144).

RETROSPECTIVE STUDY OF DECOMPRESSIVE CRANIECTOMY IN THE TREATMENT OF MALIGNANT BRAIN EDEMA

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RETROSPECTIVE STUDY OF DECOMPRESSIVE CRANIECTOMY IN THE TREATMENT OF MALIGNANT BRAIN EDEMA (Abstract): The aim of this paper is to study the evolution of patients with MCA stroke who developed MBE during hospitalization and underwent decompressive craniectomy after a failed conservative treatment. **Material and methods:** This is a retrospective a single-center study of 20 patients with malignant brain edema following an ischemic stroke who were surgically managed with decompressive craniectomy at Sibiu County Emergency Hospital between January 1st, 2015 and April 30th, 2019. **Results:** The sample was comprised of 7 women (35%) and 13 men (65%). 7 patients (35%) were under 60 years old and 13 (65%) were over 60 years old. We observed the three highest-ranking risk factors are: high blood pressure (20 patients - 100%), males and elderly both represented an equal risk category (13 patients-65%) and smoking (12 patients-60%). No patient had any surgery-related complications: 4 patients (20%) had bronchopneumonia, 1 patient (5%) had pneumonia, 1 patient (5%) had a urinary tract infection and 11 patients (55%) had irreversible cardiorespiratory arrest. At discharge, 9 patients (45%) had an ameliorated state and 11 patients (55%)died. **Conclusions:** Decompressive craniectomy is not a panacea and it is well known that patients will not return to the state before the pathology. It is unanimously accepted that patients who undergo surgery have a higher chance of life even if, in some patients, the quality of life is low. **Keywords:** ISCHEMIC STROKE, MALIGNANT BRAIN EDEMA, CRANIECTOMY, NEURO-SURGERY.

Malignant brain edema (MBE) occurs only in certain patients who have suffered ischemic stroke of the middle cerebral artery (MCA) and is characterized by neurological damage due to progressive edema, increased intracranial pressure and cerebral hernias. Only a certain subgroup of patients develops MBE. Although there are certain factors that can predict the development of edema, it is currently impossible to identify patients who will develop MBE. This leads to a worsening of the patient's health and poses a risk of

death. (1)

According to the World Health Organization (WHO), stroke affects 6.2 million lives annually and high blood pressure (HBP) is the main risk factor. In the U.S., stroke is the fifth leading cause of death, with approximately 795,000 deaths per year. (2)

By increasing life expectancy, it is estimated that in 2025, 1.5 million Europeans will suffer one stroke per year. Recently, it has been shown that the incidence of ischemic stroke is increasing among the young population in Europe (3). Several explanations for this trend have been proposed, including increasing the prevalence of diabetes, hypercholesterolemia, obesity, smoking, alcohol abuse, and illegal drug use among the young population (4).

Although there are many scales for the emergency patient (Canadian Neurological Scale, European Stroke Scale, NIHSS, Scandinavian Stroke Scale, etc.) there is no patient evaluation scale that could indicate the evolution to MBE of the MCA, the most common cause of death in ischemic stroke patients.

MATERIAL AND METHODS

Patient population

We performed a retrospective single-center study of 20 patients diagnosed with MBE who were surgically managed with craniectomies and subsequent cranioplasties at Sibiu County Emergency Hospital between January 1st, 2015 and April 30th, 2019.

Of all 3,591 patients admitted with ischemic stroke, 2,989 were in the territory of the Middle Cerebral Artery (MCA) with either a thrombotic or embolic origin. During their hospitalization at the Neurology Department, 20 patients with MCA stroke complicated with MBE confirmed by imaging explorations (CT-scan, Angio-CT and Angio-MRI). Those patients could not be treated conservatively and were transferred to the Neurosurgical Department for decompressive craniectomies.

Our exclusion criteria are:

- ischemic stroke in other territories vascular territories,
- Glasgow Coma Scale (GCS) lower than 3 points,
- refusal of surgical treatment by the patient's family

Data collection

A retrospective chart review was conducted. Demographic data (sex, age, origin community), risk factors (high blood pressure (HBP), dyslipidemia, diabetes mellitus, arterial fibrillation, toxic substance consumption), clinical data and diagnosis, paraclinical diagnosis (bio-humoral surveys, CT-scans), evolution and complications were collected.

RESULTS

The sample was comprised of 7 women (35%) and 13 men (65%). 7 patients (35%) were under 60 years old and 13 (65%) were over 60 years old. 12 patients (60%) were from an urban household and 8 patients (40%) from a rural household.

Analyzing the distribution of age in relation to origin community (tab. I) we observe that elderly patients (> 60 years old) have an urban origin. Patients under 60 years old have an equal distribution regarding origin community.

TABLE I.

Origin community in relationship to age

Urban		**Rural**	
<60 years	>60 years	<60 years	> 60 years
5 (25%)	7 (35%)	4 (20%)	4 (20%)

We divided risk factors in two categories: changeable and unchangeable (tab. II, III).

We observed the three highest-ranking risk factors are: HBP (20 patients - 100%), males and elderly both represented an equal risk category (13 patients - 65%) and smoking (12 patients - 60%).

TABLE II.
Changeable risk factors

	Frequency	Percentage
High Blood Pressure	20	100%
Atrial Fibrillation	7	35%
Other cardiac diseases	18	90%
Dyslipidemia	11	55%
Smoking	12	60%
Obesity	7	35%
Diabetes mellitus	11	55%
Alcoholism	5	25%

TABLE III.
Unchangeable risk factors

	Frequency	Percentage
Age		
< 60 years old	7	35%
> 60 years old	13	65%
Gender		
Male	13	65%
Female	7	35%
Previous vascular episodes		
Ischemic Stroke	5	25%
Transitory Ischemic Stroke	2	10%
Peripheral Vascular Disease	12	60%

The topographic localization of the ischemic stroke was established following a CT-scan. The distribution is equal: 10 patients with ischemic stroke on left MCA and the other half with stroke on the right MCA.

State of consciousness at admission in the Neurosurgical Department was assessed using the GCS. 10 patients (50%) were unconscious (GCS < 8), 8 patients (40%) were obtunded (GCS 9-12) and 2 patients (10%) were conscious (GCS > 12).

The average patient hospitalized in the Neurological Department worsened in a period of 12 to 48 hrs. In the first 12 hrs., 6 patients (30%) had deteriorated their state of consciousness, by developing MBE (confirmed by CT-scan) and was transferred to the Neurosurgical Department for surgical management. At 24 hrs., 5 patients

(25%) were transferred for surgery and predominately at 48h the rest of the patients (9 cases - 45%) were transferred.

No patient had any surgery-related complications. All complications are related to the patient's associated diseases, length of hospitalization and rapid cerebral edema uncontrollable even after decompression. Thus, 4 patients (20%) had bronchopneumonia, 1 patient (5%) had pneumonia, 1 patient (5%) had a urinary tract infection and 11 patients (55%) had irreversible cardiorespiratory arrest.

At discharge, 9 patients (45%) had an ameliorated state and 11 patients (55%) died. Death occurred preponderant in elderly patients (63.63%), respectively male (36.36%) (fig. 1).

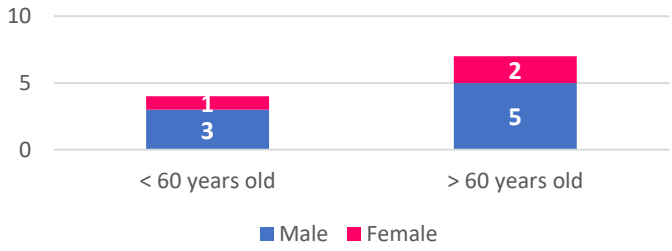

Fig. 1. Representation of deceased patients based on sex and age

DISCUSSION

According to literature, women have a higher chance of having a stroke in their lifetime but also a higher rate of post-stroke death, a higher rate of disability, dementia and depression compared to men. This is attributed to their longer life expectancy, which suggests that age is the strongest non-modifiable risk factor and is a negative predictor of outcome (5, 6).

Of the total number of patients who were transferred to the Neurosurgery Department developing MBE, mainly 60% come from urban areas, which may call into question the greater extent of risk factors in them (disorganized diet, unhealthy lifestyle , daily stress).

Ischemic stroke is a heterogeneous, multifactorial pathology influenced by modifiable and non-modifiable risk factors. In addition to the etiology of ischemic stroke, the risk factors for the underlying disease must be identified and treated at the same time. In our study all patients had at least one type of changeable risk factor. This suggests that lifestyle changes can have an impact in preventing MBEs.

Chronic consumption of toxins determines the acceleration of atheromatosis of the cerebral vessels. Tobacco is a risk factor by acting on the vascular caliber causing vasoconstriction. It affects the heart rate, causing tachycardia. Smoking also affects the lipid metabolism and increases the number of circulating catecholamines. The risk is present in both active smokers and former smokers for about 3 years after abstinence (7).

Some studies have shown the protective effect of moderate alcohol consumption, but chronic alcohol consumption is associated with the risk of hemorrhagic stroke. Chronic toxic substances consumption has the effect of inducing endotheliosis and hyalinization of the capillary wall (8).

Cardiovascular insufficiency, ischemic

heart disease, heart rhythm and conduction disorders, dysmetabolic diseases, ischemic brain events in the past are target points for the patient with stroke and MBE.

HBP is involved in the etiology of stroke by changing the quality of vascular walls. In our study, all patients were known to have HBP at the time of admission. Also, 7 patients had a personal history of atrial fibrillation, a pathology known for the major risk in stroke that increases with age. Cohort studies have shown that arterial fibrillation is a major contributing factor to stroke and a strong predictor of mortality after the first episode of stroke. Another fact observed is that this rhythm disorder is more common in older women (9, 10).

Decompressive craniectomy in MBE is a controversial topic because this life-saving maneuver is burdened by multiple complications and an increased mortality rate. However, studies have shown a lower mortality rate of patients undergoing this surgical maneuver compared to those treated only conservatively. Another important aspect is the reintegration of the patient with disability into society depending on its degree. There are studies that do not recommend decompressive craniectomy in patients older than 60 years due to adverse outcomes, but we should also consider the studies that have shown a survival rate between 6 months and one year in patients over 60 years (11, 12, 13).

CONCLUSIONS

Massive and non-responsive cerebral edema was the main post-operative complication (during hospitalization in the intensive care unit), which eventually led to irreversible cardiorespiratory arrest and death (11 cases).

The precocity with which the stroke patient is transferred to the Neurosurgery Department after a positive diagnosis of MBE is the key to saving them. Emergency imaging examination remains computed tomography, shortening the patient's path to life-saving intervention - decompressive craniectomy.

The aim of a fast, "ultra-early" operative intervention is to reduce the mortality rate, not the disabilities that are permanent consequences of stroke.

Decompressive craniectomy remains the only chance of survival for patients who develop MBE after an episode of stroke, most commonly of MCA.

ABBREVIATIONS

CT-scan – Computed Tomography Scan
GCS – Glasgow Coma Scale
HBP – High Blood Pressure
MCA – Middle Cerebral Artery
MBE – Malignant Brain Edema
NIHSS – National Institutes of Health Stroke Scale
WHO – World Health Organization

DISCLAIMER

The authors declare that they do not have any conflict of interests.

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NEWS

LIMITED INTERNAL COMBINED WITH EXTERNAL FIXATION OR SIMPLE EXTERNAL FIXATION IN TREATMENT OF OPEN TIBIAL DIAPHYSEAL FRACTURES ?

The conclusion of a recent article published in BMC Musculoskeletal Disorders is that the limited internal fixation combined with external fixation had good results compared with simple external fixation for managing open fractures. The researchers evaluated that additional internal implants did not significantly increase the risk of infection. In contrast, the combined fixation technique was beneficial for initial reduction and subsequent fixation stability, thereby permitting early rehabilitation and rapid healing with a low incidence of postoperative complications. More prospective comparative studies with larger samples and longer follow- up periods are required to accurately evaluate the clinical outcomes of this combined technique (Hao ZC, Xia Y, Xia DM, Zhang YT, Xu SG. Treatment of open tibial diaphyseal fractures by external fixation combined with limited internal fixation versus simple external fixation: a retrospective cohort study. *BMC Musculoskelet Disord* 2019; 20(1): 311. Published 2019 Jul 3. doi:10.1186/s12891-019-2679-9).

RETARDUL STATURO-PONDERAL ŞI MALNUTRIŢIA PROTEIN-CALORICĂ LA COPILUL DIALIZAT

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STATURE-WEIGHT RETARDATION AND PROTEIN-ENERGY MALNUTRITION IN CHILDREN WITH CHRONIC DIALYSIS (Abstract): Growth retardation is an important problem in children with chronic renal disease, and malnutrition is a determinative factor. **Aim**: The study intends to assess the relationship between protein-energy malnutrition and stature-weight retardation in children enrolled in chronic dialysis program. **Material and method**: The study group was composed of 16 children (5 boys and 11 girls - sex ratio of 2.2) hospitalized in the IVth Nephrology Clinic at Clinical Emergency Hospital "St. Maria" Iaşi, 13 rural and 3 urban, aged between 9 and 17 years, with chronic dialysis program. This was a follow-up study during a period of 4 years (2006-2009), resulting in correlations between anthropometric paremeters, biochemical, BIA and DEXA data. **Results**: The stature-weight deficiency of the 16 patients was as follows: after an average period of 61.7 months of HD and 32.7 months of PD, in children older than 12 years (mean age 15.27 years), 7 of 10 had stature-weight deficits higher than (-3DS) or (-4DS). The group with less than (-2DS) stature-weight deficits showed the same mean age of 15.2 years, the protein energy-malnutrition was present in 2 cases (33%) and was attributed to a dialysis period shorter than 13.8 months. **Conclusions**: The late diagnosis of the disease (at an average age of 13 years), the long period of chronic dialysis program (over 39.5 months on average) and the early debut of malnutrition are favoring or worsening factors of stature-weight retardation. After correlating ESG with biochemical, BIA and DEXA data, in our group were identified 4 cases of moderate malnutrition and 9 cases of severe malnutrition. **Key words**: MALNUTRITION, DIALYSIS, CRF, DEXA

GIANT MALIGN OCULAR MELANOMA TREATED BY SELECTIVE ARTERIAL EMBOLIZATION AND EXENTERATION. CASE REPORT

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GIANT MALIGN OCULAR MELANOMA TREATED BY SELECTIVE ARTERIAL EMBOLIZATION AND EXENTERATION. CASE REPORT (Abstract): Although rare**,** uveal melanoma is the most frequent primary malign tumor of the eye in adult population. If discovered in early phase, several conservative therapies are available, which can help the patient to preserve the eye and even the visual function: brachytherapy, proton beam radiotherapy, stereotactic radiotherapy, laser ablation or endo photocoagulation and limited resection of the tumor. In up to 40% of cases, enucleation is required due to tumoral increased size, while exenteration is required only exceptionally, in loco-regional advanced tumors. The paper presents the case of a patient diagnosed with ocular melanoma 12 years ago but refused surgery. She returns with a giant tumoral mass, complete disorganization of the eyeball and extensive extraocular lateral invasion, but no metastatic disease. Selective arterial embolization under angiographic control followed by exenteration were performed, with no local or at distance recurrence in a 2-year follow-up period. The disease course was favorable, despite many risk factors for poor prognosis. The patient is still checked regularly for metastatic disease, which remain a high risk for the following 10-15 years. **Conclusions**: Angiographic embolization may reduce the intraoperative risk of hematogenous dissemination and improve survival in regionally advanced ocular melanomas. The case was challenging due to increased size of the tumor, yet the predominantly lateral extension, spearing the orbital bones and intraconal space were positive factors for favorable outcome. **Keywords:** CILIARY BODY MELANOMA, SELECTIVE ARTERIAL EMBOLIZATION, EXENTERATION.

Although rare**,** uveal melanoma is the most frequent primary malign tumor of the eye in adult population. It is more common in individuals with fair skin and light-colored eyes and has an incidence of approximately 6 per million per year in white race. Other general factors are considered exposure to UV rays and inherited conditions like ocular melanocytotic, dysplastic nevus syndrome(1, 2). Most of them (90%) origin from the choroid, the remaining may arise from melanocytes of the iris and ciliary body (3). The main modalities of tumor dissemination are: transscleral extension, extension via optic nerve of by hematogenous dissemination, most frequently to liver. It is estimated that 40% of patients with ocular melanoma will develop hepatic metastasis in 10 years, with a poor prognostic of survival (4).

If discovered in early phase, several conservative therapies are available, which can help the patient to preserve the eye and even the visual function: brachytherapy,

proton beam radiotherapy, stereotactic radiotherapy, laser ablation or endo photocoagulation and limited resection of the tumor(3, 5-7). In up to 40% of cases, enucleation is required. Determining factors for this operation include large tumor size, extensive involvement of the ciliary body or iris, and proximity to the optic disc. Exenteration is required only exceptionally, in loco-regional advanced tumors (7).

CASE REPORT

The paper presents the case of a female patient, from an urban area, aged 47, who presents to the ophthalmologist for a progressive decrease in vision in her right eye (RE). At the ophthalmological examination, a visual acuity of light perception is found in the right eye and a normal vision of 6/6 in the left eye. Applanation tonometry revealed normal intraocular pressure of 14 mmHg in both eyes.

Fundus and ultrasound exam highlighted large tumoral mass, which fills 2/3 of the posterior pole, originating in the supero-temporal ciliary body, with surface hemorrhages, cloudy vitreous and partial retinal detachment, in the lower sector. The probable diagnosis was malignant melanoma of the ciliary body in the right eye.

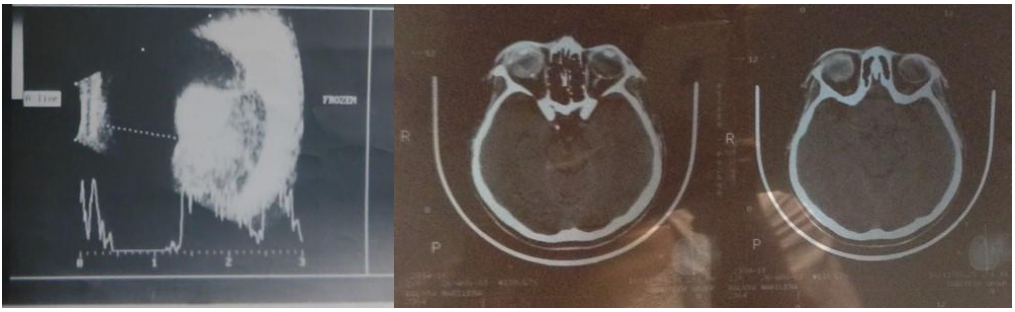

Fig. 1. a) Right eye: Ultrasound exam reveals the hyperechogenic tumoral mass in the posterior segment of the eye; b) CT aspect: increased hyperdense mass in the right eye, adjacent to the lens

The native and contrast-enhanced CT examination reveals a hyperdense formation with an irregular contour, with calcification ranges at the level of the ocular posterior pole, which makes a common body with the lens, but which does not infiltrate the adjacent sclera (fig. 1). Ultrasound and radiological screening of liver and lung metastases is negative.

The patient is recommended enucleation, for the cure with oncological visa of the eye tumor, but she refuses on her own responsibility, declaring that she wants to follow only natural treatments and has not returned to regular control.

12 years later, the patient returns to the clinic for gigantic oculo-orbital tumor, with areas of necrosis, spontaneous bleeding, the eyeball being completely disorganized and superior eyelid of purplish color and dilated tortuous veins (fig. 2).

The CT examination reveals this time a voluminous expansive process located at the level of the right orbit, developed mainly in the extra orbital area, with heterogeneous necrotic tissue structure and areas of hematic density, measuring overall 43/47 mm in axial plane and 52 mm cranio-caudal, with polycyclic contours without demarcation limit to the right

lacrimal gland and encompassing the anterior slope of the eyeball without extension to the intraconal space; disorganized eyeball with multiple microcalcifications, without bone, endosinusal or endocranial involvement (fig. 3).

Fig. 2. Giant tumor with complete disorganization of the right eye: areas of necrosis and spontaneous bleeding

Liver ultrasound and pulmonary radiological evaluation do not show the presence of metastases, despite its long evolution and very large size.

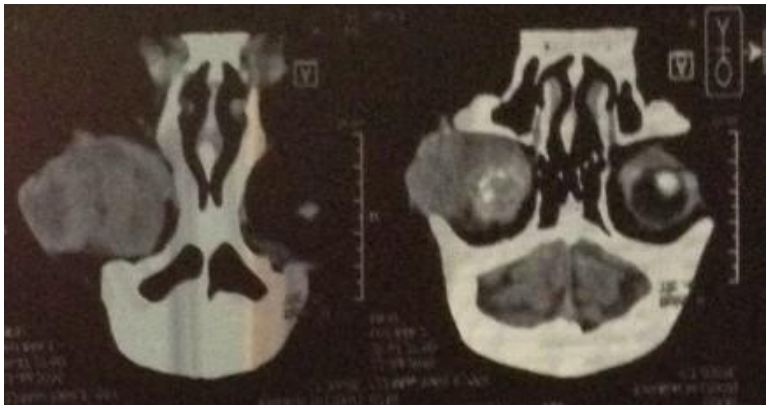

Fig. 3. CT aspect after 12 years, with important increased in size of the tumor, and antero-lateral extension to the adjacent soft tissue

The patient decides that she agrees with the hospitalization for surgical treatment. Cerebral angiography is performed by transfemoral approach that highlights pathologically marked loading on the projection area of the right eye and periorbital globe from multiple terminal branches of the right ophthalmic artery and right maxillary artery. Selective embolization of the terminal branches of the right maxillary artery with 700-micron particles is practiced, with optimal postembolization result and lack of load at the level of the tumor (fig. 4).

Fig. 4. 24 hours post-operatory aspect, with mild eyelid edema and serous-sanguinolent secretion

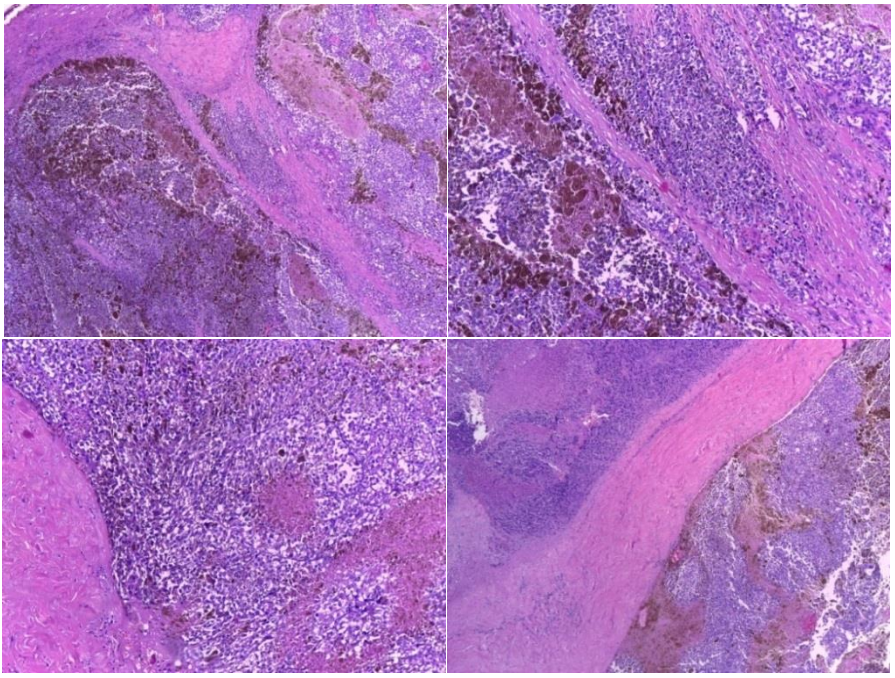

Fig. 5. Giant orbital melanoma: histopathological aspects: a) HE staining (10X10) areas of melanic cells, oval, with hyperchromic nuclei, pleomorphic, with atypical mitoses, conjunctival invasion area (dissected collagen bundles); brown-brown melanin pigment; b) HE staining (10X20) atypical melanoma cell ranges - conjunctival invasion area, foci of tumor necrosis and melanic pigment areas intracellular and extracellular; c) HE staining (10X20) diffuse area of melanic cells with atypical, pleomorphic, vesicular nuclei, pale or clear cytoplasm, melanic pigment and 2 areas of necrosis; peripheral fragment of sclera (low cellular density, eosinophilic area); d) HE staining (10X10) sclera fragment-eosinophilic thick band and areas of malignant melanoma with foci of necrosis on either side of the sclera.

Subsequently, the exenteration of the right orbit is practiced, under general anesthesia, in the mixed ophthalmic-neurosurgical team, with a favorable postoperatory evolution. The excised piece was sent for anatomopathological examination, which highlights giant orbital malignant melanoma, with marked peri scleral extension, epithelioid type, with areas of necrosis and bone metaplasia (fig. 5).

Postoperatively, the patient was examined at one week, one month, 3 months and after that bi-annually, being in a good condition, with the absence of local tumor recurrence or hepatic metastatic dissemination in 2 years follow-up.

DISCUSSION

The particularity of the case was the decision of the patient not to follow the ophthalmologist recommendations for surgical therapy in the early phase of the disease. This allowed us to follow the natural evolution of the malignant tumor over 12 years. Another aspect is the favorable evolution, extremely rare in such advanced locoregional cases.

Prognostic factors for survival can be classified into anatomical, histopathological and genetic. Anatomic predictors include largest basal tumor diameter, tumor thickness, ciliary body involvement, raised intraocular pression, and extraocular spread. Histologic predictors include presence of epithelioid melanoma cells, closed loops as vascular mimicry patterns, presence of macrophages and lymphocytes, increased HLA expression, high microvascular density, high mitotic count, and loss of nuclear immunostaining for BAP1 protein (2, 3, 8). Genetic predictors for metastasis include chromosome 3 deletion, the class 2 gene expression profile, *BAP1* loss, chromosome 8q gain, chromosome 1p loss, and chromosome 9q loss, while presence of an *SF3B1* (splicing factor 3b subunit 1) mutation is associated with late-onset metastases. A class 1 gene expression profile, chromosome 6p gain, and the presence of an *EIF1AX* (eukaryotic translation initiation factor 1A, X-linked) mutations are associated with a good prognosis (8-10).

In the particular situation of our patient, no analysis of genetic factors was performed, but the risk for an unfavorable evolution is high due to the origin of the ciliary body, important extraocular extension, epithelium type, mitosis frequencies with cellular atypia and rich vascularization. The long-term prognosis remains poor due to the high risk of developing metastases in the coming years. There is a 40% risk of metastasizing to the liver, lungs or brain within 10-15 years of diagnosis of the primary tumor. No systemic therapy was associated with an evident clinical outcome for patients with advanced disease and overall survival rate remains poor (11-15).

Hepatic metastases, which occur in 95% of patients with metastatic uveal melanoma, result in death in almost all cases. Another risk factor is the possibility of a coexistent synchronous or metachronous melanoma or non-melanoma malignancy (NMM), the incidence being estimated between 8.7% and 14% in different studies (16, 17). The most common NMMs were epithelial tumors (69.4%, most commonly carcinomas of large bowel, breast and prostate) and hematologic malignancies (21.8%) (17).

To reduce the intraoperative hematogenous dissemination of the tumor, with locoregional extension over the scleral barrier, selective embolization under angiographic control of the tumor was performed. This technique is used in the therapeutic management of liver metastases of melanoma, but the results are conflicting. Some authors report a long-term tumor

regression, however, in other papers there was no increase in survival (18-24).

Regarding the direct embolization of the orbital tumor, in the literature was reported only one case of a giant tumor in a stranded patient, with metastatic disease present and severe anemia, in which selective arterial embolization performed for palliative purposes (25), led to a significant reduction in tumor volume in the coming weeks. In the present case, the embolization was completed with surgery with a curative visa, in a patient without metastatic disease present.

CONCLUSIONS

Angiographic embolization may reduce the intraoperative risk of hematogenous dissemination and improve survival in regionally advanced ocular melanomas.

The case was challenging due to increased size of the tumor, yet the predominantly lateral extension, spearing the orbital bones and intraconal space were positive factors for favorable outcome.

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NEWS

MULTIMODALITY IMAGING APPROACH OF HEART DISEASES

Heart diseases can be evaluated with invasive and noninvasive imaging methods. Echocardiography (transthoracic, transesophageal, 3D, contrast, and stress) is generally considered the first-line modality for cardiovascular imaging. It provides comprehensive assessment of anatomy and physiology and contributes significantly to clinical management. Cardiac magnetic resonance (CMR) imaging is already routine and well established in the diagnosis and follow-up of heart diseases and provides accurate volumetric, functional and flow analysis, is accurate with low intra- and inter-observer variability and has become the gold standard for volume and flow assessments. Unlike some other imaging modalities, quality is not affected by body habitus and it is radiation free. Presence of metallic implants or a pacemaker may represent a contra-indication to CMR but 'CMR-compatible' devices have already appeared. Occasionally, severe claustrophobia makes it impossible for a patient to undergo routine awake CMR, so sedation can be considered if CMR imaging would change the clinical management. CMR can safely be performed during pregnancy although it is general recommendation to avoid scanning during the first trimester. Multidetector CT (MDCT) assessment of cardiac diseases has increased exponentially because modern MDCT can cover large anatomic volumes with excellent spatial resolution allowing detailed evaluation of small blood vessels such as coronary arteries, pulmonary veins, collaterals, arteriovenous malformations. Acquisition time is rapid (less than 2 min) allowing patients who are unable to lie still or supine for long periods to be imaged. Pulmonary parenchymal imaging is also provided which is highly relevant for patients with pulmonary hypertension. CT is a true 3D technique, allowing more accurate measurement in all planes of dimensions and may complement assessment of mechanical heart valve dysfunction, aortic or pulmonary dissection and allows 3D visualization of abscess formation in endocarditis, and is even practical in the critically ill patient. However, CT exposes the patient to ionizing radiation and iodinated contrast agents and does not provide information on hemodynamics, flow rate, or velocity. CT can be used to acquire ventricular volumes and function but with lower temporal resolution than 2D/3D echocardiography or CMR and at the expense of additional radiation exposure. (Giovanni Di Salvo, Owen Miller et al. Imaging the adult with congenital heart disease: a multimodality imaging approach. *European Heart Journal.* https://academic.oup.com/ehjcimaging/ article/19/10/1077/ 5067134).

THE VALUE OF TELEMETRIC INTRACRANIAL PRESSURE MONITORING IN SEVERE BRAIN TRAUMA PATIENTS

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THE VALUE OF TELEMETRIC INTRACRANIAL PRESSURE MONITORING IN SEVERE BRAIN TRAUMA PATIENTS (Abstract): Severe traumatic brain injury (TBI), the "silent epidemic" is a public health concern as it contributes the most to death and disability globally among all trauma-related injuries. Brain Trauma Foundation (BTF) released in 2020 the Seattle International Sever Traumatic Brain Injury Consensus Conference (SIBICC) that consist of high-quality evidence reports and three distinct treatment protocols for the management of severe TBI (sTBI). Intracranial pressure (ICP) monitoring is valuable for neurocritical care and patient outcome. The purpose of this study was to assess the outcome of 19 patients with ICP and analyze the benefits of telemetric intracranial pressure monitoring. Traumatic brain injury patients admitted to our hospital that underwent ICP monitoring with Neurovent-P-tel, an improved way of monitoring ICP and proving neurocritical care. Analyzed variables were demographics, mechanism of injury, Glasgow Coma Scale (GCS), head injury specifics, ICP measures and days of ICP monitoring. **Material and methods:** We included in the study 19 patients from our NICU (neurointesive care unit) (15 males and 4 females). Median age was 42 (75-15) years and mean days of telemetric monitoring 2 (1-8). The majority of the patients were involved in motor vehicle accidents (12), but other causes were included (4 falls, 1 assault, 1 epilepsy related, 1 unknown). **Results:** The 19 implanted telemetric sensors functioned well during the NICU admission with no documented complications. We found that ICP telemetric monitoring had benefits to the patient outcomes. **Conclusions:** In our experience, telemetric ICP monitoring is safe to use in a traumatic neurosurgical event. Larger studies need to be made in order for this method to be used as standard care. **Keywords**: TRAUMATIC BRAIN INJURY (TBI), INTRACRANIAL PRESSURE (ICP), BRAIN TRAUMA FOUNDATION (BTF), TELEMETRIC, DECOMPRESSIVE CRANIECTOMY (DC).

Traumatic brain injury is known as the "silent epidemic" as in increases the number of global mortality and disability. In European Union, the overall incidence rate of TBI is 262/100,000 (1-3). The majority of the patients with severe TBI develop elevated intracranial pressure (ICP) that causes secondary brain injury and worse outcome (4). Besides the acute phase of intracranial hypertension, patients may also

develop late onset post-traumatic hydrocephalus, which can be difficult to diagnostic in patients who still have an altered mental state or neurological deficits from the initial injury (5-7).

The historical doctrine discovered by Monroe and Kellie centuries ago regarding intracranial pressure (ICP) is the base mechanism used in present guidelines to demonstrate dysfunctional intracranial compliance. They linked ICP to a mild positive pressure created by the brain, cerebral blood volume and cerebrospinal fluid (CSF) in a semi-rigid skull box. After a traumatic brain injury brain insults can occur and disturb the normal compensation of pressure between the components (8-10).

ICP has been first introduced in the medical word by Guillaume and Janny in 1951 but recognized in traumatic standard care in 1995 in the first guidelines published by the Brain Trauma Foundation (11, 12). The current guidelines recommend continuous ICP monitoring in all salvageable severe TBI patients (Glasgow coma scale < 8) with a computed tomography (CT) scan revealing intracranial pathology or severe TBI with a normal CT scan but who exhibit two or more of the following risk factors: age over 40years, unilateral or bilateral motor posturing, or a systolic blood pressure of < 90 mmHg (13, 14).

For continuous ICP monitoring, the external ventricular drain (EVD) and intraparenchymal monitor (IPM) have been the diagnostic and therapeutic method of choice (15), but they are not without complications and limitations.

Recently, the telemetric technique was introduced to address the disadvantages of former ICP monitoring and to make long-term and less invasive monitoring more feasible (16-18). The technology has been previously used in diagnosis and treatment of patients with hydrocephalus, as postoperative monitoring after endoscopic third ventriculostomy and corrective surgery in craniosynostosis, and in research to establish reference values for normal ICP (7). Telemetric devices usually consist of three parts: the implantable sensor, reader unit or antenna, and receiver and display unit (fig. 1).

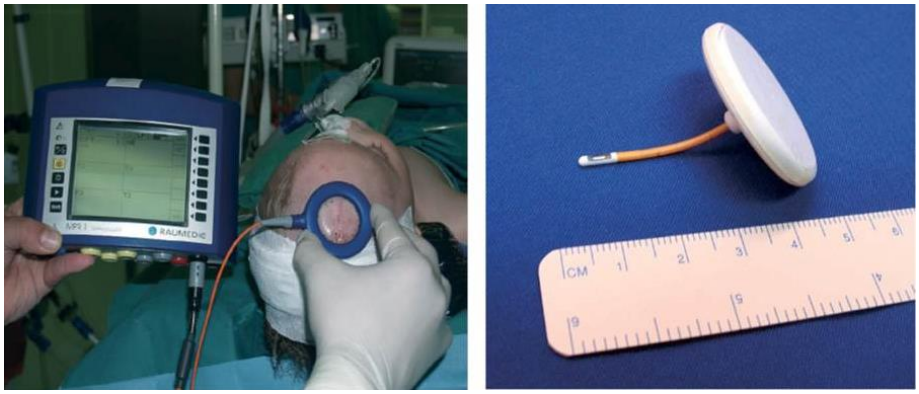

Fig. 1. Hardware for telemetric intracranial pressure monitoring; view in operating room (personal case) (left) and a close-up to the NEUROVENT P-tel Implant (right)

The purpose of this study was to assess the outcome of 19 patients with ICP telemetric monitoring that suffered a traumatic brain injury between January 2018 and

until June 2019.

MATERIAL AND METHODS

We included in our study patients with severe traumatic brain injury that required ICP monitoring with a GCS < 14 from January 2018 to June 2019. The patients underwent ICP monitoring with a telemetric device. The telemetric monitoring system consists of a passive implant sensor (Neurovent-P-tel), an active reader device (RAUMEDIC-TDT readP) and a portable recording unit (RAUMEDIC MPR1 DATALOGGER) (fig. 1). The recording device can be programmed to show different aspects of the monitoring and different alarm limits of ICP can be set. The sensor has a 5F catheter tube with a length of 25 mm, diameter of 1,7 mm, which is inserted into the brain parenchyma. The catheter is connected to a microchip enclosed in a disc-shaped ceramic housing, which is placed on the skull surface under the skin. The device is compatible with MRI scanners up to 3T. All equipment was produced by RAUMEDIC AG, Helmbrechts, Germany.

Patient demographics and clinical characteristics collected for analyzing were age, gender, GCS in admission, type of intracranial injury, mechanism of injury, ICP measures and days of ICP monitoring.

RESULTS

During the study period 19 patients met the criteria for the analysis and received ICP telemetric implant. The mean age was 42, ranging from 15 to 75 years old. The majority of the patients were males (15 patients, 78.95%) and 4 females (21.05%) (tab. I). The common cause of mechanism of injury was motor vehicle accident with 12 patients (63,16%), followed by falls with 4 patients (21.05%), 1 epilepsy related (5.26%), 1 assault (5.26%) and 1 unknown cause (5.26%) (tab. II).

GCS was measured at admission and 9 patients (47.37%) had GCS score of 3-5, 7 patients (36.84%) had GCS score of 6-8 and 3 patients (15.79%) had GCS score of 9-14. All the patients suffered from multiple brain injuries. Almost all patients had brain contusions (17 patients, 89.47%), 16 suffered from cranial fractures (84.12%), 8 had subdural hematoma (42.11%) and 4 had epidural hematoma (21.05%). From all the patients, only 2 patients underwent DC in the first 24hours and 6 patients required DC afterwards. All 19 patients were implanted telemetric ICP monitoring (tab. I).

TABLE I

Patient demographics data and results

	No. of patients
Total	19
Age	
mean	42
max	75
min	15
Sex	
male	15 (78.95%)
female	4 (21.05%)
GCS	
3	6
4	2
5	1
6	4
7	2
8	1
>8	3
Lesions	
SDH	8 (42.11%)
EDH	4 (21.05%)
contusions	17 (89.47%)
cranial fractures	16 (84.12%)
Procedures	
ICP < 24 hrs.	19
ICP > 24 hrs.	0
DC < 24 hrs.	2
DC > 24 hrs.	17
Patient Status	
Discharged	9 (47.37%)
Deceased	10 (52.63%)

The 19 implanted telemetric sensors functioned well during the NICU admission without wound infections or other complications observed. Mean days of ICP monitoring were 2, ranging from 8 days to 1. Measured ICP from the patients had a max of 60 mmHg, a min of 4 mmHg (mean value 16 mmHg) (tab. III). Despite our best effort 10 patients deceased (52.63%) and 9 patients survived (47.37%).

TABLE II
Mechanism of injury

Mechanism of injury	No. of patients /%
Fall	4 (21.05%)
Motor vehicle accident	12 (63.16%)
Unknown	1 (5.26%)
Epilepsy related	1 (5.26%)
Assault	1 (5.26%)

TABLE III
ICP monitoring

ICP MONITORING	days
Mean	2
Max	8
Min	1
	mm Hg
Mean ICP	16
Max ICP	60
Min ICP	4

DISCUSSION

ICP monitoring is regarded as the standard care for managing severe TBI in the neurointensive care unit, although in the Brain Trauma Foundation guidelines we find is as a class III evidence (13). The rationale for this recommendation is to maintain adequate cerebral blood flow and oxygenation by preventing intracranial hypertension to remain untreated (19). The negative effects of intracranial hypertension on clinical outcome following severe TBI have been known for decades (20). Early treatment of rising ICP is known to reduce the risk of secondary brain injury and therefore improvement in overall survival and neurological functional outcome (21). ICP monitoring can not only drive intervention, but also allow for evaluation of the response to various therapeutic pressure lowering intervention (19). Our study observed the beneficial effects of ICP monitoring, that allowed the treatment with non-surgical and surgical methods when needed.

Regarding the age of the patients that sustained traumatic brain injuries, there is a direct link with the risk of mortality. Fadi al Saiegh *et al.* found that there is a 1.2 times greater risk of mortality with every 10-year-increase in age regardless of whether the patients had an ICP monitor or not (22). Areas *et al.* found that age > 65 years was an independent predictor and had 2.25 higher odds of in-hospital mortality (23). Our found also that old age had a negative impact on patients outcome.

Intracranial implants for telemetric ICP monitoring have risks related to the surgery such as intracranial hemorrhage and infections (7, 24). The patients admitted to the NICU present with coagulopathies related to either the inflammatory response of the disease (e.g., in TBI), to anticoagulant drugs (stroke prophylaxis) or secondarily to intensive care interventions (e.g., fluid resuscitation). In our study no patient experienced procedure-related hemorrhage. Antes *et al.* found punctate hemorrhages without focal deficits in 9.4% of 185 patients studied, 5.6% had larger hemorrhages without focal deficits and 0.6% had a large hemorrhage with focal deficits (18).

Similarly, NICU patients are at risk for

infectious complications due to penetrating injuries and invasive procedures for treatment and monitoring purposes. The risk of wound infection of conventional ICP monitoring ranges from 7.3% to 10.4% (7, 24) and Antes *et al.* reported a risk of wound infections of 1.1% in telemetric ICP monitoring (18). In our study population, we encountered no wound or intracranial infections related to the insertion of the telemetric ICP sensors.

CONCLUSIONS

The present study evaluated the benefits of ICP telemetric monitoring in severe TBI patients in our neurointesive care unit. Our conclusion is that telemetric ICP monitoring in an acute neurosurgical patients is safe and feasible.

Further studies are needed to evaluate the long-term use of ICP telemetric monitoring and in order for this method to be used as standard care.

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NEWS

COVID-19 A CHALLENGE FOR DENTISTS?

After COVID-19 was declared a pandemic by the World Health Organization, dentists were instructed by regulatory authorities to stop the services except the emergency. Dentists are among the highest risk categories for transmission and contraction of the coronavirus, with many routine dental procedures having the potential to transmit the virus through aerosols. This was mainly for protection of dental healthcare personnel, their families, contacts, and their patients from the transmission of virus. Most dental procedures generate aerosol; preparing cavities for fillings, use of rotary instruments for root canal treatment, scaling and polishing of teeth, dental implantation and surgical removal of teeth are only some examples. Researchers should focus on developing barrier techniques and negative pressure procedures to contain and isolate the aerosol so that dental procedures to be safe for dental healthcare personnel and patients alike. (Najla Dar Odeh, *et al.* COVID-19: Present and Future Challenges for Dental Practice. *Int J Environ Res Public Health* 2020; 17(9): 3151).

CONCOMITANT LAPAROSCOPIC HIATAL HERNIA REPAIR WITH CHOLECYSTECTOMY: A MULTICENTRIC STUDY

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CONCOMITANT LAPAROSCOPIC HIATAL HERNIA REPAIR WITH CHOLECYSTECTOMY: A MULTICENTRIC STUDY (Abstract): Concomitant surgery refers to performing two or more surgical operations on one patient under the same anesthesia. **Material and methods:** We performed a retrospective multicenter study from October 2016 to October 2019, analyzing patients who underwent laparoscopic hiatal hernia repair. We extracted data of patients who underwent concomitant laparoscopic surgery for both hiatal hernia repair and cholecystectomy in the following Clinics: 1st Clinic of Surgery of Craiova Emergency Clinical County Hospital, "Colţea" Hospital, 3rd Clinic of Surgery of Cluj-Napoca and 3rd Surgery Clinic of University General Hospital of Thessaloniki and identified 20 patients who underwent hiatal hernia repair and had an added cholecystectomy. **Results:** Allocation of data by hiatal hernia type showed 6 type IV hernia (complex hernia), 13 type III hernias (mixed type) and 1 type I hernia (sliding hernia). Out of the 20 cases analyzed; 19 were chronic cholecystitis and one patient presented with acute cholecystitis. Average operating time was 168 minutes. Blood loss was minimum. Crurorraphy was performed in all cases, mesh reinforcement was added in 5 cases, and fundoplication was added in all cases: 3 Toupet, 2 Dorr and 15 Floppy-Nissen. Fundopexy was routinely added in cases with Toupet fundoplication. Cholecystectomy was performed in the following manner: 19 retrograde, 1 bipolar. All patients had favorable postoperative course. Patients follow up was at 1 month, 3 months and 6 months, with no signs of recurrence for hiatal hernia (anatomical or symptomatic) and no post cholecystectomy syndrome. **Conclusions:** Concomitant laparoscopic hiatal hernia repair and cholecystectomy is a safe and feasible option for patients with indication of surgery for both pathologies. **Keywords:** HERNIA, HIATAL, TOUPET, DORR, FLOPPY-NISSEN, CHOLECYSTECTOMY, CONCOMITANT.

Laparoscopic surgery is the golden standard for both hiatal hernia repair and cholecystectomy and offers fast recovery time with less operative time and is both

safe and effective (1). Type I hiatal hernias with impact on quality of life and refractive to medication, type II, III and IV symptomatic hiatal hernia have indication for surgery (2).

Concomitant surgery refers to performing two or more surgical operations on one patient under the same anesthesia. With the quick and widespread adoption of laparoscopic approach in surgery, the emergence of concomitant surgery has seen a rapid incline in the number of cases. It has been almost three decades since laparoscopic approach has become the gold standard for cholecystectomy (3) and the standard for hiatal hernia repair today is also by laparoscopic approach (2). Laparoscopy is not the standard for all surgical interventions, but considering that the minimally invasive approach for both hiatus hernia and cholecystectomy is the standard of care, concomitant surgery has its charm. The idea that you can combine multiple minimally invasive surgeries and perform them under one anesthesia is very appealing, as in open surgery, plus the advantages of minimal invasively: decrease in postoperative hospitalization, decrease in analgesic use, early recovery (when compared to procedures performed at different times), and increase in patient satisfaction. However we should take into account the prolongation of operative time, risk for both intraoperative complications and postoperative morbidities for both interventions.(4)

MATERIAL AND METHODS

We performed a retrospective multicenter study from October 2016 to October 2019, analyzing patients who underwent laparoscopic hiatal hernia repair.

We extrapolated data of patients who underwent concomitant laparoscopic surgery for both hiatal hernia repair and cholecystectomy in the following Clinics: 1st Clinic of Surgery of Craiova Emergency Clinical County Hospital, "Colțea" Hospital, 3rd Clinic of Surgery of Cluj-Napoca and 3rd Surgery Clinic of University General Hospital of Thessaloniki. We collected data regarding demographics, comorbidities, imaging, operative time, blood loss, intraoperative complications, postoperative evolution and follow-up. Values were imported into *Microsoft Excel* (Microsoft Office) and analyzed.

Patients presented indication for surgery for both hiatal hernia repair and cholecystectomy. In this study, no patient with completely asymptomatic gallbladder lithiasis was included.

For the diagnosis of hiatal hernia one or more of the following were performed: upper digestive endoscopy, oral and intravenous contrast enhanced computed tomography and upper digestive endoscopy. Preoperative Visick score for reflux disease was at least three. Gallbladder lithiasis was diagnosed with abdominal ultrasound.

Preoperative, all patients underwent deep venous thrombosis (DVT) and pulmonary embolism (PE) prophylaxis with *low molecular weight heparin*, bowel preparation with anti-foaming agents to reduce bloating and *nil per os* at least 12 hours before surgery.

Interventions were performed under general anesthesia, with endotracheal intubation for all patients. All patients were placed in Trendelenburg French position, both arms tucked along the upper body.

RESULTS

We identified 20 patients who underwent laparoscopic hiatal hernia repair and cholecystectomy in the same operative time.

Mean age was 63 years-old, median of 65 years, minimum of 46 years old, maximum of 75 years old and standard deviation was 7.2 years. All patients were female, and one out of 20 patients was admitted through emergency setting, while the rest were elective.

Case distribution by age group is shown in first figure.

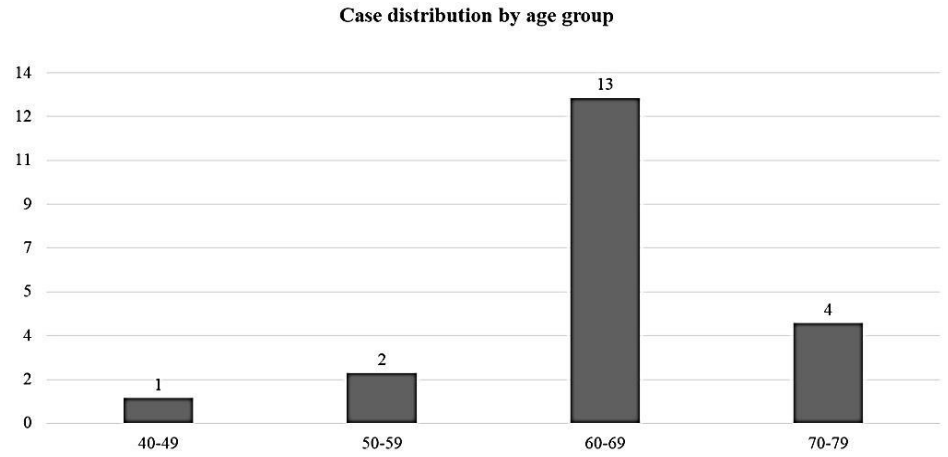


Fig. 1. Case distribution by age group is shown

Four patients (20%) were from a rural area, while 16 patients (80%) were from an urban area. Allocation of data by hiatal hernia type showed 6 type IV hernia (complex hernia), 13 type III hernias (mixed type) and 1 type I hernias (sliding hernia) (fig. 2). Out of the 20 cases analyzed, 19 were chronic cholecystitis and one patient presented with acute cholecystitis (fig. 3).

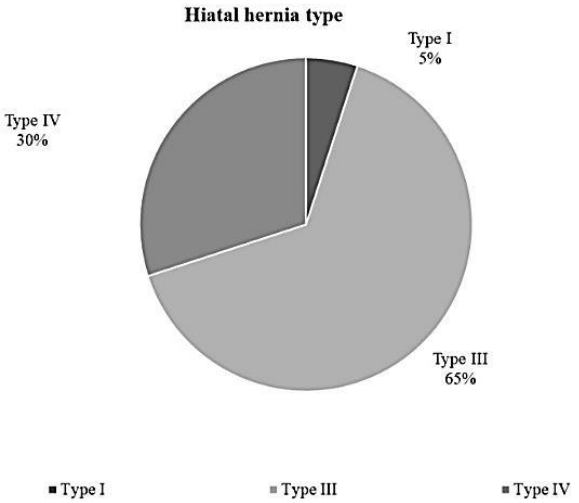


Fig. 2. Hernias type distribution

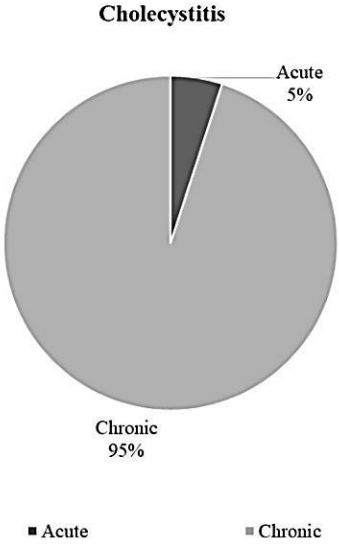


Fig. 3. Cholecystitis type

The most frequent comorbidities were obesity (10 patients) and hypertension (17 patients). Most frequent symptom was heartburn, present in 18/20 patients (90%) with a symptom duration ranging from 1 year to 20 years, under proton pump inhibitor medication, with partial symptom cessation. Regurgitations were present in 13 patients the average duration of symptoms was one year. Pain was the second most prevalent symptom and if ordered by location frequency it was: epigastric (50%); left hypochondrium (33.3%), left hemi-thorax (25%) and retrosternal pain (16.66%). Pain was postprandial in 9 patients (45%), while lying down in 7 patients (35%), ceased after eructation in 4 patients (20%) and referred pain in the left shoulder in 4 patients (20%). Other presenting symptoms with a lower incidence were dysphagia 4 cases, anemia 5 cases, coughing 5 cases (fig. 4).

The performed imaging, by order of frequency were upper digestive endoscopy (85%), barium meal (60%) and computed tomography (20%) (fig. 5).

Access in the peritoneal cavity was achieved through insufflation by Veress needle in 17 cases, 3 cases with optical access; regarding the insufflation point: 14 supra-umbilical, 6 Palmer point, with no insufflation incidents.

Average operating time was 168 minutes, median 180 minutes, minimum 120 minutes, and maximum 210 minutes. Blood loss was minimum. Crurorаphy was performed in all cases, mesh reinforcement was added in 5 cases, and fundoplication was added in all cases: 3 Toupet, 2 Dorr and 15 Floppy-Nissen. Short gastric vessels of the fundus were divided in all cases, for better mobilization. Wrap height varied between 2-3 centimeters. Fundopexy was routinely added in cases with Toupet fundoplication. Cholecystectomy was performed in the following manner: 19 retrograde, 1 bipolar. Drainage for gallbladder was added in 16 cases and was kept in place for 1-5 days. Nasogastric aspiration tube was not maintained in the postoperative period.

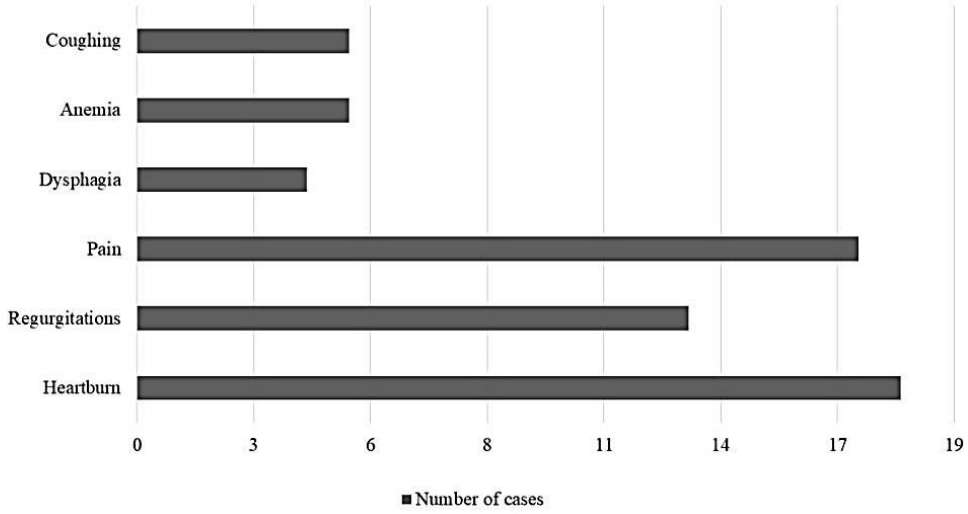


Fig. 4. Presenting symptoms

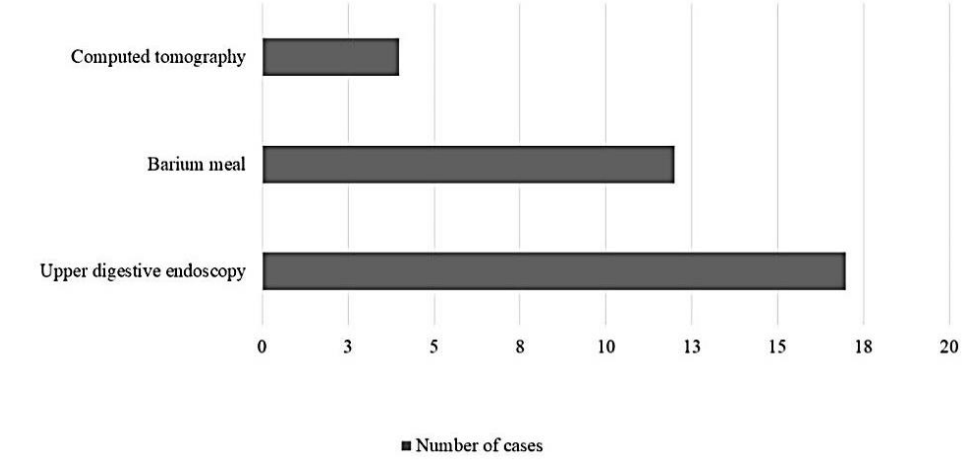


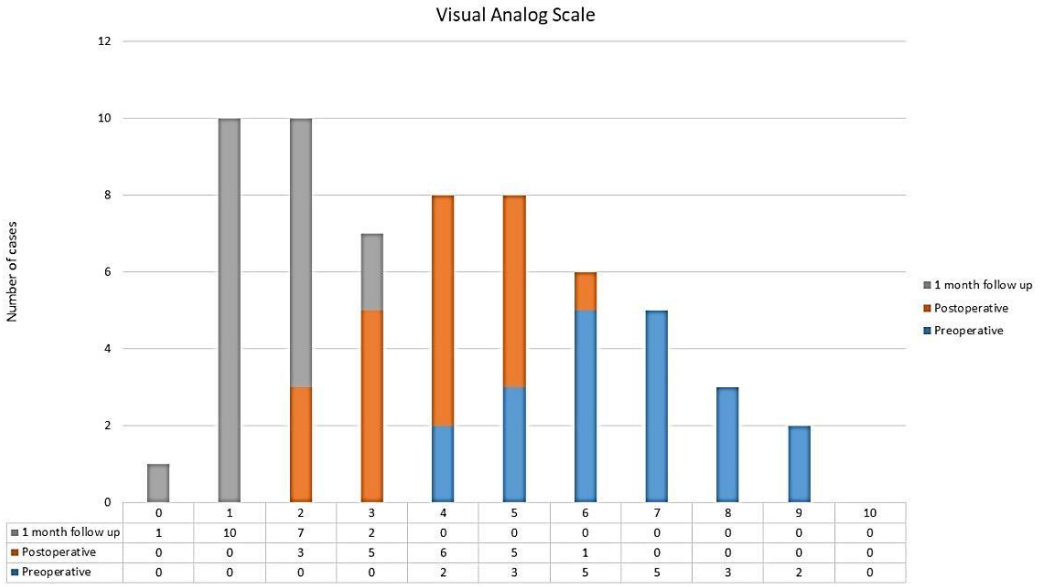
Fig. 5. Performed imaging, by order of frequency

Three patients stayed overnight in the intensive care unit. All patients had favorable postoperative course, started active mobilization by postoperative day 1, passage of flatus by postoperative day 2 and had a stool emission by discharge.

Postoperative discharge was between POD 3 and POD 9, average of 5.5 days, median 6 days. Opioid analgesics were used only during anesthesia, broad spectrum intravenous antibiotic was given intraoperatively for all patients at anesthesia induction and only one patient with acute cholecystitis received broad spectrum intravenous antibiotic 4 days. Postoperative pain management was achieved by non-opioid analgesia on demand of the patient. Pain was measured preoperatively, postop-

eratively and at 1-month follow-up using the Pain visual analogue scale (VAS) preoperative (fig. 6).

Patient follow-up was at 1 month, 3 months and 6 months, with no sign of recurrence for hiatal hernia (anatomical or symptomatic) and no minor complaints of post cholecystectomy syndrome, like fullness or dullness in the upper right quadrant, especially after some movements.



	0	1	2	3	4	5	6	7	8	9	10
1 month follow up	1	10	7	2	0	0	0	0	0	0	0
Postoperative	0	0	3	5	6	5	1	0	0	0	0
Preoperative	0	0	0	0	2	3	5	5	3	2	0

Fig. 6. Pain visual analogue scale

DISCUSSION

With the increase in safety and experience of laparoscopic surgery, the desire to perform more than one operation under one anesthesia has risen in order to take advantage of the benefits of minimally invasive surgery (4, 5). Performing both hiatal hernia repair and cholecystectomy through laparoscopic approach is feasible and safe, and by performing both surgeries under one anesthesia the need for a second hospitalization is minimized, quality of life is improved, patient has no need to take multiple work break (6).

The combined procedure needs no supplementary trocar insertion other than that for hiatal hernia repair, as trocar positioning resembles the French technique for cholecystectomy. An experienced surgeon who regularly performs hiatal hernia repair should meet no impediment in combining both surgeries, even in the presence of comorbidities such as obesity. The decision to perform this combined surgery must stem from the indications for surgery for both pathologies.

Trocar insertion must be planned beforehand, with ergonomy and triangulation for laparoscopic knot tying and suturing kept in mind. In morbidly obese patients, we must bear in mind that the umbilicus is not a reliable landmark and positioning of the landmark should be at 15 centimeters from the xiphoid on the xipho-umbilical

line (7).

Imaging plays a paramount role and should not be discredited by the desire to reduce costs, as the importance of the findings can play a vital role in the indication for surgery, procedure used and planning. Upper digestive endoscopy, the most performed investigation in our study for hiatus hernia can not only diagnose hiatal hernia but also esophagitis, Cameron ulcers and other benign or malign coexisting pathologies and can lead to a decision for surgery. Upper digestive endoscopy, in the hands of an experienced endoscopist can even rise the suspicion of a parahiatal hernia which can be confirmed by barium meal (8).

Barium meal is the gold standard for diagnosing hiatus hernia and it brings the benefit of excellent visualization of hernia sac and provides orientation for the surgeon. It was not the most performed investigation in our study, as we believe that if endoscopy provides large sizes of the hernia (large enough to classify it as a type 3), or if a type 2 paraesophageal hernia is present, barium meal is no longer mandatory. Barium meal offers a very good visual representation of the herniated stomach but is lacking in cases of complex hernias in which other abdominal organs are herniated. Computed tomography (CT) should not be overlooked as it has the potential to provide the most information, especially if enhanced with intravenous and oral contrast. It can diagnose both gallbladder stones, choledocholithiasis and all types of hiatus hernia; it can provide a better understanding of the particular anatomy of every patient. With the emergence of tridimensional reconstruction software and preoperative planning software, CT can bring a vital role in surgical planning of trocar positioning for better ergonomy for the surgeon, decreased blood loss and operating time.

Performing both surgeries provides the evident benefit of one anesthesia and one surgical time for two pathologies and is both safe and feasible in the hands of an experienced laparoscopic team (4). Operating time was 168 minutes compared to the average operating time of the standalone hiatal hernia repairs, which was 148 minutes. Operating time is similar with standalone surgery and blood loss was minimum in both cases. Oral intake, bowel movement and active mobility resumption was similar with standalone hiatal hernia surgery.

Although evidence exists regarding safety in placing mesh in clean-contaminated cases (9-14), we believe that the current level of evidence is not high enough and we advise use of mesh only in carefully selected cases if cholecystectomy is performed.

We must not forget the dreaded complications meshes can produce and we believe that it should only be used as a last resort, in cases where crural appropriation is not possible or in selected cases of recurrent hiatal hernia.

Regarding the order of the interventions, the hiatal hernia is usually performed first and then the cholecystectomy. For surgeons who regularly operate between the patients legs, the transition is easy, but for surgeons who perform hiatal hernia repair from the right side of the patient, the transition is more difficult, having to change the position of the laparoscope.

Common bile duct injuries are the Achilles heel in laparoscopic surgery versus open surgery with reported rates being higher in the laparoscopic group (14-20). Although laparoscopic cholecystectomy is

a relatively common and simple procedure, the surgeon is performing it after a hiatal hernia repair, which can lead to fatigue. The surgeon should be in a familiar position for cholecystectomy, with good operative view, good ergonomy and should bear in mind the risk of this injury especially if fatigued. Both procedures can be accompanied by intraoperative incidents and accidents, which can lead to severe morbidity. If a post cholecystectomy syndrome is suspected in the postoperative period, a careful evaluation of the patient must also include suspicion of hiatal hernia recurrence.

CONCLUSIONS

In conclusion, concomitant laparoscopic hiatal hernia repair and cholecystectomy is a safe and feasible option for patients with indication of surgery for both pathologies.

CONTRIBUTIONS

All authors have equal contributions.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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NEWS

THE BENEFITS FOR PATIENTS WITH INFLAMMATORY BOWEL DISEASE TAKING ASCVD MEDICATIONS

Chronic inflammation in patients with inflammatory bowel disease (IBD) leads to increased risk of atherosclerotic cardiovascular disease (ASCVD). The incidence of ASCVD is increased in patients with IBD as reported by multiple population-based and few retrospective case control studies. Numerous studies have examined an overlap in treatment between IBD and heart disease, with relative success. A retrospective matched case–control study (Ungaro *et al*.) assessed statin use in patients with IBD. Statin use was associated with a significantly decreased risk of IBD. In addition to studies showing benefit for patients with IBD taking ASCVD medications, there have been studies assessing the role of anti-inflammatory medications for ASCVD in the general population. In a multicenter randomized control (CANTOS) trial (Ridker *et al.*), canakinumab, a human monoclonal antibody targeted at IL-1, showed a 15% reduction in deaths from heart attacks and strokes combined. A nationwide retrospective cohort study noted that for patients with IBD taking 5-aminosalicylic acid (5-ASA), the incidence of IHD was significantly less compared to those not taking 5-ASA. Clinicians should be aware of the harmful effects of chronic inflammation on the heart. ASCVD risk is increased among patients with IBD especially during periods of active disease. (Weissman S, Sinh P, Mehta TI, *et al.* Atherosclerotic cardiovascular disease in inflammatory bowel disease: The role of chronic inflammation. *World J Gastrointest Pathophysiol* 2020; 11(5): 104-113).

CONSISTENCIES AND INCONSISTENCIES OF GLEASON SCORE AND GRADE GROUP SYSTEM IN PROSTATE BIOPSY AND RADICAL PROSTATECTOMY

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CONSISTENCIES AND INCONSISTENCIES OF GLEASON SCORE AND GRADE GROUP SYSTEM IN PROSTATE BIOPSY AND RADICAL PROSTATECTOMY (Abstract): The diagnostic of prostatic adenocarcinoma (PADK) is based on two scoring systems, namely the Gleason grading system and the grade group system - both of them being included in the pathology report. There is a discrepancy in the Gleason score given on the initial biopsy and prostatectomy specimen, respectively, that affects both patients who can benefit from active surveillance and those who cannot benefit from curative surgical treatment, and for whom therapeutic alternatives must be established. In this context, our study aimed at analyzing the diagnostic concordance between biopsies and subsequent prostatectomies, in order to establish the accuracy of prostate biopsy in predicting final Gleason score of the prostatectomy specimens. **Material and methods:** We analyzed 79 cases of PADK, initially diagnosed by prostate biopsy and subsequently treated by radical prostatectomy. The accuracy degree of the tumoral grading system assigned to the prostate biopsy, compared with the radical prostatectomy, was assessed by calculation of the Cohen's kappa concordance coefficient and calculation of sensitivity, specificity, positive prognostic value and negative prognostic value. **Results:** 37 cases (46.83%) presented the same Gleason score given for prostate biopsies and radical prostatectomies. In prostate biopsies, 32 cases (40.50%) were underestimated, compared to the Gleason score established by evaluating radical prostatectomy specimens, and 10 cases (12.65%) were overestimated. The highest agreement rate between the Gleason score in prostate biopsy and radical prostatectomy was found for Gleason score 6 (3+3) and 7 (3+4)-17 cases (21.51%) for each score category. The lowest agreement rate was observed for Gleason score 5, where no case showed a similar score for biopsy and prostatectomy. Statistical analysis indicated a kappa coefficient of 0.23, corresponding to a statistically significant satisfactory agreement ($p = 0.01$). The agreement on ISUP prognostic grade groups between prostate biopsy and radical prostatectomy indicated a slight increase in kappa coefficient -0.41, while maintaining a statistically significant moderate agreement ($p = 0.0001$). **Conclusions:** Our results demonstrate an increase in the predictive accuracy of prostate biopsy, as a result of the application of prognostic grade group system, which ensure the decrease of overestimating tendency of the old scoring system, ultimately leading to a preliminary characterization of potential lesions in prostatecto-

my specimens and a more effective treatment stratification of patients. **Keywords:** PROSTATIC ADENOCARCINOMA, GLEASON SCORE, PROGNOSTIC GRADE GROUP, PROSTATE BIOPSY, RADICAL PROSTATECTOMY.

The diagnosis of prostatic adenocarcinoma (PADK) is based on a combination of well-defined microscopic architectural, cytological and immunohistochemical features. Apparently, the microscopic identification of this neoplasia seems quite simple. However, pathologists, in their current practice, confront difficulties in recognizing and classifying histological subtypes, in line with the classification benchmarks for this pathology, which have undergone important updates in the last decade (1, 2).

The milestone in PADK diagnosis is represented by the Gleason grading system proposed by Donald Gleason in 1966 as Gleason score and constantly changed up to 2005 as a result of diagnostic experience and research on the relationship between histological, clinical and prognostic parameters (3-6). In 2005, the modified variant of the Gleason score was implemented (7), which became the most important diagnostic and prognostic tool of the PADK between 2005-2014, playing a decisive role in establishing and managing oncological and surgical protocols and in active monitoring (8-10).

The Gleason grading system modified in 2005 increased the diagnostic reproducibility and provided a better correlation between the Gleason score assigned on prostate biopsies and the final Gleason score attributed to the prostate resection specimen (11-13).

In the face of continuously accelerating scientific advancement in the medical field, under the motivation of improving the Gleason scoring system and solving its limitations, in 2014 the International Society of Urologic Pathology (ISUP) Consensus Conference validated a new prostate neoplasm grading system (2). This new prognostic grade group system is directed to the more precise stratification of prostate lesions in relation to the prognostic dictated by Gleason scoring. The five prognostic grade groups were established by reference to the probability of 5-year biochemical recurrences free progression following radical prostatectomy.

Currently, as recommended in 2016 WHO Classification for tumors of the urinary system and male genital organs (1), diagnostic evaluation of PADK is based on two scoring systems, namely the Gleason grading system and the grade group system -both of them being included in the pathology report.

A major issue on which the interest of pathologists focuses is the assessment of the discrepancy recorded in the Gleason score given on the initial biopsy and prostatectomy specimen, respectively. According to the literature, this discrepancy is present in about 50% of cases, in general the grading on the prostatectomy specimen being higher compared to the biopsy (14-17). Differences in PADK grading, in prostate biopsy and prostatectomy specimen, are important because they indicate the possibility of an underestimation or overestimation of diagnosis and implicit prognosis. This affects both patients who can benefit from active surveillance and those who cannot benefit from curative surgical treatment, and for whom therapeutic alternatives must be established.

In this context, our study aimed at analyzing the diagnostic concordance between biopsies and subsequent prostatectomies, in order to establish the accuracy of prostate biopsy in predicting final Gleason score of the prostatectomy specimens.

MATERIAL AND METHODS

The study group included 79 cases of PADK, diagnosed between January 2010 and December 2018 within the Department of Pathology of the Clinical Hospital "Dr. C.I. Parhon" Iași. Patients were initially diagnosed by prostate biopsy and subsequently treated by radical prostatectomy.

The study was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iași.

A number of 6, 12 or 24 tissue fragments were taken by ultrasound-guided prostate biopsy from both prostate lobes. In all cases, the Gleason score was assigned according to the Gleason grading system adopted by WHO in 2016 (1) and was completed by ISUP grading classification in groups (2). The cases in the grade groups 1 and 2 were considered low grade cases, those in the grade group 3 as moderate grade, and those in grade groups 4 and 5 as high grade. Under-evaluation was defined as assigning a lower Gleason score on prostate biopsy compared to the one set on the subsequent radical prostatectomy specimen, while overestimation consisted of a higher initial Gleason score on prostate biopsy.

To assess the accuracy degree of the tumoral grading system assigned to the prostate biopsy, compared with the radical prostatectomy, we applied two statistical methods: calculation of the Cohen's kappa concordance coefficient with the following interpretations: values $\leq$ 0.2 -poor agreement; values from 0.21 to 0.40 -satisfactory agreement; values from 0.41 to 0.60 - moderate agreement; values from 0.61 to 0.80 -good agreement; values above 0.8 - very good agreement; calculation of sensitivity, specificity, positive prognostic value and negative prognostic value. All data obtained were considered significant for values of $p<0.05$.

RESULTS

The most common Gleason score given on prostate biopsy fragments was 6 (3+3) in 41 cases (51.89%), followed by Gleason score 7 (3+4) in 26 cases (32.91%), while on radical prostatectomy specimens the most common was Gleason score 7 (3+4) in 37 cases (46.83%), followed by Gleason score 6 (3+3) in 22 cases (27.84 %). A number of 37 cases (46.83%) presented the same Gleason score given for prostate biopsies and radical prostatectomies, respectively. In prostate biopsies, 32 cases (40.50%) were underestimated, compared to the Gleason score established by evaluating radical prostatectomy specimens, and 10 cases (12.65%) were overestimated.

The highest agreement rate between the Gleason score attributed to prostate biopsy and that of radical prostatectomy was reported in cases evaluated with Gleason score 6 (3+3) and 7 (3+4) -17 cases (21.51%) for each score category. The lowest agreement rate was observed in cases with a Gleason score 5, where no case showed a similar score for biopsy and prostatectomy. The many cases that were underestimated (19 cases - 24.05%) belonged to the subgroup with Gleason score 6 assigned to prostate biopsy, in 16 cases being established, on the prostatectomy specimen, the Gleason score 7 (3+4), and in 3 cases - the Gleason score 8 (4+4) (tab. I).

TABLE I

Concordances and Differences between Gleason Score in Prostate Biopsy and Radical Prostatectomy

GLEASON SCORE - RADICAL PROSTATECTOMY	GLEASON SCORE - PROSTATE BIOPSY (#)						Total
	5	6	7 (3+4)	7 (4+3)	8	9	
5	**0**	5	1	0	0	0	6
6	3	**17**	2	0	0	0	22
7 (3+4)	3	16	**17**	1	0	0	37
7 (4+3)	0	1	3	**2**	1	0	7
≥8	0	2	0	0	**1**	0	3
9	0	0	3	0	1	**0**	4

- number of cases

Statistical analysis to assess the accuracy of the Gleason score given on prostate biopsy fragments in predicting the Gleason score given on the prostatectomy specimen indicated a kappa coefficient of 0.23, corresponding to a statistically significant satisfactory agreement (p = 0,01). The calculation of the kappa coefficient was based on the indicators of expected coincidence, with a value of 24.46, and observed coincidence, with a value of 37 (37 cases with the same Gleason score in prostate biopsy fragments and radical prostatectomy specimens).

By analyzing the sensitivity, specificity, positive and negative predictive values in assessing the degree of accuracy of prostate biopsy scoring in predicting the final Gleason score on the radical prostatectomy specimen, we observed the following (tab. II):

- the lowest sensitivity value of 0% was reported in prostate biopsies with Gleason score 5;

- the highest sensitivity value of 77.2% was reported in prostate biopsies with Gleason score 6;

- prostate biopsies with Gleason score 5 had zero positive predictive value, while prostate biopsies with Gleason score 7 (4+3) had the highest positive predictive value -66.6%;

- the highest specificity -100% was identified for prostate biopsies with Gleason score 9, and the lowest specificity -57.8% was calculated for prostate biopsies with Gleason score 6;

- tumors with Gleason score 8 on prostate biopsy showed the highest negative predictive value -97.36%, while prostate biopsies with Gleason score 7 (3+4) showed the lowest negative predictive value - 62.2%.

The agreement on the diagnostic classification in the ISUP prognostic grade groups, according to the initial diagnosis based on the aspects identified in prostate biopsies and radical prostatectomy specimens, respectively, indicated a slight increase in kappa coefficient -0.41, while maintaining a statistically significant moderate agreement (p = 0.0001). The calculation of the kappa coefficient was based on the indicators of expected coincidence, with a value of 60.59, and observed coincidence, with a value of 68 (68 cases with the

same classification in the ISUP prognostic grade groups in prostate biopsy fragments and radical prostatectomy specimens, according to the Gleason score) (tab. III).

TABLE II

The Accuracy of the Gleason Score on Prostate Biopsy *versus* Radical Prostatectomy

GLEASON SCORE - PROSTATE BIOPSY	SENSITIVITY	POSITIVE PREDICTIVE VALUE	SPECIFICITY	NEGATIVE PREDICTIVE VALUE
	% (# from the fraction of the calculation formula)			
5	0%(0/6)	0% (0/6)	91.7 (67/73)	91.7% (67/73)
6	77.2% (17/22)	41.4% (17/41)	57.8% (33/57)	86.8% (33/38)
7 (3+4)	45.9% (17/37)	65.3% (17/26)	78.5% (33/42)	62.2% (33/53)
7 (4+3)	28.5% (2/7)	66.6% (2/3)	98.6% (71/72)	93.4% (71/76)
≥8	33.3% (1/3)	33.3% (1/3)	100% (62/63)	97.3% (74/76)
9	0% (0/4)	-	100%(75/75	94.9% (75/79)

- number of cases, % - percentage

TABLE III

Concordances and Differences between the Prognostic Grade Groups established for Prostate Biopsy and Radical Prostatectomy

ISUP GRADE GROUP - RADICAL PROSTATECTOMY (#)	ISUP GRADE GROUP -PROSTATE BIOPSY (#)			
	1-2 (low grade)	3 (moderate grade)	4-5 (high grade)	Total
1-2 (low grade)	**64**	1	0	65
3 (moderate grade)	4	**2**	1	7
4-5 (high grade)	5	0	**2**	7
Total	73	3	3	79

- number of cases

The analysis of agreement between the prognostic grade groups established according to the Gleason score for prostate biopsies and, respectively, for radical prostatectomy specimens revealed the following aspects:

- out of the 73 cases included in the prognostic grade groups 1 and 2 in prostate biopsies, considered as low-grade cases, 9 cases (12.32%) were included in a higher-grade group after radical prostatectomy, as follows: 4 cases were classified in grade group 3, and 5 cases in grade group 4; the other 64 cases (87.67%) maintained the same prognostic grade group;

- out of the 3 cases included in the prognostic grade group 3 in prostate biopsies, considered with a moderate grade, only one case (33.3%) was re-included in the prognostic grade group 2 post surgery; for two cases, the classification was maintained;

- out of the 3 cases included in the prognostic grade groups 4 and 5 in prostate biopsies, regarded as high-grade cases, only one case (33.3%) was re-included in

the prognostic grade group 3 on prostatectomy; for two cases, the classification was maintained.

Following these permutations, the comparative analysis of the agreement of prognostic grade group classification indicated a tendency of higher grading in cases from the prognostic grade groups 1-2, reclassified in the grade group 3; a lower framing tendency, for cases from grade group 3, respectively the grade groups 4-5, reclassified in the lower grade groups.

DISCUSSION

Gleason grading system and grade group system are reference tools in the prognostic evaluation of PADK, and therapeutic decisions are dictated by their use in the diagnosis of tissue fragments obtained by prostate biopsy.

The differences between the grading of the biopsy fragment, and that of the prostatectomy specimen have as morphological substrate the multifocal feature of PADK, which determines different grading in distinct foci of the tumoral tissue. This reality may explain the oscillating rate of reported agreement between the score in prostate biopsy and radical prostatectomy with curative intent, respectively. Changes in the Gleason grading system and the introduction of the grade group system had direct implications on the concordance degree and the predictive value of the prostate biopsy, in relation to the final prostatectomy specimen.

However, the agreement rate reported in the literature is extremely variable, reflecting not only the interobserver variability, but also the different level of experience of the pathologist providing the diagnosis.

Consequently, it is imperative to validate refined criteria to ensure a high degree of accuracy and concordance between the histological grading of the biopsy and radical prostatectomy specimens. With this desideratum in mind, the efforts of specialists are also focused on identifying the factors responsible for these differences and on a better understanding of their dynamics, with the ultimate goal of reducing or even eliminating them.

Currently, routine prostate biopsy consists in 12 tissue samples, which have replaced the traditional sextant by adding biopsies of the lateral sites, thus increasing the sensitivity of prostate cancer detection. The pathology report of prostatic biopsies should include, in addition to concise description of the lesion, the percentage of tumor infiltration in each tissue fragment (expressed in millimeters or percentage), the proportion of the tumor component with the higher Gleason pattern (Gleason 4, 5), the number of tissue fragments identified with cancer, as well as their location (conditioned by sending fragments in separate containers by the clinician), the presence of perineural and capsular invasion. Numerous studies emphasize the importance of these quantitative aspects (18, 19), concluding that reporting of these parameters and grading of biopsy are indirect evidence for tumor stage and, implicitly, for tumor extension.

The extended biopsy protocol is more sensitive in detecting prostate cancer than the sextant method. In the absence of clear standardized reporting rules, some pathologists describe each biopsied tissue fragment separately, with the final pathology report including 12 Gleason scores, one score for each analyzed tissue fragment, while other pathologists prefer a general characterization of all biopsied fragments, generating a single final Gleason score

(19). These different approaches in reporting the Gleason score on prostate biopsy (in relation to the number of positive fragments and the percentage of tumor lesions) are variables with important implications in the therapeutic management of the patient.

In this regard, published studies in the literature (20-22) indicate that the highest Gleason score among the individual Gleason scores attributed partly to each biopsied tissue fragment is better correlated with the Gleason score assigned to radical prostatectomy, compared to an overall Gleason score reported for all tumor-positive prostate fragments.

Considering the possibility of multiple tumor foci with heterogeneous morphological characteristics (23), recent studies recommend the linear measurement of discontinuous tumor foci, as a more accurate method in predicting the degree of aggressiveness and tumor extension on the radical prostatectomy specimen, being better correlated with higher tumor stages (24).

How to report the percentage of a Gleason pattern 4 on prostate biopsy fragments remains a matter of controversy, given the significant link between the high incidence of Gleason pattern 4 in cases with a final Gleason score 7 on the prostatectomy specimen and high risk of disease recurrence (25). The estimation of the tumor component with Gleason 4 pattern on prostate biopsy shows interobserver variability, regardless of the used method (visual or digital), with important impact on therapeutic decision (26). However, numerous studies support the need to integrate it into the pathology report, given that the cribriform architecture (currently considered as Gleason pattern 4) is related to an adverse prognostic, at higher risk of biochemical recurrence (27) and metastatic disease (28). At the same time, reporting the percentage of Gleason pattern 4 on prostate biopsies in a final Gleason score 8 (ISUP grade group 4) on radical prostatectomy was found to be related to an increased risk of visceral and bone metastasis (29).

In addition to the lack of standardization of prostate biopsy reporting, three other major factors may be responsible for discrepancies between the Gleason score established on prostate biopsies and the final Gleason score attributed to radical prostatectomy: proportion of tumor tissue on biopsied tissue fragments, variable histopathological interpretation and sampling defects (30).

Our study revealed an agreement rate of the Gleason score in prostate biopsy and radical prostatectomy specimen of 46.83%; 40.50% of cases were underestimated and 12.22% of cases were overestimated. The accuracy rate was 0.23, corresponding to a statistically significant satisfactory agreement ($p = 0.01$). The highest agreement rate, 54.5%, was achieved in the Gleason score 7 category (3+4), while in Gleason score 6 we reported a concordance of only 44.2%. The achieved results indicated the main score change introduced by 2016 WHO classification, which is the classification of cribriform and glomeruloid architecture as Gleason pattern 4. This change led to an increase in the number of cases diagnosed with score 7 (3+4 and 4+3). Given that these aspects are relatively easy to identify by microscopic examination, a recent study indicates a sensitivity of 56.5% and a specificity of 87.29% in the recognition of cribriform pattern in prostate biopsy (31). Moreover, the use of ISUP prognostic grade groups corresponding to Gleason scores led to an increase in kappa

coefficient calculated up to the limit of 0.41 -which has a moderate agreement rate.

Our data complete the general framework of reporting in the literature, the values obtained being in line with the recognized scale of values. To support this statement, we consider it appropriate to briefly review the data on the diagnosis agreement between prostate biopsy and radical prostatectomy.

Studies published before 2014 - which marked a new age by changes to the Gleason grading system and introducing grade group system -show agreement rates of 41% (32), 43% (30), and 53% (33). The results were more often under-graded (34% or 38%) than over-graded (9%) (33, 34); 80% of cases diagnosed with unilateral disease on biopsy presented bilateral involvement in prostatectomy specimens (34). The agreement in Gleason score between biopsy and radical prostatectomy was highest among the uropathology departments that general pathology ones (33), and the accuracy of the prostate biopsy increased when at least eight cores were analyzed (34). On the other hand, the results of a much broader meta-analysis (analyzing over 14,000 cases) conducted in 2008 indicate a much higher percentage for concordance and overestimation -63% and 30%, respectively, and a lower percentage for underestimation -7% (35). The same meta-analysis stratifies the histopathological diagnosis into 3 categories, namely cases with low tumor grade, moderate tumor grade and high tumor grade, and reports the following concordances: 62% for low tumor grade, 70% for moderate tumor grade and 50% for high tumor grade (35). The accuracy of the diagnostic classification was quantified by the kappa coefficient, obtaining a value of 0.37, corresponding to a satisfactory agreement (35). Also in 2008, a study based on a much smaller number of cases (just over 1000) supports an agreement rate of 81% for Gleason score 5-6, 68% for Gleason score 7 and 70% for Gleason score 8-10 (36).

Studies published after 2016 indicate a higher level of concordance, with values of 54.5% (37), 55.4% (38), 59.3% (39), 62.5% (40) and 63.2% (41). There is also a higher rate of upgraded cases than of downgraded cases (31.1% versus 14.3%) (37). Using the population-based Surveillance, Epidemiology, and End Results (SEER) database, with a total number of 34,195 analyzed patients, Wong *et al.*, 2017 report a kappa coefficient value of 0.36, indicating a fair agreement with the following features: in Gleason score 6 the concordance was 55.3%, with 43.1% cases upgraded to Gleason score 7; in Gleason score 7, the concordance was 66.9% for 7 = 3+4 and 42.9% for 7 = 4+3; in Gleason score 8 the concordance was 24.8%, with a downgrade rate of 57.4% (38).

The level of concordance depending on the grade group is variable, the values reported being: 47.6% for group grade 1, 73.6% for group grade 2, 52.8% for group grade 3, 21.4% for group grade 4 and 68.3% for group grade 5, with upgrade in 32.3% cases and downgrade in 8.3% cases (39).

Another recent study, based on the comparison between different types of systematic biopsies, targeted biopsies and their combination with the final histopathologic diagnosis of radical prostatectomy, revealed a concordance in grade group of 49.4% for systematic biopsies, 51.2% for targeted biopsies, and 63.2% for both types of biopsies. For cases with grade group ≥ 2, the concordance was 41.2% for system-

atic biopsies, 48.3% for targeted biopsies, and 56.7% for both types of biopsies (41). As general remarks, the upgrade process is correlated with a poor outcome (40), the total number of cores and other previous biopsies were significant independent predictive factors for concordance in targeted biopsies (41), and the need of other potential factors to predict biopsy concordance is recognized (38).

CONCLUSIONS

Our results demonstrate an increase in the predictive accuracy of prostate biopsy, as a result of the application of prognostic grade group system, which ensure the decrease of overestimating tendency of the old scoring system, ultimately leading to a preliminary characterization of potential lesions in prostatectomy specimens and a more effective treatment stratification of patients.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

MULTIMORBIDITY: HOUSEHOLD AND AREA-LEVEL SOCIAL DETERMINANTS

The study aimed to systematically review the existing literature on associations between household and area-level social determinants of health (SDoH) and multimorbidity prevalence or incidence in the general population. Methods Six databases (MedLine, EMBASE, PsychINFO, Web of Science, CINAHL Plus and Scopus) were searched. The search was limited to peer-reviewed studies conducted in high-income countries and published in English between 2010 and 2019. Authors included 41 studies spanning North America, Europe and Australasia. Household income and area-level deprivation were the most explored with fairly consistent findings. The odds of multimorbidity were up to 4.4 times higher for participants with the lowest level of income compared with the highest level. Those living in the most deprived areas had the highest prevalence or incidence of multimorbidity (pooled OR 1.42, 95% CI 1.41 to 1.42). Associations between deprivation and multimorbidity differed by age and multimorbidity type. Findings from the few studies investigating household tenure, household composition and area-level rurality were mixed and contradictory; homeownership and rurality were associated with increased and decreased multimorbidity, while living alone was found to be associated with a higher risk of multimorbidity and not associated. In conclusion, improving the understanding of broader social determinants of multimorbidity, particularly at the household level, could help inform strategies to tackle multimorbidity. (Ingram E, Ledden S, Beardon S, *et al.* Household and area-level social determinants of multimorbidity: a systematic review. *J Epidemiol Community Health* 2021;75: 232-241).

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THYROGLOSSAL DUCT CYST - OLD AND NEW SURGICAL APPROACH

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THYROGLOSSAL DUCT CYST - OLD AND NEW SURGICAL APPROACH (Abstract): The thyroglossal duct cyst (TDC) results from a failure in obliterating the embryogenic duct produced during thyroid migration and it represents the most common type of developmental cyst encountered in the neck region. **Material and methods:** In the First Surgical Clinic from "Sf. Spiridon" County Clinical Emergency Hospital Iasi, Iasi, between 1998 to 2021, 21 patients with thyroglossal duct cyst were diagnosed and treated. All medical records were reviewed. **Results:** The thyroglossal duct cysts are located as midline cysts of the neck in all cases. The mean age was 39.2 years (13-66 years). The treatment performed was a variant of Sistrunk's procedure in which the thyroglossal tract was excised to a variable extent, with central hyoidectomy. The size of the cyst ranged from 1.2 to 4 cm (mean 2.6 cm). Postoperative course was uneventful in all cases. No recurrence was recorded in this series. We describe a case, a 42 years old women, with asymptomatic mass in the anterior part of the neck found a 6 months before, when the patient was referred us with lithiasic obstructive jaundice (we performed ERCP + laparoscopic cholecystectomy). The treatment performed was a variant of Sistrunk's procedure in which the thyroglossal tract was excised by minimally invasive video-assisted approach, variation of Miccoli's technique. Postoperative course was uneventful. The follow-up 1 and 2 years no recorded recurrence and thyroid scintigraphy was normal. **Conclusions:** The standard surgical approach to TDC is Sistrunk's operation with low recurrence rates. Although the results and the operative time are the same as in traditional surgery, minimally invasive video-assisted approach has better cosmetic result and a less painful course. **Keywords**: THYROGLOSSAL DUCT CYST, MINIMALLY INVASIVE VIDEO-ASSISTED APPROACH, SISTRUNK'S OPERATION.

The thyroglossal duct cyst (TDC) is a developmental abnormality who results from a failure in obliterating the embryogenic duct during thyroid migration and it represents the most common type of cyst from neck region (1). The location of the cyst can be intralingual, suprahyoid, thyrohyoid or suprasternal region or even in hyoid bone (2). TDCs are diagnosed in children but there is also a small percentage of the adult population. A cyst is a mobile, painless mass in the anterior midline of the neck and most patients present inside the cyst ectopic thyroid tissue which in rare cases can cause the development of neoplasms. The mean rate of overall recur-

rence reported in literature is 11% and the Sistrunk procedure appears to be the better choice for the therapy of TGDCs to avoid recurrences (3).

MATERIAL AND METHODS

In the First Surgical Clinic from "Sf. Spiridon" County Clinical Emergency Hospital Iasi, between 1998 to 2021, twenty-one patients with TDCs were selected and all medical records were reviewed.

In twenty cases with TGDCs we performed traditional Sistrunk's surgery and in one case the thyroglossal cyst was excised by minimally invasive video-assisted approach. The inclusion criteria were diagnosis of a TGDC by physical examination and ultrasound scan. All patients provided informed consent after the advantages and disadvantages of each procedure.

The statistical data were compared with *SPSS for Windows 10* and a p-value of <0.05 was considered statistically significant.

RESULTS

There were 18 females (85.7%) and 3 males (14.3%) with mean age 39.2 years ± 12.5 years (13-66 years) with no significance differences between female and male patients. The mean age of onset of symptoms was 24± 2.5 years. The history of the disease varied from 1 month to 36 years. 20 patients with thyroglossal duct cysts are described as midline cysts of the neck and only in one case the cyst was located laterally.

The commonest reasons for seeking treatment were cystic neck mass which moved with protrusion of the tongue and could be transilluminated (17 cases), neck swelling (2 cases), neck infection (1 case) and dysphagia (1 case). The level of the cysts in the neck was as follows: 2 in the suprahyoid region, 12 in the prehyoid region, 6 at the level of the thyroid cartilage without any statistical significance related to age, sex or size (*t-student,* χ^2, p>0.05). One case was diagnosed with an external fistula with recurrence of 10 years after cyst removal. In three cases thyroid pathology (goiter) was associated.

In this series all patients were operated on, only one case initially was treated with antibiotics for infected thyroglossal cysts. The treatment performed was a variant of Sistrunk's procedure in which the thyroglossal tract was excised to a variable extent. We performed a 4-5 cm horizontal skin incision in the cervical region and created a flap that extended 1 cm above the hyoid bone, divided the median raphe of the strap muscles, dissected and cut the hyoid bone with careful dissection of the tract, extending to the tongue base.

The size of the cyst ranged from 15 to 60 mm (mean 29 mm ± 18.33 mm). The mean blood loss was 25 mL. (ranging from 10 to 57 mL) being statistically significant related to the size of the cyst (t student, p=0,008). Postoperative course was uneventful in all cases. The mean hospital stay was 4.8 ± 2.56 days. Although there were no significant differences in terms of cyst location and hospital stay length (χ^2, p>0.05). In this series no recurrence was recorded. The cysts were successfully removed in all cases, and the pathology results indicated that all of the cysts were TGDCs.

In one case the histopathologic diagnosis was a papillary carcinoma evolving from a TDC. Initial we didn't do anything because the clinical exam, ultrasound and scintigraphy of the thyroid was normal, but after ten years the patient developed a nodular goiter and we performed total thyroidectomy.

We describe a case in view in order to highlight of the new therapeutic option for the surgical management. 42-year-old women was admitted in emergency in the First Surgical Clinic, "Sf. Spiridon" County Clinical Emergency Hospital Iasi with lithiasic

obstructive jaundice for which it was performed endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic sphincterotomy and extraction of gallstones of 1 cm and laparoscopic cholecystectomy with uneventful postoperative course. At the clinical examination of the patient we discovered an asymptomatic mass in the anterior part of the neck. Ultrasound scan revealed a cystic mass of 37 mm.

Fig. 1. Ultrasound scan - 37 mm thyroglossal cyst with a thick content.

After 6 months the patient had referred us and the clinical diagnosis discovered a cystic mass 3 cm, fibroelastic, which moved with protrusion of the tongue mobile and during swallowing. The cyst is located towards the front of the midline neck and could be transilluminated. Ultrasound scan revealed a slight increase in the size of the cyst to 37 mm with a thick content and thyroid gland was within normal limits (fig. 1). The thyroid scintigraphy was normal. CT scan was not performed.

The treatment performed was a variant of Sistrunk's procedure by minimally invasive video-assisted central neck approach, variation of Miccoli's technique. The patient was positioned on the operating table in dorsal decubitus, without neck hyperextension. A horizontal incision is made 2 cm above the suprasternal notch, in an existing skin fold, and created a working space by retraction with 2 divergent Farabeuf retractors (fig. 2). Under visual control the cyst was excised with clip application to the base of the thyroglossal tract and suturing in anatomical layers (fig. 3). The operation time was 45 minutes, blood loss was minimal and without drainage.

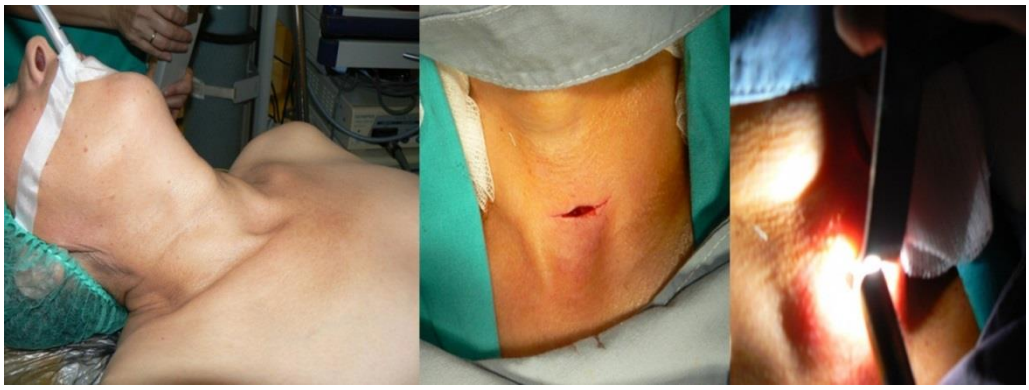

Fig. 2. The patient's position on the operating table in dorsal decubitus without neck hyperextension; a horizontal incision is made 2 cm above the suprasternal notch; created a working space by retraction with 2 divergent Farabeuf retractors.

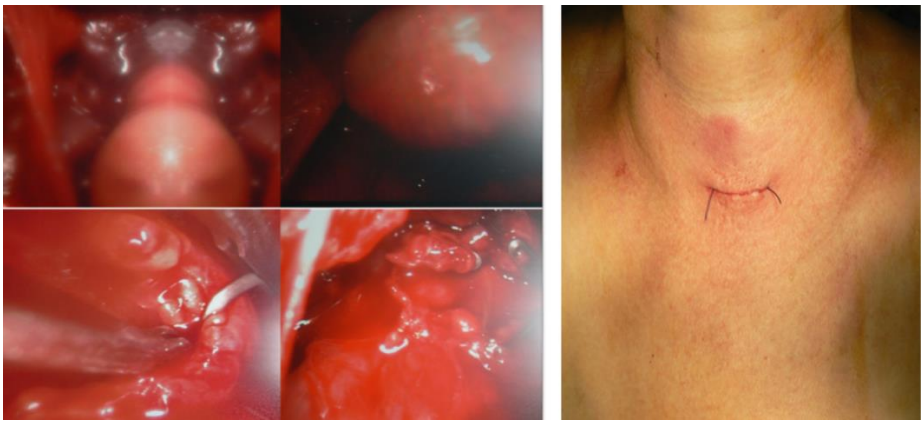

Fig. 3. Excision of the cyst under visual control with clip application to the base of the thyroglossal tract and final appearance after intradermal skin suture.

Postoperative course was uneventful and the patient was discharged after two days. The histopathologic exam revealed presence in cyst of ectopic thyroid tissue. The follow-up 1 and 2 years no recorded recurrence and thyroid scintigraphy was normal.

DISCUSSION

The failure of obliteration of thyroglossal duct in the 10th week of fetal life may result in the development of a cystic dilatation at any time in life. TDCs are localized towards the midline between the base of the tongue and the pyramidal lobe of the thyroid gland. Most of them are diagnosed in in the pediatric age group and few cases are diagnosed in adulthood (1).

The clinical diagnosis reveals a non-tender, mobile neck mass, which was painful at swallowing in the anterior midline of the neck, usually in close proximity to the hyoid bone and in rare cases the cysts are located laterally (1, 3).

Usual preoperative evaluation of thyroglossal duct cyst includes a cervical radiography, ultrasound scan, scintigraphy with 131I, thyroid function tests and for cases with malignancy and ectopic cysts, Computerized tomography has also been performed for selected cases.

The standard surgical approach to TDCs is Sistrunk's operation: removal of the mid-portion of the hyoid bone in continuity with the TDC and excision of a core of tissue between the hyoid bone and the foramen cecum. This procedure remains the gold standard for surgical management of TGDCs.

The first video-assisted neck surgery, namely a subtotal parathyroidectomy, was performed by Michel Gagner in 1996 and minimally invasive video-assisted central neck approach was first described by Miccoli in 1998 for thyroid pathology. The first thyroidectomy by minimally invasive video-assisted approach (MIVAT) in Romania was performed in 2009 at the First Surgical Clinic, "Sf. Spiridon" Hospital by Bradea and Târcoveanu (4).

About minimally invasive video-assisted approach in thyroglossal duct cyst there are few references in the literature. Huang Z *et al.* describe no recurrences after 15 endoscopic thyroglossal duct cystectomy (5). Woo SH *et al.* considering the

embryological development of TGDCs, can open a new access route to these cysts. Performed endoscope-assisted transoral removal in 13 cases with mean operation time 67.33 ± 17.26 minutes, no scars and also no recurrences (6). Han P *et al.* performed surgical resection using a submaxillary vestibular approach in 16 cases with TDCs with good results: no postoperative complications and no conversions of the operation (7). Byeon HK *et al.* describe first robot-assisted approach for synchronous occurrence of thyroglossal duct carcinoma and thyroid carcinoma (8).

When a histopathological examination reveals a malignancy, the thyroid gland must be studied radiologically and scintigraphically. Sistrunk's procedure would suffice if the thyroid gland was found to be normal (9). The occurrence of carcinoma of the thyroglossal duct is reported to be less than 1%. The clinical diagnosis of thyroglossal duct cyst is completed by ultrasound (US) scan and fine-needle aspiration cytology (FNAC) in order to plan the correct surgery for a possible carcinoma. In these cases, total thyroidectomy should be associated with Sistrunk procedure (SP) to permit the correct treatment and follow-up (10). For patients with thyroglossal duct cyst carcinoma and a normal thyroid gland, a mutational marker BRAFV600E positivity seems to be predictive of locally advanced disease mandating radioactive iodine ablation (RIA) therapy, therefore total thyroidectomy, in addition to Sistrunk's procedure (11).

CONCLUSIONS

The standard surgical approach to TDC is Sistrunk's operation with low recurrence rates. Although the results and the operative time are the same as in traditional surgery, minimally invasive video-assisted approach is a safe and effective procedure with better cosmetic outcomes and a less painful course.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

CARDIAC MYXOMA AS CYTOKINE PRODUCING TUMOUR: A REVIEW

Cardiac myxoma is the most common primary cardiac tumor. Many cytokines participate in the pathophysiology and growth of cardiac myxoma. Inflammatory cytokines, including interleukin-1, interleukin-4, interleukin-6, interleukin-8, interleukin-12, tissue necrosing factor-α, and interferon-γ, contribute to the development of inflammation and inflammation-related symptoms and further affect tumor growth. Growth factors, including vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor 1, and epidermal growth factor, contribute to angiogenesis and tumor growth and interfere with the inflammatory response. This review summarizes the current knowledge of cardiac myxomas as cytokine-producing tumors (Ajiro Y. Cardiac myxoma as cytokine producing tumor: A review. *Interv Cardiol* 2021; 13(S3): 58-63).

DIAGNOSIS OF MYOCARDIAL INFARCTION AT AUTOPSY: AECVP REAPPRAISAL IN THE LIGHT OF THE CURRENT CLINICAL CLASSIFICATION

Ischemic heart disease is one of the leading causes of morbidity and death worldwide. Consequently, myocardial infarctions are often encountered in clinical and forensic autopsies, and diagnosis can be challenging, especially in the absence of an acute coronary occlusion. Precise histopathological identification and timing of myocardial infarction in humans often remains uncertain while it can be of crucial importance, especially in a forensic setting when third person involvement or medical responsibilities are in question. A proper post-mortem diagnosis requires not only up-to-date knowledge of the ischemic coronary and myocardial pathology, but also a correct interpretation of such findings in relation to the clinical scenario of the deceased. For these reasons, it is important for pathologists to be familiar with the different clinically defined types of myocardial infarction and to discriminate myocardial infarction from other forms of myocardial injury. This article reviews present knowledge and postmortem diagnostic methods, including post-mortem imaging, to reveal the different types of myocardial injury and the clinical pathological correlations with currently defined types of myocardial infarction (Michaud K, Basso C, d'Amati G, *et al.* Diagnosis of myocardial infarction at autopsy: AECVP reappraisal in the light of the current clinical classification. *Virchows Archiv* 2020; 476: 179-194).

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COMPARATIVE STUDY ON CLASSIC *VS*. DIGITAL IMPRESSION IN FIXED PROSTHETICS

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COMPARATIVE STUDY ON CLASSIC *VS*. DIGITAL IMPRESSION IN FIXED PROSTHETICS (Abstract): Intraoral digital impression is a revolutionary breakthrough and an important step forward in dentistry due to its accuracy in achieving high-precision prosthetic work on natural teeth or dental implants. The **aim** of this study is to analyze the established differences between the digital impression and the classic impression in relation to the specific features of the clinical case. **Material and methods:** The study included 58 patients diagnosed with partially reduced edentulousness or with extensive destruction of the dental units who received fixed therapy anchored in a non-metallic registry of prosthetic restorations represented by ceramic crowns on zirconium support. **Results:** Optical impression in conventional prosthetics plays a very important role as it is the first step in the complete digital line of prosthetic construction. The accuracy and precision of the impressions are key points in the marginal fit of the prosthetic work and therefore in the success of the prosthetic treatment. **Conclusions:** Dental digital impression is a non-invasive, simple, practical, accurate and patient-friendly procedure that eliminates many of the inconveniences of conventional impressions. **Keywords:** DIGITAL IMPRESSION, CLASSIC IMPRESSION, FIXED RESTAURATION.

Impression in dentistry is an essential step in the realization of various prosthetic dental works (in fixed prosthetics - dental crowns, crowns on implants, dental bridges or in mobile and removable ones), orthodontic, or surgical impression materials have been developed and improved over time, from the classic plaster, Stents, to irreversible hydrocolloids and synthetic elastomers. Despite the impeccable quality of modern impression materials, there remain drawbacks during the impression stage that can ultimately jeopardize the quality of prosthetic restorations and their fit on the patient's prosthetic field. Given the astonishing progress of technology, this is inevitably also felt in the field of dentistry (1, 2).

Intraoral digital impression is a revolutionary breakthrough and an important step forward in dentistry due to its accuracy in achieving high-precision prosthetic work on natural teeth or dental implants. Optical impression in conventional prosthodontics plays a very important role as it is the first step in the complete digital line of prosthet-

ic construction. The accuracy and precision of the impressions are key points in the marginal adaptation of the prosthetic work and therefore in the success of the prosthetic treatment (3).

Some of the **benefits** of digital impressions are represented by:

1. Elimination of possible causes of errors due to traditional classic impressions, such as: Incorrect choice of impression tray, either of the wrong size or made of plastic material, which can easily deform; the most suitable tray for the classical procedure is the individual resin impression tray, adapted to the unique situation in each patient's mouth; since it does not require an impression tray, there is no longer the inconvenience of the impression material not adhering to the tray and deforming on removal; if the impression material is stored improperly, its setting time changes, so additional deformation may occur; it is sometimes necessary to redo the impression if areas of missing substance or incorrect registration are observed, which means wasted material and extra time spent in the office; if defects appear in the working model, the impression must be returned, but its fidelity will no longer be as it was originally; in the case of digital impressions, the information is not lost, as it is stored in the computer, so the fidelity of the impression will be fully preserved (4, 5).

2. Increased patient acceptability: The small size of the intra-oral scanner eliminates the inconvenience of holding the bulky, material-laden impression tray in the mouth, which makes many patients feel sick; at the same time, a number of patients may experience allergic reactions to impression materials, which do not occur with digital technology; the risk of swallowing impression material if handled incorrectly is eliminated. The method is advantageous for patients who have problems tolerating conventional impressions for various reasons: microstomia, exaggerated gag reflex, torus (maxillary or mandibular), other retentive areas in the prosthetic field that may interfere with the removal of the impression after the impression material has been taken (1, 6).

3. The dental digital impression is also more comfortable and safer for the clinician, as it does not need to be sanitized like a traditional impression, so contact with patient fluids and tissue debris is reduced and the risk of contamination with various pathogens is minimized; ♦ high accuracy leading to perfect adaptation of work; automatic and immediate color registration; ♦ immediate control and verification of dental preparations.

4. Reduced distortion of impression material;

5. Accuracy - any microdetail of the prosthetic field (teeth, implants, gums, fillings, work) are recorded at micron level with maximum accuracy

6. A wide range of dental indications (crowns, veneers, inlays, root canal devices, implants)

7. 3D preview of the preparation;

8. Reduced costs of impression materials, individual impression trays, plaster for model making; ♦ no need to stock and purchase impression trays and impression materials; ♦ the need for long-term storage of models is eliminated.

9. No risk of microbial contamination for the dental laboratory (no physical contact with the impression);

10. The information (impression) can be transmitted electronically immediately to the laboratory (by e-mail);

11. Clinical data related to the preparation, neighboring teeth, antagonists, intermaxillary reports, are kept in files, without

any changes, for a virtually unlimited period of time.

12. Quality treatment - an accurate impression will always generate an accurate work.

Dental digital impression is therefore a non-invasive, simple, practical, accurate, patient-friendly procedure that eliminates many of the inconveniences of traditional impressions, but for an exceptional final result, the doctor's contribution is important. Even if in the future research will continue and dentistry will rely on modern instruments, the human factor is indispensable and cannot be substituted (7, 8).

MATERIAL AND METHODS

The aim of this study is to analyze the established differences between the digital impression and the classic impression in relation to the specific features of the clinical case.

The study included 58 patients diagnosed with partially reduced edentulousness or with extensive destruction of the dental units who received fixed therapy anchored in a non-metallic registry of prosthetic restorations represented by ceramic crowns on zirconium support.

A series of parameters were analyzed: the time allocated to the registration of the impression, a particularly important stage in the clinical-technological algorithm of fixed prosthodontics, the clinical evaluation of the restorations in the frame in the case of optical impression prosthodontics and classical impression prosthodontics, as well as the patient evaluations using a visual analogue scale.

RESULTS

The results of the study are presented in first table. The time spent on the digital impression is much shorter than the classic impression, the digital technique being more convenient for both clinicians and patients.

TABLE I

The results of the study

No.	Procedure	Time spent (classical technique)	Time spent (digital technique)
1	Impression	11:14 ± 01:33	07:10 ± 02:10
2	Dentist`s assessment	47.01 ± 20.10	23.39 ± 17.03
3	Patient`s assessment	43.79 ± 26.10	6.20 ± 5.32

DISCUSSION

However, there are also **obstacles and difficulties** encountered in optical impressions. These include the following: ♦ some systems require the teeth to be powdered with a special spray prior to scanning and the uneven layer of powder can lead to inaccuracy; ♦ complexity - the equipment is sophisticated and requires instruction and training to master the technique; ♦ the time needed for the actual scanning maneuver is longer compared to traditional impressions; ♦ cost of purchasing equipment (scanner, software and/or milling machine - for closed systems); ♦ the size of scanners (e.g., iTero) can be problematic when trying to impression 2nd or 3rd molars; ♦ in the case of subgingival preparations, it is difficult to impression the preparation margins.

Even if the digital impression technique proves its superiority in many aspects, including ease of execution, it has a weakness, which can only be overcome with the help of the clinician's experience and pro-

fessionalism. We are talking about the registration of subgingival preparations and the gingival sulcus, which can compromise the marginal fit of the prosthetic piece, making treatment a failure. The scanner records the same images that the clinician can perceive with the naked eye, so for the correct reproduction of the gingival boundary it is mandatory to ensure visibility, by correctly performing the gingival eviction and by the best possible isolation of the prosthetic field (the dam being the most suitable) (9, 10).

Digital impression is performed by intraoral scanning, a method using the triangulation technique. Intraoral scanners (IOS) are devices used to take digital impressions and collect information about the shape and size of dental arches by emitting a light beam. They project a light grid (structured light or laser) onto the tooth surface and capture, through high-resolution cameras, the distortion that such a beam undergoes when it hits those structures (Fig. 1).

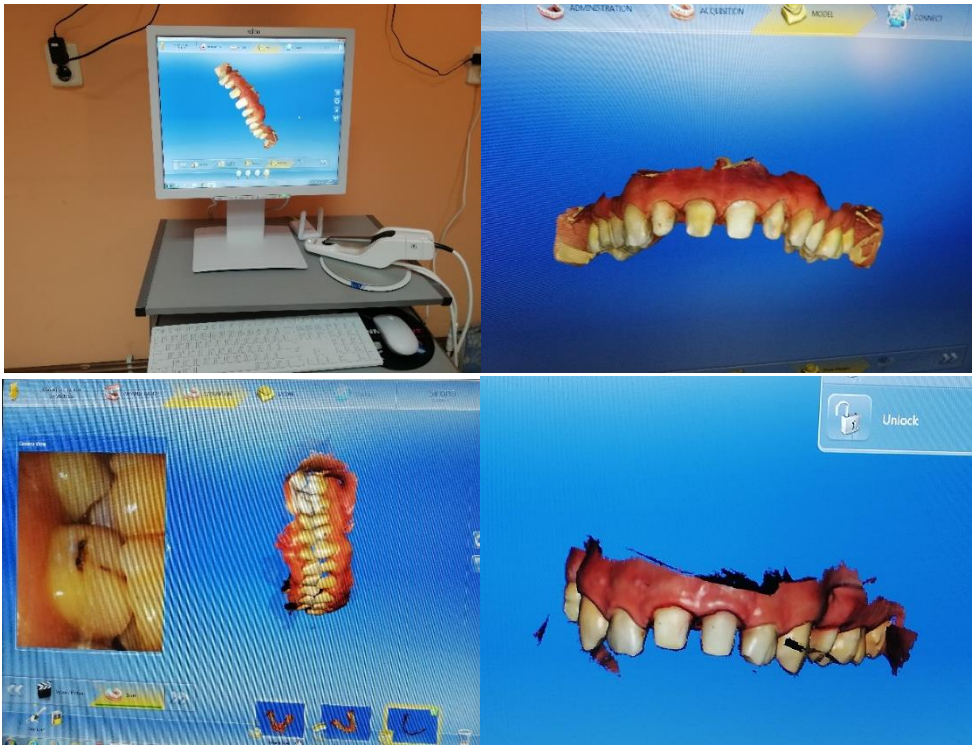

Fig. 1. Practical aspects of digital impression

The intraoral scanning protocol involves the following clinical steps: ♦ application of a retraction wire to expose the contour of the polished bridge and subgingival sills; ♦ directing the scanner to capture the image in multiple directions, specific to each type of scanner, according to the manufacturer's instructions, in order to obtain a digital impression of maximum precision (7, 11); ♦ scanning antagonists following the same principle.

Scanning of the prepared tooth or pros-

thetic field can be done by two procedures: ♦ intraoral scanning, direct and fast, the data can be sent immediately by e-mail in STL format to the CAM laboratory where the prosthetic work is made; ♦ extraoral scanning, indirect, applied on the conventional final impression or on the cast model after the impression sent to the laboratory; although it is a more time-consuming procedure and the impression can be a potential source of error, it is preferred by the doctor because of the cost of the investment for the intraoral scanner (12, 13).

Intraoral image capture is performed by shooting with multiple frames of approximately 3 teeth in the case of iTero (Align Technology), Trios Color (3 Shape) or CS 3500 (Carestream Dental LLC) scanners, respectively video shooting as found in True Definition Scanner (3M ESPE), CEREC Omnicam (Sirona), Apollo DI (Sirona), PlanScan (Planmeca) equipment (Iliescu). The registration of larger areas is done by superimposed images that are assembled by a computer program, generating a virtual 3D model in which the areas not directly imaged are completed by extrapolation. Inaccuracy of the fit of the prosthetic restoration to the tooth is due to two factors (14, 15, 16): ♦ main factors: accuracy of tooth preparation and the appropriate 3D virtual design on the computer; ♦ additional dental factors: location of the coronal prosthetic preparation margins, location of the crown equator, location of retentions; ♦ digital additional factors: quality of digitized data acquisition, quality of data processing, efficiency of noise removal filters induced by measuring systems (14, 15).

To date (2017), the scientific literature considers the accuracy of optical impressions to be clinically satisfactory and similar to that of conventional impressions in single-tooth restorations and fixed partial dentures up to 4-5 elements. However, optical impressions do not appear to have the same accuracy as conventional impressions for larger restorations (fixed partial dentures with more than 5 elements or full bridges on natural teeth or implants). However, accuracy and precision are not the only elements that can differentiate commercially available devices today. A whole series of elements (need for powder application, scanning speed, tip size, color detection capability) differentiate scanners in terms of their clinical use (17, 18, 19).

Scanners are very useful devices and are indicated in various areas of dentistry (both for diagnosis and for the fabrication of restorations): prosthodontics, implantology, orthodontics.

In 2020, Kanchan Aswani *et al*., analyzed 507 PubMed indexed articles (2018-2019). Following the application of exclusion criteria (articles published in a language other than English, review articles, articles evaluating the marginal fit of different restorations, etc.) 412 articles were eliminated. Only 95 articles that met the inclusion conditions (articles published in English, clinical or laboratory studies, etc.) were considered. Compared to conventional impressions, optical impressions eliminate several clinical and laboratory steps in the fabrication of prosthetic parts.

Thus, the clinical steps eliminated are: ♦ choice of impression tray; ♦ preparation of the material; ♦ the actual impression; ♦ decontamination of the impression; ♦ referral to the dental laboratory. The laboratory steps eliminated are: ♦ casting the model; ♦ sectioning; ♦ establishing intermaxillary ratios; ♦ scanning the models individually and in occlusion.

In 2014 Yuzbasioglu *et al.* conducted a study on 24 patients (12 males, 12 females).

The study findings were: ♦ the digital impression technique was more efficient than the conventional technique (the working time for the conventional impression was longer than that for the digital technique); ♦ compared to the conventional technique, the digital technique was more efficient; it was accepted and preferred by patients; ♦ the comfort of the digital technique was higher than that of the conventional method.

CONCLUSIONS

Due to its accuracy, short duration, patient comfort and last but not least due to the reduction of material consumption, intraoral scanning has gained increasing popularity and is expected to gradually replace the traditional impression. With the help of a high-performance intraoral scanner, it is now possible to quickly send an accurate digital impression electronically to the dental laboratory directly in the dental practice by the dentist. The introduction of digital technology as a standard procedure for the fabrication of prosthetic restorations is unfortunately still hampered by the need to train highly qualified staff and by the cost price reduction, which is still far too high compared to conventional procedures. Optical impression in conventional prosthetics plays a very important role as it is the first step in the complete digital line of prosthetic construction. The accuracy and precision of the impressions are key points in the marginal fit of the prosthetic work and therefore in the success of the prosthetic treatment.

Dental digital impression is a non-invasive, simple, practical, accurate and patient-friendly procedure that eliminates many of the inconveniences of conventional impressions. Digital impression was originally used in prosthodontics as part of CAD/CAM technology to shorten work steps and achieve a fully digital workflow. In dentistry, digitized technology is slowly but surely taking the place of traditional procedures. From diagnosis to the final tooth, any dental procedure performed digitally is more accurate, safer, and better tolerated by the body.

CONFLICT OF INTEREST AND FUNDING

Authors declare that there is no conflict of interest, and they received no funding for this study.

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NEWS

HIGH-DOSE OF ASCHORBIC ACID CAN IMPROVE SYMPTOMATOLOGY OF CORONAVIRUS INFECTION

Following the outbreak of the pandemic, whose promoters are SARS CoV-2 and MERS CoV, studies show that an attempt was made to quickly find a solution that involved immunizing the population, making a vaccine, or finding the right treatment to increase survival by administering antivirals, corticosteroids or of other substances that stimulate immunity and decrease the oxidative stress potentiated by the infection. According to studies, the oxidative way in which viruses cause damage to organs, finding that at the base is an oxidation-reduction reaction that causes severe inflammatory reactions, increasing the number of reactive oxygen species degenerating the antioxidant system of the airways. Related studies have shown the positive effect of high doses of vitamin C by significantly lowering inflammatory markers by its reducing effect that reduces reactive oxygen species that cause such serious damage. (Hoang BX, Shaw G, Fang W, Han B, Possible application of high-dose vitamin C in the prevention and therapy of coronavirus infection. *J Glob Antimicrob Resist* 2020; 23: 256-62).

CURRENT APPROACHES IN FIXED PROSTHESES ON ZIRCONIUM SUPPORT

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CURRENT APPROACHES IN FIXED PROSTHESES ON ZIRCONIUM SUPPORT (Abstract): Zirconium oxide is a superior material in terms of biocompatibility with adjacent tissues, color similar to natural teeth, resistance to daily masticatory stress and abrasion, chemical stability in high-quality restorations and last but not least translucency. The aim of the study is to individualize the therapeutic aspects of non-metallic zirconia restorations anchored in the register of contemporary non-metallic dentistry in accordance with the particularities of clinical cases and the requirements of the technological line addressed. A total of 34 zirconia-supported ceramic prosthetic restorations were made for the morpho- functional rehabilitation of various edentulous areas of the maxillary and mandibular arches. As far as the technological specifics of these types of restorations are concerned, Exocad software and MEDIT 300 scanner and Cerec CAD-CAM system were used. In dentistry, digitized technology is slowly but surely taking the place of traditional procedures. From diagnosis to the final tooth, any dental procedure performed digitally is more accurate, safer, and better tolerated by the body. **Keywords:** ZIRCONIUM, CAD/CAM, SOFTWARE, AESTHETICS.

In terms of technological developments and biomaterials required in complex oral rehabilitations, for a long time acrylic resins, metals, composites and ceramics have been chosen in combination with noble (gold or silver-palladium based), non-noble (Cr-Ni, Cr-Co, Cr-Ni-Be based), titanium alloy infrastructures. Moreover, modern all-ceramic systems (InCeram, Empress, Vita), zirconium oxide systems, all-polymer systems, and therapeutic solutions based on these materials are used in contemporary prosthetics to improve the aesthetic result (1, 2, 3).

The social impact on the patient makes the dentist's choice of biomaterial a decision based on relevant arguments, considering the particularities of the clinical case in question and the patient's aesthetic requirements (4, 5, 6).

Currently, all-ceramic restorations are the most commonly used therapeutic alternative compared to ceramic work on metal infrastructure. Although the lack of either alloy or zirconium infrastructure may cause a decrease in mechanical strength over

time, biocompatibility achieves superior aesthetic ratings, thus becoming a solution that captures the attention of patients who place a primary emphasis on aesthetics (7, 8, 9).

Another material that revolutionized dentistry and was discovered in 1789 by the German chemist Martin Heinrich Klaproth is zirconium oxide, a white-ivory-colored ceramic material noted for its variety and remarkable properties such as high flexural strength and hardness. Zirconium oxide is a superior material in terms of biocompatibility with adjacent tissues, color similar to natural teeth, resistance to daily masticatory stress and abrasion, chemical stability in high-quality restorations and last but not least translucency (10, 11, 12).

These advantages make it the material of choice in contemporary dentistry (for crowns, bridges, implants), but also in the medical field in general for the production of bone prostheses, hearing aids, etc.

There are two types of zirconium oxide restorations:

- Restorations made entirely of zirconium which have a very high resistance to mastication forces and can therefore also be used for prosthetic work in the lateral area(13, 14).
- Restorations with a ceramic layer on zirconium substructure, which can be indicated especially in the frontal areas of dental arches.

The realization of a ceramic prosthetic work on zirconium infrastructure requires the accurate completion of an algorithm that includes clinical and technological steps that follow a certain sequence.

AIM

The aim of the study is to individualize the therapeutic aspects of non-metallic zirconia restorations anchored in the register of contemporary non-metallic dentistry in accordance with the particularities of clinical cases and the requirements of the technological line addressed.

MATERIAL AND METHODS

A total of 34 zirconia-supported ceramic prosthetic restorations were made for the morpho functional rehabilitation of various edentulous areas of the maxillary and mandibular arches. As far as the technological specifics of these types of restorations are concerned, Exocad software and MEDIT 300 scanner and Cerec CAD-CAM system were used.

The clinical and paraclinical examination of the patient is the first stage of the algorithm, in which the diagnosis will be clarified, and the treatment plan will be established in accordance with the wishes of the patient, who must participate in the establishment of this therapeutic strategy, giving their consent in the case of treatment objectives. The preparation of the prosthetic field, the preparation of the organic substructures with the least possible sacrifice of amelo-dental tissue is the next extremely important stage. Special attention must be paid to the space required for the future prosthetic construction, in relation to neighboring and antagonist teeth, so as to ensure optimal retention and the possibility of insertion. The preparation of the socket is equally important as it directly conditions the marginal dento-prosthetic closure. After ticking off the specific preparations for the application of ceramic prostheses on the zirconium infrastructure, the dentist

carries out the registration of the main and peripheral elements of the prosthetic field.

RESULTS AND DISCUSSION

Prosthetic restorations made of zirconia-supported ceramics have brought together both single restorations and small-width dental bridges in keeping with the partially reduced edentulousness they have rehabilitated.

Thus, a percentage of 48.7% was recorded for single crowns that rehabilitated dental units affected by coronal lesions with varying degrees of destruction, while rehabilitation using dental bridges covered a percentage of 51.3% that ensured morphological restoration of dental arches in accordance with the parameters of the potential prosthetic spaces (fig. 1).

As regards the location of prosthetic restorations of single crowns were located in the maxilla, 57.9% in the posterior region and 85.4% in the anterior region, which was recognized by the degree of aesthetic requirements of the restorations. As regards the prevalence of prosthetic restorations made of zirconia-supported ceramic, 14.6% of the restorations were located in the anterior mandibular area and 42.1% in the posterior area, which is associated with the degree of aesthetic restoration in conjunction with the degree of visibility during the tooth movement. As regards the topography of the zirconia-supported ceramic bridges, they were found in the posterior area in a percentage of 64% for the maxilla and 36% for the mandible, while the anterior locations were in a proportion of 66.7% for the maxilla and 33.3% for the mandible. The choice of these types of restorations was correlated with the masticatory forces with the potential prosthetic spaces with the other types of restorations, an important role being played by the static occlusion and the mandibular dynamic trajectories, as well as the type of smile for each individual patient. (fig. 2).

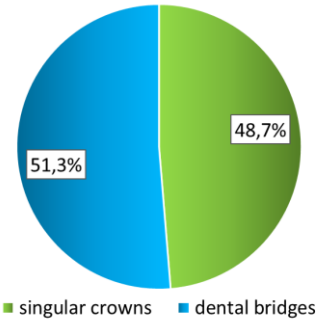

Fig. 1. Esthetic ceramic restoration on zirconia framework

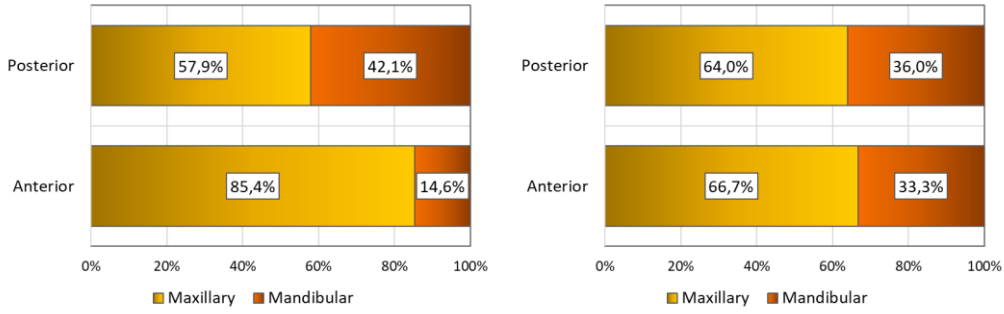

Fig. 2. Topography of single zirconia-supported ceramic crowns and bridges

Grafted on the addressed problem we selected some representative aspects. Restorative treatment begins with the preparation of the organic substructures by the dentist, according to the type of restoration, to create sufficient space for both components for optimal insertion and adaptation. From the clinical-technological algorithm we have selected the steps listed in the digital register with the identification of the working features.

The complex digital workflow uses Exocad software, DentalDB 2.2 Valletta and starts with the creation of the patient record in the Job definition window.

Scanning working models and occlusion: The Exocad software and the MEDIT 300 scanner are used to scan the entire complex. After selecting the simulator and the type of preparation, both the maxillary model, followed by the prepared prosthetic abutment and the mandibular model, together with the prepared abutments positioned according to the computer's instructions on the special holder (fig. 3) are inserted and fixed on the existing table inside the scanner. Finally, the models are scanned in occlusion, resulting in virtual models with the abutments positioned appropriately in the dental arches.

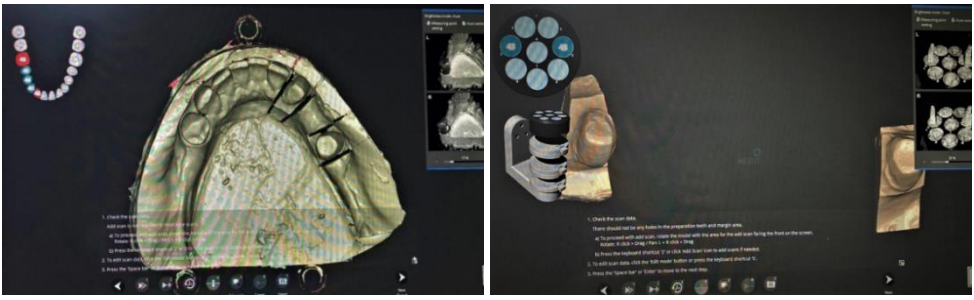

Fig. 3. Virtual representation of the lower model with movable abutments

Detection of the collars of each prepared substrate: In this case, the tracing of the collars was done automatically by making a few modifications to each abutment to obtain a more uniform and linear collar. **Determination of the unique insertion direction and thickness of the zirconia infrastructure:** For a perfect fit of the restoration, it is necessary to establish a single insertion axis for both the lower bridge and the individual upper crown, without certain differences in the proximal and occlusal faces. In the same step we select the space required for the luting cement of about 0.05 mm and the thickness of the zirconia infrastructure which is 0.6 mm in general (fig. 4).

Following these steps, the actual design of the zirconia caps and intermediate is carried out with the help of the software, which renders a general shape of a tooth gallery, according to previously established parameters.

Modifications are made to this shape using add, smooth, remove and also gingival adaptation of the bridge body. Once the optimal shape of the zirconia support has been established, the connectors are applied to connect the caps and the bridge body to each other.

A final step after completion of the zirconia infrastructure design is to save the design and import the file into the CAM software, where it will be placed on the zirconia disc belonging to CeraDirect, Super High Translucent Multilayer and programmed for milling .

Fig. 4. Establishing the insertion axis

Milling of the zirconium support: The disc is inserted and clamped in the 51D Roland enclosure, which mills the zirconia substructure in 5 axes using three types of milling cutters of 0.6 mm, 1 mm and 2 mm (figs. 5 and 6). After completion of the whole process, the milling machine is vacuumed, and the disc is removed and cleaned of impurities with an air jet.

Then the connectors supporting the caps are sectioned using special diamond cutters and the infrastructure is adapted to the level of the prepared organic substructure

Fig. 5. Fixing the zirconium disc

Fig. 6. Milling of zirconium infrastructure

Sintering: This stage aims to increase the strength and translucency of the previously adapted infrastructure. The prosthetic restorations, i.e., zirconium caps, are placed in ceramic vessels and then in the Nabertherm LHT 01/17D sintering oven .

They are subjected to a special sintering regime, which is usually specified by the zirconium disc manufacturer, rising to temperatures of 1,450°C over 6-18 hours.

Following the sintering process, the zirconium caps will have a different, undersized and colored appearance from the original appearance when removed from the disc. At this level a first sample of the infrastructure can be made in the dental surgery.

Application of ceramic masses: The layering technique is used, and the application of ceramic bodies is done with special brushes. A layer of Ivo color glaze is brushed onto the surface of the zirconium framework prepared for layering and powdered with a ceramic or silicate powder on

the lateral and occlusal sides to achieve better retention.

The caps are then placed on a refractory support and placed in the firing furnace at temperatures above 800°C for 14 min.

The ceramic layers shall be applied by the technique mentioned as follows:

- Application of IPS e. max Ceram dentin layer, D2 shade, with the help of a brush, in a thicker layer, achieving an oversized morphology. Place the work in the burning oven at 745°C for 15-20 min. After removal from the oven, wait for cooling and if necessary, the work can be processed.

After processing, the restoration is prepared for the glazing stage by removing impurities with the steamer and drying the work with an air jet.

- Application of the final glaze layer consists of brushing a liquid of viscous consistency over the entire surface of the lower dental bridge and the individual upper crown. Firing in the special furnace is carried out at a temperature of 745°C for 13-15 min. This final layer facilitates the highlighting of the chosen shade in agreement with antagonist and adjacent teeth and gives a glossy tint in order to achieve a superior aesthetic.

In order to create micro-retentions that will facilitate the definitive cementation in the patient's oral cavity, after the proper application of the ceramic masses, the internal faces of the ceramic crowns are sandblasted on zirconium infrastructure, using a corundum jet conveyed by compressed air.

The relevant aspects selected from the clinical-technological algorithm of the clinical case highlight the importance of choosing an optimal solution in accordance with the therapeutic principles, the clinico-biological indications of the prosthetic field to be restored and the patient's wishes at the aesthetic-functional level.

CONCLUSIONS

CAD/CAM systems are nowadays widely used in dentistry for making dentures. The introduction of these systems in dentistry has made it possible to meet the expectations of patients, who, with the help of chair-side systems, can benefit from high quality and precise prosthetic restorations in a single treatment session.

CAD/CAM systems have significantly revolutionized conventional techniques and protocols for prosthetic work, economic efficiency combined with the high quality of restorations has made this technology a subject of interest for doctors and technicians as well as for patients, who benefit from painless treatment and visible results in the shortest possible time.

In dentistry, digitized technology is slowly but surely taking the place of traditional procedures. From diagnosis to the final tooth, any dental procedure performed digitally is more accurate, safer, and better tolerated by the body.

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NEWS

EFFICACY OF SUBCUTANEOUS CLOSED SUCTION DRAIN IN REDUCTION OF POSTOPERATIVE SURGICAL SITE INFECTION

Surgical site infections (SSIs) are the infections of wound after an invasive operative approach. It remains to be a major morbidity for patients undergoing surgeries although there have been tremendous improvements in the surgical techniques. Different interventions to suppress the selective serotonin reuptake inhibitors have been proposed. Many of them have been routinely used by surgeons like minimizing shaving, hand washing, and preoperative antibiotics and these are well accepted. Drains are used in major abdominal surgeries, hernia repairs, breast surgeries reducing collections in closed areas. Hematoma, serous fluid and dead space in surgical incision wounds raise the risk of infection as they serve as the platform for microbial growth. Studies have proved that the usage of subcutaneous drains has lowered the chances of infection. According to this study the comorbid patients, emergency cases and colorectal surgeries had a higher incidence of developing SSI. The most common organism isolated was E. coli followed by S. aureus . The assessment of these variables therefore helps in providing a prophylactic treatment to reduce the mortality in the high-risk patients. (Harish R., Kazi F. N., & Sharma J. (2021). Efficacy of Subcutaneous Closed Suction Drain in Reduction of Postoperative Surgical Site Infection. *Surgery journal (New York, N.Y.)*, *7*(4), e275–e280.)

KEY ISSUES OF THE CHEMICAL PROPERTIES OF DRINKING WATER IN TIMIS COUNTY, ROMANIA

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KEY ISSUES OF THE CHEMICAL PROPERTIES OF DRINKING WATER IN TIMIS COUNTY, ROMANIA (Abstract): The **aim** of the performed study was to identify the key issues with the chemical properties of the drinking water in Timis County, Romania. **Material and methods:** The observational case-study was conducted for two years (2016-2017) on a sample of 1,086 water sources (2,159 water tests) in Timis County. **Results:** The majority of the water chemical parameters fell under the Maximum Admitted Concentrations (MAC). Main key issues identified were excessive concentrations of manganese in Timisoara (mean-208 µg/L), of iron in Timis County (rural) (mean-239 µg/L) and of ammonia in Timis County (urban: Lugoj) (mean-0.96 mg/L) public wells. Manganese mean concentrations surpassed MAC (50 µg/L) in non-chlorinated water, for companies, individual and public wells, in both areas Timisoara and Timis County. Iron mean concentrations surpassed MAC (200 µg/L) in public wells in Timis County and Timisoara, in non-chlorinated water. Ammonia means concentrations exceeded MAC (0.5 mg/L) in chlorinated water, in Timis County, for companies and public sources, and in Timis County (rural) in public wells. In Timisoara from 444 water tests in public wells, only 269 were harvested for manganese, 274 for iron**. Conclusions:** excessive concentrations of manganese, iron and ammonia are the key issues with the chemical properties of drinking water in Timis County. **Keywords:** WATER CHEMICAL PARAMETERS, IRON, MANGANESE, AMMONIA, OBSERVATIONAL CASE-STUDY.

Timisoara (the capital of Timis County) and Timis county have a good representation of confined (wells with a depth over 80 meters) and surface water. Pumps are used to extract and conduct ground water to the surface and central water systems are used to treat and deliver surface water to consumers. Research studies indicated high values of nitrates, iron, and manganese in the neighbor areas with reduced drinking water quality (1, 2) and with pathology occurrence. There is a gap of knowledge in the area about the concentration of certain chemical elements in the water offered and consumed by the population. The aim of the performed study was to identify the key issues with the chemical composition of drinking water in Timis County and Timisoara.

MATERIAL AND METHODS

Material of study consisted of a sample of 1,086 drinking water sources (2,159 water tests) disinfected with chorine and non-disinfected, used by the general population in Timis County and Timisoara,

during two years of study (2016 and 2017) (fig. 1).

Monitoring and audit control was done by the Public Health Direction Timis (PHDT), especially for non-chlorinated water (927 sources).

For chlorinated water (159 sources) having as source surface waters (Bega and Timis rivers) the water control done by PHDT followed after a daily monitoring performed by the treating water plants in their own laboratories.

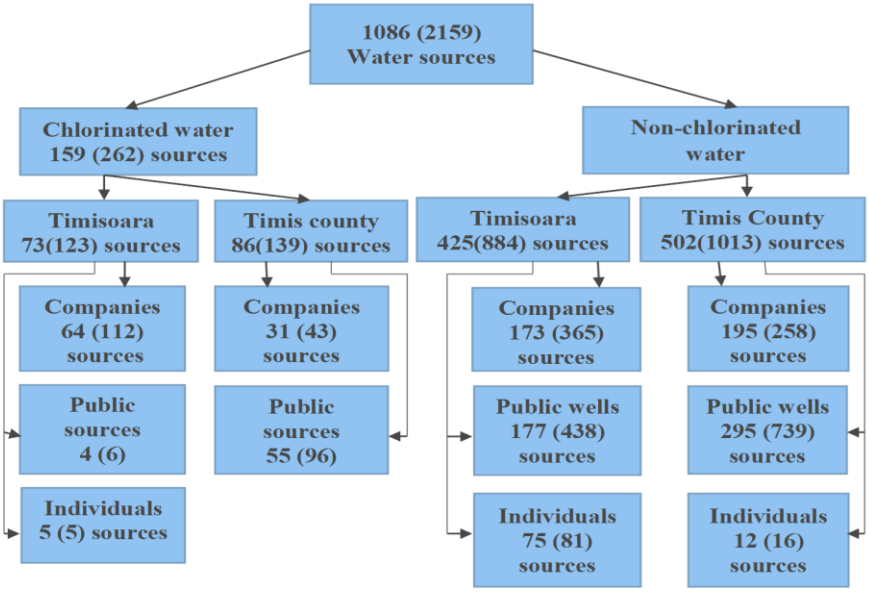

Fig. 1. Study sample of the water sources (number of water tests in brackets)

The method consisted of an observational case-study (combined approach: cohort and cross-sectional) of the chemical composition of drinking water (usual parameters: pH, turbidity, conductivity, ammonia, nitrites, nitrates, chlorides, residual chloride, total hardness, iron, manganese, and oxidability), in Timis County and Timisoara, Romania from 01.01.2016 to 31.12.2017. The methods of analysis and maximum admitted concentrations in accordance with law 458/2002 republished (2011) (3) are mentioned in the PHDT analysis bulletins and they are in accordance with national guidelines (4). Statistical analysis (mean, median) was performed with the aid of *SPSS version 20.0* software.

The agreement to access primary evidence, while keeping this evidence confidential and respecting the personal data protection law of the owners, was asked and received from the Leadership of the Public Health Direction Timis. The agreement of the "Victor Babes" University of Medicine and Pharmacy's Ethical Commission was requested and obtained for this study.

RESULTS

Chemical parameters in drinking water for entire sample

Mean concentrations of the chemical parameters of water demonstrated after water tests analysis can be seen in tab. I. First result that could be noticed is that the

majority of the water chemical parameters fell under the Maximum Admitted Concentrations (MAC), except for manganese and iron (for non-chlorinated water in both areas) and ammonia (for chlorinated water in Timis County) (tab. I).

First key issue identified was **manganese mean concentrations** that frequently surpassed MAC (50µg/L) in non-chlorinated water, for companies, individual and public wells, in both areas Timisoara and Timis County (tab. I).

TABLE I.

Mean concentrations of the chemical parameters of water for the entire sample

Area, property and chlorination			Mean concentrations of the chemical parameters										
			pH (≥6.5/ ≤9.5i)	**Turbidity** (5 F.T.U)	**Conductivity** (2500 µs/cm)	**Ammonia** (0.5 mg/l)	**Nitrites** (0.5 mg/l)	**Nitrates** (50 mg/l)	**Chlorides** (250 mg/l)	**Hardness** (min 5°G)	**Iron** (200 µg/L)	**Manganese** (50 µg/L)	**Oxidability** (5 mg/l)
T	C	0	7.40	1.44	611	0.19	0.050	3.49	23.74	9.29	147	**76.0**	1.3
		1	7.45	0.54	414	0.12	0.020	4.16	30.47	7.77	61.0	35.0	1.54
	P	0	7.55	0.70	706	0.19	0.010	1.57	21.51	11.43	97.0	**208**	1.01
		1	7.80	0.14	334	0.10	0.010	5.16	21.94	6.00	28.0	0.00	0.82
	I	0	7.31	4.67	774	0.42	0.070	11.25	50.25	12.57	**495**	**145**	1.98
		1	7.28	0.95	382	0.10	0.010	6.54	40.02	5.00	42.0	30.0	1.62
TC	C	0	7.57	0.70	698	0.20	0.020	9.12	30.31	11.22	111.0	**58.20**	1.7
		1	7.63	0.55	532	**0.71**	0.012	3.63	22.47	6.35	68.2	34.60	2.21
	P	0	7.60	0.84	731	0.37	0.018	8.80	28.05	13.52	**207**	**59.40**	2.31
		1	7.77	0.63	558	**0.72**	0.016	9.50	24.02	8.89	77.50	37.33	1.96
	I	0	7.71	0.72	589	0.15	0.015	7.99	23.81	8.42	113.0	**57.50**	1.25

T- Timisoara, TC-Timis County; C-companies; P-public sources; I-Individuals;
1-Chlorinated; 0-Non-chlorinated.

Iron mean concentrations were proven to be another problematic issue that surpassed MAC (200 µg/L) in public wells in Timis County (207 µg/L) and in public wells in Timisoara (495 µg/L), in non-chlorinated water (tab. I). Another parameter that surpassed MAC (0.5mg/l) was **ammonia mean concentrations** in chlorinated water, in Timis County, for companies (0.71 mg/l) and public sources (0.72 mg/l) (tab. I). Ammonia exceeded MAC in Timis County (rural) (0.55 mg/l) in public water sources, too. Another aspect that needs attention for these key issues is **the number of water tests** for each chemical parameter. In Timisoara from 444 (438 non-chlorinated water) water tests in public wells, only 269 were harvested for manganese, 274 for iron and 442 for ammonia. There were public wells that were not monitored for manganese (169), and iron (164). In Timis-County from 741 public sources (657 non-chlorinated water), only 544 were harvested for manganese, 716 for iron and

527 for ammonia. Only the monitored water sources have been considered in this analysis.

DISCUSSION

Although drinking water quality is regulated and monitored in many countries there is a need to review standards, to assess accurately the population exposure through robust epidemiological studies (5). In the performed study the resulted sample was complex, and it was a misbalance between monitored chlorinated and non-chlorinated water sources. First obtained results offer us a picture of the chemical quality of drinking water. Therefore, averages of the majority of chemical parameters were included in the intervals prescribed by guidelines. It is necessary to consider in this kind of assessment not only one chemical, but especially the mixture of contaminants, particularly at low exposure range (6).

The resulted key issues in the performed study were **manganese, iron and ammonia**. Usually manganese intake from drinking-water is low (20 μg/L) and it could be provided from rocks and soil or from water pollution of improperly constructed wells. Manganese could also be provided for organism through food and air (at different order of magnitude milligrams and nanogram, respectively) (7). Manganese mean concentration and median were found as very high in Timisoara public wells (majorities of the tests performed for manganese surpassed the MAC-50 μg/L). Elevated levels of dissolved manganese are often found in deep wells. Since 2011, the World Health Organization considered manganese concentrations in drinking water were not necessary to derive a formal guideline value, the maximum admitted concentration (400 μg/L) considered before being too high to adequately protect public health (8). In the performed study public wells with the resulted mean concentrations of manganese higher than 200 μg/L can be found in the entire city of Timisoara. In Timis County very high concentrations of manganese were also found. The enrichment of soil with manganese resulting from industrial inputs may threaten human beings (through environmental factors: water, food and air) (9). In research literature there are described different ways to remove manganese from drinking water by physical, chemical, and biological processes or by a combination of these methods (10).

Another discovered key issue of drinking water in rural Timis County (in non-chlorinated water and in public wells) was the **iron** concentration, recorded with a mean concentration that surpassed MAC (200 μg/L) and a median that fell in the admitted interval. Public and individual wells water that exceeded MAC for manganese, iron and other metals proved to be with reduced quality in another study (11). Iron mean concentration was also high in 23 public wells from Timisoara, but the calculated median was included in the admitted interval. Both substances (iron and manganese) impart a strong metallic taste to the water and both cause staining. Water rich in iron and/or manganese may appear initially without color, but orange-brown (iron) or black (manganese) stains or particles quickly appear as the water is exposed to oxygen (12). A better monitoring of public wells was noticed in Timis County than in Timisoara (great number of missing tests for manganese and iron determination

in water). In literature research iron and manganese proved to have an impact on human health (possible mild cognitive impairment and Alzheimer disease) (13), and not only the effect of altering sensorial (taste) and physical (color) quality of water.

Ammonia was found in high concentrations in chlorinated water in Timis county (urban: Lugoj and Faget) and in non-chlorinated water, in public wells from Lugoj after 2 successive determinations (March and July) in 2016. While ammonia itself is not noxious, it is an indicator of organic water pollution. Mean concentrations of ammonia in the majority of sources from Timis County and Timisoara fell under MAC (0.5 μg/L). In deep wells ammonia concentrations could increase in an anaerobic environment. In surface water the aerobic environment decreases ammonia. In another study nitrification (ammonia being transformed in nitrites) was analyzed in the sediment of a surface drinking water supply in Hong Kong (Dongjiang River) and it was frequently detected (14).

The study's results represent a starting point for future investigation of the factors that determine such high concentrations of manganese in public wells from Timisoara, of iron in public wells from (rural) Timis County and of ammonia in chlorinated water and public wells from Lugoj in 2016. A better monitoring of public wells from Timisoara resulted as being necessary. A limit of this study is that it is an observational one.

CONCLUSIONS

Drinking water supplied in Timis County and Timisoara over the 2016-2017 period corresponded as chemical composition to recommended guidelines for most usual chemical parameters, except manganese, iron and ammonia.

Manganese was found as a key issue with the chemical properties of drinking water in Timisoara and in certain villages from Timis County. Implications of this found key issue are important due to the extensive use of public wells by the population of Timisoara.

Iron was another key issue with the drinking water chemical concentrations in Timis County (rural) and in Timisoara public wells. Implication of this key issue is that its presence could be associated with color change and high concentrations of other chemicals, such as manganese in public wells from Timisoara.

Last identified key issue was ammonia in Timis County (urban - Lugoj), in chlorinated (companies and public sources) and non-chlorinated water (public wells).

Public well monitoring and control was discontinuous, and it was better performed in Timis County than in Timisoara. New investigation regarding the causes of these key issues and their health impact will be necessary as well as an intervention plan to solve them.

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CONFLICT OF INTEREST AND FUNDING

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MAGNITUDE AND VARIATION OF THE MANGANESE, IRON AND AMMONIA IN DRINKING WATER FROM TIMIS COUNTY, ROMANIA

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MAGNITUDE AND VARIATION OF THE MANGANESE, IRON AND AMMONIA IN DRINKING WATER FROM TIMIS COUNTY, ROMANIA (Abstract): The **aim** of the performed study was to investigate the magnitude and variation of the manganese, iron and ammonia, as identified key issues, considering presence/absence of chlorination, area, property and time when the samples were collected. **Material and methods:** The observational case-study was conducted for 2 years (2016-2017) on a sample of 1,086 water sources in Timis County. A statistically significant difference ($p<0.001$) resulted between chlorinated and non-chlorinated sources for manganese and for iron mean-concentrations, Timisoara and Timis County (rural) public wells for manganese and for iron mean concentrations, and public and companies` wells in Timisoara for manganese. **Results:** Statistically significant differences of ammonia mean concentrations were also found between Timis County and Timisoara for public wells ($p<0.001$) and for companies ($p=0.002$), in non-chlorinated water. Calculated medians of manganese concentrations in well water were high in Timisoara (200 µg/L) and in Timis County (95 µg/L) and exceeded MAC (50 µg/L). The highest concentration of manganese (500 µg/L) was recorded in Timisoara, in 2016. Median of iron (93 µg/L) concentrations in public wells in Timis-County (rural) was much higher than median (50 µg/L) in public wells in Timisoara. Both median values didn`t exceed MAC (200 µg/L). Still, the highest mean concentration of iron (3,780 µg/L) was found in a public well from Timisoara, in 2016. Median of ammonia concentrations in public wells (non-chlorinated water) in Lugoj (0.6 mg/L) also surpassed MAC (0.5 mg/L). **Conclusions:** excessive concentrations of manganese, iron and ammonia were found in drinking water in Timis County, with significant variation in space, time and depending on property and chlorination. **Keywords:** MAGNITUDE, VARIATION, IRON, MANGANESE, AMMONIA, OBSERVATIONAL CASE-STUDY.

Drinking water is an important element of the diet for the population served and its chemical composition could have a strong impact on health. Health effects due to the modified chemical quality (heavy metals or fluoride) of drinking water were demonstrated in numerous studies (1, 2) with a special approach in the area of inorganic arsenic (3, 4). Drinking water supply in Timis County is provided from surface and groundwater, through central and local systems of water treatment. Key issues (excess of manganese, iron and ammonia) were identified in drinking water in Timis County in a previous study (5). The aim of the performed study was to investigate the

magnitude and variation of the manganese, iron and ammonia considering concentration levels, water chlorination and non-chlorination, areas, property (companies, public and individual) and time when the samples were taken.

MATERIAL AND METHODS

This case-study is based on 2159 water tests taken from 1086 water sources over the two years period of 2016 and 2017. The case-study investigated water sources disinfected with chorine and water sources not disinfected with chorine, from Timisoara and Timis County. These different water sources are monitored by the Public Health Department of Timis and serve individuals, companies, and the general public.

Magnitude and variation (depending on area, time, property and chlorination) of the three key issues (high concentrations of manganese, iron and ammonia, in Timis County and Timisoara) identified in a previous study (5) were analyzed considering the same data base. The sample structure can be seen in first figure.

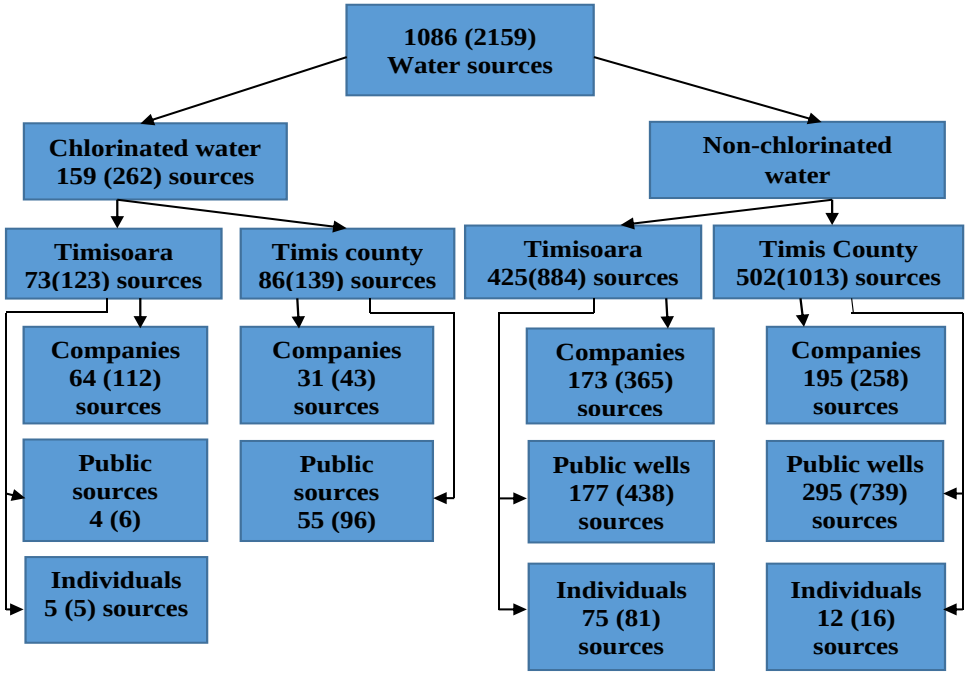

Fig. 1. Study sample of the water sources (number of water tests in brackets)
Retrieved from C. Petrescu, 2022 (5)

Water tests were performed once (cross-sectional) or several times (cohort) for all the variation types considered inside the chosen sample. A misbalance between the numbers of chlorinated and non-chlorinated water sources was noticed inside the sample, the real number of chlorinated water sources being much higher. The methods of analysis and maximum admitted concentrations of manganese, iron and ammonia

were in accordance with national guidelines (6).

Statistical analysis (mean, median, t-test for independent samples) was performed with the aid of *SPSS 20.0* software. The t-test for independent samples was used to compare the chemical parameters' average concentrations among the considered sample elements. Median and mean concentrations were considered when we analyzed magnitude and variation of the key issue parameters. All the necessary approvals (data protection law and ethic agreement) for this study were requested and received from the leadership of County and from the "Victor Babes" University of Medicine and Pharmacy Timisoara's Ethical Commission.

RESULTS

A comparison of the chemical parameters` mean concentrations (T test for independent samples) considering chlorination, area and the property revealed statistically significant differences of the identified key issues (5) between (tab. I):

TABLE I.

Comparison results of the averages of chemical parameters between the considered sample elements (number of water tests in brackets)

The study sample elements (number of water tests)	Parameters	t[1]	df	*p* (2-tailed)
Chlorinated (262)/non-chlorinated (1,897) water – Entire sample	Iron	**-6.643**	28.761	<0.001
	Manganese	-7.398	27.512	<0.001
Timisoara (112)/Timis (43) chlorinated water - Companies	Iron	4.416	126.51	<0.001
Timisoara (365) /Timis (258) non-chlorinated water - Companies	Ammonia	-3.104	230.29	0.002
Timisoara (438) /Timis (739) non-chlorinated water - Public wells	Ammonia	**-9.245**	479.61	<0.001
	Iron	**-5.733**	791.31	<0.001
	Manganese	**19.749**	513.82	<0.001
Public wells (438) / Companies (365) Timisoara non-chlorinated water	Manganese	**17.175**	579.4	<0.001
Public wells (438) / Individual sources (81) Timisoara non-chlorinated water	Manganese	3.124	87.383	0.002
Companies (365) / individual sources (81) Timisoara non-chlorinated water	Manganese	-3.503	85.01	0.001

[1] Levene's test with equal variances not assumed.

- **Chlorinated and non-chlorinated water** (manganese and iron higher in non-chlorinated water);
- **Timisoara and Timis County** (rural) for chlorinated water in companies (iron higher in Timisoara) and for non-chlorinated water in companies (ammonia - higher in Timis County) and in public wells (manganese higher in Timisoara and iron and ammonia higher in Timis County);
- **Public wells and Companies** with non-chlorinated water in Timisoara (manganese higher in public wells);
- **Public and Individual wells** with

non-chlorinated water in Timisoara (manganese higher in public wells);

- **Companies and Individual wells** with non-chlorinated water in Timisoara (manganese higher in individual wells) (tab. I). A limit of this statistical analysis is the different number of water tests for different types of sources.

Magnitude and variation of Manganese in drinking water

Calculated medians of manganese concentrations in well water were high in Timisoara (200 µg/L) and in Timis County (95 µg/L). Both median values exceeded MAC. In Timisoara public wells, in 145 from 269 water tests manganese concentrations exceeded 200 µg/L. Only 24 tests fell under national guideline standards (50 µg/L). Spatial distribution of manganese concentrations in public wells from Timisoara could be seen in figure 2.

The highest mean concentrations of manganese in public wells from Timisoara were found in the following streets: Podgoriilor (500 µg/L-1water test), Piata Unirii (450 µg/L-1 water test), Petofi Sandor (400 µg/L -3 water tests), and Tacit (367 µg/L -3 water tests) (fig. 2). In several localities in Timis County (14 from 502 water sources) were also recorded high concentrations (over 200 µg/L) of manganese, with the highest values in: SanMihaiu German (483µg/L), Utvin (325µg/L), Mosnita Noua (300 µg/L) and Nemesti (283 µg/L). Manganese concentrations exceeded MAC and recorded high monthly concentrations in public wells in Timisoara, in April (500 µg/L), June-August (234 µg/L, 218 µg/L and 216 µg/L), November (228 µg/L) and December (155 µg/L) in 2016, and in March-June (209 µg/L, 163µg/L, 201 µg/L and 250 µg/L) in 2017.

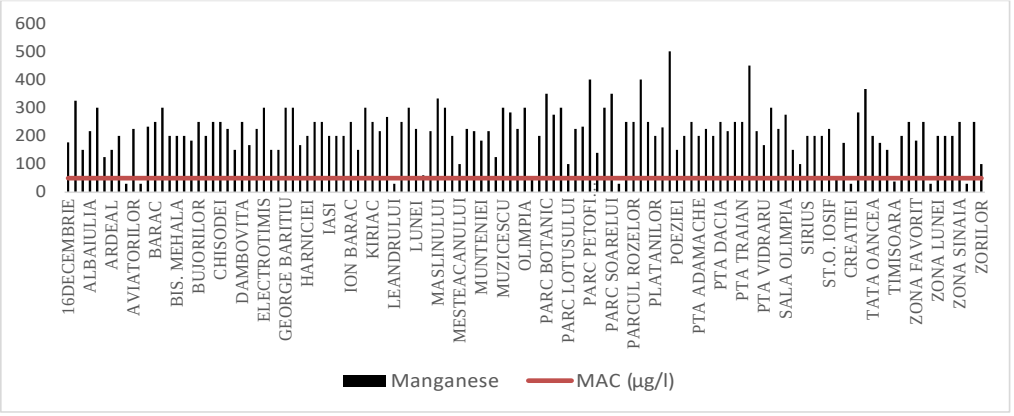

Fig. 2. Spatial distribution of the manganese mean concentrations (≥ 50µg/L) in public wells from Timisoara

Magnitude and variation of Iron in drinking water

Median of iron (93 µg/L) concentrations in public wells in Timis County (rural) was much higher than median in public (50 µg/L) and individual (50 µg/L) wells in Timisoara. Medians of iron in Timis County and in Timisoara did not exceed MAC. In (rural) Timis County public wells, in 207 from 657 water tests iron mean con-

centrations surpassed 200 µg/L. Spatial distribution of iron mean concentrations (≥200 µg/L) in public wells from Timis County can be seen in figure 3.

The highest mean concentrations of iron were found in the following villages in (rural) Timis County: Bazosu Nou (1,720 µg/L - 5 water tests), Mosnita (1,715 µg/L -water pollution -3,760 and 3,420 µg/L and other 9 water tests under 50 µg/L), SanMihaiu German (1,730 µg/L- water pollution 4,580, 680, 410 µg/L in 3 water tests) and Grabati (1,195µg/L -water pollution – 1,710, 1,560 and 1,420 µg/L and 90 µg/L in 4 water tests). Still the mean concentration was 176.7µg/L and the median was 71 µg/L, when considered all public wells from Timis County. There were no missing water tests.

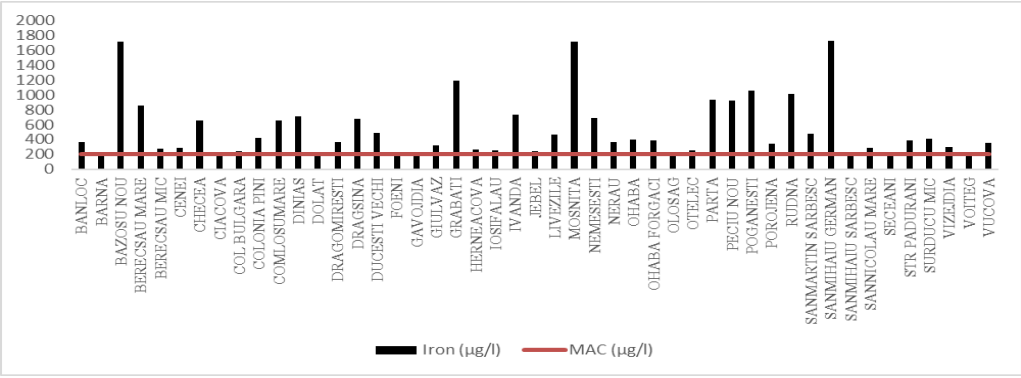

Fig. 3. Spatial distribution of the iron mean concentrations (≥ 200µg/L) in public wells from (rural) Timis County

In Timisoara the number of water tests with iron higher than 200 µg/L was 23, with the highest mean concentrations in 3 public wells: Podgoriilor street (3,780 µg/L), Clabucet Park (1,969 µg/L), Rozelor Park (1,520 µg/L). It is interesting that the levels of manganese were also high in these wells (500, 350, 350 µg/L, respectively). When we considered comparatively the mean and median values of iron in Timisoara for public wells, we noticed a great difference: mean - 95.35µg/L; median-40 µg/L, due to the presence of these very high values in few places.

Iron monthly concentrations were high in public wells in Timis County in January (885 µg/L), February (460µg/L) and July (393 µg/) in 2016, in July (200 µg/L), August (314 µg/L) and December (493 µg/L) in 2017. In May 2017, high monthly concentrations of iron (436 µg/L) were recorded in Timisoara.

Magnitude and variations of Ammonia in drinking water

Ammonia was found with high mean concentrations in public wells (non-chlorinated water) in Lugoj (0.96 mg/L). Medians of ammonia concentrations in public wells (non-chlorinated water) in Lugoj (0.6 mg/L) and in Timis County - rural (0.4 mg/L) also indicate ammonia excess in Lugoj. Ammonia regarding the chemical composition of the drinking water supply in Lugoj (44 public wells and 74 water tests) recorded the highest monthly concentrations in March (0.94mg/L) and July (1.02 mg/L) 2016.

DISCUSSION

In the performed study groundwater sources belong to the city or town halls, companies or individual people. First obtained results offer us a picture of the significant differences of the identified key issues (5) between chlorinated and non-chlorinated water, Timisoara and Timis County, companies, public and individual sources. The spatial and temporal approach was proven to be associated with the aquifer types and to be effective and necessary for correctly measuring or estimating the population's exposure to the tested chemicals (7). Manganese and iron mean concentrations and medians were found to have significant spatial and temporal differences, chlorination and the water ownership being also important variation factors. In research literature there is described a way to remove manganese from drinking water by using of oxidant substances (8), such as chlorine that explain partially the difference of manganese concentration between chlorinated and non-chlorinated water that was shown in the performed study. Magnitude of chemical compounds concentrations was high in public wells from Timisoara for manganese and iron (certain wells), in public wells from Timis County (rural) for iron and manganese (certain wells) and in Lugoj for ammonia in public wells and disinfected water (certain places). In 2016, seasonal variation indicates high monthly concentrations along entire year for manganese (no variation) and first part of the year for iron, and during the spring and summer for ammonia. In 2017, high monthly concentrations were found during the spring and the summer for manganese and during the second part of the year for iron. A study of the seasonal and spatial variability of anthropogenic and natural factors performed in China revealed as determinants for groundwater parameters: water-rock interaction, geogenic iron and manganese, agricultural pollution and organic pollution (9). Another study showed that residents were more willing to accept high metal (manganese and iron) concentration than international guidelines assume (10). In Timisoara a great part of population seems to prefer groundwater, thus explaining the great number of wells used free by-passing people, by individuals or companies. In Timis County (rural) the population has to use groundwater, this being the main source of the water supply.

A limit of this study is that it is an observational one.

CONCLUSIONS

In conclusion, the magnitude as the very high concentrations of the manganese, iron and ammonia, considered as key issues of drinking water supply, and the variation in the concentration of these key elements depend upon the presence or absence of chlorination, the area, property and time of the sampling. Very high concentrations of manganese, iron and ammonia were found in certain public wells from Timisoara, Timis County (rural) and Lugoj. During 2016 the levels of these key issues concentrations were higher than in 2017.

CONFLICT OF INTEREST AND FUNDING

The author declares that there is no conflict of interest, and she received no specific funding regarding this scientific research.

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NEWS

THE USE OF SELF-ADHESIVE CEMENTS IN THE ADHESION OF FIBER POSTS AFTER THE APPLICATION OF *EDTA*

The objective of this study was to evaluate the effects of the application of EDTA at different concentrations such as 17%EDTA and 24%EDTA on the parietal dentine before the use of a self-adhesive resin cements: RelyX U200 and Multilink Speed. This type of the cements is used for the adhesion of fiber post which are indicated in the reconstruction of teeth with extensive coronal destruction. This study was performed on 78 extracted human single-rooted premolar teeth, divided in six groups, also ten roots of each group were used for bond strength and three roots for microhardness evaluations. The data obtained were statistical analyzed by two-way ANOVA and post-hoc Turkey's test (α=0.05). The use of EDTA at different concentrations is not recommended as pretreatment for fiber post cementation using self-adhesive cements (Gruber YL, Jitumori RT, Bakaus TE, Reis A, Gomes JC, Gomes GM. Effect of the application of different concentrations of EDTA on the adhesion of fiber posts using self-adhesive cements. *Braz Oral Res* 2020; 35: e012 / doi: 10.1590/1807-3107bor-2021.vol35.0012).

VALIDATION OF THE ROMANIAN VERSION OF MULTIPLE SCLEROSIS QUALITY OF LIFE (MSQOL-54) INSTRUMENT

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VALIDATION OF THE ROMANIAN VERSION OF MULTIPLE SCLEROSIS QUALITY OF LIFE (MSQOL-54) INSTRUMENT (Abstract): **Aim**: To validate the Romanian version of MSQOL questionnaire. **Material and methods**: The questionnaire was translated and culturally adapted in the Romanian space for the Romanian population and the questionnaire was validated in 50 patients with multiple sclerosis (MS) at the Neurology Clinic at of the Iasi Rehabilitation Hospital. The following indicators were analyzed: Cronbach's alpha coefficient, Pearson's (r) correlation coefficient, used to investigate the relationship between the composite scores and the main clinical and demographic variables, and the Bartlett Sphericity Test, which shows the correlation between the questionnaire items. **Results**: The psychometric standards met, and the general demographic data are illustrated by the following results. The group consisted of 50 patients, 35 women (70.0%) and 15 men (30.0%), mean age 39.76 ± 10.493 years, age range 18 to 65 years old, with no significant gender differences between the sexes (p = 0.627). Significance level of the Bartlett Sphericity Test, p = .000 - which shows that the items in of the questionnaire are correlated with each other. The Pearson correlation coefficients, all correlation coefficients obtained are statistically significant and moderate or strong in intensity. Median EDSS score was 3 in women, 4 in men, total score was 3. **Conclusions**: From now on, this adequate psychometric questionnaire will which allows an accurate measurement of quality of life (QoL) in MS patients with multiple sclerosis which is from now will be available for all medical professionals dealing with MS patients in Romania. **Keywords:** MULTIPLE SCLEROSIS, DEMYELINATING DISEASE, MSQOL54, VALIDATION PROCESS.

Multiple sclerosis (MS) is chronic immune-mediated of the central nervous characterized by neurodegeneration and neuroinflammation. The main consequence of this processes is the progressive alteration of neuronal transmission, clinically manifest through a wide variety of heterogeneous symptoms (1).

Focal demyelinating lesions are highlighted by magnetic resonance imaging (MRI) as a technique of high accuracy for the lesions detection. MS is the most com-

mon cause of primary non-traumatic neuromotor disability in young adults (2).

Epidemiological data shows that an estimated 2.3 million MS population is living worldwide, with the higher prevalence of multiple sclerosis in Europe and North America and a lower prevalence in sub-Saharan Africa and East Asia. The prevalence of MS is 30-80/100,000 among European population and around 40/100,000 in Romanian population (3, 4). The mean age of diagnosis is 32 years and females are twice as likely to live with MS as males. The lowest incidence is recorded in the equatorial regions, less than 1/100,000 (5).

Nowadays there are a multitude of available quality of life instruments in MS (HRQoL): Hamburg Quality of Life Questionnaire in Multiple Sclerosis (HAQUAMS), Functional Assessment of Multiple Sclerosis (FAMS), Multiple Sclerosis Quality of Life Inventory (MSQLI), Multiple Sclerosis International Quality of Life (MUSIQOL), and Multiple Sclerosis Impact Scale (MSIS). All these scales are necessary for both clinicians and patients to customize the medical care plan and, also, to improve doctor-patient communication (6).

The validation of the MSQOL-54 questionnaire is useful both in current clinical practice and research, the aim being to find out if this psychometric instrument reliably and validly measures the level of quality of life in a population in a specific culture (7). At this point of time only MSQOL-54 and MSQLI contain a series of more specific questions with the role of highlighting both the clinical manifestations and the therapeutic management (8).

MSQOL-54 questionnaire is a multidimensional health-related QoL measure that combines both generic and MS-specific items into a single instrument for the multiple sclerosis patients. It is a structured, self-report questionnaire that the patient can generally complete with little or no assistance, which includes eight elements that measure the physical components and five elements that measure the mental component related to QoL (9).

Currently the MSQOL 54 questionnaire is not validated in Romanian language. At the present time, physicians and researchers have to use the original English version which is a difficult process for most of the patients. Our goal is to translate and culturally adapt the specific MSQOL-54 instrument, pursuing the validation of the Romanian version. In this process of questionnaire validation our objective is to adapt it to the Romanian cultural, social, religious and linguistic space. In our study we focused on the evaluation and the assessment of the quality of life in MS patients using the MSQOL 54 questionnaire (7).

MATERIAL AND METHODS

Baseline characteristics of the population

To be eligible for this study, the patients had to meet the following inclusion criteria: MS diagnosis according to McDonald 2017 criteria; age over 18 years; EDSS (The Expanded Disability Status Scale) score >1. Exclusion criteria were patient's refusal to participate in this study, patient with terminal cancer, terminal illness, or any other severe diseases that could affect QoL and mental health conditions associated with inability to discern and consent to participate in this study.

The investigators were trained how to correctly apply the questionnaire and to calculate the final score. We took into consideration the possible confusion that may be encountered in the application of the MSQOL54 questionnaire due to the slight possibility of patient’s lack of correctly understanding the terminology. To avoid this specific bias due to patients’ incorrect

terminology understanding, the questionnaire was completed under the close supervision of a research team member.

The questionnaire validation process is performed according to the questionnaire validation methodology. We have obtained author's consent for the and use of the questionnaire in this research paper and its validation. The translation process was conducted as follows: translation from English into Romanian, two versions by two different translators, then the translation from Romanian into English by a native English speaker who also speaks Romanian. The validation process is conducted on a group of 50 subjects diagnosed with MS at the Neurology Clinic of the Iași Rehabilitation Hospital who met the inclusion/exclusion criteria and agreed to participate at the time of their presentation to the clinic for various care related problems.

Statistical analysis

The data were collected in a database created in Excel 2010 (Microsoft Corporation, USA) and statistically processed using the *SPSS version 23.0* (Statistical Package for the Social Sciences).

The following indicators were analyzed: demographic data -median, Min-Max, SD (women/men; age, level of education; EDSS score); Cronbach's alpha coefficient (α) -subscale; Kaiser-Meyer-Olkin (KMO) statistics-subscale correlations; Bartlett's sphericity test-subscale correlations) (9).

RESULTS

General characterization of the study group: the group consisted of 50 patients, 35 women (70.0%) and 15 men (30.0%), with mean age of 39.76 ± 10.493, age range from 8 to 65 years old, with no significant differences between the sexes (p = 0.627), first table. Half of the patients had high school education, two primary school education, and the remaining patients higher education.

The average EDSS score was 4.302 ± 6.9326, with scores ranged between 1.0 and 7 slightly higher in women than in men, although even in this case the difference was not statistically significant (p = 0.130). This study shows that 84.0% of patients had EDSS <5.5, 4 patients (8.0%) had an EDSS score between 5.6 ÷ 6.5, in one patient (2.0%) EDSS score was between 6.5 ÷ 8 and in another patient (2.0%) the EDSS score was 8 (tab. II).

TABLE I.

Age of the subjects included in the study. Baseline characteristics of the study population

Sex	N	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Median
F	34	39.26	10.509	1.802	18	64	38.50
M	15	40.87	10.736	2.772	25	65	44.00
Total	49	39.76	10.493	1.499	18	65	39.00

TABLE II.

EDSS score (The Expanded Disability Status Scale) of the study population

Sex	N	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Median
F	33	4.500	8.3423	1.4522	1.0	50.0	3.000
M	15	3.867	1.4075	.3634	2.0	6.0	4.000
Total	48	4.302	6.9326	1.0006	1.0	50.0	3.000

MSQOL-54 questionnaire has 14 quantitative 0-100 scales, based on them 2 global scores being calculated: the physical health component, respectively the mental health component.

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy, a statistic that indicates the variance of the investigated variables, caused by the identified factors, had a value of 0.868, close to 1, which means that factor analysis is adequate. The same was true for the significance level of Bartlett Sphericity Test, p = .000 - which shows that the items in the questionnaire are correlated with each other (tab. III).

TABLE III.

Kaiser-Meyer-Olkin Measure and Bartlett's Test of MSQOL-54 questionnaire.

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.	0.868
Bartlett's Test of Sphericity	Approx. Chi-Square 330.559 df 66 Sig. .000

The Pearson correlation coefficients between the used scales and the two components, the physical and the mental, were also calculated to check for correlations. All correlation coefficients obtained were statistically significant and moderate or strong in intensity, which on the one hand shows that the two global indicators are correctly defined, and, on the other hand, that the scales are interlinked, so the questionnaire is valid (tabs. IV and V).

The high values seen in this column show that the scales are well correlated - the weakest correlated being for "Sexual function" scale, with a communality of 0.482.

The extracted communalities are estimates of the variability of the scales explained by the identified factors. In this case high values should be obtained; low values are indicating the scales that are not satisfactorily covered by the factorial solution (percentages above 50% are generally acceptable). In our case, this category includes "Sexual function" (0.366), "Cognitive function" (0.475) and "Overall Quality of Life" (0.488) (tab. VI.)

TABLE IV.

Pearson correlation coefficients of MSQOL-54 physical scales

Pearson correlation coefficients		Physical component	Physical function	Health perceptions	Energy	Role limitations – PHYSICAL	Pain	Sexual function	Social function	Health distress
Physical component	R	1	.777**	.703**	.853**	.814**	.708**	.613**	.766**	.779**
	P		.000	.000	.000	.000	.000	.000	.000	.000
Physical function	R	.777**	1	.359*	.641**	.589**	.563**	.331*	.567**	.459**
	P0	.000		.011	.000	.000	.000	.019	.000	.001
Health perceptions	R	.703**	.359*	1	.496**	.602**	.273	.348*	.500**	.578**
	P	.000	.011		.000	.000	.055	.013	.000	.000
Energy	R	.853**	.641**	.496**	1	.600**	.678**	.498**	.651**	.672**
	P	.000	.000	.000		.000	.000	.000	.000	.000
Role limitations – PHYSICAL	R	.814**	.589**	.602**	.600**	1	.477**	.454**	.529**	.475**
	P	.000	.000	.000	.000		.000	.001	.000	.000

Pearson correlation coefficients		Physical component	Physical function	Health perceptions	Energy	Role limitations – PHYSICAL	Pain	Sexual function	Social function	Health distress
Pain	R	.708**	.563**	.273	.678**	.477**	1	.323*	.497**	.528**
	P	.000	.000	.055	.000	.000		.022	.000	.000
Sexual function	R	.613**	.331*	.348*	.498**	.454**	.323*	1	.408**	.552**
	P	.000	.019	.013	.000	.001	.022		.003	.000
Social function	R	.766**	.567**	.500**	.651**	.529**	.497**	.408**	1	.553**
	P	.000	.000	.000	.000	.000	.000	.003		.000
Health distress	R	.779**	.459**	.578**	.672**	.475**	.528**	.552**	.553**	1
	P	.000	.001	.000	.000	.000	.000	.000	.000	

TABLE V.

Pearson correlation coefficients of MSQOL-54 mental scales

		Mental components	Health distress	Overall Quality of Life	Emotional well-being	Role limitations - EMOTIONAL	Cognitive function
Mental components	R	1	.777**	.651**	.743**	.845**	.688**
	P		.000	.000	.000	.000	.000
Health distress	R	.777**	1	.685**	.461**	.480**	.528**
	P	.000		.000	.001	.000	.000
Overall Quality Of Life	R	.651**	.685**	1	.361**	.371**	.342*
	P	.000	.000		.010	.008	.015
Emotional well-being	R	.743**	.461**	.361**	1	.488**	.437**
	P	.000	.001	.010		.000	.001
Role limitations – EMOTIONAL	R	.845**	.480**	.371**	.488**	1	.448**
	P	.000	.000	.008	.000		.001
Cognitive function	R	.688**	.528**	.342*	.437**	.448**	1
	P	.000	.000	.015	.001	.001	

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

TABLE VI.

Communalities of principal scales of MSQOL 54

	Initial	Extraction
Physical function	.586	.527
Role limitations – PHYSICAL	.624	.555
Role limitations – EMOTIONAL	.528	.509
Pain	.577	.529
Emotional well-being	.599	.511
Energy	.756	.764

	Initial	Extraction
Health perceptions	.675	.650
Social function	.543	.558
Cognitive function	.556	.475
Health distress	.701	.634
Sexual function	.482	.366
Overall Quality of Life	.555	.488

DISCUSSION

Clinical validity of the scales was assessed by comparing the mean values of the scores recorded for the demographic characteristics of the group: sex, background, level of education, EDSS score, and treatment (10). Parametric tests (t-Student and ANOVA) were used to make the comparisons when the normal distribution law occurred or when the data were normally distributed, and non-parametric tests (Mann-Whitney and Kruskal-Wallis) were otherwise used.

In general, most of the investigated scores were higher in men than in women, with a few exceptions: Role limitations – physical, Health perceptions, Social function, Sexual function and Satisfaction with sexual function. The observed differences are quite important, but they reach statistical significance only in the case of Cognitive function scale, with higher values in the male than in female patients and Satisfaction with sexual function scale, with higher values in women (fig. 1).

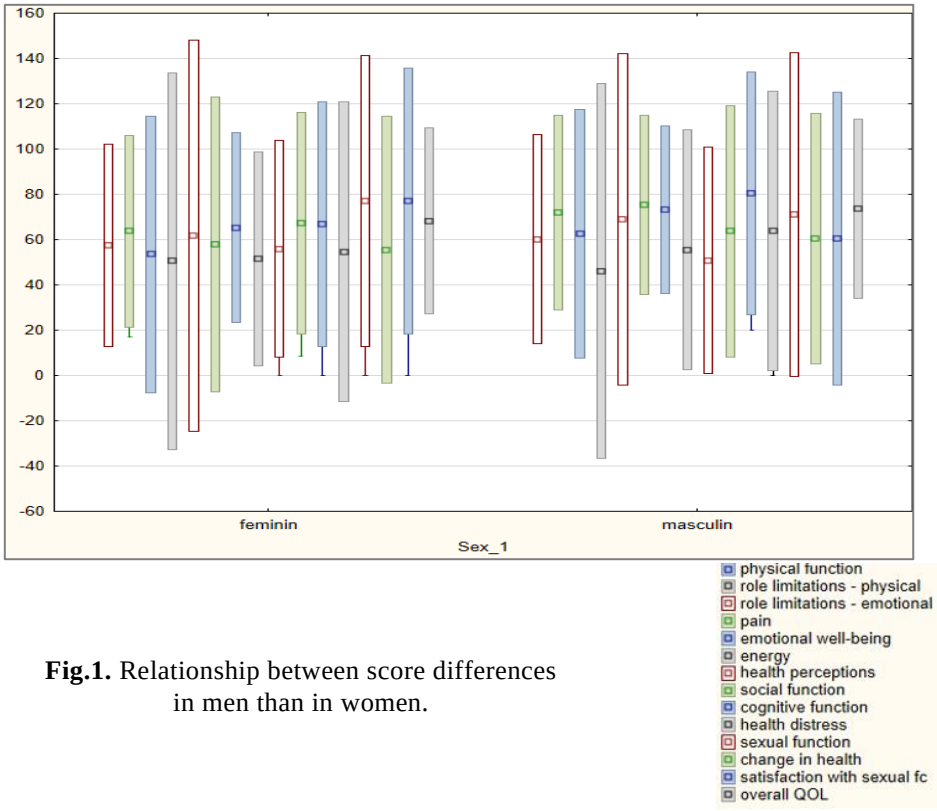

Fig.1. Relationship between score differences in men than in women.

We have also found a tendency of Physical Health Composite (PHC), mental health composite (MHC), and overall quality of life (QOL) to increase as patients have a more advanced level of education (this is the case of Physical Health Components. Mental Health Components, Change in health and Overall Quality Of Life) / that the higher the education level the higher the scores on Physical Health Composite (PHC), mental health composite (MHC), and overall quality of life (QOL); however, the differences between the 3 categories are not statistically significant (fig. 2).

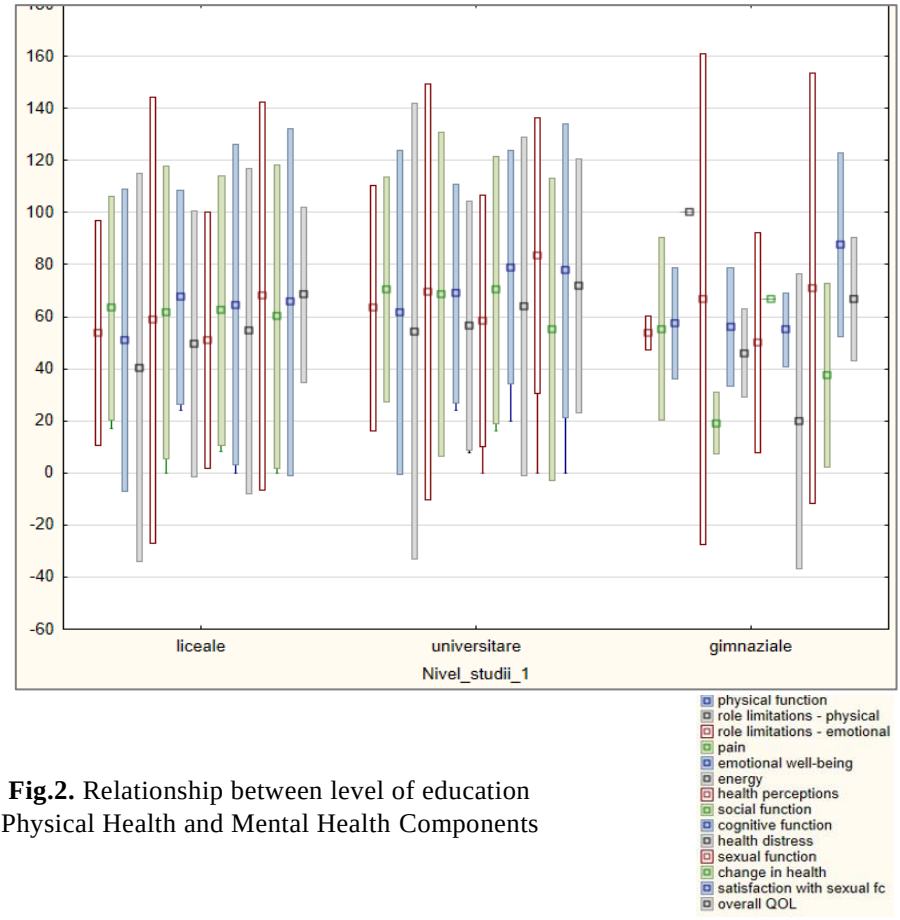

Fig.2. Relationship between level of education Physical Health and Mental Health Components

On the other hand, the scores tend to decrease as EDSS score increases, but even in this case the variations do not exceed the statistical significance threshold. One possible explanation is that we could only compare the EDSS categories <= 5.5 (the most numerous in the group) and EDSS <= 6.5 (4 cases) for in which the scores were quite high; for the other 2 EDSS categories we only had one patient so, no statistical tests could be applied. There was a significant decrease in all scores in the patient with EDSS> 8, but the analysis could not go deeper because only one study patient presented this characteristic (fig. 3).

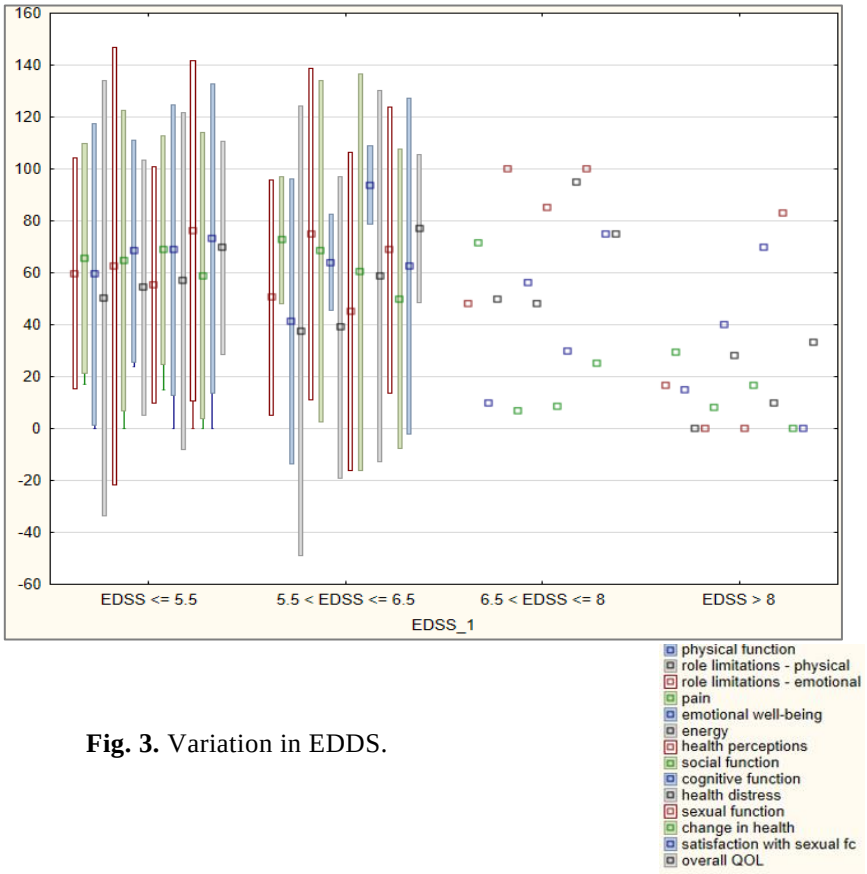

Fig. 3. Variation in EDDS.

In this study, the correlation between the scores on the MSOQL 54 questionnaire and the clinical and demographic factors used, such as age and EDSS score did not show a significant correlation (11). Similar results were found in the validation study of this questionnaire in Turkey, with no significant correlations between the following scores: age, sex, level of education (12). Also, in this study the male patients showed a 100% rate of sexual satisfaction. Despite this discordant result. medical data showed an increased rate of sexual dysfunction problems, but in this study, the females showed a high rate of sexual satisfaction. This aspect underlines the difficulty of talking about male sexual function, which can be attributed to cultural and social perceptions or characteristics (12).

In our study younger patients with a higher level of education scored higher on the mental and physical components, while the MSQOL-54 validation study in Bosnia and Herzegovina showed that younger patients with a higher level of education, shorter disease duration had lower scores in some areas of HRQoL: such as role limitations due to physical problems, physical health, and role limitations due to emotional problems (13).

CONCLUSIONS

This adequate psychometric question-

naire which allows an accurate measurement of QoL in MS patients is from now on available for all medical professionals dealing with MS patients in Romania. Using this instrument in medical practice could provide additional information about treatment effectiveness and the impact of this disease on the physical and mental components of QoL in these patients.

This study demonstrates that the Romanian version of MSQOL-54 is well accepted by MS patients and this instrument will become a routine in medical practice.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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DESIGN AND *IN SILICO* STUDIES ON DIFFERENT DENGUE VIRAL PROTEINS OF SOME COMPOUNDS BASED ON ACETOPHENONE SKELETON

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DESIGN AND *IN SILICO* STUDIES ON DIFFERENT DENGUE VIRAL PROTEINS OF SOME COMPOUNDS BASED ON ACETOPHENONE SKELETON (Abstract): **Aim:** In this study, we performed the evaluation of anti-Dengue potential of some acetophenone derivatives and diazine with dihydroxyacetophenone skeleton derivatives, using molecular docking approach, as potential candidates for anti-dengue virus drug discovery. **Material and methods:** For this study 20 synthetic ligands were used. Docking studies were conducting using iGEMDOCK (Generic Evolutionary Method for molecular Docking) software. **Results:** The obtained results revealed that all the 20 compounds show good affinity with all the proteins; significant affinity was observed between the tested compounds and Dengue transmembrane domain of NS2A protein. **Conclusions:** The conclusion drawn from our virtual screening and docking result reveals that the investigated compounds have a good binding affinity with most of the proteins and some of they can be used as an effective drugs target for Dengue virus. **Keywords:** BINDING INTERACTION, MOLECULAR DOCKING, DENGUE VIRUS, ACETOPHENONE SKELETON, DRUG DESIGN.

Highly functionalized acetophenone derivatives are an important class of organic compounds which exhibiting interesting biological properties, such as antimicrobial (1) and anticancer activities (2) being a valuable compound for supramolecular or medicinal chemistry (3). In last years, 1,2-diazine derivatives have demonstrated to possess a large variety of biological activities: antihypertensive (4) diuretic (5) antiplatelet (6) cardiotonic (7) anticancer (8) anti-HIV (9) anti-inflammatory (10) antimicrobial (11) anti-tuberculosis (12) etc.

Dengue virus is part of the family *Flaviviridae*, genus Flavivirus (13, 14). The Dengue virus causes a range of clinical manifestations, the most severe forms being Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (15, 16). Currently, there has been no vaccine or effective antiviral drug available against dengue infection. Therefore, many researchers are trying to find an efficient antiviral agent against this virus (17).

There are ten proteins in Dengue virus, three are structural proteins, including

envelope (E), capsid (C) and PreM, and seven are nonstructural proteins. The process of viral replication and pathogenesis of Dengue virus is associated with the seven non-structural proteins, namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (18,19).

NS proteins reshape the inner organization of the cell, mature the polyprotein, replicate the viral RNA and help the virus evade the immune system. Among the seven non-structural proteins, NS5 is the largest protein (102 kDa) that encoded by *Flavivirus* genome (18). NS5 encodes methyltransferase (Mtase) on N-terminal and RNA-dependent RNA polymerase (RdRp) on C-terminal and it can also down-regulate the host immune interferon response (20).

The effective and efficient methods in designing and developing a novel potential drug candidates can be completed through *in silico* approach because the identification of potential drugs can be obtained more quickly (21).

In recent times, computational drug chemistry is proving to be an efficient tool for identification and prediction of compounds that can have anti dengue activity (22). Docking analysis is an important aspect for getting the information on the interaction profile of the protein with the ligands. The interaction of the protein with the ligands is given by the binding energy (23).

The compounds containing acetophenone skeleton and diazine with dihydroxyacetophenone scaffold derivatives have wide interest due to their diverse pharmacological properties. In particular, these biological activities make these derivatives more attractive and testing as novel therapeutic compounds.

As part of our aim in research of biologically active acetophenone derivatives, the free hydroxyl groups attached on the acetophenone ring has allowed us to introduce some radicals that can improve the biological activity. Looking to the medicinal importance of the aceto-phenone skeleton, we performed the evaluation of anti-Dengue potential of some acetophenone derivatives and diazine with dihydroxyacetophenone skeleton derivatives, previously synthesized, using molecular docking approach.

MATERIAL AND METHODS

Chemistry

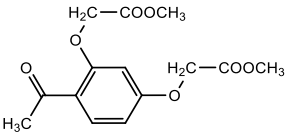
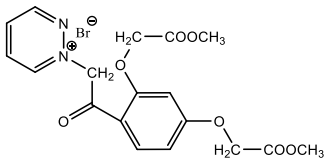
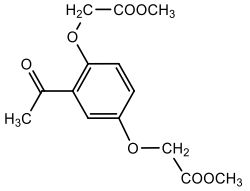
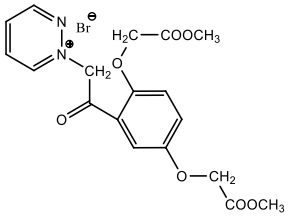
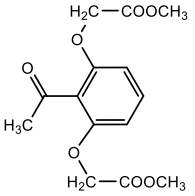
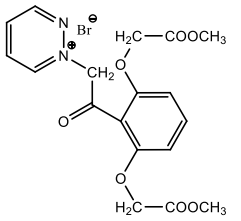
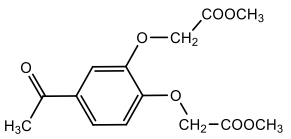
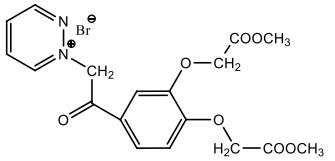
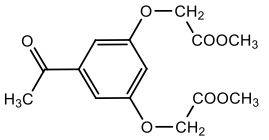
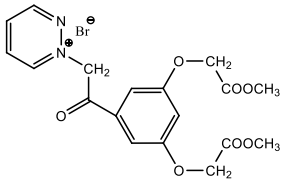
The synthesis of the target compounds which were used as ligands were previously reported (24, 25). A series of dihydroxyacetophenone were treated with methyl chloroacetate at room temperature. When the reaction occurred in a molar ratio of 1:2, dialkylated derivatives were obtained (**1a-e**). These derivatives lead to the bromo-bisetherificated acetophenone derivatives (**2a-e**) in the presence of copper bromide in a mixture of chloroform/ethyl acetate. In order to obtain cycloimmonium salts, different nitrogen heterocyclic compounds have been treated with halogenated acetophenone skeleton derivatives with increased reactivity (**3a-e** and **4a-e**).

Spectral characterization

In Table I we present the chemical structure and the name for the synthesized compounds. Table II shows some physico-chemical and spectral characteristics of the derivatives. Synthetic pathway of compounds with acetophenone skeleton is presented in figure 1.

TABLE I.

Chemical structure of compounds with acetophenone skeleton (1-4)

Comp.	Structure	Comp.	Structure
1a	 dimethyl 2,2'-((4-acetyl-1,3-phenylene) bis(oxy)) diacetate	**3a**	 1-(2-(2,4-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) pyridazin-1-ium bromide
1b	 dimethyl 2,2'-((2-acetyl-1,4-phenylene) bis(oxy)) diacetate	**3b**	 1-(2-(2,5-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) pyridazin-1-ium bromide
1c	 dimethyl 2,2'-((2-acetyl-1,3-phenylene) bis(oxy)) diacetate	**3c**	 1-(2-(2,6-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) pyridazin-1-ium bromide
1d	 dimethyl 2,2'-((4-acetyl-1,2-phenylene) bis(oxy)) diacetate	**3d**	 1-(2-(3,4-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) pyridazin-1-ium bromide
1e	 dimethyl 2,2'-((5-acetyl-1,3-phenylene) bis(oxy)) diacetate	**3e**	 1-(2-(3,5-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) pyridazin-1-ium bromide

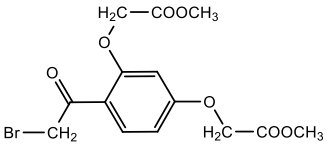
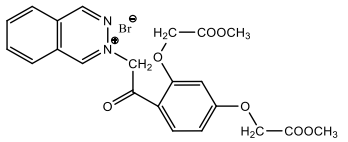
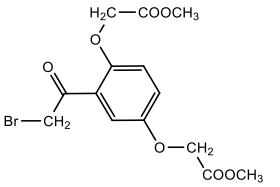
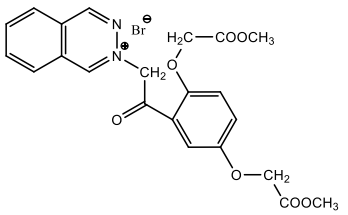
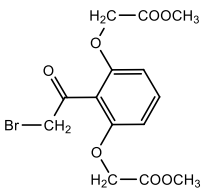
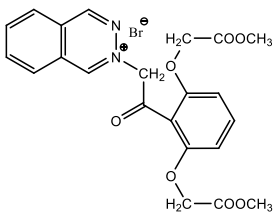
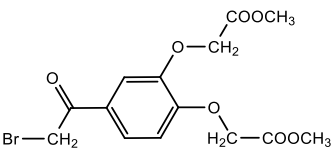
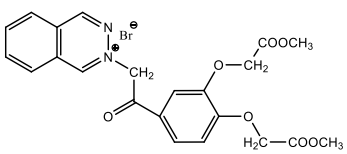
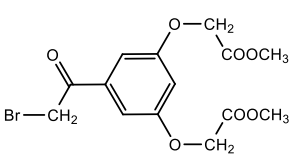
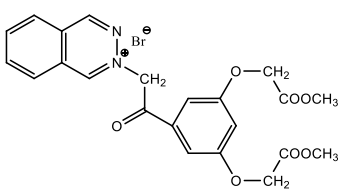
Comp.	Structure	Comp.	Structure
2a	 dimethyl 2,2'-((4-(2-bromoacetyl)-1,3-phenylene) bis(oxy)) diacetate	**4a**	 2-(2-(2,4-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) phthalazin-2-ium bromide
2b	 dimethyl 2,2'-((2-(2-bromoacetyl)-1,4-phenylene) bis(oxy)) diacetate	**4b**	 2-(2-(2,5-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) phthalazin-2-ium bromide
2c	 dimethyl 2,2'-((2-(2-bromoacetyl)-1,3-phenylene) bis(oxy)) diacetate	**4c**	 2-(2-(2,6-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) phthalazin-2-ium bromide
2d	 dimethyl 2,2'-((4-(2-bromoacetyl)-1,2-phenylene) bis(oxy)) diacetate	**4d**	 2-(2-(3,4-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) phthalazin-2-ium bromide
2e	 dimethyl 2,2'-((5-(2-bromoacetyl)-1,3-phenylene) bis(oxy)) diacetate	**4e**	 2-(2-(3,5-bis(2-methoxy-2-oxoethoxy)-phenyl)-2-oxoethyl) phthalazin-2-ium bromide

TABLE II.

Physicochemical and spectral characteristics for synthesized compounds

Comp.	Melting point (°C)	IR (cm^{-1})
1a	114–115	3102, 3077, 3050 (C–H arom), 2963, 2945, 2920 (C–H aliph), 1765 (C=O ester), 1668 (C=O keto), 1600, 1492, 1451, 1427(C–C arom), 1265, 1250, 1225, 1198 (C–O–C)
1b	95–96	3070, 3048, 2997 (C–H arom),2953, 2928 (C–H aliph), 1765, 1742 (C=O ester), 1665 (C=O keto), 1590, 1545, 1499, 1429 (C–C arom), 1303, 1282, 1224, 1203, 1169 (C–O–C)
1c	80-81	3100, 3048, 3011 (C–H arom), 2957, 2917 (C–H aliph), 1767, 1751 (C=O ester), 1697 (C=O keto), 1599, 1470, 1441 (C–C arom), 1248, 1211, 1130, 1086 (C–O–C)
1d	89–90	3077, 3036, 3011 (C–H arom),2982, 2959, 2920 (C–H aliph), 1777, 1763 (C=O ester), 1674 (C=O keto), 1591, 1521, 1437, 1412 (C–C arom), 1298, 1276, 1202, 1144 (C–O–C)
1e	102–103	3094, 3045, 3007 (C–H arom),2974, 2959, 2943 (C–H aliph), 1755 (C=O ester), 1684 (C=O keto), 1595, 1460, 1439, 1381 (C–C arom), 1303, 1276, 1219, 1167, 1088 (C–O–C)
2a	71–72	3093, 3072, 3018 (C–H arom), 2965, 2918 (C–H aliph), 1763, 1738 (C=O ester), 1674 (C=O keto), 1606, 1502, 1452, 1433, 1417 (C–C arom), 1296, 1215,1165, 1080 (C–O–C), 608 (C–Br)
2b	84–85	3094, 3071, 3017 (C–H arom), 2963, 2920 (C–H aliph), 1763, 1737 (C=O ester), 1674 (C=O keto), 1601, 1503, 1451, 1435, 1417 (C–C arom), 1296, 1215, 1165, 1080 (C–O–C), 608 (C–Br)
2c	57-58	3093, 3052 (C–H arom), 2994, 2947, 2911 (C–H aliph), 1749 (C=O ester), 1676 (C=O keto), 1595, 1470, 1435, 1377 (C–C arom), 1220, 1182, 1145, 1124 (C–O–C), 613 (C–Br)
2d	89–90	3091, 3022 (C–H arom), 2956, 2915 (C–H aliph), 1745 (C=O ester), 1637 (C=O keto), 1615,1514, 1433, 1400 (C–C arom), 1246, 1155, 1116, 1063 (C–O–C), 617 (C–Br)
2e	75–76	3090, 3013 (C–H arom), 2964, 2951 (C–Haliph), 1751 (C=O ester), 1705 (C=O keto), 1595, 1460, 1439, 1379 (C–C arom), 1219, 1166, 1093 (C–O–C), 654 (C–Br)
3a	83–84	3093, 3028 (C–H arom), 2972, 2955 (C–H aliph), 1741, 1739 (C=O ester), 1690 (C=O keto), 1598, 1502, 1492, 1438, 1423 (C–C arom, C–N arom), 1213, 1176, 1084, 1061 (C–O–C)
3b	127–128	3098, 3028 (C–H arom), 2970, 2952, 2920 (C–H aliph), 1748, 1739 (C=O ester), 1690 (C=O keto), 1599, 1513, 1495, 1437, 1423 (C–C arom, C–N arom), 1213, 1177, 1084, 1060 (C–O–C)
3c	127-128	3095, 3032 (C–H arom), 2969, 2949 (C–H aliph), 1753 (C=O ester), 1690 (C=O keto), 1597, 1470, 1437, 1423 (C–C arom, C–N arom), 1253, 1219, 1130 (C–O–C)
3d	188–189	3071, 3044 (C–H arom), 2978, 2931 (C–H aliph), 1767, 1748 (C=O ester), 1682 (C=O keto), 1597, 1586, 1514, 1433 (C–C arom, C–N arom), 1279, 1219, 1180, 1159 (C–O–C)
3e	100–101	3096, 3065, 3018 (C–H arom), 2978, 2955, 2931 (C–H aliph), 1749, 1732 (C=O ester), 1692 (C=O keto), 1601, 1438, 1387 (C–C arom, C–N arom), 1294, 1230, 1169 (C–O–C)
4a	148–149	3091, 3061 (C–H arom), 2990, 2957 (C–H aliph), 1751 (C=O ester), 1680 (C=O keto), 1614, 1591, 1495, 1452, 1423 (C–C arom, C–N arom), 1288, 1209, 1173, 1076 (C–O–C)
4b	130–131	3087, 3055, 3011 (C–H arom), 2976, 2955 (C–H aliph), 1757, 1737 (C=O ester), 1686 (C=O keto), 1605, 1573, 1495, 1435, 1418 (C–C arom, C–N arom), 1283, 1215, 1176, 1082 (C–O–C)
4c	130-131	3080, 3056 (C–H arom), 2955, 2936 (C–H aliph), 1751 (C=O ester), 1687 (C=O keto), 1597, 1470, 1437, 1395 (C–C arom, C–N arom), 1281, 1219, 1130, 1080 (C–O–C)
4d	151–152	3090, 3039 (C–H arom), 2990, 2938 (C–H aliph), 1768, 1748 (C=O ester), 1676 (C=O keto), 1597, 1582, 1528, 1441 (C–C arom, C–N arom), 1298, 1256, 1193, 1139 (C–O–C)
4e	165–166	3096, 3065, 3019 (C–H arom), 2978, 2932 (C–H aliph), 1749, 1732 (C=O ester), 1692 (C=O keto), 1601, 1473, 1439, 1387 (C–C arom, C–N arom), 1294, 1263, 1231, 1169, 1086 (C–O–C)

Molecular docking

Preparation of Dengue viral proteins

The protein data bank (PDB) was used to obtain the three-dimensional structure of the macromolecule. PDB contains large number of proteins which are experimentally determined and stored in this site. The structures are downloaded and saved either in mm CIF or PDB format. Proteins of dengue virus were used for this study. The 3D structure of all the seven proteins were downloaded from PDB and saved in PDB format. The downloaded proteins were viewed in Py-Mol viewer.

Preparation of ligands

The compounds selected from both natural and synthetic sources can be considered as ligands. 20 synthetic ligands were used for this study (tab. I). Ligands were constructed using ChemSketch. The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol for docking analysis and named as A, B and C respectively.

Docking study

Docking studies were conducting using iGEMDOCK software. IGEMDOCK (Generic Evolutionary Method for molecular Docking) is a graphical-automatic drug design system for docking, screening and post-analysis. The proteins and the ligands were loaded, and the out path was set. Standard docking parameters were used for docking (population size=200, generations=70 and Number of solutions=2). The docking process was initiated. After the docking process, the best docking pose for the individual ligands can be obtained for all the seven dengue viral proteins. The best binding pose, the binding affinity and the total binding energy values were saved in the output folder. The saved files were visualized in Py-Mol viewer.

RESULTS AND DISCUSSION

Chemistry

The designed dihydroxyacetophenone derivatives (**1a-e**, **2a-e**) and diazine with dihydroxyacetophenone skeleton derivatives (**3a-e**, **4a-e**) were synthesized according to figure 1.The procedures and conditions of the reactions were previously presented (24, 25).

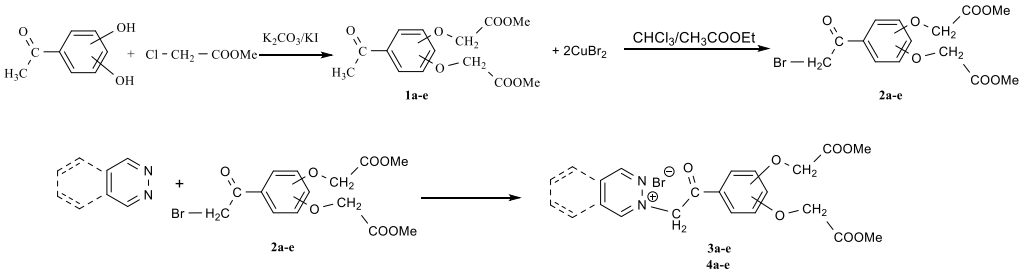


Fig. 1. The reaction pathway in synthesis of the target compounds

***In silico* molecular docking**

Using molecular docking approach, we performed the evaluation of anti-Dengue potential of five bis-etherificated acetophenone derivatives, five α-bromo-bisetherificated acetophenone derivatives and ten cycloimmonium salts with acetophenone skeleton, previously synthesized. So, 20 compounds, which were used as ligands, were selected for this study (tab. I).

The fitness and the interaction profile (the total binding energy, Van der Waal's Force and H-bond energy, in kcal/mol) of the dengue virus proteins with the ligands are presented in tables III-V. The lower energy scores represent better target protein-ligand binding affinity compared to higher energy score.

TABLE III.

The total binding energy (kcal/mol) profile for Dengue virus proteins with the investigated ligands

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
1a	-89.7	-89.06	-106.15	-635.01	-89.87	-94.95	-98.66
1b	-87.9	-85.47	-88.3	-580.07	-101.2	-89.7	-96.04
1c	-94.48	-89.71	-104.68	-564.22	-95.4	-106.8	-97.45
1d	-86.76	-87.5	-100.04	-629.62	-107.78	-94.87	-103.4
1e	-94.67	-88.84	-100.94	-589.19	-97.33	-96.63	-90.41
2a	-88.09	-86	-104.58	-613.36	-98.19	-96.92	-94.53
2b	-103.8	-95.49	-110.91	-591.18	-95.85	-94.08	-97.45
2c	-84.3	-82.71	-94.61	-566.75	-98.64	-101.93	-97.61
2d	-86.46	-86.94	-106.3	-588.14	-95.65	-93.21	-111.26
2e	-86.66	-92.06	-100.77	-637.089	-100.83	-101.88	-99.91
3a	-95.37	-104.65	-102.33	-655.9	-119.72	-106.6	-98.68
3b	-88.9	-104.62	-104.19	-648.73	-97.6	-113.61	-113.87
3c	-94.27	-106.92	-106.62	-672.03	-110.38	-109.35	-99.27
3d	-107.53	-104.59	-106.82	-714.91	-100.03	-110.31	-107.81
3e	-98.29	-102.47	-118.08	-692.88	-103.11	-125.27	-108.72
4a	-110.76	-99.69	-112.89	-719.34	-124.58	-102.09	-118.23
4b	-104.18	-105.47	-125.63	-732.67	-103.07	-100.71	-112.29
4c	-107.54	-98.52	-104.44	-673.19	-118.92	-115.73	-133.29
4d	-102.82	-96.26	-130.32	-701.34	-111.48	-100.82	-103.09
4e	-100.03	-113.81	-105.08	-677.67	-98.56	-114.16	-102.55

TABLE IV.

Van der Waal's Force values (kcal/mol)

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
1a	-87.58	-68.2	-89.65	-582.54	-69.11	-79.71	-88.33
1b	-70.42	-71.77	-75.32	-575.02	-87.24	-74.77	-85.54
1c	-81.47	-72.27	-73.67	-535.97	-81.4	-89.5	-89.18
1d	-78.45	-62.39	-77.73	-258.57	-81.92	-83.18	-82.99
1e	-80.94	-76.35	-82.5	-557.23	-76.69	-80.15	-72.85
2a	-78.32	-77.24	-81..35	-491.54	-72.68	-76.67	-78.05
2b	-88.16	-73.27	-80.78	-466.02	-81.26	-77.76	-71.15

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
2c	-62.02	-66.38	-87.36	-562.92	-88.14	-80.94	-95.11
2d	-71.09	-70.43	-99.3	-588.14	-82.3	-74.72	-101.76
2e	-69.62	-68.53	-79.58	-580.68	-81.34	-82.23	-84.05
3a	-81.39	-84.05	-85.71	-655.9	-89.62	-89.94	-78.78
3b	-73.65	-91.81	-93.07	-648.73	-85.06	-96.4	-98.62
3c	-73.46	-99.92	-87.54	-617.15	-94.55	-87.85	-85.89
3d	-98.25	-86.32	-84.57	-714.91	-87.56	-88.29	-93.47
3e	-92.02	-83.38	-97.23	-686.79	-92.62	-103.94	-89.06
4a	-94.99	-82.04	-102.15	-668.27	-100.67	-80.4	-102.41
4b	-95.96	-87.36	-106.51	-655.21	-98.59	-94.03	-97.92
4c	-96.82	-79.71	-85.18	-568.33	-103.69	-89.5	-105.1
4d	-83.71	-89.26	-105.77	-697.84	-105.14	-87.25	-98.61
4e	-80.3	-99.27	-85.85	-624.79	-85.06	-97.99	-89.29

TABLE V.

H-bond energy (kcal/mol) profile for Dengue virus proteins with the investigated ligands

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
1a	-2.08	-20.85	-16.5	-52.47	-20.75	-15.25	-10.33
1b	-17.48	-13.71	-12.99	-5.05	-14	-14.93	-10.5
1c	-13.01	-17.44	-31.01	-28.25	-14	-17.3	-8.27
1d	-8.31	-25.11	-22.31	-47.05	-25.86	-11.69	-20.41
1e	-13.73	-12.5	-18.44	-23.96	-20.64	-15.4	-17.55
2a	-9.77	-8.76	-23.23	-121.82	-25.51	-17.25	-16.48
2b	-15.64	-2.22	-30.13	-125.17	-14.59	-16.31	-26.3
2c	-22.28	-16.33	-7.25	-3.83	-10.5	-20.99	-2.5
2d	-15.36	-16.51	-7	0	-13.35	-18.49	-9.5
2e	-23.04	-23.53	-21.19	-56.41	-19.49	-19.65	-15.85
3a	-13.98	-20.61	-16.62	0	-30.1	-16.66	-19.9
3b	-15.36	-12.81	-11.12	0	-12.9	-17.29	-15.25
3c	-20.81	-7	-19.08	-57.89	-15.83	-21.5	-13.38
3d	-9.28	-18.27	-22.24	0	-12.47	-22.01	-14.34
3e	-6.27	-19.09	-20.84	-6.09	-10.5	-21.33	-19.65
4a	-15.77	-17.65	-10.74	-51.07	-23.92	-21.69	-15.82
4b	-8.22	-18.11	-19.13	-77.46	-10.48	-6.69	-14.37
4c	-10.73	-18.81	-19.26	-104.86	-15.23	-26.23	-27.88
4d	-19.11	-7	-24.56	-3.5	-6.34	-13.57	-4.49
4e	-19.73	-14.54	-19.23	-52.85	-13.5	-22.17	-13.26

The cluster interaction tables which include H-Bond profile and amino acid position profile for the dengue virus proteins with the ligands are presented below (tabs. VI and VII).

A close-up view of binding mode of ligands with the seven selected proteins is shown in the figures 2-8.

TABLE VI.
H-bond profile for Dengue virus proteins with the investigated ligands

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
1a	-	H-S H-M	H-S H-M	H-M	H-S	H-S H-M	H-S
1b	H-S H-M	H-S H-M	H-M	H-S	H-S H-M	H-S H-M	H-S H-M
1c	H-S H-M	H-M	H-S	H-S	H-S	H-S	H-S
1d	H-S H-M	H-S H-M	H-S	H-S H-M	H-S H-M	H-S	H-S H-M
1e	H-S H-M	H-S	H-S	H-S	H-S H-M	H-S H-M	H-S H-M
2a	H-S	H-M H-S	H-S H-M	H-M	H-S H-M	H-S H-M	H-S H-M
2b	H-S	H-S H-M	H-S H-M	H-M	H-S H-M	H-S H-M	H-S H-M
2c	H-S H-M	H-S	H-M	H-S	H-S	H-S H-M	H-S
2d	H-S H-M	H-M	H-M	-	H-S H-M	H-S	H-S
2e	H-S H-M	H-S H-M	H-S H-M	H-S	H-S H-M	H-M	H-S
3a	H-S H-M	H-M	H-S H-M	-	H-S H-M	H-S H-M	H-M
3b	H-S H-M	H-M	H-S H-M	-	H-S H-M	H-S H-M	H-S H-M
3c	H-S H-M	H-S H-M	H-S	H-S H-M	H-S H-M	H-S H-M	H-S H-M
3d	H-S	H-M	H-S H-M	-	H-S H-M	H-S H-M	H-S H-M
3e	H-S	H-S H-M	H-S	H-S H-M	H-S H-M	H-S H-M	H-S H-M
4a	H-S	H-M	H-S H-M	H-M	H-S H-M	H-S H-M	H-S
4b	H-S	H-S H-M	H-S	H-S	H-S	H-S	H-S H-M
4c	H-S	H-S H-M	H-S	H-M	H-S H-M	H-S H-M	H-S H-M
4d	-	H-M	H-S H-M	H-S	H-M	H-S	H-M
4e	H-S H-M	H-S H-M	H-S H-M	H-S H-M	H-S H-M	H-S	H-S H-M

H-M is the hydrogen bonding between the viral protein and the main chain of the aminoacid;
H-S is the hydrogen bonding between the viral protein and the side chain of the aminoacid

TABLE VII.

Amino-acid position/profile for Dengue virus proteins with the investigated ligands

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
1a	-	ARG(619)/ -11.9 SER(577)/ -3.3	LYS(221)/-7 SER(185)/ GLY(199)/- 3.5	PHE(15)/ -3	ARG(60)/-7	THR(289)/ THR(317)/-2.5 ALA(290)/ THR(289)/-3.5	ASN(69)/ ARG(581)/ -3.5
1b	ARG(41)/-3.5 LEU(44)/ LYS(45)/ LEU(46)/ PHE(47)/-3.5	LYS(625)/-7 ILE(618)/-3.5	GLY(259)/-7	ASP(1)/ -2.5	ASN(152)/-7 LEU(149)/- 3.5	ASP(470)/-2.5 ARG(463)/ ASN(464)/-3.5	ARG(352)/ -3.5 THR(270)/ -3.5
1c	ARG(41)/-7 LEU(44)/-3.1	GLY(628) /-7	ARG(294)/ -12.9	THR(7)/ -2.5	ASN(152)/ -10.5	ARG(460)/-7	LYS(253)/ -8.3
1d	ARG(41)/-3.5 PHE(47)/-4.6	ARG(619)/ -11.1 PRO(635)/ -2.9	GLN(253)/ -9.9	ASP(1)/ -2.5 GLY(3)/- 2.9	ASN(105)/ -9.5 SER(75)/ -3.5	ASN(329)/-3.5	LYS(253)/-7 ASP(254)/ -3.5
1e	ARG(32)/-4 LEU(29)/-2.7	THR(667)/-5	ARG(294)/- 9.4	ARG(18)/- 7	ARG(107)/ -7 LEU(74)/ GLU(90)/ ASN(105)/ -3.5	THR(450)/-2.8 THR(450)/-3.5	ASN(272)/-7 ASP(596)/ -2.8
2a	ARG(68)/-6.3	TYR(578)/ -3.5 THR(667)/ -2.5	TRP(232)/ -6.2 TRP(210)/ -3.5	GLY(5)/-7	ARG(24)/-7 ASP(58)/ -3.5	HIS(485)/-6.9 THR(389)/-3.5	ARG(361)/ -6.1 THR(270)/ -3.5
2b	ARG(41)/- 10.3	THR(634)/- 6.7 GLY(628) /-5	GLN(253)/-6 LYS(227)/- 6.9	GLY(5)/-7	TRP(83)/- 3.5 LEU(149)/- 3.5	ARG(387)/-6.7 PHE(610)/-2.6	ASP(131)/ -9.2 LYS(105)/- 5.5
2c	THR(25)/-3.9 ARG(22)/-4.5	ARG(619)/- 10	GLY(259)/- 3.5	ARG(18)/- 3.8	ASN(152)/-7	ARG(418)/- 11.8 ASN(464)/-3.5	TYR(90)/- 2.5
2d	ARG(41)/ -12.2 PHE(47)/-3.2	GLY(628)/ ILE(630)/ ARG(629)/ THR(634)/ -3.5	AS(180)/ SER(185)/- 3.5	-	ASN(152)/- 3.5 LEU(149)/- 3.5	ARG(463)/ ASN(464)/-3.5 GLY(462)/ ARG(463)/-3.5	ARG(581)/A SN(69)/-3.5
2e	THR(62)/-5 VAL(23)/ SER(24)/ THR(25)/-3.5	LYS(625)/ -3.5 ARG(629)/-7	GLN(253)/ -6.2 LYS(227)/ -3.3	THR(7)/- -4.5	ARG(107)/ -10.1 SER(75)/ -3.4	ARG(418)/-7 ARG(418)/-3.5	HIS(53)/ -9.9

Ligand	Capsid protein	Envelope protein	NS1 protein	NS2A protein	NS3 protease protein	NS3 helicase protein	NS5 protein
3a	LYS(67)/-7 ARG(100)/-7	ARG(629)/ ILE(630)/-7	ASN(234)/-7 SER(228)/-3.5	-	ARG(107)/ -9.5 ASN(105)/-7	ARG(418)/-8.8 GLY(196)/-5.4	TYR(606)/ -10.5
3b	THR(71)/-6.3 ARG(100)/-6.9	GLY(628)/ ILE(630)/-3.5	ARG(336)/ -15.3 GLY(292)/ -6.2	-	ASN(152)/ -5.9 ALA(166)/ ASN(167)/ -3.5	ARG(418)/-3.5 GLY(196)/-8.3	THR(104)/ -5.7 THR(105)/ -5.6
3c	ARG(85)/-10.5 ASN(93)/-3.3	LYS(625)/ -3.5 GLY(628)/-3.5	ASN(234)/-5.3	THR(7)/-2.7 TYR(8)/-3.2	ASN(105)/-6.3 SER(75)/ ASN(105)/-3.5	THR(289)/-12 THR(289)/-3.5	HIS(110)/-3.9 GLU(111)/ VAL(132)/-3.5
3d	ARG(68)/-9.3	GLY(628)/ ILE(630)/ ARG(629)/ THR(634)/ -3.5	THR(262)/ -4.5 ILE(242)/ LYS(245)/ -3.5	-	THR(118)/ THR(120)/ -2.5 ALA(166)/ -3.5	ARG(387)/-10.3 ASP(603)/-2.9	HIS(52)/-3.5 THR(50)/ THR(51)/ -3.5
3e	ARG(55)/-6.3	LYS(625)/ -6.2 ILE(618)/-2.9	ARG(294)/ -8.3	ASP(1)/ -3.5	LYS(74)/ ASN(152)/ -3.5 LEU(149)/ -3.5	ASP(284)/-4.6 LEU(193)/-3.5	THR(51)/ -4.7 THR(51)/ GLN(693)/ TRP(694)/ -3.5
4a	ARG(41)/-12.3	ILE(630)/-6.2	ARG(336)/-5.6 ASN(293)/-7	GLY(5)/-3.2	ARG(107)/-4.5 ASN(105)/-7	ARG(463)/-4.3 GLY(196)/-6.9	THR(51)/-7.5
4b	ARG(55)/ LYS(45)/-3.5	THR(644)/ -4.7 LEU(636)/ GLU(638)/-3.5	ASN(234)/ -6.5	THR(7)/-8	ARG(55)/ -6.1	ARG(427)/-3.8	HIS(52)/ ASP(690)/ -2.5 ASP(690)/ -2.9
4c	ARG(41)/-10.7	THR(634)/ -5.3 GLY(628)/ ILE(630)/ ARG(629)/ -3.5	SER(228)/ -4.2	GLY(5)/-7.4	ARG(55)/ -3.5 VAL(59)/ GLU(86)/ VAL(146)/ -3.5	ARG(387)/-10 VAL(544)/-3.5	THR(51)/ -9.5 THR(51)/ -3.5
4d	-	GLY(628)/ ILE(618)/-3.5	SER(239)/-2.5 GLU(238)/-5.6	ARG(18)/-3.5	VAL(155)/-2.8	ARG(274)/-10.1	GLY(743)/-2.6
4e	THR(25)/-3.4 VAL(23)/ SER(24)/-3.5	LYS(625)/ -3.5 ILE(618)/-3.5	ARG(336)/ -6.7 SER(228)/ -3.5	THR(7)/ -2.5 TYR(8)/-3	ARG(107)/ -6.9 SER(75)/ -3.5	ARG(387)/ -10.3	TRP(418)/ -6.3 GLY(743)/ -3.5

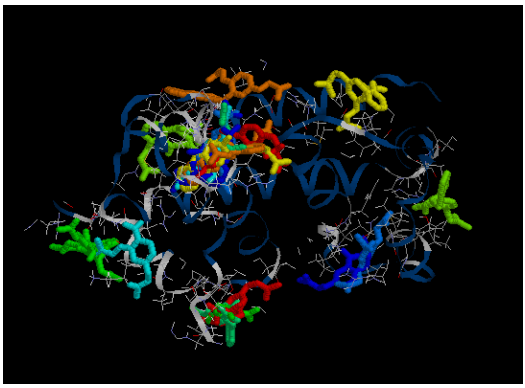


Fig. 2. Interaction of all compounds with Capsid protein

Fig. 3. Interaction of all compounds with Envelope protein

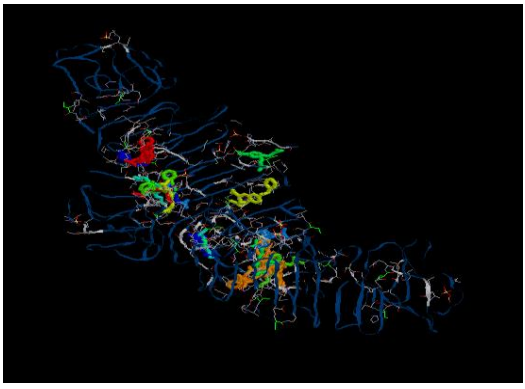


Fig. 4. Interaction of all compounds with NS 1 protein

Fig. 5. Interaction of all compounds with Transmembrane Domain of NS2A protein

Fig. 6. Interaction of all compounds with NS3 Protease protein

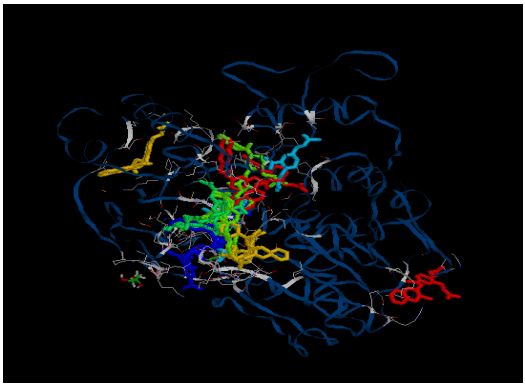


Fig. 7. Interaction of all compounds with NS3 Helicase protein

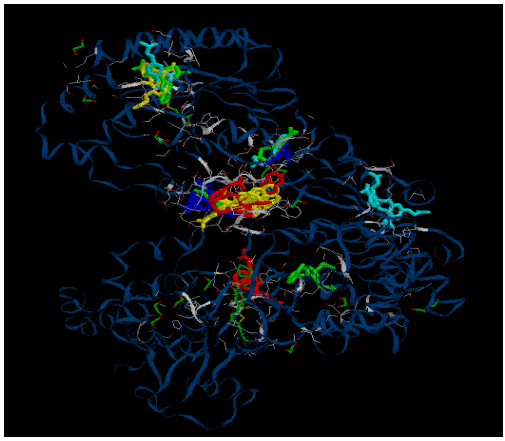


Fig. 8. Interaction of all compounds with NS 5 protein

From tables III-V, the 3D structure coordinates of protein of Dengue virus is optimized and 20 compounds are identified. Their total binding energy was calculated using iGEMDOCK. Evaluations of binding conformation of 20 compounds with dengue viral proteins are performed using iGEMDOCK. From docking study, we listed binding affinity of 20 compounds based on ligand binding energy. The binding pose for each ligand molecule into the dengue viral protein is analyzed and the one having lowest ligand binding energy with these proteins among the different poses are generated. The lower energy scores represent better protein-ligand target binding affinity compared to higher energy score.

The obtained results showed significant affinity between the tested compounds and Dengue transmembrane domain of NS2A protein. NS2A protein, a membrane protein, is one of the proteins found in the replication complex of flaviviruses. This protein possesses specific recognition sites for certain proteases (26). Compound **4b** was found to have the lower ligand binding energy compared to the binding energy at the other analogs for transmembrane domain of NS2A protein (binding energy value = -732.67 kcal/mol),

The amino acid positions for each ligand were studied (Table VII). Interaction analysis of binding mode of compound **4b** for transmembrane domain of NS2A protein revealed that it forms one hydrogen bonds at positions THR(7) with bond energies -8 kcal/mol,

Among the 20 analogs, compound **4a** was found to have lower ligand binding energy than other analogs for Capsid protein (binding energy value = -110.76 kcal/mol), and for NS3 Protease protein (binding energy value = -124.58kcal/mol).

Evaluations of the results showed that, the compound **4a** 2-(2-(2,4-bis(2-methoxy-2-oxoethoxy)phenyl)-2-oxyethyl)phthalazin-2-ium bromide forms one hydrogen bond at positions ARG(41), with bond energies, -12.3kcal/mol. We further analyzed the docked pose for finding the binding mode of compound **4a** into Capsid protein and for NS3 Protease protein to validate the reasonable binding conformations.

Compound **4c** was found to have the lower ligand binding energy for NS5 protein with a value of -133.29 kcal/mol and it appear two hydrogen bonds at positions THR(51) with different bond energies (-9.5 kcal/mol and -3.5 kcal/mol respectively).

For Envelope protein compound **4e**was found to have lower ligand binding energy (binding energy value = -113.81kcal/mol), than other analogs. Compound **4e** 2-(2-(3,5-bis(2-methoxy-2-oxoethoxy)phenyl)-2-oxyethyl)phthalazin-2-ium bromide forms two hydrogen bonds at positions LYS(625) and ILE(618), with bond energies, -3.5kcal/mol.

NS3 protein is a particularly interesting molecular target for antiviral compounds because of it central role in the viral life cycle. Dengue virus NS3 is a multifunctional protein in which the N-terminal 180 amino acids encode a protease that mediates viral polyprotein processing (27). This protein endowed with protease, helicase, adenosine triphosphatase (ATPase) and RNA 5' triphosphatase (RTPase) activities (28).

In examining the binding interaction and position of the compound **4a** with NS3 Protease protein ligand binding site predicted, it was found that two hydrogen bonds are formed, at positions ARG(107) and ASN(105) with bond energies, -4.5 kcal/mol and -7 kcal/mol, respectively.

The lower ligand binding energy for NS3 Helicase protein was proven to be compound **3e**. The evaluation of amino acid positions reveals that this compound, 1-(2-(3,5-bis(2-methoxy-2-oxoethoxy)phenyl)-2-oxyethyl)pyradazin-1-ium bromide, forms two hydrogen bonds at positions ASP(284) and LEU(193).

Dengue virus NS1 is a glycoprotein essential for viral replication with an unknown mechanism (29). NS1 is considered a unique "viral toxin" in dengue disease (30). Compound **4d** was found to have lower ligand binding energy (binding energy value= -130.32kcal/mol), for NS1 protein. The compound **4d** 2-(2-(3,4-bis(2-methoxy-2-oxoethoxy)phenyl)-2-oxyethyl)phthalazin-2-ium bromide forms two hydrogen bonds at positions ARG(336) and SER(228) with bond energies, -6.7 kcal/mol and -3.5kcal/mol respectively.

CONCLUSIONS

Our molecular docking studies explored the possible binding modes of 20 synthesized compounds, as ligands, with different Dengue virus proteins which include capsid protein, envelope protein, NS1 protein, transmembrane domain of NS2A, NS3 protease protein, NS3 helicase protein and NS5 protein. Several interactions were observed between the ligands and the protein binding pocket like the total binding energy, Van der Waal's Force and H-bond energy, in kcal/mol. The obtained results revealed that all the 20 compounds show good affinity with all the proteins; significant affinity was observed between the tested compounds and Dengue transmembrane domain of NS2A protein.

On comparing the binding energy and the binding site residues, we found that all compounds differ either in their binding modes or with the binding site residues for hydrogen bond formation. The conclusion drawn from our virtual screening and docking result was that the compound **4a** has highest binding affinity with most of the proteins and it can be used as an effective drug target for Dengue virus. Though, there are many reports on the *in vitro* analysis of these compounds and its antioxidant properties, but there are no *in silico* studies that predict the binding and active regions especially with these proteins.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

ABBREVIATIONS

DVP: Dengue Viral Proteins, AD: Acetophenone derivatives, iGEMDOCK: Generic Evolutionary Method for molecular Docking, NS2A protein: nonstructural protein 2A, HIV: human immunodeficiency

virus, DHF: Dengue hemorrhagic fever, DSS: Dengue shock syndrome, E: envelope, C: capsid, PreM: premembrane, NS1 protein: nonstructural protein 1, NS2B protein: nonstructural protein 2B,NS3 protein: nonstructural protein 3,NS4B protein: nonstructural protein 4B,NS5 protein: nonstructural protein 5,NSA protein: nonstructural protein, RNA: ribonucleic acid, Mtase: methyltransferase, PDB: Protein Data Bank, ATPase: adenosine triphosphatase, RTPase: ribonucleic acid 5' triphosphatase.

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IMPLICATION OF HUMAN FC RECEPTORS POLYMORPHISM IN CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA

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IMPLICATION OF HUMAN FC RECEPTORS POLYMORPHISM IN CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA (Abstract)**:** Immune thrombocytopenic purpura (ITP) is the most frequent form of autoimmune disease in childhood, characterized by thrombocytopenia (platelet count $<150 \cdot 10^9$/L) with otherwise normal cell lineages. One of the main mechanisms is the platelet destruction by phagocytic cells, covered by human Fc receptors (FCGR). The FCR family involves 3 classes of receptors: FCGR1A, 1B, and 1C; FCGR2A, 2B, and 2C; FCGR3A and 3B, all present on chromosome 1q23-24. The response to ITP treatment may be influenced by the polymorphism of the FCGR3A gene, which contributes to different levels of destruction of antibody - bound platelets. **Material and methods:** We report the results of FCGR2/3 polymorphic genotypes in 16 children with ITP admitted at the "Sf. Maria" Emergency Children's Hospital, Iasi, Romania, treated with corticosteroids, intravenous immunoglobulin (IVIg) or both, or conservative treatment, with a minimum of one year follow up. **Results:** We identified 9/16 patients with FCGR2C ORF haplotypes, 8 in heterozygous state and one in homozygous state, and 15/16 patients with FCGR2A-His166 variant. While it was previously reported that classical FCGR2C-ORF and 2B.4 variants correlate with the transient form of ITP and a good response to IVIg, in our sample it was present only in 3 patients, who had limited course of disease and a good response to IVIg. 5 patients were heterozygotes for FCGR2C-p.Gln62Trp, all of them developing an acute form, a good response to IVIg and no relapses. Furthermore, it has been demonstrated an association between FCGR genotypes characterized by increased immunoglobulin binding activity (FCGR3A allele V158 and FGCGR3B allele NA1) and chronic children ITP. In our study, only 2/16 patients express NA1 in homozygous state, one of them developing persistent ITP, while the other responded well to the treatment and did not relapse. **Conclusions:** Genetic influences contribute to the pathogenesis of ITP in children and response to IVIg. We evaluated the genetic risk factors in the FCGR2/3 locus that are associated with predisposition to limited course and a favorable response to IVIg in ITP in children. However, confirmation in a larger prospectively recruited cohort of children with ITP is needed. **Keywords:** FCGR POLYMORPHISM, IMMUNE THROMBOCYTOPENIC PURPURA, CHILDREN, PLATELETS.

Immune thrombocytopenic purpura (ITP) is the most frequent form of autoimmune disease in childhood, characterized by thrombocytopenia (platelet count <150 · 10^9/L) with otherwise normal cell lineages. The incidence ranges from 2-8 cases / 100,000 people, with a higher frequency in boys, with maximum between 2-5 years old (1, 2). The diagnosis is based on history, clinical exam, cell blood count, with the elimination of other clinical conditions that may lead to thrombocytopenia - malignancies, immunodeficiencies or immunologic diseases. Most often, the disease is self-limited, having spontaneous resolution in several weeks. However, approximately 20% of children with ITP still have decreased platelet counts 6 months after the clinical onset (1, 3). The main mechanisms explaining thrombocytopenia is the platelet destruction by phagocytic cells. Human Fc receptors (FCGR) are glycoproteins that bind the Fc portion of IgG which links to platelet-specific membrane antigens. Consequently, these platelets are phagocytized by reticuloendothelial cells, that express Fc receptors. The FCR family involves 3 classes of receptors: FCGR1A, 1B, and 1C; FCGR2A, 2B, and 2C; FCGR3A and 3B, all present on chromosome 1q23-24 (1,4). Loss of inhibitory FCGR2B expression, altered function due to single nucleotide polymorphisms (SNPs), or overrepresentation of FCGRs produce a disturbed immunity and subsequent auto-inflammation.

The role of Fc receptors is underlined by the fact that intravenous immunoglobulin (IVIg) treatment and splenectomy are efficient treatment choices, although the mechanism of action of IVIg in ITP is not clear. Several explanations have been proposed, including increased surface expression of FCGR2B, blockade of the Fc receptor for IgG or the acute interaction activating FCGRs on dendritic cells (5). The response to ITP treatment may be influenced by polymorphism of the FCGR3A gene, which contributes to different levels of destruction of antibody- bound platelets (6).

We report the results of FCGR2/3 polymorphic genotypes in children with ITP followed at the "Sf. Maria" Emergency Children's Hospital, Iasi, Romania, during a two-year period.

MATERIAL AND METHODS

We included in the current study all children diagnosed with ITP during a two-year period and a follow up of minimum 12 months. The study was approved by the Ethics Committee of "Sf. Maria" Emergency Children's Hospital, Iasi, Romania (Ref No. 503/08.01.2020). ITP was diagnosed based on platelet count <150·10^9/L. Secondary causes of thrombocytopenia were excluded. Clinical manifestations such as ecchymosis and petechiae, epistaxis, hemorrhagic bullae on the oral mucosa, platelets count, and the therapeutic schemes were recorded. Treatment decision (corticosteroids, IVIg, no treatment) were taken by the individual hematologists, based upon their experience. Bleeding severity was classified according to Buchanan Score (7). We collected blood samples (5 ml into a P100 tube) with informed consent. All patients had a minimum one-year follow-up. Persistent ITP was considered when low platelet count persisted after 3 months of follow up, while chronic ITP - when platelet count continued to remain below the threshold after 12 months of follow up.

The PureLink™ Genomic DNA kit (Thermo Fisher Scientific) was used to extract the genomic DNA, following the recommended protocol. To evaluate both the copy number variations (CNV) and the

SNPs in FCGR 2/3 locus, we performed multiplex ligation-dependent probe amplification (MLPA) with two complementary probe mixes P110 / P111. MLPA probes were designed specifically to cover a distance of 180 kb in the locus FCGR 2/3, located on the long arm of chromosome 1 (1q23.3). For the correct assessment of the CNVs or rearrangements, both probe mixes contain 14 probes, including FCGR2/3 locus and the thermal shock proteins HSPA6 and HSPA7 (fig. 1).

The protocol consisted of denatured 100 nanograms of genomic DNA followed by hybridizing with MLPA probes at 60°C for approximately 20 hours in a SensoQuest thermocycler (Sen-soQuest GmbH, Göttingen Germany). Reference DNA SD038 were used as reference DNA samples. The ligation was done at 54°C for 20 minutes. PCR amplification was performed using Cy5 labelled primers. The fluorescent amplicons were separated by capillary electrophoresis in CEQ 8000 GeXP Genetic Analysis System (Beckman Coulter, USA), and the raw data were analyzed by using Coffalyser.NET V9 software (MRC-Holland, Amsterdam) (8). Because the copy number variation in FCGR2/3 locus, the cut-off values probes were established between 0.8-1.2 for normal copy numbers (from reference DNA) and raised and decreased proportionately for more or fewer copies.

Institutional Review Board Statement. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of "Sf. Maria" Children's Hospital, Iasi, Romania (Ref. No. 503/08.01.2020).

Informed Consent Statement. Informed consent was obtained from all subjects involved in the study.

RESULTS

Sixteen children with ITP, aged 1 to 15 years old (10 boys and 6 girls) were included in the study. 15/16 patients had a clinical onset of the disease following an episode of acute viral infection prior to presentation. All cases presented platelet number below 20000/mmc. The assessment of bleeding severity according to Buchanan score revealed 3 patients with mild hemorrhage (grade 2), 6 with moderate (grade 3) and 7 patients with severe hemorrhage (grade 4). The therapeutic scheme was the following: 5 patients received corticosteroids, 8 patients received corticosteroids associated with IVIg, 2 patients received only IVIg and one patient was monitored without any specific medication. 6 patients developed persistent ITP and none of them progressed towards chronic ITP (tab. I).

Deletions/duplications

The analysis of deletion /duplication in the low-affinity FCGR locus identified one patient with CNR1 type deletion (copy number region 1), including the FCGR2C, HSPA7 and FCGR3B genes (fig. 1) and two patients with CNR4 (fig. 2) that includes the FCGR3B, HSPA7 genes and the fusion between proximal FCGR2B and distal FCGR2C genes.

The presence of the CNR4 duplication (fig. 2) with the fusion between the proximal part of FCGR2B (at least 3 exons) and the distal part of FCGR2C (two exons) is supported also by the presence of an additional copy (respectively three copies) for probes upstream of FCGR2B, and those for intron 2, exon 3 and intron 3 that detect the ORF allele from the wild-type FCGR2B gene (FCGR2B-wt) and the presence of 3 copies of probes that detect mutations in intron 7 of FCGR2C, respectively (fig. 2. Panel III).

TABLE I.
Patients' sample description

Patient	Age at diagnosis (years)	Gender	Platelet count at onset (/ml)	Bleeding severity (Buchanan grading)	Genetic mutations	Treatment	ITP outcome
P1	3	M	12,000	Grade 2	NA1 Del CNR1 Val/Phe	corticosteroids	persistent
P2	1	F	1,000	Grade 3	NA1/NA2 Classic ORF Val/Phe	corticosteroids +IV Ig	acute
P3	16	F	3,000	Grade 3	NA1	corticosteroids +IV Ig	acute
P4	9	M	5,000	Grade 2	NA1/NA2 Val/Phe	IV Ig	persistent
P5	15	M	1,000	Grade 3	NA1/NA2 Val/Phe Classic ORF	corticosteroids	acute
P6	3	M	15,000	Grade 3	NA1/NA2	No specific treatment	acute
P7	1	F	3,000	Grade 3	NA2 Dup CNR4 Classic ORF	corticosteroids + IV Ig	persistent
P8	5	M	15,000	Grade 4	NA1/NA2	corticosteroids	acute
P9	11	M	4,000	Grade 4	NA1/NA2 Val/Phe Classic ORF	corticosteroids	acute
P10	6	F	6,000	Grade 3	NA1/NA2	corticosteroids + IV Ig	acute
P11	4	M	8,000	Grade 4	NA2	corticosteroids	acute
P12	2	M	4,000	Grade 2	NA1/NA2/SH Dup CNR4 ncORF/ncORF/classic ORF	corticosteroids + IV Ig	acute
P13	6	F	1,000	Grade 4	NA1/NA2 Val/Phe Classic ORF	corticosteroids + IV Ig	acute
P14	5	M	1,000	Grade 4	NA2 Classic ORF	corticosteroids + IV Ig	persistent
P15	2	F	4,000	Grade 4	NA1/NA2 Val/Phe	corticosteroids + IV Ig	persistent
P16	7	M	20,000	Grade 4	NA1/NA2 Val/Phe Classic ORF	IV Ig	persistent

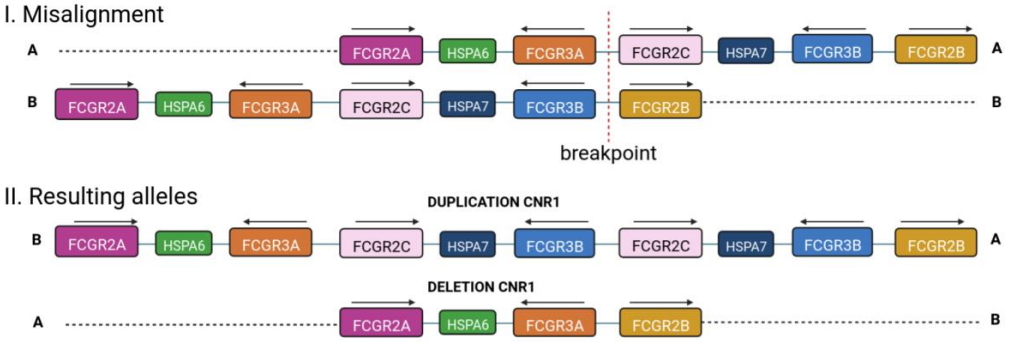

Fig. 1. Schematic representation of non-allelic homologous recombination (NAHR) creation of CNR1: Panel I: Misalignment of homologous chromosomes A and B. Red vertical lines indicate crossover breakpoints. Panel II: The resulting chromosomes, one with a CNR1 deletion and the other with a CNR1 duplication.

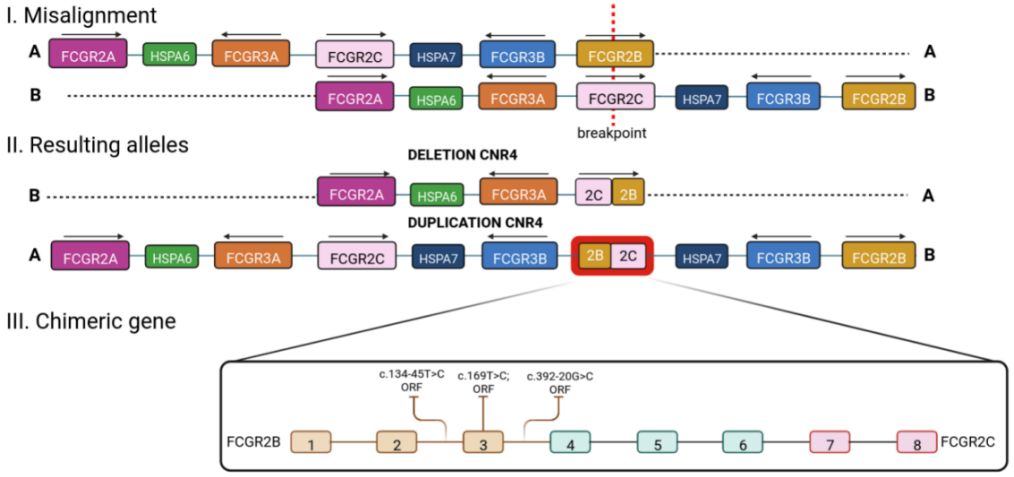

Fig. 2. Schematic representation of non-allelic homologous recombination (NAHR) creation of CNR4. Panel I: Misalignment of homologous chromosomes A and B due to an 82 kb repeat unit. Red vertical lines indicate crossover breakpoints. Panel II: The resulting chromosomes, one with a CNR4 deletion and the other with a CNR4 duplication.

Panel III: *FCGR2B* chimeric allele created by the fusion of the first three exons of the *FCGR2B* gene (in orange) and the last two exons of the *FCGR2C* gene (in pink); for exons 4 to 6 (in blue), there were no MLPA probes to specify their origin.

FCGR2C STOP/ORF haplotypes

To establish FCGR2C STOP/ORF haplotypes, 3 probes were evaluated for *FCGR2C*, in intron 2 (c.134-45T STOP), exon 3 (c.169T STOP) and intron 3 (c.392-20G STOP) respectively, in comparison with three nonspecific variants in the same positions for FCGR2B-wt or FCGR2C-ORF: c.134-45T>C ORF (intron 2), c.169T>C (exon 3), c.392- 20G>C ORF (intron 3) (fig. 3. Panel I). To obtain the

ORF/STOP copy number, we subtracted the *FCGR2B* gene copy number according to the genomic rearrangements previously determined using CNV-specific probes. We identified 9 patients with FCGR2C ORF haplotypes, 8 in heterozygous state and one in homozygous state (patient P12 with CNR4 duplication).

We further evaluated the four probes common for *FCGR2C* and *FCGR2A*-wt (fig. 3), in order to establish the classical or nonclassical ORF haplotypes: two probes located in the splicing site at the border between exon 7 and intron 7 (c.798+1A>G for classical ORF and c.798+1A presented in both nonclassical ORF 1 and ORF 2) and two probes in the splicing site at the border between intron 7 and exon 8 (c.799-1C>G ORF for classical ORF and nonclassical ORF1 and c.799-1C present only in nonclassical ORF2). Considering the number of copies of the *FCGR2A* gene we have determined the presence of classic and nonclassic ORF in *FCGR2C*. We set up the classic ORF in the *FCGR2C* gene in the presence c.798+1A>G and c.799-1C>G variants. In our study we identified 8 patients with classic ORF in heterozygous status.

The presence of c.798+1A with c.799-1C>G variant was considered nonclassical ORF1 and c.798+1A with c.799-1C was considered nonclassical ORF2. The nonclassical ORF1 was the most frequent haplotype identified in 14/16 patients, 6 cases in heterozygous status with classical ORF. Nonclassical ORF patients do not express FCGR2C.

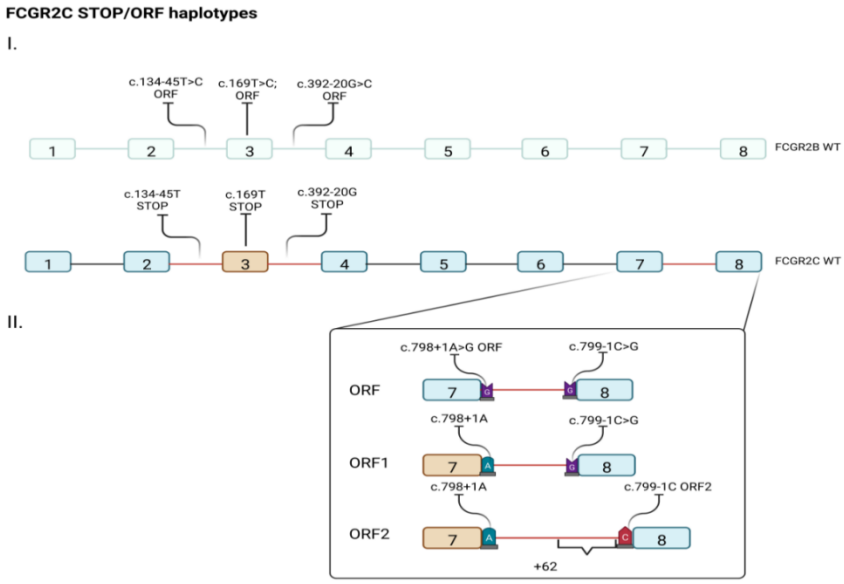

Fig. 3. Graphic representation of the FGCR2C STOP/ ORF haplotype. Panel I: Location of MLPA probes for ORF/STOP haplotype in FCGR2B and FCGR2C. Panel II: The position and combination of probes in classical and nonclassical ORF in FCGR2C gene

FCGR2B haplotypes

We identified the most frequent haplotype 2B.1 (9/16 patients) and the fewest haplotype 2B.4 in 3 patients, either in

combination with 2B.2 (2/16) or with 2B.2/2B.1 Furthermore, one of these patients had a CNR4 duplication.

FCGR3B haplotypes

The NA1, NA2 and SH haplotypes in *FCGR3B* are found in three polymorphic forms and determine the allotypic variants of the Human Neutrophil Antigen1 (HNA1). We constructed the haplotypes considering copy number variation using six probes in exon 3 in *FCGR3B* and *FCGR3A* gene (fig. 4). In our study we found two patients homozygous for NA1 (P1 and P3), three homozygous for NA2 (P7, P11, P14), the rest being heterozygous NA1/NA2, only one patient (P12) with CNR4 duplication was found heterozygous for NA1/NA2/SH.

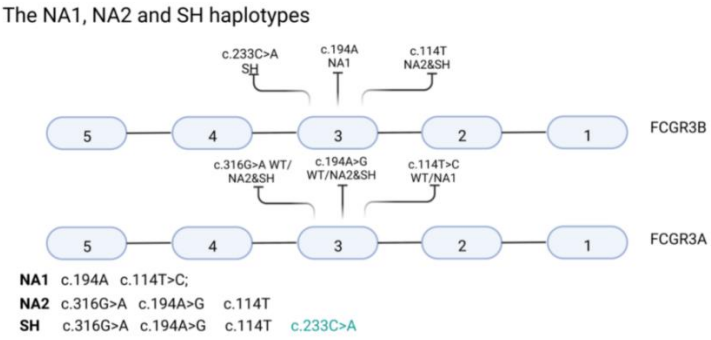

Fig. 4. Schematic representation of MLPA probes in *FCGR3B* and distinguishing between FCGR3B NA1/NA2/SH haplotypes.

Mutations

Several MLPA probes were designed to identify the mutations in FCGR2/3 locus. In our study we identified in FCGR2A gene, 9 patients with p.His166Arg, one patient in homozygous state (P15) and 5 patients heterozygous for p.Gln62Trp mutation.

The presence of valine at position 158 in protein FCGR3A (p.Val158Phe) was observed in 10 patients, two patients being in the homozygous state (P7 and P12, both with CNR4 duplication. Related to p.Ile232Tre mutation in the FCGR2B gene, we identified only 4 heterozygous patients.

Regardless the presence of mutations, these patients had a rather favorable evolution with good response to treatment (intravenous immunoglobulins and cortisone) and did not evolve to the chronic form. Thus, none of the genetic particularities of our patients correlated to the response to treatment or clinical outcome (tab. II)

DISCUSSION

Within the mechanism of ITP, FCGR determine effects on many levels, such as on antigen presentation, B-cell activation threshold, platelet clearance through FCGR on myeloid and/or natural killer (NK) cells, and it seems that the presence of certain polymorphisms influence response to different therapies. Fc receptors are glycoproteins that are found on different cells (neutrophils, macro-phages, lymphocytes, platelets) and are involved in creating connections between the humoral and cellular immune responses. The human FCGR family is represented by three major classes of receptors encompassing

12 transcripts derived from eight genes: FCGR1A, 1B and 1C; FCGR2A, 2B and 2C; FCGR3A and FCGR3B (10), all of which are present on chromosome 1. FCGRI is a high-affinity receptor for monomeric IgG, but the other receptors FCGR2 and FCGR3 need to form of immune complexes with IgG to express their function (low-affinity receptors). FCGR2 class is encoded by three genes (FCGR2A, 2B and 2C) and FCGR3 is encoded by two genes (FCGR3A and 3B).

The FCGR2 and FCGR3 genes show multiple genetic variants including functionally relevant SNPs and copy number variations (CNVs) that may influence antibody-mediated phagocytosis and antigen presentation activity.

These SNPs and CNVs have been associated with various autoimmune diseases (like systemic lupus erythematosus (SLE), multiple sclerosis, Addison disease, heparin-induced thrombocytopenia, PTI), infectious diseases, efficacy of immunotherapy and they have been shown to be involved in disease susceptibility (3, 10-12) and it is likely that other disease associations will be identified in the future.

After testing cohorts of patients using MLPA technique, it was possible to identify the CNRs present at this locus, now describing four distinct CNRs (13). Also, there were discovered chimeric genes at the locus and rearrangements resulted from the fusion of a part of either FCGR2A or FCGR2B to other parts of the locus (although their copy number is never increased or decreased). These modifications of genes have important consequences for expression and function (4, 13).

In our study we used the MLPA technique to identify the presence of FCGR2 and FCGR3 polymorphisms in patients diagnosed with immune thrombocytopenia.

The results of our study are marked by the limitations of a single-center study with a small number of patients, as a consequence of the rarity of the disease (0.2 – 0.4/100,000 inhabitants) and the inclusion of pediatric patients only. Therefore, we considered to provide a presentation of clinical evolution of our patients in relation with the particularities identified FCGR2/3 genes.

TABLE II.

Molecular findings in our study

MLPA probemixes P110/P111			P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
CNV			del CNR1						DUP CNR4					DUP CNR4?				
Haplotypes	FCGR2C STOP/ORF haplotypes	INTON 2 (c.134-45T>C;ORF)	STOP	STOP/ORF	STOP/STOP	STOP/ORF	STOP/ORF	STOP/STOP	STOP/STOP/ORF	STOP/STOP	STOP/ORF	STOP/STOP	STOP/STOP	ORF/ORF/ORF	STOP/ORF	STOP/ORF	STOP/STOP	STOP/ORF
		EXON 3 (c.169T>C; p.Ter57Gln; ORF)	STOP	STOP/ORF	STOP/STOP	STOP/ORF	STOP/ORF	STOP/STOP	STOP/STOP/ORF	STOP/STOP	STOP/ORF	STOP/STOP	STOP/STOP	ORF/ORF/ORF	STOP/ORF	STOP/ORF	STOP/STOP	STOP/ORF
		INTRON 3 (c.392-20G>C; ORF)	STOP	STOP/ORF	STOP/STOP	STOP/STOP	STOP/ORF	STOP/STOP	STOP/STOP/ORF	STOP/STOP	STOP/ORF	STOP/STOP	STOP/STOP	STOP/STOP/ORF	STOP/ORF	STOP/ORF	STOP/STOP	STOP/ORF
	FCGR2C nonclasic haplotypes	ORF/ORF1/ORF2	ORF1	ORF/ORF1	ORF1	ORF1/ORF2	ORF/ORF2	ORF1/ORF2	ORF/ORF1/ORF1	ORF1	ORF1/ORF	ORF1/ORF2	ORF1/ORF2	ORF/ORF2/ORF2	ORF/ORF1	ORF/ORF1	ORF1	ORF/ORF1
	FCGR3B haplotypes	NA1/NA2/SH	NA1	NA1/NA2	NA1	NA1/NA2	NA1/NA2	NA1/NA2	NA2	NA1/NA2	NA1/NA2	NA1/NA2	NA2	NA1/NA2/SH	NA1/NA2	NA2	NA1/NA2	NA1/NA2
	FCGR2B haplotypes	2B.1 (-386G/-120T) / 2B.2 (-386C/-120T) / 2B.4 (-386C/-120A)	2B.1	2B.2/2B.4	2B.1	2B.1	2B1/2B.2	2B.1	2B.1	2B.1	2B.1/2B.2	2B.1	2B.1	2B1/2B.2/2B.4	2B.2/2B.4	2B.1/2B.2	2B.1	2B.2
Mutations	FCGR2A	c.497A>G; p.His166Arg	His/His	His/His	His/His	His/Arg	His/Arg	His/Arg	His/His	His/Arg	His/Arg	His/His	His/Arg	His/Arg	His/Arg	His/His	Arg/Arg	His/His
		c.184C>T; c185A>G; p.Gln62Trp	WT	HZ(CA>TG)*	WT	WT	HZ(CA>TG)*	WT	WT	WT	WT	WT	WT	HZ(CA>TG)*	WT	HZ(CA>TG)*	WT	HZ(CA>TG)*
	FCGR3A	c.526G>T; p.Val158Phe	Val/Phe	Val/Phe	Phe/Phe	Val/Phe	Val/Phe	Phe/Phe	Val/Val	Phe/Phe	Val/Phe	Phe/Phe	Phe/Phe	Val/Val	Val/Phe	Phe/Phe	Val/Phe	Val/Phe
	FCGR2B	c.695T>C; p.Ile232Thr	Ile/Ile	Ile/Ile	Ile/Ile	Ile/Ile	Ile/Ile	Ile/Ile	Ile/Ile	Ile/Thr	Ile/Ile	Ile/Thr	Ile/Thr	Ile/Ile	Ile/Ile	Ile/Thr	Ile/Ile	Ile/Ile

Various studies depicted the role of Human Fc Receptors in childhood immune thrombocytopenic purpura (tab. III).

FCGR2A is polymorphic and has two codominant expressed alleles, FCGR2A-H166 and FCGR2A-R166 (p.His166Arg). This polymorphic variation of FCGR2A results from a single base substitution at nucleotide position 497, (A or G) which codes for the amino acids histidine (H) or arginine (R), respectively, at position 166 and was formerly known as p.His131Arg. This amino acid substitution is associated with an important impaired ability of the receptor to bind human IgG2 (14). The allele H166 is the variant with a high affinity for human IgG2, whereas the other allele R166 arginine has a low affinity for human IgG2.

The gene for FCGR3A is mainly expressed on natural killer (NK) cells and monocytes. It has two polymorphic variant alleles due to a single base substitution of thymidine to guanine (T to G) at nucleotide 526 (c.526G>T). expressed in protein as p.Val158 (V158) and p.Val158Phe (F158) (13,15,16).

The FCGR3A-V158 variant (currently known as p.Val176Phe) has a higher affinity for IgG1 and IgG3 in comparison with FCGR3A-F158 (17). Inheritance of either higher or lower affinity FCGR variants may determine certain predispositions of individuals to FCG-dependent immune disorders (18-20).

Li *et al.*, conducted a meta-analysis of relevant studies to research the effects of FCGR polymorphism on susceptibility to ITP. FCGR2A H131R polymorphism was significantly associated with childhood-onset ITP, FCGR3A-158V polymorphism was significantly associated with both childhood-onset ITP and adult-onset ITP. Thus the authors proved that FCGR2A H131R polymorphism may serve as a potential genetic biomarker for childhood onset-ITP while FCGR3A-158V polymorphism for both childhood and adult-onset ITP (21).

The presence of variant FCGR3A V158 in ITP - was found by Amorim *et al.* (1) and Carcao *et al.* (22), but not by Papagianni *et al.* (23) Zakaria *et al.* (24), investigated the contribution of FCGR2A, and FCGR3A genes polymorphism to the risk of ITP and their association with disease characteristics in 80 patients and 80 controls. The combined His166/R166 and F158/V158 genotype was found be more prevalent in ITP patients compared with controls. The results showed no significant difference between frequency distribution of the FCGR2A and FCGR3A alleles and genotypes on children newly diagnoses ITP compared with chronic ITP (24).

In our study, 15/16 patients expressed FCGR2A-His166 variant. We found the heterozygous variant FCGR3A – V158/F158 (Val/Phe) in 8/16 patients; 2 patients with homozygous variant that present also CNR4 duplication. Our results are similar with the findings of Breunis *et al.* (2008) who analyzed the DNA from 116 patients with ITP and 100 controls and found that FCGR3A-V158/F158 are risk factor for childhood ITP, compared with FCGR3A-F158/F158 variant (25).

Breunis *et al.* found that FCGR2C-ORF is significantly associated with susceptibility to both adult and childhood ITP (25). Several molecular studies described FCGR2C-ORF (Stop/ORF/nc-ORF). In most people, the FCGR2C gene is not expressed due to a stop codon at c.169T in exon 3 (FCGR2C-p.Ter57). FCGR2C-ORF is scored in case of the presence of FCGR2C c.134-45T>C in intron 2, in exon 3 the FCGR2C c.169T>C (rs759550223) polymorphism converting the stop codon

into a glutamine and thereby enabling expression of the gene (FCGR2C-p.Ter57Gln) (8) and c.392-20G>C alteration in intron 3 (13).

The classic ORF haplotype consists of nucleotide changes in FCGR2C-ORF and absence of the intron 7 splice variant (c.798+1A; rs76277413). In the case of FCGR2C-ORF and c.798+1A, the haplotype was scored as FCGR2C-nc-ORF (nonclassic open reading frame).

In FCGR2C-nc-ORF1 and 2, presence of an A at the splice donor site c.798+1 result in splicing out of exon 7 and loss of expression due to a frameshift and activation of a novel stop codon in exon 8. In FCGR2C-nc-ORF 2, presence of C at 799-1 activates a cryptic acceptor splice site within intron 7 causing re-introduction of a 62 bp sequence of intron 7 into the transcript (fig.4, Panel II).

The MLPA technique cannot establish if two given mutations are on the same chromosome. As shown by Nagelkerke *et al.*, between FCGR2C-ORF variant in exon 3, c.169T>C, p.Ter57Gln (formerly known as p.57Q) and c.798+1G exist a very strong linkage disequilibrium, suggesting that these two variants are located on the same chromosome. If c.169T>C (p.Ter57Gln) is accompanied by c.798+1A the haplotype is designated "nonclassic" FCGR2C-ORF (9).

Taking this into account, we detected seven samples that were attributed to classical ORF haplotype (P2, P5, P7, P9, P12, P13, P14) and in which we appreciated that FCGR2C-ORF is expressed (tab. II). For patients P4 and P12, the alleles expressing the FCGR2C-ORF variant (p.Ter57Gln) are associated with the presence in intron 3 of the STOP variant (c.392-20G) and nonclassical ORF2 (in intron 7), which probably leads to low to absent expression of FCGR2C protein.

TABLE III.

Original studies depicting the role of Human Fc Receptors in childhood immune thrombocytopenic purpura

Study / Author	Study type and population	Aim	Results
Schmidt *et al.*, 2019 (26)	Randomized controlled trial (TIKI study) 180 children with newly diagnosed with ITP, aged 3 months - 16 years and 180 healthy controls.	To evaluate whether the known FCGR 2/3 polymorphism are a risk factor for ITP, and to what extent it is related to clinical outcome and response to IVIg.	The variant FCGR2C-ORF and FCGR2A- p Gln62Trp allele were overrepresented. The FCGR2C-ORF and FCGR2A- p. Gln62Trp are associated with a transient I course of ITP and a favourable response to IVIg. A combined deletion of FCGR2C/FCGR3B (CNR 1 deletion) was associated with prolonged disease courses and nonresponse to IVIg.
Papagianni *et al.*, 2013 (23)	Prospective multicentre study 53 children aged 6 months old- 15 years with newly diagnosed and chronic ITP and 45 controls.	To assess the link between FCGR polymorphism and therapeutic response to IVIg.	No significantly difference between allelic frequencies FCGR2A, FCGR3A in children with ITP compared with healthy controls. No association between the two FCG receptors and the therapeutic response to IVIG. The genotype FCGR2A - 158VF had a significant higher frequency in children with ITP

Foster *et al.*, 2001 (15)	Prospective study 37 Caucasian children with chronic ITP and 218 healthy controls	To investigate the distribution of common polymorphisms in 11 candidate genes. This included three structural variants in low affinity FCGRs: FCGR2A, FCGR3A, FCGR3B.	Strong association with ITP was noticed in FCGR3A, FCGR3B, TNF and LTA. 81% of patients with chronic ITP possessed one or both higher risk variant genotypes: FCGR3A V/F or FCGR3B NA1/NA1
Heitink-Pollé *et al.*, 2018 (27)	Prospective, multicentre, stratified open, parallel group study on 200 children aged 3 months -16 years.	To investigate whether IVIg treatment decrease the risk of chronicity in ITP. Among baseline characteristics of the study samples, genetic variations in FcgR genes FCGR2A, FCGR2B, FCGR2C, FCGR3A, and FCGR3B, was sought.	The patients with genotype FCGR2B – 232I responded to IVIg. The homozygous FCGR2B-232T patients did not respond.
Amorim *et al.*, 2012 (1)	Prospective study 39 subjects' children with acute and chronic ITP, aged 1-10 years and 78 children – control group.	To investigate the receptor gene polymorphism in children with ITP	An association of the polymorphic allele FCGR3A with ITP was found.
Carcao *et al.*, 2003 (22)	Retrospective study 98 children, aged 6 months old – 16 years, with acute and chronic ITP, and 130 healthy adult controls	To report the occurrence of polymorphic genotypes FCGR2A and FCGR3A childhood ITP.	An over-representation of the homozygous FccRIIa-131H/H genotype in ITP children *vs.* control group was found.
Bruin *et al.*, 2004 (2)	Prospective study 60 children, aged 6 mo-16 years newly diagnosed with ITP.	To investigate which patient variables, including FGR2B-232I/T predict the development towards chronic ITP.	Female gender was 3 times higher more susceptible to develop chronic ITP. Lack of infection at onset correlate with a 3.6 higher possibility for chronicity. The presence of genotype FCGR2B-232I/T had a six times higher risk for chronicity compared with FCGR2B- 232I/I.
Zakaria *et al.*, 2023 (24)	Case control study 80 children with primary ITP and 80 healthy controls	To investigate the contribution of FcyRIIa and FcyRIIIa genes polymorphism to the risk of ITP and their association with disease characteristics in Egyptian children	A significant difference genotype distribution and allelic frequencies for FcyRIIa, and FcyRIIIa polymorphism in ITP patients versus controls ($p < 0,05$) was found.

The nonclassical ORF1 was the most frequent haplotype identified in 14/16 patients, 6 cases in heterozygous status with classical ORF. Nonclassical ORF patients do not express FCGR2C.

Concerning ITP susceptibility, Caucasian Europeans seem to express an important disequilibrium among FCGR2A-

p.Gln62Trp, FCGR2C-ORF, FCGR3A-V158, and the 2B.4 promoter (4, 13, 16). Classical FCGR2C-ORF and 2B.4 seem to be correlated with the transient forms of ITP and a good response to IVIg (26).

We establish the haplotypes for *FCGR2B* and *FCGR2C* promoters as follows: the 2B.4 haplotype is considered in the presence of -386C/-120A and was assigned only to the *FCGR2B* as previous studies demonstrated (2); haplotype 2B.1 is considered in the presence of -386G/-120T and haplotype 2B.2 in the presence of -386C/-120T and was assigned to the *FCGR2C* gene. Only 3 of our patients present associated haplotype 2B.4 and classical FGCR2C-ORF (P2, P12, P13) and had the acute form of the disease and good response to IVIg, even though there are not significant differences between their clinical presentation and the other patients from the study group.

In the study performed by Schmidt *et al.*, probably the largest one in children with ITP (TIKI trial), the presence of FCGR2A – p.Gln62Trp allele significantly associated with transient ITP and a good response to IVIg. Also, the variant FCGR2C-ORF and FCGR2A- p.Gln62Trp allele were overrepresented (26). In our study 5 patients were heterozygous for FCGR2C-p.Gln62Trp (patients P2, P5, P12, P14, P16), all of them presenting a short course of disease, good response to cortisone and IVIg and no relapses. Also, the CNR1 deletion was found significant for the susceptibility of developing the chronic form of the disease and with a lower response to IVIg (26). As opposed to this, our patient that we found with the CNR1 deletion, responded well both to cortisone and IVIg, had a few relapses, but did not develop chronic ITP.

In Bruin's study (2) over 60 children with newly diagnosed ITP, the presence of heterozygote FCGR2B- p.Ile232Tre mutation (Ile232/Tre232) increased six times the risk of developing chronic ITP comparing with the genotype FCGR2B – p.Ile232 the homozygous state (Ile232/Ile232) (2). In our sample, we identified only 4 heterozygous patients (P8, P10, P11, P14) with the mutation Ile232/Tre232 in the FCGR2B gene, but unlike Bruin's study, their outcome was very good, with a positive response to corticosteroids and no relapses.

Apart from the risk of chronicity, Heitink-Pollé *et al.* (28) indicated that patients with homozygous FCGR2B-Tre232/Tre232 did not respond to IVIg treatment while all complete responders presented FCGR2B –Ile232 allele (27). Neither of the patients in our study group presented FCGR2B- Tre232/Tre232 variant which could be consistent with their good clinical evolution.

Nagelkerke *et al.*, investigated the genetic variation in the FCGR2/3 locus in auto-immune and auto-inflammatory disease, and how the FcyR variants relate to treatment response and outcome. FCG2B - p.232Thr allele expression is increased in adult ITP and in chronic childhood ITP. In adult autoimmune diseases there is an association of classic FCGR2C-ORF with ITP, while in childhood autoimmune diseases, classic FCGR2C-ORF confers susceptibility to childhood ITP as well as Kawasaki disease. Classic FCGR2c-ORF and 2B.4 correlate positively with the response to intravenous immunoglobulins in childhood ITP. Moreover, these variants are associated with transient disease course, and not to chronic thrombocytopenia (28).

Other candidate gene polymorphisms have been studied by Foster *et al.* (2001) in

a cohort of children with chronic ITP. The genes include three structural variants in low-affinity FCGRs (FCGR2A, FCGR3A and FCGR3B) and regulatory variants in eight cytokine genes (TNF, LTA, IL1A, IL1B, IL1RN, IL4, IL6 and IL10). A strong association with ITP was observed in four loci (FCGR3A, FCGR3B, TNF and LTA). The heterozygous V158/F158 genotype of FCGR3A was increased in chronic ITP patients compared with controls (62% vs 41%). 81% of children with chronic ITP possessed one of both of the higher risk variant genotypes of FCGR3A V158/F158 and FCGR3B NA1/NA1) *versus* 50% of controls (15).

Furthermore, it has been demonstrated an association between FCGR genotypes characterized by increased immunoglobulin binding activity (FCGR3A allele V158 and FGCGR3B allele NA1) and chronic children ITP (15). In our study, only 2/16 patients express NA1 in homozygous state, one having V158/F158 (P1) and one F158/F158 (P3). The first patient that associated also CNR1 deletion presented persistent ITP, while the other responded well to the treatment and did not relapse.

In our sample, three patients were homozygous for NA2, the rest (12 patients) being heterozygous NA1/NA2 and only one patient, which presents CNR4 duplication was found heterozygous for NA1/NA2/SH. This patient had a good response to IVIg and did not have any relapses.

CONCLUSIONS

Several studies showed the genetic involvement of the FCGR2/3 locus presenting many functional genetic variants in complex linkage (13, 16, 26). A variety of other illnesses (e.g., Kawasaki disease), also linked to this polymorphism, could further provide us with important information about fundamental variations in pathophysiologic mechanisms (13). The locus is also promising in terms of genetic therapeutic efficacy prediction, particularly for cancer immunotherapy, even though this is not yet applicable. A complete genetic analysis could be helpful for research on the locus, given its intricacy and errors in the current databases containing reference sequences. This kind of technique has recently been investigated for FCGR3A employing long-range sequencing with Oxford Nanopore technology, allowing a thorough analysis of polymorphism locations within the gene (29).

The best course of treatment for ITP in children needs to consider medical factors such as platelet count, FCG polymorphism, clinical history, bleeding severity but also the moral values such as beneficence, non-maleficence, and respect for parents/patient' autonomy. Further research on ITP in children, particularly focused on genetic interference with disease outcome are needed. However, this research on a potentially vulnerable population like this needs to be conducted in a safe and respectful of the child's rights and welfare, such as obtaining informed consent from the child and their parents or guardians, minimizing potential harms, and ensuring confidentiality (30). A thorough analysis of all SNPs and CNVs together is required to take full use of genetic diversity at the FCGR2/3 locus. The MLPA analysis is useful to give an overview of the genetic alterations of this locus as it is shown in our study. Our findings were overall concordant with other studies' results except for the CNR4 duplication, which would need to be studied in a larger lot of patients to establish

whether it is connected to the predisposition to develop ITP.

With this study we aimed to evaluate the genetic risk factors in the FCGR2/3 locus that were identified in childhood ITP. All the variations detected in our patient group were associated with limited course of the disease and good response to IVIg. These findings could suggest a specific population particularity. The results must be interpreted with caution due to the small sample and confirmation in a larger prospectively recruited cohort of children with ITP is needed.

CONFLICTS OF INTEREST AND FUNDING

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All authors had equal contributions.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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EXPLORING THE RELATIONSHIP BETWEEN PSYCHOSOCIAL RISKS, BURNOUT LEVELS, AND EMOTIONAL INTELLIGENCE AMONG MEDICAL AND NON-MEDICAL PERSONNEL IN ROMANIAN MEDICAL UNITS

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EXPLORING THE RELATIONSHIP BETWEEN PSYCHOSOCIAL RISKS, BURNOUT LEVELS, AND EMOTIONAL INTELLIGENCE AMONG MEDICAL AND NON-MEDICAL PERSONNEL IN ROMANIAN MEDICAL UNITS. (Abstract). Emotional intelligence implies the ability to perceive, understand, manage, and express emotions effectively, both in oneself and in others. Burnout is a state of emotional, physical, and mental exhaustion caused by prolonged and excessive stress, particularly related to one's job or work environment. Psychosocial risks at work refer to factors in the work environment that can influence employees' psychological and social well-being. **The objective** of this study was to investigate the differences between medical and non-medical personnel regarding burnout, emotional intelligence, and perceived psychosocial risks at work in healthcare facilities in Romania. **Material and methods**: A cross-sectional study was conducted in February-March 2023 on a sample of 500 healthcare specialists, including 217 nurses, 98 physicians, and 55 respondents with other medical specialities (e.g., psychologists, biologists, pharmacists, physiotherapists etc.), along with 130 non-medical personnel from public and private healthcare units in Romania. Data was collected using the Maslach Burnout Inventory (MBI HSS), UNIPSICO Battery, Trait Meta-Mood Scale (TMMS-24), and Eco System. **Results:** For medical personnel, burnout scores were as follows: emotional exhaustion (M = 19.73 ± 13.68), depersonalization (M = 4.44 ± 4.99), and personal accomplishment (M = 38.67 ± 7.45). Non-medical personnel showed higher values in emotional exhaustion (M = 27.33 ± 14.16), depersonalization (M = 6.59 ± 5.99), but lower scores in personal accomplishment (M = 35.87 ± 7.92). Organizational justice scores were lower for non-medical personnel (M = 21.63 ± 5.61) compared to medical personnel (M = 25.87 ± 5.86). Regarding protective factors against burnout, in terms of emotional intelligence, emotional attention had values of (M = 30.30 ± 5.87) for medical personnel and (M = 31.63 ± 5.05) for non-medical personnel. For emotional clarity, medical personnel scored (M = 31.34 ± 4.96) and non-medical personnel scored (M = 31.93 ± 4.59), while for emotional repair, medical personnel scored (M = 29.97 ± 4.28) and non-medical personnel scored (M = 29.68 ± 4.30). Role conflict showed higher scores for non-medical personnel (M = 9.61 ± 4.34) compared to medical personnel (M = 7.25 ± 4.27). Role clarity scores were higher for medical personnel (M = 16.25 ± 3.61) and lower for non-medical personnel (M = 16.06 ± 3.51). Workload scores were higher for non-medical personnel (M = 24.19 ± 8.02) and lower for medical personnel (M = 21.97 ± 7.90). **Conclusions:** Medical personnel experiences a higher sense of organizational justice and personal accomplishment compared to the non-medical staff. In the case of the neg-

ative dimensions of burnout, higher levels of emotional exhaustion and depersonalization have been recorded among non-medical personnel. Additionally, for emotional attention dimension, higher scores have been registered among the non-medical staff. Regarding other psychosocial risks, role conflict has recorded higher values among administrative personnel, as well as workload. **Keywords:** MEDICAL PERSONNEL, NON-MEDICAL PERSONNEL, BURNOUT, EMOTIONAL INTELLIGENCE, ORGANIZATIONAL JUSTICE, PSYCHOSOCIAL RISKS.

Burnout is a psychological response that occurs when individuals experience prolonged exposure to stress (1). The concept of burnout was first introduced by Herbert Freudenberger in the early 1970s and 1980s, who noticed that exhausted colleagues showed a lack of motivation at work. He described burnout as a feeling of exhaustion resulting from striving excessively to meet idealistic expectations. He believed burnout could stem from unmet self-imposed or socially influenced expectations, leading to physical and emotional exhaustion. This state can cause individuals to feel ineffective, drained, and detached from their work, thus hampering productivity and success (2).

The European Agency for Safety and Health at Work categorizes psychosocial risks into factors like excessive workload, conflicting demands, and uncertainty about the worker's role and responsibilities (3). *Role conflict*, also known as role-related conflict, refers to a scenario where a worker finds it challenging to meet conflicting role expectations simultaneously. It occurs when an individual receives contradictory demands or responsibilities from multiple sources, making it difficult to reconcile these conflicting roles. Another aspect of role conflict is when a worker faces tasks that require resources they lack, hindering their ability to fulfil the assigned duties adequately. Role clarity refers to the extent to which there is a lack of clear information or clarity regarding the expectations associated with a particular role (4). Numerous studies have demonstrated a positive correlation between role clarity and burnout (5). *Emotional intelligence* was introduced to the psychology literature in the 20th century by Salovey and Mayer in 1990. Mayer and Salovey define emotional intelligence as the ability to recognize, understand, and regulate one's own emotions and the emotions of others, to differentiate between them, and to use this information to guide thoughts and actions (6). In 1995, Daniel Goleman wrote the best-selling book "Emotional Intelligence" which popularized the concept of emotional intelligence worldwide (7). Emotional intelligence acts as a protective factor against the impact of psychosocial factors. According to the model of the four branches of emotional intelligence, having appropriate intrapersonal and interpersonal qualities predicts better emotion regulation and effective emotional management. Perceiving emotions involves acknowledging and recognizing emotional information, while using emotions requires activating rational thinking. Understanding and analysing emotions involves cognitive processing, from labelling emotions to distinguishing between words and actual emotions. Lastly, managing emotions reflects the ability to handle one's own emotions and the emotions of others (8).

Healthcare specialists, including nurses, doctors, pharmacists, physiotherapists, biologists, psychologists, and social work-

ers, face challenging tasks, heightened difficulty, significant responsibility, and a strong sense of dedication (9). The primary psychosocial hazards encountered by personnel in the healthcare sector are related to workload, organizational justice issues, emotional regulation, conflicting demands, and role conflicts (10).

Healthcare professionals also include the administrative staff of a general hospital, who play a crucial role in providing and processing various data necessary for managerial decision-making. They efficiently manage the workforce, facilities, and budgets while coordinating and handling conflicting interests among different departments, such as medical treatment, healthcare, and support services departments (11). Healthcare professionals face daily challenges at work, including psychosocial factors that can make their job demanding. These psychosocial risks have been associated with burnout (12). The impact of psychosocial risks can influence both psychological and physical health through psychophysiological mechanisms triggered by stress. An overload of demands and emotional requirements, coupled with inadequate personal or material resources to cope with them, can lead to this adverse psychological state in workers (13).

Despite the significant relevance of psychosocial risks and emotional intelligence in influencing burnout, there is a notable scarcity of research focusing both medical and non-medical personnel in healthcare units. After conducting a thorough review of existing literature, no studies were found that specifically investigated the psychosocial risks faced by both categories of personnel mentioned earlier and their association with emotional intelligence, considering burnout.

Hence, the present study aims to bridge this gap in the literature by conducting an analysis of the impact of psychosocial risks and emotional intelligence on burnout levels for medical and non-medical personnel among Romanian healthcare specialists.

MATERIAL AND METHODS

Participants and data collection

Between February and March 2023, a cross-sectional study was conducted using the snowball sampling method. An online questionnaire was developed and distributed through Google Forms. The distribution of the questionnaire was facilitated by the Order of General Medical Assistants, Midwives, and Medical Assistants in Romania, as well as the heads of departments from public and private medical facilities located in Iasi, Constanta, Oradea, Cluj, Bucharest, and Timis. Additionally, the questionnaire was made available through the Pharmacists' College in Romania, the College of Psychologists in Constanta, the Association of Legal Advisors from Public Hospitals, the Association of Quality Management Officers in Public Hospitals, and the Sanitas Constanta Trade Union.

The group of participants in the study comprised 370 medical staff respondents, including doctors, nurses, physical therapists, biologists, pharmacists, psychologists, social workers, and others. Additionally, there were 130 non-medical (administrative) staff respondents who were employed in hospitals and health facilities across Romania. So, a total of 500 subjects were included in the research. The participants worked in various types of hospitals, including municipal, clinical, regional, county, or polyclinic hospitals, in both the private and public sectors.

To be eligible for inclusion in the

study, participants had to be currently employed in Romanian hospitals, clinics, or state/private institutions. Any participants who submitted their answers after the specified deadline were excluded from the analysis.

In the study, the respondents were categorized into two groups based on their professions, to gain insights into sector-specific challenges. Medical and non-medical personnel often encounter distinct challenges within their respective fields, and identifying sector-specific factors contributing to burnout was a primary aim. Moreover, there is a lack of research comparing these different categories of personnel, with non-medical staff being particularly underrepresented in healthcare studies, especially in Romania. By analyzing both medical and non-medical personnel, the study offers an opportunity to compare their experiences. This comparative analysis helped shed light on the differences in symptoms and the impact of burnout across different fields. Previous research has indicated that non-medical personnel may face higher levels of workload, emotional exhaustion, and depersonalization, as well as lower scores for personal accomplishment (14). Moreover, another study analysing in comparison Romanian medical and non-medical personnel illustrated that wellbeing and organizational justice, are higher also for medical personal (15).

Instruments

Burnout—The 22-item Maslach MBI Human Services Survey (MBI-HSS) measuring instrument (16) was used to measure the level of burnout. MBI-HSS is the original and most widely used variant of the tool. The instrument contains 22 items, to which participants are asked to respond on a Likert scale from 0 to 6, where 0 = never and 6 = always. The instrument contains three dimensions: emotional exhaustion, depersonalization, and personal accomplishment. Examples of scale items are *I feel emotionally drained from my work* (emotional exhaustion); *I feel very energetic* (personal accomplishment); and I'*ve become more callous towards people since I took this job* (depersonalization). High scores for the emotional exhaustion and depersonalization dimensions and low scores for personal accomplishment correspond to higher levels of burnout (17). In the research sample, the Cronbach Alpha consistency coefficients for each subscale range from acceptable to excellent, namely $\alpha = .94$ (emotional exhaustion), $\alpha = .79$ (personal accomplishment), and $\alpha = .70$ (depersonalization).

The Trait Meta-Mood Scale (TMMS-24) was used to evaluate emotional intelligence (18). It is a shorter version of the TMMS-48 (19), containing 24 items that are categorized into three dimensions: attention, clarity, and repair. Participants responded to the items using a five-point Likert scale, ranging from 1 (strongly disagree) to 5 (strongly agree). Examples of the items include: "I pay a lot of attention to my feelings" (attention), "My feelings are clear to me" (clarity), and "Even when I feel sad, I try to think about pleasant things" (repair). Psychometric properties of this instrument were found to be good in previous studies (all Cronbach's alphas > 0.85) (18) In our study, attention $\alpha = .91$, clarity $\alpha = .91$, repair $\alpha = .78$.

The UNIPSICO Battery (Unidad de Investigación Psicosocial de La Conducta Organizacional) (20) was used to evaluate psychosocial risks. This battery assesses the psychosocial risks faced by workers, considering three key factors: demands, resources, and consequences. Participants

provided responses to all items using a five-point Likert scale, ranging from 0 (never) to 4 (very often: every day). Previous studies have demonstrated that this instrument possesses good psychometric properties (Cronbach's alphas > 0.86) (20). Specifically, the scales of the UNIPSICO Battery utilized in this research were:

The Role Conflict Scale measures the instances where an individual faces challenges in satisfying conflicting role expectations that they are involved in. It serves to assess psychosocial demands. The scale includes five items, with example statements such as "I receive incompatible requests from two or more people." The internal consistency of the scale for this study's sample was determined to be Cronbach's alpha = .85.

The Role Clarity Scale was assessed using the UNIPSICO subscale (20). This subscale consists of 5 items with a Cronbach's alpha of .82. The items pertain to aspects like knowledge of job attributions and competencies, objectives, tasks, responsibilities, and job content, as well as understanding how to perform the job and the worker's expected role (e.g., "I know exactly what is expected of me at work").

ECO system (21) was used for measuring organizational justice via the dimension of organizational justice (8 items) to measure the psychosocial risks at the organizational level. It was measured on a 5-point Likert scale, where 1 = to a very small extent and 5 = to a very large extent. An example of items is "The salary I receive is correct relative to the work I am doing". The scale was used in organizations in Romania in the past; it has its own specificity, where employees are under the influence of similar professional pressures and working conditions but hold different positions in an organization, as in our study (medical and non-medical staff). On the research sample, an acceptable internal consistency coefficient is found for the organizational justice sub-scale, $\alpha = .74$. The ECO system was also used to measure workload - with the dimension Workload (8 items) to measure psychosocial risks at the organizational level. The dimension of workload presented adequate psychometric properties in previous studies (Cronbach's alphas > 0.78) and organizational justice (Cronbach's alphas > 0.81), (22) and the present one. On the research sample, a very good internal consistency coefficient was found for the workload sub-scale, $\alpha = .90$.

Statistical analyses. The *IBM Statistical Package for Social Sciences* (SPSS) and Statistics for Windows, *version 29* (SPSS Inc., Chicago, IL, USA) were used to test the hypotheses. The first group included medical personnel (doctor, nurse, physical therapist, psychologist, pharmacist, biologist, chemist, and social worker), and the second one included non-medical personnel (administrative staff).

Ethical approval. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Board of the Association of Legal Advisors from Public Hospitals in Romania, Research Ethical Agreement No. 54/15, September 2022.

RESULTS

Socio-Demographic and Job-Related Data

The study included 370 medical staff respondents and 130 administrative staff respondents. Table I presents the demographic data. Most of the participants were female (85.2%). About 80.20% of the participants were in a relationship, 75.80%

had children, and 41.80% had more than 20 years of work experience. Additionally, 72.40% of the respondents worked in a public institution, and 35.00% worked in an emergency unit. Moreover, 24.40% held a management position, 37.40% had a bachelor's degree, and 29.60% reported suffering from chronic diseases.

TABLE I.
Characteristics of the study sample (n = 500)

Characteristics	N	%
Gender		
Male	74	14.80%
Female	426	85.20%
Marital status		
Single	99	19.80%
In a relationship	401	80.20%
Children		
No	121	24.20%
Professional category		
Nurse	217	43.40%
Administrative personnel	130	26.00%
Physician	98	19.60%
Other medical personnel	55	11.00%
Job tenure in years		
5 years or less	89	17.80%
between 6 and 10 years	81	16.20%
between 11 and 20 years	121	24.20%
more than 20 years	209	41.80%
Type of organization		
Public	362	72.40%
Private	62	12.40%
Public and private	76	15.20%
Emergency unit		
Yes	175	35.00%
Team coordinator		
Yes	123	24.40%
Education		
High school/post-secondary studies	141	28.20%
Certificate or qualification diploma	10	2.00%
Bachelors' degree	187	37.40%
Master's degree	131	26.20%
PhD studies/Post doc	31	6.2%
Chronic disease		
Yes	148	29.60%

Descriptive statistics

The data was entered into SPSS version 29 for hypothesis testing and statistical analysis. The research sample consisted of 426 women (85.2%) and 74 men (14.8%). Among them, 98 were physicians (19.6%), 217 were nurses (43.4%), 130 are administrative staff (26%), and 55 (11%) belonged to other professions (such as physiotherapists, psychologists, pharmacists, biologists etc.).

TABLE II.

Descriptive and correlation analysis

	M (SD)	1	2	3	4	5	6	7	8	9
Medical										
1. Emotional exhaustion	19.73(13.68)	-								
2. Personal accomplishment	38.67 (7.45)	-.27**	-							
3. Depersonalization	4.44 (4.99)	.50**	-.33**	-						
4. Organizational justice	25.87 (5.86)	-.45**	.26**	-.31**	-					
5. Emotional Attention	30.30 (5.87)	-.05	.14**	-.20**	.07	-				
6. Emotional Clarity	31.34 (4.96)	-.22**	.42**	-.32**	.25**	.44**	-			
7. Emotional Repair	29.97 (4.28)	-.24**	.41**	-.33**	.26**	.31**	.55**	-		
8. Role conflict	7.25 (4.27)	.52**	-.22**	.41**	-.53**	-.02	-.19**	-.16**	-	
9. Workload	21.97 (7.90)	.76**	-.25**	.29**	-.38**	-.01	-.17**	-.22**	.40**	-
10. Role clarity	16.25 (3.61)	-.39**	.48**	-.40**	.47**	.20**	.47**	.32**	-.38**	-.27**
Non-Medical										
1. Emotional exhaustion	27.33 (14.16)	-								
2. Personal accomplishment	35.87 (7.92)	-.11	-							
3. Depersonalization	6.59 (5.99)	.39**	-.14	-						
4. Organizational justice	21.63 (5.61)	-.28**	.08	-.24**	-					
5. Emotional attention	31.63 (5.05)	-.05	.23**	.08	.02	-				
6. Emotional clarity	31.93 (4.59)	-.04	.23**	.00	.07	.60**	-			
7. Emotional repair	29.68 (4.30)	-.04	.24**	.00	.11	.25**	.49**	-		
8. Rol conflict	9.61 (4.34)	.45**	-.03	.41**	-.56**	-.02	-.08	-.01	-	
9. Workload	24.19 (8.02)	.76**	.04	.45**	-.23**	-.05	-.10	-.06	-.06	-
10. Role clarity	16.06 (3.51)	-.19*	.29**	-.23**	.24**	.08	.18*	.10	-.30**	.07

Nmedical = 370; Nnon = 130; *p < .05; **p< .001

Inferential Data Analysis

H1: There are statistically significant differences in psychosocial risks, burnout, and emotional intelligence between medical and non-medical staff.

To investigate this hypothesis, an independent samples T-test was applied. The results indicate that there were signif-

icant differences between the two groups concerning emotional exhaustion, professional accomplishment, depersonalization, organizational justice, emotional attention, role conflict, and workload (tab. III).

TABLE III.
T test for independent sample

	Medical		Non-medical		t	Cohen's d
	M	SE	M	SE		
Emotional exhaustion	19.73	0.71	27.33	1.24	-5.39**	-0.55
Personal accomplishment	38.67	0.39	35.87	0.69	3.63**	0.37
Depersonalization	4.44	0.26	6.59	0.52	-3.99**	-0.40
Organizational Justice	25.87	0.30	21.63	0.49	7.17**	0.73
Emotional attention	30.30	0.30	31.63	0.44	-2.29*	-0.23
Role conflict	7.25	0.22	9.61	0.38	-5.39**	-0.55
Workload	21.90	0.41	24.19	0.70	-2.83*	-0.30
Emotional clarity	31.34	0.25	31.94	0.40	-1.19	
Emotional repair	29.97	0.22	29.68	0.37	0.66	
Role clarity	16.25	0.18	16.06	0.30	0.52	

Nmedical = 370; Nnon = 130; *p < .05; **p< .001

H2: Psychosocial risks (organizational justice, role conflict, workload, and role clarity) have a statistically significant correlation with burnout.

According to the correlation analysis (Table 2), it can be observed that the dimensions of burnout correlate with different psychosocial risks for both professional domains. For the medical staff, statistically significant correlations were found between the dimensions of emotional exhaustion and organizational justice ($r = -.45$, $p < .01$), role conflict ($r = .52$, $p < .01$), workload ($r = .5776$, $p < .01$), and role clarity ($r = -.39$, $p < .01$). The second dimension, personal accomplishment, correlates significantly and positively with organizational justice ($r = .26$, $p < .01$) and role clarity ($r = .48$, $p < .01$), and negatively with role conflict ($r = -.22$, $p < .01$) and workload ($r = -.25$, $p < .01$). On the other hand, depersonalization correlates negatively with organizational justice ($r = -.31$, $p < .01$) and role clarity ($r = -.40$, $p < .01$), and positively with role conflict ($r = .41$, $p < .01$) and workload ($r = .29$, $p < .01$).

Similarly, for the non-medical staff, significant correlations were observed between emotional exhaustion and organizational justice ($r = -.28$, $p < .01$), role conflict ($r = .45$, $p < .01$), and role clarity ($r = -.19$, $p < .01$). Regarding personal accomplishment, there was a significant correlation only with role clarity ($r = .29$, $p < .01$). The last dimension, depersonalization, correlates negatively with organizational justice ($r = -.24$, $p < .01$) and role clarity ($r = -.23$, $p < .01$), and positively with role conflict ($r = .41$, $p < .01$).

H3: Emotional intelligence will positively correlate with the dimension of personal accomplishment and negatively cor-

relate with the dimensions of emotional exhaustion and depersonalization.

The correlation coefficients indicate that personal accomplishment significantly and positively correlates with all three dimensions of emotional intelligence (emotional attention, emotional clarity, and emotional repair) for both groups. However, in the case of medical personnel, stronger correlations (medium or large effect sizes) were observed with clarity ($r = .42$, $p < .01$) and emotional repair ($r = .41$, $p < .01$) compared to non-medical personnel, who showed smaller or medium correlations with these dimensions of emotional intelligence.

Regarding emotional exhaustion, significant and negative correlations were observed only for healthcare personnel, with clarity ($r = -.22$, $p < .01$) and emotional repair ($r = -.24$, $p < .01$). For non-medical personnel, there were no significant correlations. Similarly, depersonalization presented significant and negative correlations only for healthcare personnel, with all relationships being negative.

Thus, the dimensions of burnout were closely related to the dimensions of emotional intelligence, but only for healthcare personnel. For non-medical personnel, only personal accomplishment was affected by emotional intelligence.

DISCUSSION

Regarding burnout, non-medical personnel face higher values for emotional exhaustion as well as depersonalization than medical personnel, and higher values for personal accomplishment for medical personnel. Scholars in the field have reached an agreement that burnout tends to manifest gradually rather than showing immediate symptoms. It develops as a response to emotional exhaustion and prolonged exposure to stressors. As individuals experience emotional exhaustion and face continuous stressors, this can contribute to an acceleration in the degree of dehumanization and a sense of dissatisfaction with their professional roles (23, 24). The administrative personnel seem to undergo increased emotional exhaustion and depersonalization, leading to symptoms like weariness, diminished motivation, and a cynical attitude towards colleagues or stakeholders. These adverse consequences may be intensified by heavy workloads, scarce resources, and stress in the workplace (15). Emotional exhaustion and depersonalization results for administrative personnel may be caused by a lack of control because this category of stuff may have limited decision-making power in critical situations, leading to feelings of vulnerability and frustration. At the same time, administrative personnel might feel undervalued or underappreciated for their contributions to patient care, leading to a sense of depersonalization and disengagement, known as a lack of recognition.

Regarding psychosocial risks, administrative staff face higher levels of workload and lower levels of organizational justice. Concerning workload and resource disparities: non-medical staff may face heavier workloads and fewer resources than medical personnel, leading to higher stress levels and reduced job satisfaction. Based on previous studies, it has been observed that non-medical staff often perceive a lower level of organizational justice. This perceived injustice might be attributed to salary disparities between the two categories of staff in healthcare in Romania (14).

Role conflict is also higher for non-medical personnel. Role conflict has

emerged as a significant predictor of burnout. In previous research, it was revealed that behind this evidence is the fact that the levels of the burnout syndrome are significantly and positively correlated with role conflict. The relationship between role conflict and burnout suggests that as role conflict increases, so does the likelihood of experiencing burnout (4). The lack of organizational justice can also cause emotional exhaustion (25). In the case of emotional intelligence, the protective factor of burnout, significant differences between the two subgroups with higher values for administrative stuff were registered only for emotional attention. Hence, one reason could be team dynamics since administrative personnel might need to coordinate and cooperate with various healthcare teams and departments. Higher emotional attention could facilitate better teamwork, communication, and conflict resolution, which are essential for effective healthcare administration. Another reason could be represented by job stressors: while medical personnel face stress linked to patient care and medical decisions, administrative personnel may experience stress from managing administrative tasks, handling complex logistics, and coordinating multiple tasks. Higher emotional attention might help them manage and cope with these stressors successfully.

For the medical staff, statistically significant correlations were found between the dimensions of emotional exhaustion and organizational justice, role conflict, workload, and role clarity. In the case of non-medical staff, significant correlations were observed between emotional exhaustion and organizational justice, role conflict, and role clarity. Regarding the correlation between burnout and psychosocial risks, in the case of emotional exhaustion, a negative correlation was found with organizational justice, suggesting that employees who perceive fairness and equity in the organization are less likely to experience emotional exhaustion. When they believe that they are treated fairly, this can act as a barrier against burnout. Physicians who face a heavy workload and extended working hours are at higher risk of experiencing burnout (26).

Additionally, role conflicts and emotionally demanding jobs further contribute to feelings of emotional exhaustion and depersonalization. In previous studies, emotional exhaustion showed a significant and strong positive correlation with workload as well as with role conflict (27).

In case of medical personnel, emotional exhaustion is also positively corelated with workload which means that a higher workload may lead to increased emotional exhaustion as it can be physically and emotionally draining for medical staff to handle a heavy workload. Experiencing a heavy workload, increased work intensity, and irregular work schedules leads to a lack of concentration, memory issues, and hinders the ability to think clearly and make decisions. When employees perceive the need to handle multiple tasks simultaneously and be innovative, it can result in sleep disturbances and contribute to burnout. Furthermore, the presence of high emotional demands in the workplace, including dealing with emotionally challenging situations and personal problems of others, along with the need to maintain emotional coherence with work, can also lead to sleep disturbances, stress, and somatic problems (28).

The negative correlation of both groups with emotional exhaustion and role clarity could mean that clear and well-defined

roles and duties may mitigate emotional exhaustion as they reduce ambiguity and uncertainty. The research found a statistically significant correlation between emotional exhaustion and role conflict. This means that medical and non-medical personnel staff members who experience higher levels of role conflict (e.g., managing multiple tasks, conflicting burdens etc.) are more likely to experience emotional exhaustion. The demanding and high-pressure nature of medical professions can contribute to this phenomenon. Clarity about their tasks and responsibilities may help non-medical staff handle their work more effectively, leading to lower levels of pressure and emotional exhaustion.

In the case of medical personnel, the second dimension, personal accomplishment, correlates significantly and positively with organizational justice and role clarity and negatively with role conflict and workload. Results are consistent with previous studies, where role conflicts had a positive correlation with burnout in the case of nurses and may contribute to higher levels of burnout registered for this category of employees (29).

In the case of administrative staff, personal accomplishment corelates only with role clarity. In the case of medical personnel, there is also a positive correlation with organizational justice: when employees feel that they are rewarded fairly and recognized for their achievements, it enhances their sense of personal accomplishment. The positive correlation of personal accomplishment with role clarity means that clearly defined roles allow medical and non-medical staff to better understand their responsibilities and contribute to a sense of accomplishment when performing their roles successfully. The negative correlation of personal accomplishment with role conflict for medical personnel suggests that an environment where healthcare professionals experience conflicting tasks or expectations could affect the sense of personal accomplishment felt by them (28). As expected, another negative correlation was established between personal accomplishment and workload in the case of medical personnel because high overload can negatively impact the feeling of personal accomplishment, as it may be difficult to achieve a sense of success when overwhelmed with tasks.

The third dimension of burnout, depersonalization, correlates negatively with organizational justice for both subgroups: when employees feel that they are treated unfairly, they might respond by developing a sense of detachment toward their work and patients.

The negative correlation between depersonalization and role clarity for the two groups could lead to misunderstandings and a sense of detachment from one's tasks, potentially contributing to depersonalization. The lack of role clarity is a psychosocial risk factor that significantly impacts workers' well-being and performance levels. When employees are uncertain about their roles and responsibilities, they tend to invest less effort in their tasks and perform less effectively. Consequently, these workers show reduced engagement and, as a result, experience lower levels of well-being and job satisfaction. Studies have demonstrated that the presence of unclear goals is associated with diminished performance, job satisfaction, and overall engagement among healthcare professionals and other workers (30). Having well-defined roles can help foster a more personal connection with the work and pa-

tients. The correlation between depersonalization and role conflict for both groups is that role conflict may create emotional strain, leading to a more impersonal and detached approach to work and patient interactions. On the other hand, a positive correlation with workload in the case of non-medical personnel suggests that workload can be emotionally overloading and lead to a lack of emotional investment in interactions, which might contribute to depersonalization. Findings are supported by previous research where we can see strong correlations between psychosocial risk factors and burnout (31).

Previous results indicated that healthcare professionals with higher levels of emotional intelligence were less prone to experiencing emotional exhaustion and depersonalization. Additionally, they were more likely to experience a sense of personal accomplishment in their roles. This suggests that emotional intelligence plays a significant role in influencing the experience of burnout among health care professionals, positively impacting their well-being and job satisfaction (32).

Personal accomplishment correlates with the three dimensions of emotional intelligence. Healthcare professionals who possess higher levels of emotional intelligence are more likely to experience a sense of personal accomplishment in their roles. There are several reasons why emotional intelligence contributes to this feeling of accomplishment: improved patient relationships by establishing better connections with their patients. They can understand and empathize with patients' emotions, concerns, and needs, leading to more effective communication and a higher level of patient satisfaction. When healthcare professionals see the positive impact of their care on patients' lives, their sense of accomplishment is enhanced. A person with higher level of emotional intelligence could develop effective conflict resolution, so they can resolve disagreements with patients, colleagues, or supervisors in a respectful and empathetic manner. Successfully resolving conflicts augments their sense of competence and accomplishment in handling difficult interactions.

Another positive aspect of emotional intelligence is personal growth and development. Emotional intelligence involves continuous self-improvement and learning. Healthcare professionals who enthusiastically work on developing their emotional intelligence are more open to feedback, receptive to learning opportunities, and eager to adapt and grow in their roles. This dedication to personal development contributes to a greater sense of accomplishment in their professional journey.

Regarding emotional exhaustion and emotional intelligence, significant and negative correlations are observed only among medical staff with emotional clarity and emotional repair. In contrast, there are no significant statistical correlations among non-medical staff. Similarly, depersonalization shows significant statistical correlations only among medical staff, with all relationships being negative. However, a health care professional who possesses the ability to understand and regulate their emotions during these service operations is more likely to reduce their experiences of emotional exhaustion and depersonalization. Moreover, they are more likely to enhance their sense of personal accomplishment. This indicates that emotional intelligence and the capacity to effectively handle emotions in the context of their work can play a crucial role in minimizing

burnout and enhancing job satisfaction among health care professionals (32). This finding is in line with Salovey *et al.*'s (33) assertion that individuals who possess the ability to regulate their emotional states tend to be healthier. They achieve this by accurately perceiving and evaluating their emotional states, knowing how and when to express their feelings, and effectively managing their mood states. This suggests that health care professionals with higher emotional intelligence are better equipped to handle the challenges and stressors of their profession, leading to lower emotional exhaustion levels and potentially better overall well-being (32).

In our study, significant negative correlations were found between emotional intelligence and the two negative dimensions of burnout, namely, emotional exhaustion and depersonalization, only in the case of medical personnel. These results are confirmed by previous literature, which revealed significant correlations between emotional intelligence and the dimensions of burnout among nurses. Specifically, there was a significant inverse relationship between emotional intelligence and emotional exhaustion, indicating that higher levels of emotional intelligence were associated with lower levels of emotional exhaustion among nurses. Similarly, there was a significant inverse relationship between emotional intelligence and depersonalization, suggesting that higher emotional intelligence was linked to lower levels of depersonalization. Personal accomplishment significantly correlates with emotional intelligence. Results are in line with other studies that revealed a significant direct correlation between emotional intelligence and personal accomplishment among nurses (34). This means that higher emotional intelligence scores were associated with a greater sense of personal accomplishment among healthcare personnel. In a study investigating the connection between Emotional Intelligence (EI) and burnout among healthcare professionals with a focus on demonstrating how EI serves as a protective factor against the risk of burnout, a significant relationship between EI and burnout was highlighted, emphasizing the potential role of emotional intelligence in mitigating burnout among healthcare professionals. The sample for this survey consisted of health professionals, including doctors, nurses, and other caregivers, totaling 148 employees (35).

Strengths and limitations of the study

This is one of the few studies conducting a comparative analysis of medical and non-medical staff in Romania. However, there are certain limitations that need to be acknowledged. Firstly, the use of the snowball sampling method to select participants could introduce bias into the results. Additionally, some variables were measured with only a few items, and only specific dimensions of the scales were considered, which may have restricted the scope of the research. Another limitation is the reliance on self-reporting, which could lead to overestimated correlations and misinterpretations of the data. To obtain a more comprehensive understanding of healthcare institutions, future studies should also include auxiliary staff from both public and private medical units in Romania, as this group was not examined in the current research. It would be valuable to investigate whether the proposed intervention model yields different results for these employees. To accurately reflect reality, longitudinal research is necessary to ex-

plore how various factors impact the perception of burnout and psychosocial risks among healthcare employees at different points in time.

CONCLUSIONS

Based on the findings of this study and consistent with previous scientific literature (36-39), it can be concluded that psychosocial risks and emotional intelligence play a significant role in predicting burnout. It is important to note that emotional intelligence is not limited to specific job roles or professions; both medical and administrative personnel can benefit from evolving their emotional intelligence to enhance their interactions with patients, colleagues, and themselves. Moreover, these distinctions might not apply generally to all individuals within these roles, as emotional intelligence can vary greatly from person to person, regardless of their job title. The medical staff experiences a higher sense of organizational justice and personal accomplishment compared to the non-medical staff. Regarding the negative dimensions of burnout, higher levels of emotional exhaustion and depersonalization have been recorded among non-medical personnel. Additionally, higher scores have been observed among administrative staff for the dimension of emotional attention or emotional intelligence. As for the other psychosocial risks, role conflict has been reported to have higher values among administrative personnel, as has workload. In both groups, when there is a high level of emotional exhaustion or depersonalization and an increased sense of role conflict or workload, perceived organizational justice and role clarity are lower. On the other hand, when personal accomplishment is high, there is also a higher level of role clarity (for both groups) and a greater sense of organizational justice (for medical personnel).

CONFLICT OF INTEREST AND FUNDING

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PHYSICAL VIOLENCE IN THE HEALTHCARE SYSTEM – AN EPIDEMIC WITH DIRE CONSEQUENCES ON NURSES

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PHYSICAL VIOLENCE IN THE HEALTHCARE SYSTEM - AN EPIDEMIC WITH DIRE CONSEQUENCES ON NURSES (Abstract): Workplace violence against nurses is a serious problem, affecting healthcare systems worldwide. Physical violence is a visible form of abuse, although little is known about its prevalence and consequences. Nurses, acting as intermediaries between doctors and patients, are particularly vulnerable due to their frontline roles, engaging with diverse individuals in emotionally charged environments. The aim of this study was to analyze the incidents of physical violence against nurses in Romania, assessing their frequency, characteristics and consequences and the victims' sociodemographic and occupational profiles. **Materials and methods:** We conducted a quantitative, prospective study, using the Romanian version of the questionnaire developed by International Labor Organization, International Council of Nurses, World Health Organization, and Public Services International regarding workplace violence in healthcare. The survey, distributed online to Romanian nurses and doctors, collected data on the workplace violence incidents, the victims and aggressors, and the consequences of these occurrences. **Results:** 2.4% of the 7,203 nurses who participated in our study declared that they experienced physical abuse in the previous year. Most incidents were committed by patients and their relatives, inside medical facilities. Both physical and psychological consequences of the violence incidents were mentioned in varying degrees and significant correlations were found between the assaulted nurses and their gender, professional experience, work sector, and working shifts or nights. **Conclusions:** This research reveals the reality of the physical violence against nurses in Romania and emphasizes the importance of addressing this phenomenon by the healthcare system. Furthermore, our findings indicate the need for preventative initiatives and measures towards workplace violence in healthcare. **Keywords:** WORKPLACE, HEALTHCARE SYSTEM, PHYSICAL VIOLENCE, NURSES, CONSEQUENCES.

The World Health Organization defines workplace violence as "incidents in which staff are abused, threatened, or attacked in circumstances related to their work, including during travel to or from home, and which involve a threat to their safety, well-being, or health" (1).

Even though workplace violence in the medical field is common, it is often underreported, and its consequences are difficult

to estimate. Physical violence is one of the many forms of workplace violence and it is the most noticeable because of its consequences, which can range from minor injuries to serious ones. Moreover, in addition to physical injuries victims of physical abuse may suffer from psychological consequences, including anxiety, depression and burnout, and may be unable to perform their duties or they may need time off work (2). The threat of workplace violence against any member of the medical personnel can be translated to the inability of the healthcare system to protect its employees and endangers the quality of the medical care (3).

Nurses are particularly vulnerable to workplace violence, ranging from verbal abuse to physical assaults, due to the fact that they act as a vital intermediary between the doctors and the patients and their families. Moreover, nurses have to provide care in an emotionally dominated environment and to constantly engage with different types of people. Therefore, physical violence against nurses is a serious and widespread concern for healthcare systems around the globe (4, 5).

One study conducted in Germany reported that 70.7% of the medical staff had been the victim of physical violence and 89.4% of verbal abuse (2). Similar results were observed in a study conducted in the emergency room of a Turkish hospital, where 91.4% of the nurses reported verbal abuse and 74.9% reported physical abuse during the previous 12 months (6).

A Chinese study that documented the violent episodes against medical personnel during a time span of 10 years reported 29 murders and 52 serious injuries(5), while 70% of the Singaporean medical personnel experienced physical abuse, even though these actions were under-reported (7).

Workplace violence in healthcare has been under-investigated in Romania. According to data provided by a 2015 study conducted by the College of Physicians from Bucharest, 10.2% of physicians reported experiencing physical aggression(8). Furthermore, a study involving resident physicians from the “Carol Davila” University of Medicine and Pharmacy, Bucharest, revealed that 8.4% of the participants experienced physical aggression from a patient during their residency (9). The scarcity of the studies that explore workplace violence in the Romanian healthcare system, particularly that of physical violence against nurses, emphasizes the need for further research.

The aim of this study was to analyze the incidents of physical violence against nurses in Romania, assessing their frequency, features and consequences (both physical and psychological) and also the victims’ sociodemographic and professional characteristics.

This study is part of the doctoral research entitled "Research on violence against medical personnel in Romania". The doctoral research has been carried out at the Doctoral School of “Grigore T. Popa” University of Medicine and Pharmacy from Iasi and aims to investigate violence against medical personnel (doctors and nurses) in Romania.

MATERIALS AND METHODS

Study design

We carried out a quantitative, retrospective study in which data was collected with a questionnaire that was distributed to all nurses and doctors in Romania.

Data Collection

The survey instrument used is the

Workplace Violence in the Health Sector Country Case Studies Research Instruments - Survey Questionnaire (10). The questionnaire was developed by International Labor Organization, International Council of Nurses, World Health Organization, and Public Services International in 2003 and it was translated by the authors of this study to Romanian with the approval of the World Health Organization. The complete questionnaire includes 130 questions, divided into 7 sections: the first one regarding the sociodemographic and professional characteristics of the participants, the next 5 sections exploring different types of physical and psychological violence (i.e., verbal abuse, intimidation, racial harassment, and sexual harassment) experienced by the participants at the workplace in the previous year, with a focus on prevalence, perpetrators' characteristics, effects, consequences and actions that were taken by the participants and by the institutions; the last part of the questionnaire investigates workplace policies and working conditions, and it also contains questions about the participants' opinions about the factors contributing to violent incidents. Each section starts with the definitions of the investigated notions, in order to limit the personal interpretations of the participants.

The questionnaire was distributed through Google Forms (access link: https://forms.gle/JtzMi3sfa2Yd6a6k9) to all doctors and nurses in Romania, who were affiliated with the national professional associations, regardless of the medical institution where they worked. In the nurses' case, invitations to participate to this study were sent via e-mail to all members of the Romanian Order of General Nurses, Midwives and Medical Assistants, that included the link to our questionnaire. Afterwards, the access link was distributed online in the main Facebook group of Romanian nurses with approximately 40,000 members. The access link was valid for 2 months, between 10.02.2022-10.04.2022.

Data Analysis

Data was analyzed with the IBM *SPSS Statistics 20* software, using descriptive statistics to calculate the prevalence rates, frequencies and percentages and also univariate analyses. The threshold for statistical significance was set at a p-value below 0.05.

Ethical Considerations

The doctoral research was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iași, as indicated by the Approval No. 52/21.02.2021. Prior to accessing the questionnaire, each participant was asked to read the information about the study mentioned on the first page of the questionnaire and to consent to participation. The responses provided by the participants were anonymous.

RESULTS

In this article the authors present the results concerning the incidents of physical violence against nurses and their consequences, as well as the aspects related to the organizational dynamics of their work environments.

Participants

The study group consisted of 7,203 nurses, 6,675 (92.7%) of them being women. The majority of the participants worked in public healthcare facilities (75.2%), in shifts (66.1%) and had direct interactions with patients during their work (81.7%). Their workplace is usually the hospital (59%), 31.8% of the participants having

had over 20 years of experience in the healthcare system. The socio-demographic and professional characteristics of the participants are further detailed in first table.

TABLE I.
Socio-demographic and professional characteristics of the study population

Characteristics		N =7203	%
Sex	Male	528	7.3
	Female	6675	92.7
Professional experience	Less than 5 years	1844	25.6
	Between 6 and 10 years	1113	15.5
	Between 11 and 15 years	941	13.1
	Between 16 and 20 years	1016	14.1
	More than 20 years	2289	31.8
Work sector	Private institutions	1782	24.7
	Public institutions	5415	75.2
	Not disclosed	6	0.1
Work in shift	No	2439	33.9
	Yes	4764	66.1
Night shift (between 18:00 and 7:00)	No	3258	45.2
	Yes	3945	54.8
Direct interactions with patients	No	1319	18.3
	Yes	5884	81.7
Workplace	Hospital	4253	59
	Other medical institutions including palliative care, hospice, military units, school medical office	2950	39.7
	Not disclosed	94	1.3
Work status	Employee of the staff	6711	93.2
	Head nurse/Senior nurse	222	3.1
	Volunteer	185	2.6
	Nurse privately employed	46	0.6
	Senior Manager	33	0.5
	Not disclosed	6	0.1

Prevalence and characteristics of the violent incidents

According to the definition provided in the questionnaire, physical violence is "the use of physical force against another person or group, that results in physical harm, sexual or psychological harm. It can include beating, kicking, slapping, stabbing, shooting, pushing, biting, and/or pinching, among others".

Among the nurses participating in this study, 172 were victims of a physically violent incident at the workplace, which amounts to 2.4% of the total number of participants. However, 335 (4.7%) of the nurses participating in our study witnessed physical violence at work (tab. II). Moreover, the prevalence of incidents differs between males and females, with a higher prevalence observed among males (4.4%)

compared to females (2.2%).

Most of the physical violence events occurred within the medical institutions (79.7%) and the aggressors were either the patients (66.3%) or their relatives (11%).

Half of the violent incidents could have been avoided according to the nurses involved. The main characteristics of the physical violence events mentioned are summarized in table III.

TABLE II.

Frequency and percentage of physical violence in the study group

Physical violence at the workplace		N (7203)	%
Experienced at least a physical violent incident at work in the last year	No	7031	97.6
	Yes	172	2.4
Witnessed at least one physically violent incident in the last year	No	6868	95.3
	Yes	335	4.7

TABLE III.

Characteristics of the reported physical violence incidents

Characteristics		n (172)	%
Location	Within the medical institution	137	79.7
	At the patient's home	15	8.7
	Outside the medical institution - on the way to work	7	4.1
	Outside the medical institution - at your home	6	3.5
	Unspecified location	7	4.1
Perpetrator	Patient	114	66.3
	Patient's relatives	19	11
	Staff member, superior or colleague	23	13.4
	Member of the general public	11	6.4
	Not disclosed	5	2.9
Opinion on whether the event could have been prevented	No	86	50
	Yes	86	50
Presence of Physical Injuries	No	126	73.3
	Yes	46	26.7
Time off work needed	No	141	81
	Yes	31	18

Significant results from the Chi-Square statistical analysis revealed associations between the physical violence and some of the characteristics of the study population. Thus, significant correlations were observed for gender ($p = .002$), professional experience ($p = .028$), work sector ($p = .014$), working in shifts ($p = .000$), night shifts ($p = .000$), and working in hospitals ($p = .000$) (tab. IV). In our study female nurses, with little work experience, that worked in public institutions, in shifts and at night and that interacted directly, constantly with patients have experienced physical violence at their workplace.

TABLE IV.
Socio-demographic, professional characteristics of the victims of physical violence - results of the chi-square statistical test

Characteristics		n=172	%	p-value
Gender	Male	23	13.4	.002
	Female	149	86.6	
Professional experience	Less than 5 years	49	28,8	.028
	Between 6 and 10 years	34	19.8	
	Between 11 and 15 years	28	16.3	
	Between 16 and 20 years	22	12.8	
	More than 20 years	39	22.7	
Work sector	Private institutions	143	83.1	.014
	Public institutions	29	16.9	
	Not disclosed	0	0	
Work in shifts	No	35	20.3	.000
	Yes	137	79.7	
Night shift (between 18:00 and 7:00)	No	35	20.3	.000
	Yes	137	79.7	
Direct interactions with patients	No	12	7	.000
	Yes	160	93	
Workplace	Hospital	129	75	.000
	Other medical institutions including palliative care, hospice, military units, schools medical office	43	25	
	Not disclosed	0	0	
Work status	Employee of the staff	153	89	.292
	Head nurse/Senior nurse	10	5.8	
	Volunteer	6	3.5	
	Nurse privately employed	2	1.2	
	Senior Manager	1	0.6	
	Not disclosed	0	0	

Consequences of the violent incidents on victims

Among the nurses who sustained a physically violent incident, 46 nurses (26.7%) suffered from physical injuries after the violence, and 31 (18%) needed days off work because of them (tab. 3).

In fifth table, we present the psychological effects of physical violence events reported by the participants. The table displays the frequencies and percentages of responses to questions assessing the extent of psychological impact, categorized into “Not at all”, “Slightly”, “Moderately”, “Quite a lot”, and “Very much”. Our results show that 8,7% of the victims experienced very often distressing thoughts or memories. Nurses reported feeling very much “on guard” (11.6%) or being very much affected by the feeling that their activities require additional effort (7.6%). Overall, responses indicate varying levels of psychological distress, with most participants reporting some degree of psychological impact of the violent incident.

TABLE V.
Psychological consequences of physical violence

Psychological Consequences	Not at all (N, %)	Slightly (N, %)	Moderately (N, %)	Quite a lot (N, %)	Very much (N, %)
(a) Repeated memories, thoughts, or images	57 (33.1%)	37 (21.5%)	41 (23.8%)	22 (12.8%)	15 (8.7%)
(b) Avoidance of thoughts or discussions	58 (33.7%)	40 (23.3%)	45 (26.2%)	19 (11.0%)	10 (5.8%)
(c) Feeling "super-alert" or "on guard"	51 (29.7%)	30 (17.4%)	47 (27.3%)	24 (14.0%)	20 (11.6%)
(d) Feeling that activities require extra effort	56 (32.6%)	32 (18.6%)	51 (29.7%)	20 (11.6%)	13 (7.6%)

DISCUSSION

Workplace violence in healthcare affects all categories of staff, with nurses being at the forefront. The aim of this study was to evaluate the prevalence, characteristics and consequences of the physical violence incidents the nurses in the Romanian healthcare system, and also the socio-demographic and professional characteristics of the victims.

Our study included a diverse group of nurses working in various environments, with different professional experiences, in order to provide a global perspective of the workplace violence phenomenon in the Romanian healthcare system. The results showed that 2.4% of participants claimed they had been a victim of physical violence at work in the previous year, with 4.7% of them witnessing it. The difference in the percentages highlights the fact that incidents of physical violence happen even when coworkers are around.

According to a study conducted in the USA, 25% of the participating nurses reported having had experienced physical violence (11). A single-hospital study conducted in Great Britain showed that 63% of the medical staff had missed work due to physical abuse in the last month (12). Studies pinpointed hospitals as environments associated with higher prevalence of physical violence against medical staff (6, 13).

The majority of the physical violence incidents reported by nurses in our study occurred in public medical institutions, with a significant statistic correlation between being employed in hospitals and the risk of being a victim of physical workplace violence. This is consistent with findings of other studies, which emphasize the role of the organizational deficiencies - such as the lack of security personnel, stressful work environment, insufficient staffing and prevention policies, that contribute to the occurrence of violent incidents against medical staff (12, 14).

Our study revealed that most of the physical violence incidents were initiated by patients (66.3%) and their relatives (11%). This finding is mentioned also in studies conducted in other countries, with patients and their relatives being the primary source of workplace violence against medical personnel, especially physical violence (6, 14).

The nurse - patient relationship is influenced by the emotional vulnerabilities of the patients and their families, but also by the social constructs regarding the position of nurses. Most nurses interact directly with patients, as demonstrated by our

study, and this dynamic may result in a mutually frustrating relationship between them and patients and their relatives (14). The perceived role of the nurse is that of an employee meant to satisfy both the doctor's and the patient's requirements; therefor the patients and their families often consider the healthcare staff, and nurses in particular, as nothing more than service providers that must be responsive to their needs (15). This view overlooks and ignores the importance of internal policies, organizational procedures, and medical judgments that are involved in the patient-medical personnel relationship.

Another important factor that influences the nurse - patient relationship is the different perspectives regarding the medical situation. While nurses may view the medical process as a routine, patients and their families perceive it as extremely stressful and even life-threatening. This difference can be interpreted by the patients as a proof of nurses' lack of compassion, empathy and engagement (16).

According to our results, half of the violent incidents could have been avoided. This proves that nurses can identify viable solutions that could decrease the risk of physical violence at their workplace. However, this aspect requires further research in order to implement efficient policies and initiatives to prevent and address instances of physical violence in healthcare institutions.

In terms of socio-demographic characteristics of the victims, significant correlations were found between the occurrence of the physical violence and the victims' gender, professional experience, and work sector. Thus, our study showed that the newly hired employees (28.8%) are the most commonly involved in violent incidents. This happens because nurses with little experience in the field might find it more difficult to communicate with patients and their families, which could be perceived as insecurity and a lack of good patient care (15).

Our study shows that both female and male nurses can experience physical workplace violence; almost 90% of the victims were female, yet the male nurses that participated in our study reported physical violence more often (4.4% of men compared with 2.2% of women). On the one side, female nurses might be subjected to stereotypes about their role; in our society, this translated also to the way the victims think about the incident: female victims often state the unpredictable nature of the assaults and think of them as aspects of their professional role, while male nurses describe them factual, including data about the witnesses and the consequences (17). This result must be interpreted cautiously due to the difference in sample size, i.e., there are fewer men involved in our study than women.

A particularity of working in the medical field is the work in shifts and at night. These two parameters were found in our study to be significantly correlated with the risk of nurses to be assaulted at work. This finding is supported by other studies that also correlate them with longer waiting times and lower tolerance levels of patients and their relatives (18, 19). Moreover, the night shifts are frequently understaffed and this aspect adds to the unique characteristics of the patients examined at night, i.e., alcohol consumption, altered general status, and agitation (18). This finding of our study suggests the need of preventive actions and special training actions, emphasizing the role of the institutional policies (18).

There are many different types of consequences that can arise from physical violence. First, there are the immediate physical consequences. In our study, about one-third of the attacked victims suffered physical injuries, and 18% of them even needed time off. This finding is in line with the results of other studies that mention that these traumatic events are more often associated with work interruption (6,20). The physical consequences are the most commonly mentioned in the literature, and they have an impact not only on victims, but also on the relationship between coworkers, patients, and their families as well as the work environment (2, 14). These effects are also linked to increased levels of stress and insecurity in the workplace (7, 21).

In terms of psychological consequences experienced by nurses who have been physically harmed, our study showed that constant memories and uncomfortable thoughts, along with the avoidance of conversations about the incident appear as a result of the violence incidents. This type of consequences is also revealed by the literature, especially in situations involving physical violence. Nurses frequently express feelings of frustration, rage, and anxiety (2). Other studies showed that these feelings regarding the violent incidents can be viewed as post-traumatic stress symptoms (22, 23), reaffirming the need for further change.

The constant feeling of being "on guard" that some nurses stated they felt after the physical incident further supports the idea that they perceive their workplace as unsafe, and any interaction as a possible source of violence (22). This increased alertness can have a negative effect on the nurses' relationships with patients, family members, and coworkers, adding to stress and burnout (2, 3). Physical violence in the workplace can also lead to a decrease in motivation, engagement, and overall job satisfaction of nurses, which can impact the quality of life as well as the quality of the medical care as a whole (20,21). Burnout syndrome and the desire to quit the workplace are also linked with physical violence sustained at work (24).

Strengths and limitations

One strength of our study is the large number of participants and the national coverage of the study. The large number of information collected on the violent incidents at the workplace and their consequences, coupled with the large number of participants may provide a quite accurate picture of the current situation in our country.

We attempted to reduce the memory-related bias by restricting our analysis to events that occurred in the last year and gathering detailed information. We encouraged participants to be more open about their experiences with workplace violence by choosing an anonymous survey. One limitation of our study is that we did not select representative samples of nurses from various medical specialty, as the questionnaire was distributed online by the professional association to all nurse in Romania. As a result, the study might not capture the nuanced variations that could exist among different medical specialties, since the experiences, perspectives and practices of nurses may vary across different branches in healthcare.

CONCLUSIONS

The findings of this study show that workplace violence against nurses, and in particular physical violence, is a serious problem of the Romanian healthcare sys-

tem, providing an analysis of its prevalence, features and consequences.

In our study, nurses who work at night, in shifts, or in a hospital, with limited work experience were more prone to physical workplace violence; the perpetrators were usually the patient and their relatives, contributing to the nuances of this problem. The consequences of the violent events span from physical ones, that might even need time off work, to psychological ones (distressing thoughts and memories, increased alertness), which are often difficult to measure, but all with a definite impact on the quality of life of nurses and the quality of care provided to patients.

To address this issue effectively, the results of our study can be further expanded and serve as the starting point for discussions regarding initiatives, policies and programs targeted at preventing and solving workplace violence against healthcare personnel. On the one side, it becomes essential to promote a culture where workplace violence is not tolerated, starting with organizational change, in terms of security levels, facilities improvement, and policies dedicated to reporting workplace violence. In addition, the wide range of consequences mentioned in our study emphasizes the urgency of providing support to victim; offering professional care becomes crucial in order to minimize the impact of workplace violence on their work and overall well-being.

In conclusion, tackling workplace violence calls for actions initiated from the medical institutions and expanded in order to encompass the whole healthcare system. To build work environments where the safety and wellbeing of healthcare professionals are promoted, society as a whole must also be involved.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the *Helsinki Declaration* of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the participants included in the study. No funding was received for this study.

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THE INFLUENCE OF SYNTHESIS TEMPERATURE ON A POLYURETHANE CARRIER USED FOR THE TRANSFER OF 2'-DEOXYCYTIDYLIC ACID

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THE INFLUENCE OF SYNTHESIS TEMPERATURE ON A POLYURETHANE CARRIER USED FOR THE TRANSFER OF 2'-DEOXYCYTIDYLIC ACID (Abstract): 2'-Deoxycytidylic acid, a nucleotide based on cytosine, deoxyribose and phosphate group, is a fundamental unit of nucleic acids. Various supplements containing nucleosides or nucleotides have been formulated for patients with genetic defects resulting in the presence of incorrect nucleotides. The **aims** of the current research were to obtain and to characterize polyurethane transporters that can be used for the transfer of nucleotides through body membranes. **Materials and methods:** Three samples have been developed based on different synthesis temperatures and the effects of this parameters on the properties of the final products were assessed; there were measured the solubility, pH, refractivity index, the encapsulation efficacy and release profile, size distribution and surface charge, cells proliferation and irritation tests on mice skin. **Results:** The obtained mean values show that were obtained almost neutral acido-basic particles with sizes range from 126 to 814 nm; Zeta potential values indicate the tendency to form clusters in samples with synthesis temperatures above 40°C, while UV-Vis evaluations show an approx. 60% encapsulation efficacy. Regarding the biosafety profile of the samples, the assays on cells culture and mice skin did not elicit important side effects at tested concentration. **Conclusions:** The findings of this preclinical study suggest that polyurethane carriers exhibit promise as a therapeutic or preventative measure for a range of diseases by addressing genetic irregularities, demonstrating their safety for such applications. **Keywords:** CELLS CULTURE; DRUG DELIVERY; MICE SKIN; UV-VIS; ZETASIZER.

The monomers of DNA, that contain a base (adenine, cytosine, guanine, thymine), a sugar (deoxyribose) and phosphate residues, are called nucleotides. They present similar structures with nucleosides, biological molecules known as glycosylamines,

but lacking phosphate functional groups (1). These compounds serve as units in the macromolecular chains of the nucleic acids, the most important molecules for the continuity of life. 2'-Deoxycytidylic acid, also known as 2'-Deoxycytidine-5'-monophosphate (fig. 1), is formed by the reduction of cytidine-monophosphate, the product of hydrolysis of cytidine triphosphate and cytidine diphosphate; it may be *de novo* synthesized in a series of steps via aspartate transcarbamylase and dihydroorotate as other pyrimidines, but it also be derived from RNA-rich dietary sources (2).

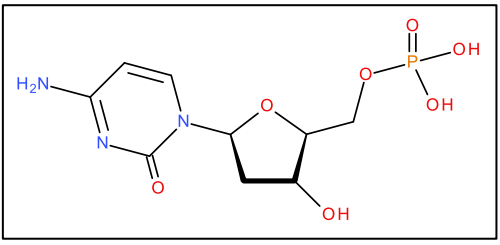


Fig. 1. The chemical structure of 2'-Deoxycytidylic acid

Recent studies present a correlation between nucleosides and brain diseases: modified adenosine levels can cause seizures, while changes of the astrocyte-based adenosine cycle were found in epileptic brain (3), guanosine decrease the number of seizures determined by glutamatergic agents and it is neuroprotective in brain hypoxia and ischemia (4). Inosine is discharged from neurons in the presence of extracellular calcium depending on the N-methyl-d-aspartate receptor (5), while cytidine and uridine have an impact in neurodegenerative conditions due to their contribution to brain phosphatidylethanolamine and phosphatidylcholine levels via the Kennedy pathway (6). D. Boison has described the nucleoside metabolism and several nucleoside-based therapies to treat brain diseases (7), but he also mentioned that nucleoside transporters represent a very interesting research theme.

The majority of naturally occurring nucleotides, as well as many nucleotide medications, possess a strong affinity for water and do not spontaneously cross lipid bilayers. Such transportation, if it occurs at all, typically requires mediation or active transport mechanisms (8). The carriers used for the transport of various biologically active substances are the results of the connection between nanotechnology, medicine and pharmacy; organic and inorganic delivery systems, based on nanocapsules, nanodots, nanofibers, dendrimers and matrices, were developed and characterized in the last decades (9). Polyurethane (PU) drug delivery systems were developed starting with the end of the last century; their first syntheses were based on raw materials with aromatic character such as methylene-diphenyl diisocyanate and toluene diisocyanate and the obtained structures were large and presented low penetration rates. At the beginning of this century, K. Bouchemal and her team (10-12) described the synthesis of PU capsules with much smaller sizes based on an interfacial polyaddition process combined with a spontaneous emulsification.

Our research team has modified the previous recipes in order to avoid the use

of raw materials with carcinogenic potential, to decrease the number of the chemical compounds, to enhance the encapsulation efficacy and the release profile; thus, we have synthesized and characterized PU carriers applicable for various extracts and drugs (13-15). The synthesis temperature represents a very important parameter that can have a huge impact on the properties of the final products; R. Fiorio *et al.* (16) showed in a study on the impact of synthesis temperature on PU samples that the glass transition was not modified, but activation energy (E_a) has changed and many side reactions (other than formation of urethane groups) have appeared. The objectives of the current research were to synthesize and to characterize PU structures obtained at different temperatures and to evaluate the impact of the temperature on the transport potential of nucleosides.

MATERIALS AND METHODS

Chemicals, cells culture, and mice

Sorbitane trioleate or Span® 85 was purchased from Sigma (Taufkirchen, Germany), while isophorone diisocyanate (IPDI), polyethylene glycol (PEG 400), polycaprolactone diol (PCL Mn~530), 1,6-hexanediol (HD), tris(hydroxymethyl)aminomethane (THAM), inorganic salts (NaCl, KCl and $MgCl_2$, Na_2HPO_4, K_2HPO_4, KH_2PO_4 and $NaHCO_3$) and 2'-Deoxycytidylic acid as sodium salt were acquired from Sigma Aldrich (St Louis, USA) and acetone from Honeywell Riedel-de Haen (Seelze, Germany). All the reagents were analytical grade purity and they were used without any previous purification; a JP Selecta Dest-4 distiller was involved to obtain double distilled water.

For the cytotoxicity evaluation, HDFa (Human Dermal Fibroblast) cell line was purchased from Invitrogen (Waltham, USA), while all the reagents (culture media, antibiotics, FBS, PBS, trypsin-EDTA and MTT solution, DMSO) and culture supplies (culture flasks, Pasteur pipettes, pipette tips, microplates) were achieved from Thermo Fischer Scientific (Waltham, USA).

Female, 6-8-week-old CB17SCID mice were purchased from Charles River (Sulzfeld, Germany). They were kept under standard conditions - temperature (24± 2°C), humidity (55±5%), 12h light/dark cycle.

Synthesis of the transporter

The PU synthesis is based on a polyaddition reaction between an active hydrogen-containing phase (ether- and/or ester-polyols) and a di- or tri-isocyanate. In the current research, an aliphatic isocyanate (IPDI) was used as the organic phase. In the aqueous phase, the ratio between PEG 400 and PCL was established according to our previous results (17,18) and taking into consideration that this balance has a huge impact on the drug release kinetics.

The inorganic phase (1.8 mL HD, 3.5 g PEG 400, 3.0 g PCL, 1.2 g THAM, 1.5 mL Span® 85 and 0.5 g 2'-Deoxycytidylic acid sodium salt) was dissolved in 35.0 mL double distilled water and stirred at 375 rpm and 35±0.2°C overnight. In parallel, three isocyanate solutions obtained from 6.0 mL IPDI in 25.0 mL acetone were homogenized in different flasks at 345 rpm and 40±0.2°C for 30 minutes. Then, the aqueous phase was divided equally among the three flasks and their contents continued to be stirred at 350 rpm using different temperatures (**Tab. I**) for 3 hours to complete the synthesis of the macromolecular chains; the temperatures were precisely monitored using a P700 Universal thermometer from Dostmann electronic GmbH

(Wertheim, Germany). In the end, the content of the three flasks was sonicated for 5 min using a Bandelin Sonorex Digitec bath sonicator (Berlin, Germany).

TABLE I.
The synthesis temperature used for every sample

Samples code	Synthesis temperature °C
PU_T1	25
PU_T2	40
PU_T3	55

There were obtained white suspensions that were passed through 0.22 μm PVDF sterile syringe filters from Merck Millipore (Darmstadt, Germany) during a washing process with a mixture of water-acetone (1: 1.3, v/v) and finally they were centrifuged at 2,200 G using an Ika Mini G microcentrifuge (Staufen, Germany) in order to concentrate the samples; the water-acetone mixture used during the washing process was later analyzed in order to established the free drug content. In the end, the samples were dried in borosilicate glass Petri dishes at room temperature to constant mass (approx. 120 hrs.).

Samples Stability

The samples stability was assessed by using an aliquot of every sample that was stored at different temperatures: 8±0.5°C, 25±0.5°C and 40±0.5°C with 65±3% relative humidity for one month; color as maximum absorbance and electrical conductivity of samples were recorded every third day using a SI Analytics UVi Line 9400 spectrophotometer (Mainz, Germany), respectively a Jenway Bench 4010 Conductivity meter (Staffordshire, UK) at 25°C in aqueous solutions (0.8 mg mL^{-1}).

pH, Refractive index
and Solubility Measurements

pH of every sample was measured using a Mettler Toledo FiveGo F2 portable pH meter (Schwerzenbach, Switzerland), In-Lab® Expert Go Sensor and aqueous solutions with the same concentration (0.8 mg mL^{-1}) at 25°C.

2-3 drops of every aqueous solutions and a Kern digital refractometer (Balingen, Germany) were used to assess the refractive index of the synthesized samples at room temperature.

For the solubility in distilled water, acetone, and DMSO, based on a procedure already described by our team (13), 5.0 mg dried product were dissolved in the respective solvent in 20 mL glass vials with screw caps at room temperature; the volume of every solvent was registered when a single, clear liquid phase with no distinct solid or gel particles was recorded.

Drug Loading

The successfully entrapped amount was found by reporting the quantity of the free drug to the total added amount, Equation 1:

$$DL\% = \frac{TA - FD}{TA} \cdot 100$$

where DL% is the drug loading percentage, TA and FD are the total amount and the amount of free drug (mg).

These amounts were calculated using the Beer-Lambert law: a calibration curve (the absorption values *vs*. drug concentration) was initially drawn.

Drug Release Profile

The cumulative drug release (CDR) was obtained by monitoring the amount of 2'-Deoxycytidylic acid sodium salt that was released during the PU transporter exposure to a simulated body fluid (SBF) prepared according to F. Baino and S. Yamaguchi (19): 6.6 g NaCl, 2.3 g $NaHCO_3$, 0.4 g KCl, 0.1 g Na_2HPO_4 were mixed with 960 mL double distilled water in a 1000 mL-capacity flat bottom flask and heated at

37°C; then 0.3 g $MgCl_2$ hexahydrate, 0.4 g $CaCl_2$ dihydrate, 0.1 g Na_2SO4, 6.1 g Tris were added and the pH was adjusted to 7.4 with HCl 1M. The samples were maintained inside SBF for 5 days at 37.0±0.5°C and 150 rpm; 1.0 mL SBF was replaced daily with fresh degrading environment and UV-Vis spectroscopy was employed to quantify the released drug.

The Penetrability Rate

A Franz-cell system (Φ 15 mm, diffusion area of 1.77 cm^2 and the receptor volume of 12.0 mL) and a PVDF artificial membrane Spectra/Por® were used to test the transporter permeation. The receptor chamber was filled with PBS and the experiment was done at 25±1°C; at every 10 hours, 1.0 mL liquid from the receptor chamber was replaced with fresh buffer and spectral analyzed.

Zetasizer Tests

The size and surface charge of PU transporter were assessed through dynamic light scattering technique on a Cordouan Technol. system (Pessac, France); the main input parameters that were set for the determination of particles size and its distribution were the temperature (25°C), time interval (18 μs), channels number (460), laser power (80%), acquisition mode (continuous) and analysis mode (Pade-Laplace). The following parameters were set to detect the surface charge: temperature (25°C), applied field (automatic), medium resolution (0.8 Hz), measures number/sequence (3), laser power (80%), Henry function (Smoluchowski), cuvettes (quartz).

Cells Viability

HDFa cells (passage 3) were used as follows: the cells were seeded in a culture medium supplemented with 10% FBS and 1% penicillin-streptomycin; the medium was changed at 2-3 days and the proliferation was determined by the MTT technique. Cells from the HDFa line (1x10^5 cells/mL) were seeded in 100 μL medium in 96-well plates. Cells were allowed to adhere for 24 hrs. in the incubator. After 24 hrs., the growth medium was changed with the test compounds (90 μL medium+10 μL sample); the tests were performed in triplicate at 24 and 72 hrs. after the treatment.

Skin Irritation Evaluations

The irritation potential on sensitive mice with and without hair is often used as a parameter that describes new synthesized chemical compounds. In the current study, 20 mice were divided into five groups, labeled as the samples code (tab. I) and two special groups (control group – mice treated only with the solvent, and SLS group – mice treated with a 2.0% sodium lauryl sulfate solution, a recognized skin irritant). The hair from the back of CB17SCID mice (approx. 3 cm^2) was shaved at the beginning of experiment and then every 5 days. The application of the studied samples (40±5 μL once) was done on the back skin every third day for 15 days and the measurements of parameters were performed 30 minutes later. The changes of skin erythema and hydration were monitored using a Multiprobe Adapter System (MPA5) from Courage-Khazaka (Koln, Germany) equipped with a Mexameter® MX 18 and a Corneometer® CM 825 probes.

Statistics

Data analyses were performed using IBM *SPSS Software version 27.0.0.0* (Armonk, NY, USA). The continuous variables are presented as mean and standard error (SE). Non-parametric tests were used to evaluate the significance due to the small datasets. $p< 0.05$ was considered statistically significant. The charts were modeled in Excel - Microsoft® Office Professional Plus

2019 from Microsoft Corp. (Washington, USA).

Ethics approval

The proposal of this research was checked and approved by the Ethics Committee of "Victor Babes" University of Medicine and Pharmacy Timisoara (Approval No. 42, 29th of October, 2021).

RESULTS

Stability of PU transporter

It is well known that polymeric materials present a huge stability in time; however, a few degradative processes based on the thermal and/or UV radiation, the microorganisms attack and hydrolytic conditions can alter the properties of macromolecule-based biomaterials (20). The stability of a pharmaceutical formulation represents a key parameter, that indicates the storage-controlled conditions such as humidity, temperature, sun exposure. The modifications of samples color and electrical conductivity are displayed in table II.

pH measurements

The pH monitoring shows the almost neutral character of tested samples. The following results were obtained: 6.68±0.18 (sample PU_T1), 6.78±0.11 (PU_T2), and 6.71±0.14 (PU_T3).

TABLE II.

The changes of maximum absorbance and electrical conductivity levels in 30 days

Sample	Percentual changes at different temperature, %					
	8±0.5°C		25±0.5°C		40±0.5°C	
	abs.	cond.	abs.	cond.	abs.	cond.
PU_T1	4.3	7.5	4.0	7.0	4.1	6.5
PU_T2	4.5	7.9	4.2	7.7	4.3	7.6
PU_T3	4.4	8.3	4.2	7.9	4.2	7.8

Refractivity index

The values of refractivity index were: 1.59 for samples PU_T1, respectively 1.60 for samples PU_T2 and PU_T3.

Sample solubility

Table III displays the solubility of synthesized samples in different solvents.

TABLE III.

Comparative solubility of samples

Sample	Solubility (mg mL^{-1})		
	water	acetone	DMSO
PU_T1	0.87	1.07	1.09
PU_T2	0.79	0.99	0.92
PU_T3	0.75	0.93	0.86

Drug loading

The encapsulation efficiency represents the percentage of the biologically active compound that was successfully entrapped within the structures of the transporter / delivery system; the encapsulation efficiency and the controlled release behavior are the main parameters in this field and they depend most on the chemical structure and on the forces between molecules. In the current study, the average encapsulation efficiencies are equal to 63.7% (PU_T1), 58.1% (PU_T2) and 58.5% (PU_T3).

Drug release profile

The cumulated drug release is presented in second figure.

Penetrability rate

The penetrability through membranes represents the major rate-limiting step which have an impact on the release profile and further on the bioavailability of the active compound. A proper permeation through an artificial membrane was ob-

served for all samples (fig. 3), but the best results were recorded in the case of sample PU_T1 (more than 80% passed through the membrane in the first 2 days).

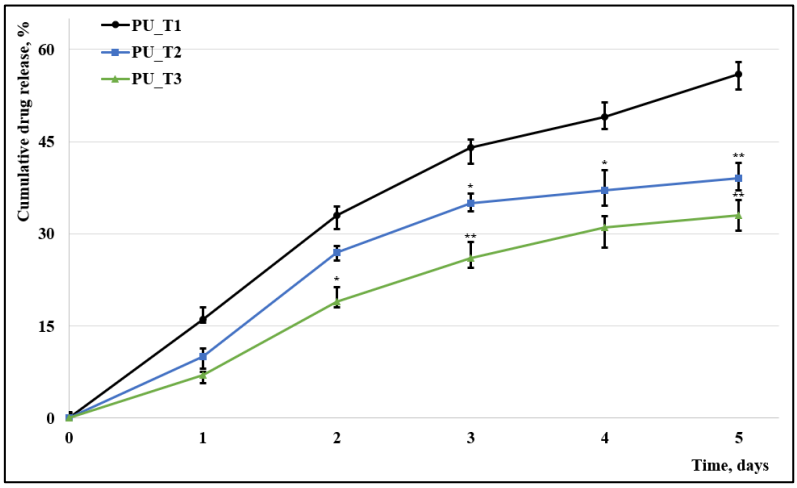


Fig. 2. Comparative cumulated drug release (mean ± SE); * for p< 0.05 and ** for p< 0.01 vs. PU_T1.

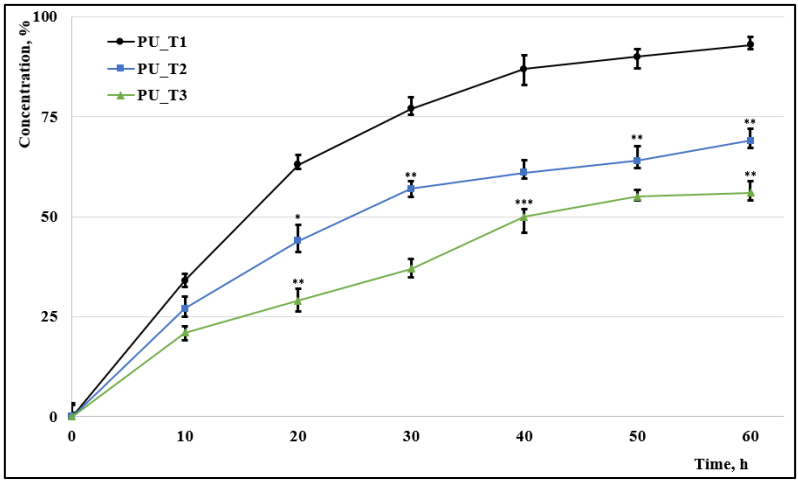


Fig. 3. Comparative penetrability rates (mean ± SE); * for p< 0.05, ** for p< 0.01, and *** for p< 0.001 vs. PU_T1.

Zetasizer

The analysis based on the dynamic light scattering is often used to characterize the colloidal suspensions and solutions; particles' size, their dispersity and surface charge represents important parameters in drug delivery too. Table IV shows the values obtained in the Zetasizer evaluations.

Table IV presents the formation of polydisperse systems containing two particle populations for samples PU_T2 and PU_T3, respectively a more homogeneous

system based on a single population of particles in the case of sample PU_T1; the average values of Zeta potential are also specific to systems with low resistance against the tendency to form particles cluster (for samples PU_T2 and PU_T3), while the value for PU_T1 is situated between medium and strong resistance (+34.4 mV).

TABLE IV.
Comparative Zetasizer characterization of samples

Sample	Analyzed parameters		
	Size (nm)	PDI	Zeta potential (mV)
PU_T1	162±7 (100%)	0.31	+34.4
PU_T2	126±11 (40%) 614±13 (60%)	0.87	+20.9
PU_T3	192±19 (29%) 814±23 (71%)	0.95	+17.6

Cells viability

In figure 4 are presented the HDFa cells proliferation rate.

The cells proliferation rates were studied at 24 and 72 hours (fig. 4). After 24h, a reduced inhibition was observed in samples PU_T2 and PU_T3, while sample PU_T1 exhibited an increased cell proliferation. At 72h incubation, in all samples, the same trend of stimulated cell proliferation was observed.

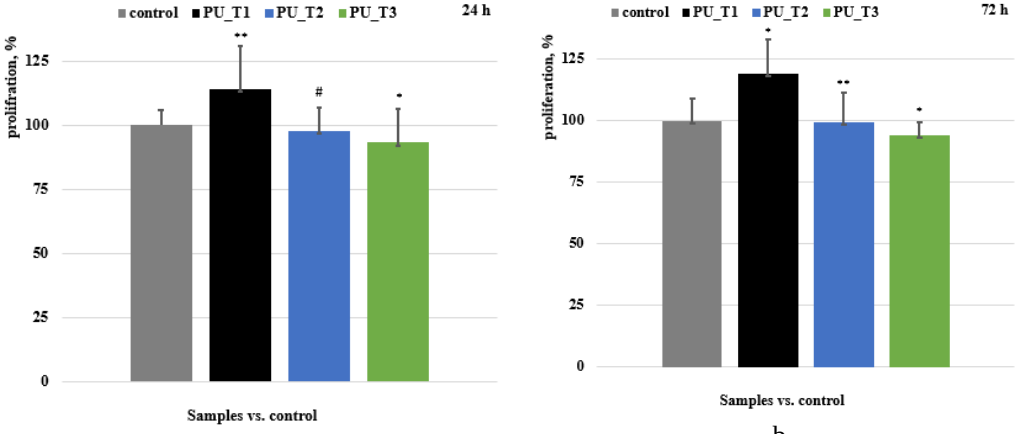
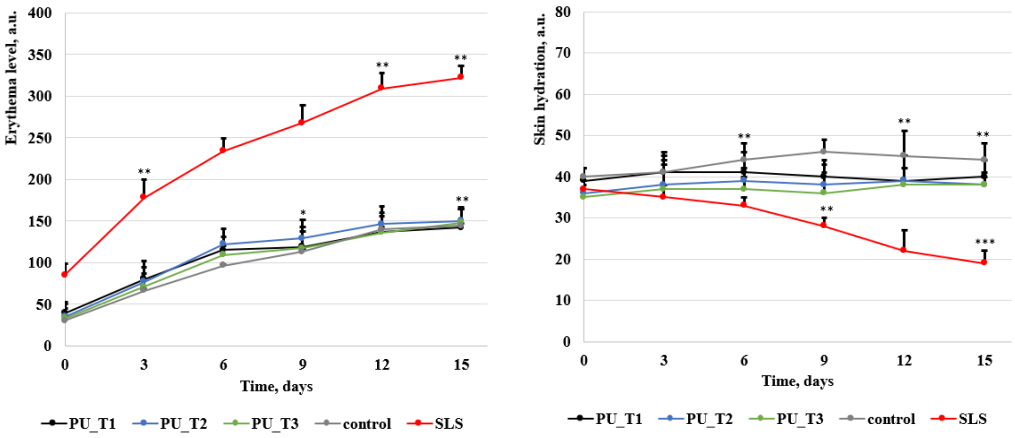


Fig. 4. Comparative cells proliferation (mean ± standard error) at (a) 24 and (b) 72 hrs.; # p= 0.0565, * p< 0.05, ** p< 0.01.

Skin Irritation Evaluation

Figure 5 presents the evolutions of two main skin parameters (erythema and skin hydration) during the 15-day experiment.

It can be noticed that the levels of erythema increased for all samples but these raises were much lower to that recorded in the case of sodium lauryl sulfate; on the other hand, the levels of the skin hydration (fig. 5b) have been modified only slightly in the case of mice treated with the synthesized samples.



a b

Fig. 5. Comparative changes of erythema and skin hydration (mean ± standard error); * p< 0.05, ** p< 0.01, *** p< 0.001.

DISCUSSION

A huge variety of polymers are obtained and tested as drug delivery systems in the administration of various extracts and drugs with low solubility and bioavailability (21, 22). Many PU carriers, as nanocapsules, dendrimers, and matrices, were developed in the last two decades; they were tested as delivery systems for ginger (13), garlic and mistletoe extracts (14), tocopherol (10-12), anti-tuberculosis drugs (23).

The temperature and pressure represent the main factors that have an important impact on the chemical synthesis and on the materials with special properties; the temperature influences the reaction rate and the equation between these parameters was proposed by Svante Arrhenius in 1889 (24). On the other hand, the temperature has a great impact on the volume: Jacques Charles stated that volume / temperature = constant (experiments on gases in the 1780s); in a similar manner, the most materials increase their volume when the temperature increases (they expand), except a few anomalies - the water between 0 and 4°C, super hard materials (25), multilayer steel (26), ceramics (27), rare-earth intermetallic with cobalt and iron (28).

Polymers present a wide range of linear thermal expansion; their coefficients are correlated with the type of intermolecular bonds. Their synthesis temperature depends on the exo- *vs*. endo-thermic processes that occur; thus, the requirements for polymerizations range from room temperature (29) till 100-200°C (30), polycondensation at 35°C (31), while polyadditions frequently exceed 90°C (32, 33). The synthesis of polyurethanes is based on a fast and exothermic reaction (34), so low synthesis temperatures, that were used in this study, can be an advantage.

The stability of polymer materials is well known and it is often the subject of waste issues. However, a few external factors, such as bacteria, water, temperature, and UV radiations, can accelerate their degradation. The results of the current study show that the obtained PU transport-

ers are stable between 8 and 40°C for 30 days, the changes of samples' maximum absorbance were between 4.0 and 4.5% for PU_T1 at 25°C and PU_T2 at 8°C, while the modifications of electrical conductivity were between 6.5 and 8.3% for PU_T1 at 40°C and PU_T3 at 8°C.

The pH of the samples, between 6.68 and 6.78, determined in aqueous solutions, is specific to nearly neutral acido-basic products. A narrow range of refractivity index (1.59-1.60) was found and it is very similar with other data reported in the literature (35).

The solubility is a significant drawback for all polymers; the current research revealed low values ranging from 0.75 mg mL^{-1} for PU_T3 in water to 1.09 mg mL^{-1} PU_T1 in DMSO. Generally, PU delivery systems present low solubilities which are often associated with a delayed release (36).

Important differences were found in the tested samples for the encapsulation efficacy, a key parameter in the design of drug transporters: PU_T1 sample has encapsulated almost 64% of the active agent, while the other two samples just 58%; the literature presents various values of the encapsulation efficacy between 40-91% (37).

The drug release profile is similar for the three samples, but higher values were found in the case of PU_T1: after 5 days, almost 60% of the encapsulated compound was released, while the other two samples present just 39 and 33%. The permeability of the PU transporters through an artificial membrane has revealed once again that the first sample (PU_T1), synthesized at the lowest temperature, is the best; almost the entire amount has passed through membrane within 60 hours.

Dynamic light scattering is often used as a technique proper to measure the particles' velocity to find their size, respectively the electrophoretic mobility to analyze their tendency to form clusters. The Zetasizer measurements (tab. IV) present a higher tendency to form particles' agglomerates and heterogeneous populations in the case of samples that were synthesized at higher temperature (PU_T2 and PU_T3).

The analysis of the proliferation of Human Dermal fibroblasts has indicated different trends among the samples: in the case of the first sample higher values was found (114% at 24 hrs. and 119% at 72 hrs.) compared to the second sample (98% at 24 hrs. and 99% at 72 hrs.) and the third one (93% at 24 hrs. and 94% at 72 hrs.). Based on these results, the samples can be considered as non-cytotoxic and more than that, in the case of PU_T1, it can be related to other studies that presents PU as friendly products with cells proliferation (38).

Due to their extremely sensitivity, mice are often used in the assessment of cosmetics, dermatological products, but also in testing of the irritation effects for newly synthesized pharmaceutical formulations. The levels of erythema have increased from approx. 35 to 145 arb. units; despite these increases, the three samples can be considered as safe products compared to the difference observed in the case of SLS (from 85 to 320 arb. units / same period). The decrease of hydration along with the increase of erythema and trans-epidermal water loss represent the main indicators of a skin disease or of an irritation effect; however, the amplitude of the change per time unit is important to be used in such assessment; the results show a maintained level of hydration in the case of samples with PU transporters, while the level de-

crease from 37 to 19 arb. units for SLS; based on the literature data (39), it can be stated that synthesized PU not alter the epidermal function.

CONCLUSIONS

Polyurethane materials are often used in medical and pharmaceutical applications as wound dressing, artificial skin, bone prostheses, heart valves, due to their biocompatibility. In the present study, a polyaddition reaction combined with a simultaneous emulsification was involved for the obtaining of PU particles that can be used as a carrier for nucleotides; the synthesis was done at three different temperatures in order to evaluate the impact on the final properties. The results indicate the preparation of a carrier that encapsulate large amounts of the active substance (around 60%), with a good release profile and a proper penetrability rate.

The physicochemical analysis showed that the sample prepared at 25°C (PU_T1) present the best encapsulation efficacy and the lowest tendency to form clusters that were observed compared to the other samples in Zetasizer characterization. Moreover, the sample prepared at the lowest temperature offers the best results in the cells viability, respectively in non-invasive tests on mice skin. Thus, the core finding of the current research is that a synthesis temperature below 40°C is suitable in the obtaining of a PU carrier for nucleotides.

Based on all the promising features previously described, the newly synthesized PU samples can be considered suitable candidates for the development of pharmaceutical formulations for delivery of nucleotides.

CONFLICT OF INTEREST AND FUNDING

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EXPLORING MYOCARDIAL BRIDGES CHARACTERISTICS IN NORTH-EASTERN ROMANIA. A RETROSPECTIVE ANALYSIS

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EXPLORING MYOCARDIAL BRIDGES CHARACTERISTICS IN NORTH-EASTERN ROMANIA: A RETROSPECTIVE ANALYSIS (Abstract): Myocardial bridges are defined by a segment of the coronary artery taking an intramuscular route, diverging from its usual superficial path over the myocardial tissue and, instead, traversing through the myocardium itself. Currently, their true prevalence remains uncertain and challenging to estimate accurately. Their dynamic presentation shapes the diversity of clinical manifestations, resulting in highly variable expressions among individuals. **Materials and methods:** We conducted a retrospective analysis of myocardial bridge characteristics in patients undergoing coronary computed tomography angiography (CCTA) at the "Prof. Dr. George I.M. Georgescu" Institute of Cardiovascular Diseases in Iasi, Romania, from 2012 to 2022. Additionally, we sought to investigate any potential correlation between the presence of myocardial bridges and the development of atherosclerosis and coronary artery disease. **Results:** Out of the 66 patients with myocardial bridges, 44 were males, accounting for 66.7% of the total cases. The average age was of 59.42 years (± 11.84 years). In 97% of cases, the left anterior descending artery (LAD) was affected, primarily at its middle segment (60.6%). The mean width measured 3.02±1.22 mm and the mean length was 20.33±9.25 mm. A significant elevation in the average calcium score level was observed among males (p=0.025). Individuals with myocardial bridge thickness over 5 mm showed significantly higher average calcium scores (p=0.025). **Conclusions:** Using CCTA improves the detection of clinically significant myocardial bridges, with the middle segment of the left anterior descending artery being the primary site in most cases. The study suggests a potential link between myocardial bridges thickness and higher coronary artery calcium scores, prompting further exploration. Correlations with atherosclerosis and hypertension require deeper investigation for a comprehensive understanding of myocardial bridges clinical impact. **Keywords:** MYOCARDIAL BRIDGES, CORONARY ARTERY ANOMALIES, ROMANIAN POPULATION, CARDIAC IMAGING.

INTRODUCTION

Initially documented by Reymann in 1737, myocardial bridging represents a congenital cardiac anomaly wherein a layer of cardiac muscle overlays a section of a coronary artery passing through the myocardium, forming a bridge-like configuration. This intramural portion of the artery, referred to as the "tunneled" artery, is surrounded by cardiac muscle tissue (1, 2). The prevalence of myocardial bridges varies depending on the diagnostic method employed. Post-mortem investigations yield the highest detection rate for myocardial bridges, ranging up to 86%, with an average of 25% (1). Invasive procedures like conventional coronary angiography can detect approximately 12% of myocardial bridges. Given the current inclination towards non-invasive approaches for identification, coronary computed tomography angiography (CCTA) emerges as the most accurate for assessing myocardial bridges. It provides superior capabilities in delineating the bridge's dimensions, as well as evaluating the degree of the induced compression (1). With various methods available to detect these anomalies, each differing in accuracy and specificity, the true prevalence of myocardial bridges remains uncertain and challenging to estimate accurately. However, there is a high likelihood that the actual prevalence of myocardial bridges exceeds current estimates (1). The predominant localization of myocardial bridges is typically within the left anterior descending artery (LAD), particularly evident in its mid-segment (3). When assessed using CCTA, myocardial bridges are categorized based on their depth: superficial (≥1 mm), deep (≥2 mm), and very deep (≥5 mm). Additionally, myocardial bridges are classified as short if their length is <25 mm and long if their length is ≥25 mm. This classification criterion aids in distinguishing between benign and clinically significant myocardial bridges based on their depth and length within the cardiac muscle (4). While the superficial and short myocardial bridges might usually be asymptomatic, those with a depth ≥2 mm and a length ≥25mm can have clinical manifestation that might vary from angina, heart block to acute coronary syndrome, syncope and even rarely sudden death (5). At the same time, recent studies aim to discern whether the presence of myocardial bridges exhibits a potential protective or detrimental effect on various cardiac conditions. Researchers analyzed the values of Ki-67 marker, assessing artery inflammation and atherosclerosis, and macrophages among the bridged segment of the coronary arteries (6). This study revealed their lower levels in the segments with myocardial bridges, compared to the other areas of the artery. Nevertheless, there are authors who contradict this hypothesis, suggesting that the existence of myocardial bridges could be a factor in determining coronary artery disease and therefore, ischemia (1). The diverse array of clinical expressions associated with myocardial bridges are significant given the absence of standardized guidelines for their identification, monitoring and treatment (7).

AIMS. Our study aimed to explore the characteristics of myocardial bridges observed in patients seeking care at "Prof. Dr. George I.M. Georgescu" Institute of Cardiovascular Diseases, over a decade within the North Eastern region of Romania. Additionally, we sought to investigate any potential correlation between the presence of myocardial bridges and the development of atherosclerosis and coronary artery disease. The motivation for our investigation started from the limited existing research on

the prevalence and attributes of myocardial bridges within the Romanian population. This gap in knowledge highlights the importance of our study in contributing to a deeper understanding of cardiovascular anomalies within this specific demographic.

MATERIALS AND METHODS

Study design and patients

Our research investigated a cohort of 12,758 patients with cardiovascular diseases who underwent coronary computed tomography angiography (CCTA) from 2012 to 2022 at "Prof. Dr. George I.M. Georgescu" Institute of Cardiovascular Diseases from Iasi, Romania. The information employed in this study was obtained from the medical files of the patients. The acquired data facilitated the categorization of the enrolled cohort into various groups based on anomalies of the coronary arteries, with a primary focus on myocardial bridges. Our database included demographic variables including the age and gender (female/male) of the patients, all of whom were adults. Simultaneously, we documented the presence of coronary artery disease, chronic cardiac failure, dyslipidemia, hypertension and coronary angioplasty with stents. The study design and protocol were approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania (no. 115/15.10.2021).

CCTA Assessments (Myocardial Bridges and Agatston score)

In this investigation, we initially incorporated data concerning the affected coronary artery by myocardial bridges, along with detailed characteristics regarding the depth and length of these structures. All measurements pertaining to these parameters were recorded in millimeters (mm). Subsequently, our classification scheme stratified myocardial bridges into three distinct categories based on depth: superficial (<2mm), deep (≥2 and <5), and very deep (≥5 mm). Regarding length, we defined two categories relative to a threshold of 25 mm: those falling below this threshold were categorized as short, whereas those equal to or exceeding it were classified as long. Concurrently, we documented the presence of atherosclerosis and consecutively assessed the degree of stenosis in the affected coronary artery with myocardial bridge, classifying stenosis in non-critical if the diameter of the coronary artery was <70% and critical if it was ≥70%.Furthermore, we integrated the Agatston calcium score into our database, categorizing it into four different groups: 0. no atherosclerosis (0 Agatston units), 1. mild (1-100Agatston units), 2. moderate (101-400Agatston units) and 3. severe (>400Agatston units).

Statistical analysis

Data were collected and subjected to analysis using the *Statistical Package for Social Sciences version 27.0* (IBM Corp., Armonk, NY, USA). Specifically, the analysis utilized the ANOVA test, a widely recognized method for examining variance across multiple groups. Primary indicators such as the minimum and maximum values, along with frequency distributions, were examined to gain insights into the range and distribution of the data. Indicators of central tendency including the simple arithmetic mean and median were calculated to understand the average or typical value of the dataset. Indicators of dispersion, such as the standard deviation and standard error, were computed to assess the spread or variability of the data points around the mean. Additionally, confidence intervals of the mean were determined to quantify the precision of the estimated

population parameters. Qualitative significance tests were conducted to examine the significance of categorical variables within the dataset. All analyses maintained a significance level of $p < 0.05$.

RESULTS

Among the examined patients, 350 (2.74%) exhibited coronary artery anomalies, and among them, 66 were diagnosed with myocardial bridges (18.9%). Out of the total cohort, 44 were males, constituting 66.7% of the patients, while 22 were females, accounting for 33.3% (fig. 1).

The age ranged from 22 to 84 years, with an average level of 59.42 years ± 11.84 years, which is relatively close to the median value (60.50 years) (fig. 2).

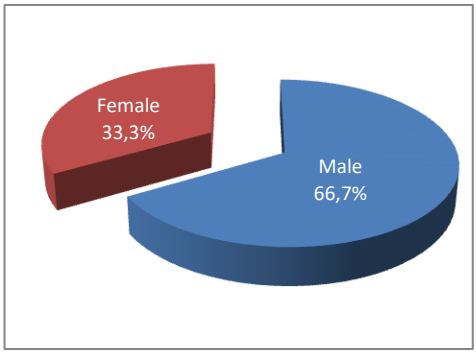

Fig. 1. Gender distribution among patients with myocardial bridges

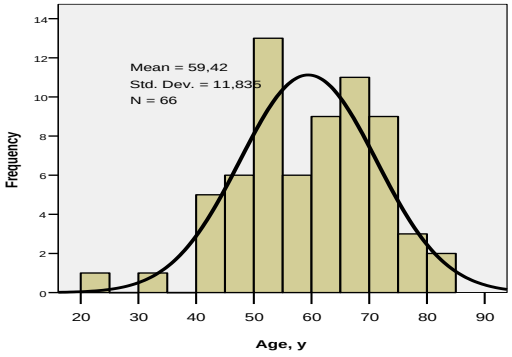

Fig. 2. Diagnostic age histogram for patients with myocardial bridges

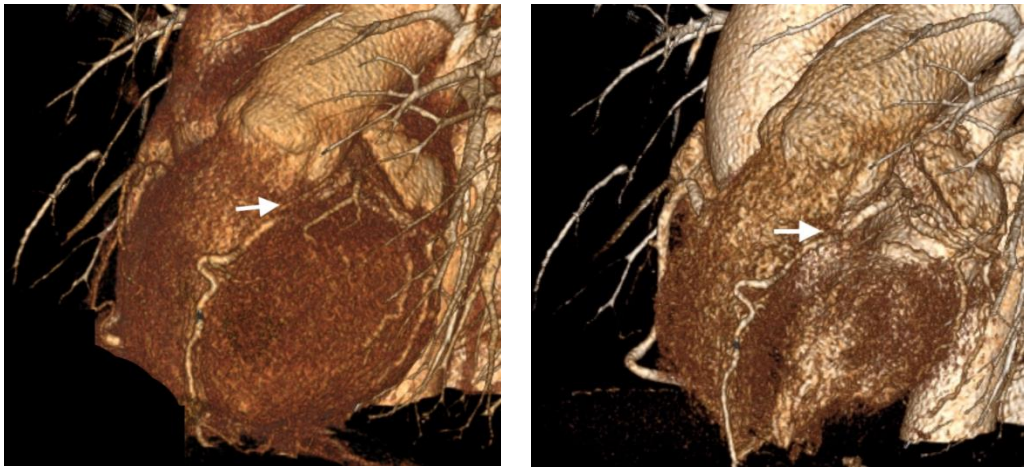

Fig. 3. 3-dimensional volume-rendered images obtained from coronary computed tomography angiography of a myocardial bridge in the middle segment of the left anterior descending artery in a female patient

In 97% of cases, the primary coronary artery affected was the left anterior descending artery (LAD) (fig. 3). Only one instance of myocardial bridge was observed

on the right coronary artery (RCA) and another one on the left main (LM).

The middle segment of the LAD was the most common site of myocardial bridges localization, accounting for 62.5% of total bridges found (tab. I).

TABLE I.

Localization of myocardial on the left anterior descending artery (LAD), left main (LM) and right coronary artery (RCA)

Characteristics	Coronary		
	LAD (n=64)	LM (n=1)	RCA (n=1)
Location			
proximal	11 (17.2%)	1 (100%)	1 (100%)
medial	40 (62.5%)	0 (0%)	0 (0%)
distal	12 (18.8%)	1 (100%)	0 (0%)

Furthermore, our investigation explored the primary characteristics of myocardial bridges, examining their morphology concerning length and width, as well as the presence of stenosis (tab. II). Analysis of myocardial bridge depth revealed that the majority of patients (69.7%) exhibited deep myocardial bridges, with a smaller proportion presenting very deep myocardial bridges (16.7%), and a further subset displaying superficial myocardial bridges (13.6%) (tab. II). In terms of length, data indicated that a significant portion of patients (74.2%) had short myocardial bridges, while the remaining 25.8% presented long ones.

TABLE II.

Characteristics of myocardial bridges

Myocardial bridges Coronary Artery	N	%
LAD	64	97.0
LM	1	1.5
RCA	1	1.5
Location on the coronary artery		
Proximal	13	19.7
Medial	40	60.6
Distal	13	19.7
Width (mm)		
Mean ± SD	3.02±1.22	
median/limits	2.50/1.3-6.0	
Thickness (mm)		
< 2	9	13.6
2-5	46	69.7
≥ 5	11	16.7
Length (mm)		
Mean ± SD	20.33±9.25	
median/limits	18/8-50	
Dimensions		
Short	49	74.2
Long	17	25.8

The mean width measured 3.02 ± 1.22 mm, indicating a relatively consistent range. Conversely, the mean length was 20.33 ± 9.25 mm, demonstrating a wider dispersion of values. Simultaneously, 24 patients exhibited stenosis on the LAD, with only one case showing involvement of the RCA (tab. 3). When evaluating the degree of the stenosis of the affected coronary arteries, the results revealed a mean value of 13.26±21.13. There were 12 patients (18%) who presented stenosis ≥50%, and just one patient with stenosis >70%. Nevertheless, in our study, only 3 male patients (5%) underwent stent placement.

TABLE III.

Morphological aspects of myocardial bridges

Characteristics	Coronary		
	LAD (n=64)	LM (n=1)	RCA (n=1)
Stenosis	24 (37.5%)	0 (0%)	1 (100%)
Thickness			
< 2 mm	9 (14.1%)	0 (0%)	0 (0%)
2-5 mm	44 (68.8%)	1 (100%)	1 (100%)
≥ 5 mm	11 (17.2%)	0 (0%)	0 (0%)
Length			
Long	17 (26.6%)	0 (0%)	0 (0%)
Short	47 (73.4%)	1 (100%)	1 (100%)

Our analysis of the coronary artery calcium score revealed a heterogeneous distribution among patients. While a substantial portion (42.4%) exhibited a mild score, a significant proportion (40.9%) presented zero scores, indicating absence of calcification. Additionally, a smaller percentage (9.1%) exhibited severe scores, while moderate scores were observed in just 7.6% of cases (fig.4). This spectrum of findings underscores the differences of calcium deposition within the coronary arteries with myocardial bridges, suggesting varying degrees of cardiovascular risk among the studied population.

Our observations revealed a significant elevation in the average calcium score level among males (p=0.025) (fig.5).

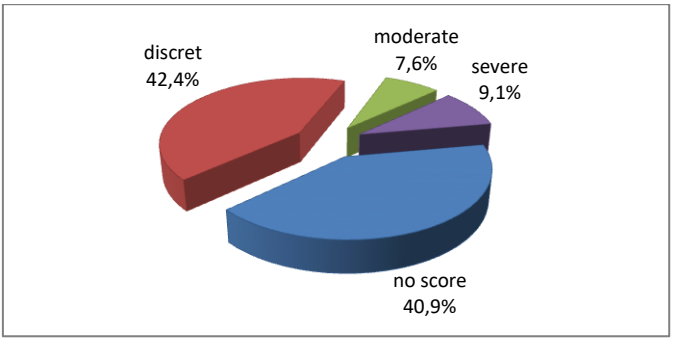

Fig. 4. Coronary Artery Calcium Score in patients with myocardial bridges

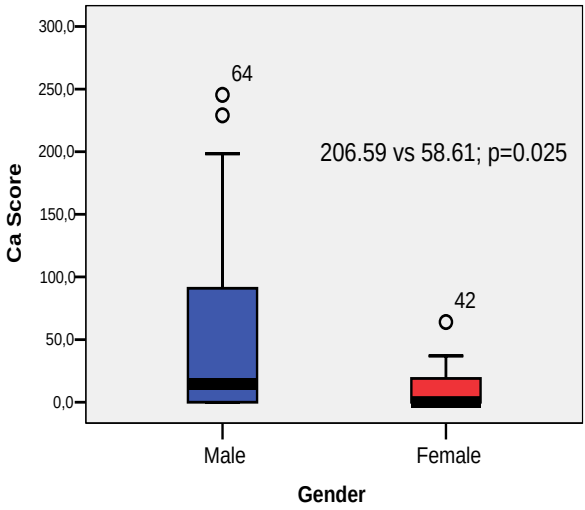

Fig. 5. Average calcium score levels by gender

Simultaneously, the average calcium score level was significantly higher in individuals with a myocardial bridges thickness exceeding 5 mm (p=0.025) (fig. 6).

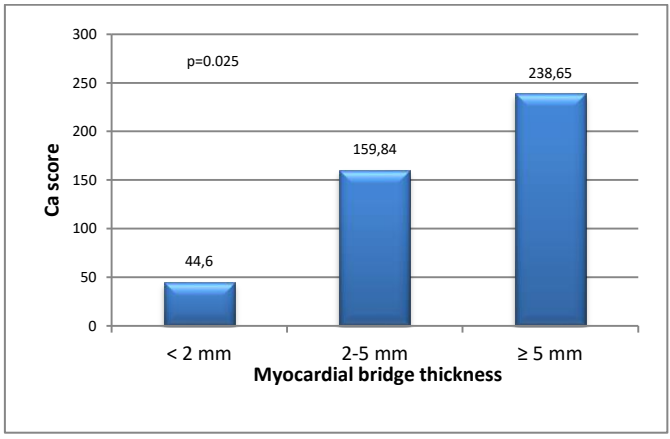

Fig. 6. Comparison of Average Calcium Score Levels between Myocardial Bridge Thickness Groups

Our investigation into the prevalence of hypertension, dyslipidemia, coronary artery disease (CAD), and chronic heart failure among these patients offered promising insights, observing a predominant occurrence of these conditions in males. Hypertension manifested in 29 patients (44%), with a noteworthy observation that half of the male cohort was affected by this condition. Dyslipidemia was observed in 9 male individuals and 6 female patients. Furthermore, CAD was identified in 25 patients (38%), while chronic heart failure was diagnosed in 17 individuals (26%) (tab. IV).

TABLE IV.
Associated conditions by gender in patients with myocardial bridges

Comorbidities	Male (n=44)		Female (n=22)		Chi² Test p	RR	IC95%
	n	%	n	%			
Hypertension	22	50.0	7	31.8	0.157	1.68	0.79-3.57
Dyslipidemia	9	20.5	6	27.3	0.537	1.14	0.73-1.80
Coronary artery disease	18	40.9	7	31.8	0.470	1.31	0.62-2.76
Chronic cardiac failure	14	31.8	3	13.6	0.098	2.20	0.74-6.51

DISCUSSION

The large spectrum of coronary artery anomalies comprises of a variety of rare congenital conditions, exhibiting diverse morphologies, clinical presentations, and potential hemodynamic implications (8).

Myocardial bridges, included within this category, are distinguished by a segment of the coronary artery taking an intramuscular route, diverging from its usual superficial path over the myocardial tissue and, instead, traversing through the myocardium itself. Therefore, this anomaly may impact not only the anatomy of the coronary arteries, but also the blood flow distribution among the myocardium(9).The extent of this effect fluctuates depending on the degree of compression in the bridged region during contraction, creating a 'milking' action that reduces blood flow and could predispose to ischemia. This dynamic presentation not only influences myocardial perfusion, but also shapes the occurrence and diversity of clinical manifestations in patients with myocardial bridges, resulting in highly variable expressions among individuals (10).

Their identification is now achievable through both invasive methods, such as Intravascular Ultrasound (IVUS) or coronary angiography, and non-invasive approaches like echocardiography, CCTA and Cardiac Magnetic Resonance Imaging (CMRI) (5). Echocardiography serves as a valuable tool for detecting myocardial bridges, although its specificity is limited-it may reveal irregularities in the septal wall motion from end-systole to early-diastole, alongside preserved heart function in the apex(4).Non-invasive imaging techniques, particularly CCTA, offer the highest accuracy and specificity in detecting myocardial bridges. CCTA alone can detect up to 86% of cases, emphasizing its efficacy. In contrast, the prevalence of myocardial bridges detected via coronary angiography is a mere 1.7%. This discrepancy underscores the superiority of non-invasive methods in identifying these anomalies (5). At the same time, CMRI, due to the lack of contrast administration or radiation exposure, is preferable for younger athletes and patients with kidney failure. Recent studies show its superiority over MDCT in detecting calcified coronary artery lesions, but its use in detecting myocardial bridges is still limited due to the poor spatial resolution (11, 12). Our study revealed a detection rate of myocardial bridges at 0.51% among all patients scanned (12758). Remarkably, among all coronary artery anomalies observed, myocardial bridges ranked as the third most common, accounting for up to 18.9% of cases (13).

In our study, we included patients aged 22 to 84 years, with a mean age of 59.42 years ± 11.84, reflecting the diverse age range at which myocardial bridges are

identified. Furthermore, we noted a predominant presence of male patients with myocardial bridges (66.7%), consistent with findings from prior studies, thereby underscoring their heightened prevalence among men (2).

While myocardial bridges may occur across any of the primary coronary arteries, prevailing research underscores their most common occurrence along the left anterior descending artery (LAD), especially within its middle segment (10). Our study corroborates this notion, particularly evident in the predominant presence of myocardial bridges in the LAD in 97% of cases. Additionally, a small minority of cases (3%) featured myocardial bridges on the right coronary artery (RCA) and left main coronary artery (LM). However, our findings reveal a distribution pattern within the myocardial bridges localization: the middle portion was most frequently affected, observed in 40 patients, followed by the distal and proximal segments, each exhibiting nearly comparable prevalence rates (18.8% and 17.2%, respectively).

Debates persist over the role of myocardial bridges within the structure of the coronary artery, with discussions focusing on their potential protective or detrimental impacts. Coronary atherosclerosis involves the formation of plaques into the main arteries of the heart, predisposing to the development of thrombotic events triggered by their rupture (14). Though some theories propose that myocardial bridges may offer protection against atherosclerosis development, additional research is necessary to validate this hypothesis. Several studies revealed that the vascular segments with myocardial bridges exhibit a notable reduction in the occurrence of atherosclerotic lesions compared to those without it (6).

In our study, 38% of the patients exhibited stenosis on the coronary artery affected by the myocardial bridge, with a majority of 24 cases involving the LAD. Simultaneously, we examined the calcium score, also referred to as the Agatston Calcium Score, to assess the presence and extent of calcium buildup within the coronary arteries while utilizing CCTA. This score serves as a predictive instrument for imminent cardiovascular disease progression and helps in shaping therapeutic strategies. At the same time, it is an important non-invasive tool that could not only be used in evaluating the patients with clinical manifestations, but also in asymptomatic individuals with low to moderate risk of developing CAD (14). The observation that 41% of patients in our study recorded a zero-calcium score, indicating a reduced likelihood of developing CAD in the near term, alongside 42% displaying mild scores, could further highlight the potentially protective function of myocardial bridges in the coronary arteries. At the same time, only a small percentage of patients (18%) presented stenosis ≥50%. Despite only 17% of patients exhibiting moderate and severe calcium scores, careful consideration is warranted. Concurrently, our study revealed a noteworthy increase in the average calcium score level among males ($p=0.025$). These findings underscore the necessity for ongoing surveillance of the patients, given the unpredictability of myocardial bridge manifestations and clinical outcomes.

Two primary characteristics of myocardial bridges are their length, spanning between four and forty millimeters, and their depth, ranging from one to ten millimeters (15).A meta-analysis study indicated that, through angiography and CCTA, the average length of myocardial bridges was 21

millimeters, with a depth of 3 millimeters. Autopsy findings revealed a mean length of 19.3 millimeters and a depth of 3.2 millimeters for myocardial bridges. Nevertheless, it is evident that even subtle variations may arise, influenced by the method of investigation-whether invasive (angiography) or non-invasive (CCTA), as well as through autopsy (1). Despite these differences, the dimensions remain remarkably similar across the various modalities. In our study implying the evaluation of myocardial bridges through CCTA the mean length was 20.33±9.25, while the width was 3.02±1.22.

Based on their depth, we classified myocardial bridges as superficial (1-2mm), deep (≥2 and <5mm), and very deep (≥5 mm). The majority of myocardial bridges were deep, with 44 individuals presenting it on the LAD and two patients on the RCA, respectively on the LM. Superficial and very deep myocardial bridges were present in almost the same proportions, with just two more patients presenting superficial compared to very deep. Simultaneously, regarding their length, myocardial bridges were categorized as short (<25mm) and long (≥25 mm), with only 25.8% presenting long myocardial bridges. Clinical significance in myocardial bridges is typically defined by a depth of≥2 mm and a length of≥25 mm (4). In our study, we found that 26% of the patients met this criterion, with 6 individuals exhibiting long and deep myocardial bridges (9%) and 11 individuals displaying long and very deep myocardial bridges (17%). At the same time, a higher value of the depth is associated with increased chance for the development of cardiovascular events (4). This theory is further supported by one of our findings, which indicated a higher average calcium score level in individuals with a myocardial bridge thickness exceeding 5 mm (p=0.025). This suggests an elevated risk of potential rupture of atherosclerotic plaques, thereby predisposing individuals to acute coronary syndromes.

Typically, most myocardial bridges are benign and asymptomatic, particularly when their depth is less than one millimeter. However, they can occasionally become clinically significant. Their initial presentation may manifest as angina (stable, unstable or vasospastic), often occurring during systole when the myocardial bridge is compressed (5, 11).Unstable angina was more frequently identified in patients affected by this anomaly than troponin-positive myocardial infarction (5). Nevertheless, there are no correlation between the severity of the angina and the dimensions of the myocardial bridge (1).More severe manifestations include Takotsubo syndrome, heart blocks, or, in extremely rare cases, sudden death (5). Research indicates that male patients affected by this anomaly are at elevated risk of experiencing ventricular tachycardia or premature ventricular contractions during physical exertion. This heightened risk is attributed to the occlusion of the coronary artery, potentially leading even to sudden death, scenario also observed in high-performance athletes with myocardial bridges (1, 16).

Currently, there are several hypotheses regarding the development of myocardial ischemia in these patients. In healthy individuals, 80% of coronary flow occurs during diastole, but in those with myocardial bridges, systolic compression disrupts this normal flow pattern. Concurrent tachycardia can further reduce diastole, exacerbating myocardial bridge manifestations and

increasing oxygen consumption, potentially leading to ischemia. Another phenomenon is the “branch steal" in myocardial bridges, resulting in lower diastolic pressures in the affected segment and more local ischemia in mild-moderate bridges compared to distal myocardium. Additionally, the continuous milking effect of the anomaly exposes sub-endothelial tissue and activates the coagulation system, thereby increasing the risk of intracoronary thrombogenesis and raising questions about the true extent of their contribution to cardiovascular mortality (11, 16).

In our research, we explored the presence of hypertension as a potential accelerator of atherosclerosis and, consequently, CAD. Our findings revealed that hypertension was prevalent in 44% of the patients, notably affecting 50% of male participants. Similarly, when investigating the presence of CAD, we observed its occurrence in 38% of the patients, predominantly affecting 18 male patients compared to only 7 female patients. Additionally, fewer patients exhibited chronic cardiac failure, with a majority of affected individuals being male. These observations underscore a gender disparity in the prevalence of cardiovascular conditions among individuals with myocardial bridges, with men exhibiting a higher frequency of hypertension, CAD, and chronic cardiac failure compared to women. Nevertheless, in our study, dyslipidemia was present only in 15 patients (23%).

In such clinical scenarios, pharmacological treatment is necessary to alleviate symptoms arising from concurrent disorders such as arrhythmias, hypertension, or hypertrophy (5). In the therapeutic management of myocardial bridges, beta-blockers are prioritized to optimize hemodynamic function, followed by non-dihydropyridine calcium channel blockers, which have a vasodilator effect and are able to improve symptomatology. Additionally, antiplatelet therapy should be considered to prevent and mitigate acute events in hypertensive patients with coronary artery disease (CAD) (5, 1).

In cases where pharmacological interventions prove inefficient and the affected coronary artery exhibits significant stenosis, stent implantation may emerge as the preferred solution. Prior studies have demonstrated enhanced clinical outcomes following coronary stenting in patients with myocardial bridges. However, in situations where neither medical treatment nor coronary stenting are feasible, surgical myotomy may be considered. Alternatively, another surgical option entails the anastomosis of the internal mammary artery (IMA) to the LAD (2). Finally, coronary artery bypass grafting may be considered in severe cases of myocardial bridges with significant hemodynamic impact and dimensions exceeding 25 millimeters (1). In our study we observed that only three male patients needed coronary angioplasty with stents, comprising just 5% of the total patient population, suggesting that only in certain situations myocardial bridges may lead to severe enough hemodynamic compromise to necessitate invasive interventions.

CONCLUSIONS

Utilizing a sensitive and precise investigative technique such as CCTA improves the detection of clinically significant myocardial bridges, with the middle segment of the left anterior descending artery being the primary site in most cases. The study suggests a potential link between myocardial bridges thickness and higher coronary artery

calcium scores, prompting further exploration. Correlations with atherosclerosis and hypertension require deeper investigation for a comprehensive understanding of myocardial bridges clinical impact.

CONFLICT OF INTEREST AND FUNDING

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URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS IN NORTH-EASTERN ROMANIA

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URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS IN NORTH-EASTERN ROMANIA (Abstract): Urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL) is currently proposed as an improved diagnostic tool for urinary tract infection (UTI). Both normal and pathological standardized values are lacking. There is some evidence that children with recurrent UTI may have lower uNGAL values, thus promoting it as a prognostic biomarker. **Aim**: To compare baseline uNGAL values in patients with recurrent UTI to controls without history of UTI. **Materials and methods**: Patients and/or their caretakers signed an informed consent prior to participation. Recurrent UTI (rUTI) was defined as history of at least 2 UTI episodes in the last 6 months or 3 in the last year, confirmed by positive urine culture. The correspondent control group had no history of UTI. Obtained urine samples were frozen and kept until uNGAL assay measurement was performed as per the manufacturer's recommendations. Data analysis was performed using IBM® SPSS Statistics (version 29.0.20 (20)). **Results**: We found a significant difference in uNGAL values between the rUTI group and the control group (p=0.01), AUC = 0.815, cutoff = 5.27 ng/mL (TPR =1, FPR =0.3). uNGAL values in the rUTI group were not correlated with age (Pearson r = 0.243, p =0.423). uNGAL values were higher in the rUTI group. **Conclusions**: Patients with history rUTI have higher uNGAL values than those without history of UTI. uNGAL does not seem to be a protective factor and low values do not seem to be a predictive diagnostic tool for pediatric UTI. Further studies should take into consideration the patient's history of UTI regarding cut-off values. **Keywords**: NGAL, PEDIATRIC, RECURRENT URINARY TRACT INFECTION.

Urinary tract infection (UTI) in infants and young children is an important bacterial infection that can lead to permanent renal function loss. Current diagnosis options have low sensitivity and specificity and definitive diagnosis may postpone antibiotic administration up to 48 hours. Neutrophil gelatinase-associated lipocalin is a relatively new renal biomarker that has shown promise in early diagnosis of UTI and potential renal scarring. Baseline values of uNGAL have been proposed (1, 2), but a recent meta-analysis (2) has shown that underlying patient characteristics may influence determining cut-off values. Our study aims to compare the values of uN-

GAL in a population with history of rUTI, with a control group of patients without history of UTI from pediatric patients in Northeastern Romania, in order to check if baseline uNGAL values remain the same after having suffered UTI and to test if uNGAL can be considered a prognostic factor for UTI.

MATERIALS AND METHODS

This study was approved by the "Sf. Maria" Emergency Clinical Hospital for Children Ethics Committee (no. 5075/17 February 2021) and the "Grigore T. Popa" University of Medicine and Pharmacy Iași's Ethics Committee (no. 8753/28 May 2020) and informed consent was obtained prior to participation. Cases were patients with ages between 3 months and 18 years with a history of recurrent UTI (rUTI), from the Department of Pediatric Nephrology at "Sf. Maria" Emergency Clinical Hospital for Children from March 2021 to March 2022. RUTI was defined by two or more culture proven UTIs with at least 100,000 CFU/mL of a single urinary pathogen in the last 6 months, respectively three or more in the last year. Control patients were a convenience sample enrolled from the General Pediatrics Department at "Sf. Maria" Emergency Clinical Hospital for Children. Patients who provided urine samples for routine clinical purposes were approached for inclusion in this study as controls. None of the control patients required fluid replacement, resuscitation or hospital admission, or had any clinical parameters to suggest dehydration, such as tachycardia or decreased urine output. Controls were excluded if they had a history of a UTI, evidence of a UTI at the time of enrollment (proven UTIs with at least 100,000 CFU/mL of a single urinary pathogen), known or suspected renal dysfunction, or had any urologic history.

The following parameters were studied: patient diagnosis at the moment of admission, number of UTI recurrences, urinalysis and presence of pyuria, urine culture, presence of malformation, age and sex of the patient and presence of antibiotic prophylaxis in relation to the values of urinary NGAL.

A minimum of 5 mL of urine were collected from cases and controls. Samples were centrifuged, aliquot and stored at -80 degrees Celsius within 12 hours of collection. UNGAL measurements were performed as per manufacturer's recommendations in triplicate using *Human Neutrophil Gelatinase-Associated Lipocalin / NGAL (LCN2) ELISA Kit* from producer Abbexa®. Patient data was collected from the electronic medical record. Data was introduced in IBM® SPSS Statistics (version 29.0.20 (20)). Grouping variable statistics was analyzed. Non-parametric distributed variables, i.e. uNGAL in the risk and healthy patients were compared using the Mann-Whitney-U test. The area under the receiver operator characteristic (ROC) curve for identifying rUTI involvement was calculated using logistic regression models for uNGAL. The optimal uNGAL cut-off point for the identifying rUTI involvement, as well as the associated sensitivity and specificity were calculated. A p value of < 0.05 was considered statistically significant. The datasets generated during the current study are available from the corresponding author on reasonable request.

RESULTS

A total of 13 patients (10 female, 3 male) aged between 3 months and 18 years with recurrent UTI were introduced in the study, with 10 case controls with appropriate age/sex distribution.

Of the 13 patients, 2 had no demonstrated underlying malformation, while 11 presented different types of congenital anomalies of the kidney and urinary tract CAKUT (6 presented with different degrees of VUR (1-5) including one secondary to posterior urethral valve, 2 presented right kidney hypoplasia, 2 presented kidney hypoplasia, 1 neurogenic bladders). 7 of the 13 patients had received antibiotics as prophylaxis for UTI prior to admission.

All patients had history of at least 2 infections previous to the current admission. Of these 5 had a recurrence of 2 infections, 1 presented at least 3 infections, 2 had 4 recurrences and 2 had at least 6 infections by the time of admission. 3 of these patients had presented with more than 6 infections.

Pyuria (defined as above 10 leucocytes/ high-power field) was not found in any patients. Urine culture was negative (<1000 CFU/mL) in all patients. Creatinine values varied from 0.35 to 0.86 mg/dL. Creatinine clearance varied from 82.67 to 172.85 mL/min/1.73m^2 with a median of 111.27 mg/dL.

Of the 13 patients with rUTI, only 5 had undergone DMSA scan to confirm scarring. 3 of 5 presented 1 kidney scar, the rest presented 3 or more kidney scars.

uNGAL values varied from 5.78 ng/mL to 31.93 ng/mL in the rUTI cases, with a mean value of 16.23 ng/mL and a median of 13.74 ng/mL. The control group uNGAL values were between 0.17 ng/mL and 19.38 ng/mL, with a mean of 6.49 ng/mL and median of 3.39 ng/mL. Further description of uNGAL (ng/mL) value distribution can be seen in first table.

TABLE I.

uNGAL (ng/mL) distribution descriptive values

		uNGAL_rITU	uNGAL_control
Number of patients	Statistic	13	10
Range	Statistic	26.15	19.21
Minimum	Statistic	5.78	.17
Maximum	Statistic	31.93	19.38
Mean	Statistic	16.2377	6.4920
	Std. Error	2.62642	2.15737
Std. Deviation	Statistic	9.46970	6.82220
Variance	Statistic	89.675	46.542
Skewness	Statistic	.857	1.053
	Std. Error	.616	.687
Kurtosis	Statistic	-.689	-.454
	Std. Error	1.191	1.334

Mann-Whitney U and Wilcoxon W test were applied to test whether rUTI and control groups have identical (H0) or different uNGAL distributions (H1). uNGAL values were significantly higher in the rUTI group than in the case control (p =0.011) (Asymp. Sig. (2-tailed) = 0.011) (tab. II). uNGAL value distribution in the rUTI risk group and case control group are listed in first figure.

TABLE II.
Statistical test

	uNGAL
Mann-Whitney U	24.000
Wilcoxon W	79.000
Z	-2.544
Asymp. Sig. (2-tailed)	.011
Exact Sig. (2*(1-tailed Sig.)) (not corrected for ties)	.010

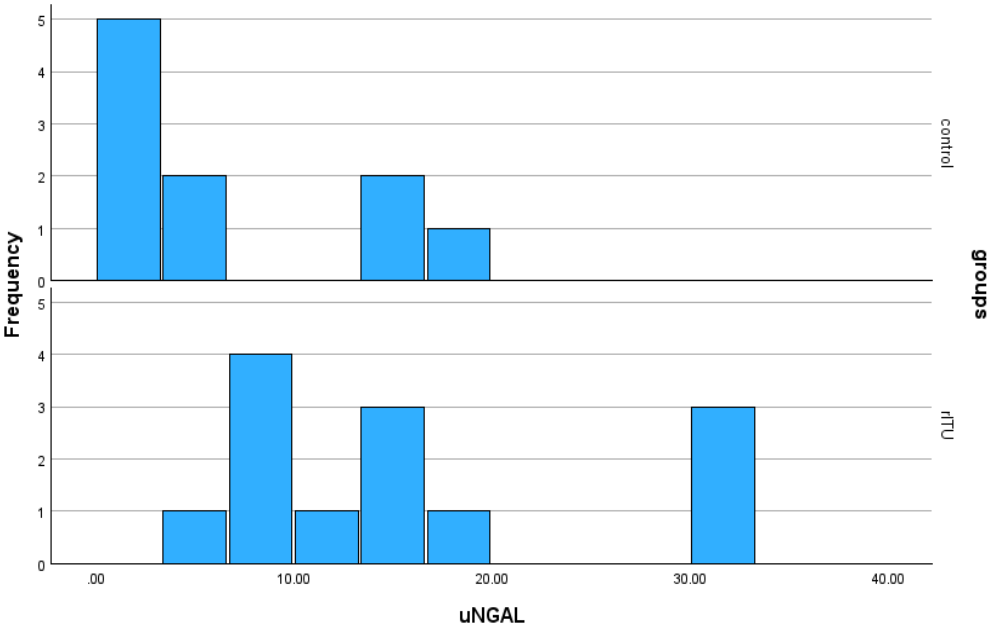

Fig. 1. uNGAL (ng/mL) value distribution in the rUTI risk group and case control group

ROC-AUC curve for NGAL values suggested the best cutoff points of 5.27 ng/mL FPR = 0.3, TPR = 1 with AUC = 0.815 (fig. 2).

Seven patients had various degrees of VUR (grade 1-5). For these patients, no significant correlation was found between uNGAL values and VUR degree (Pearson Correlation -0.173, p=0.711).

No significant correlation was found between uNGAL values and age expressed in months (Pearson Correlation 0.243, p=0.423), height expressed in cm (Pearson Correlation 0.098, p=0.750) or weight expressed in kg (Pearson Correlation 0.202, p=0.508).

uNGAL values did not significantly correlate with creatinine (Pearson Correlation 0.088, p=0.775) or creatinine clearance (Pearson Correlation -0.171, p=0.577). Creatinine clearance was calculated by the Schwartz formula ((k*height(cm))/serum creatinine (mg/dL).

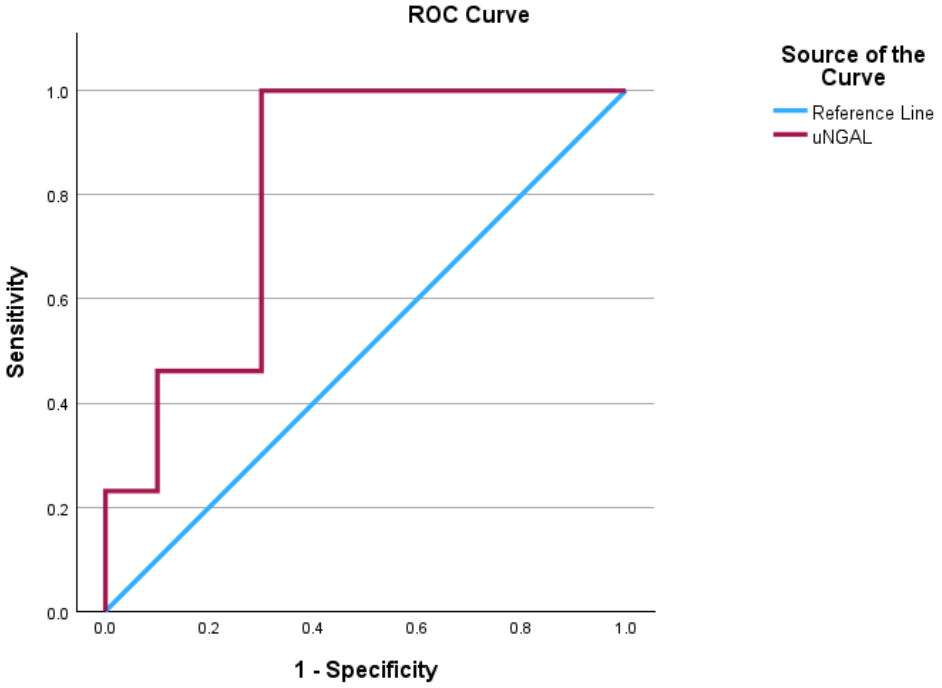

Fig. 2. ROC curve for uNGAL. AUC 0.815. p=0.002, (95% CI 0.62-1.01), cutoff uNGAL = 5.27 ng/mL, TPR = 1, FPR = 0.3.

DISCUSSION

Urinary tract infection (UTI) in infants and young children is an important bacterial infection with an incidence of 2% during the first 2 years of life. The prevalence of UTI is higher (5-24.2%) among children aged < 2 years presenting with fever (1-3). Early detection and proper management of UTIs is important since UTIs involving the kidney may cause permanent renal scarring and be a risk factor for future development of renal insufficiency (4). Other later-life risks related to renal scarring include hypertension, preeclampsia during pregnancy and proteinuria (5). Recurrent UTIs (rUTI) occur in approximately 12% of patients following an initial UTI (6). Acute pyelonephritis, especially in recurrent episodes may lead to renal parenchymal involvement and scarring with high prevalence rate (15%-60%) among children (7). Furthermore, it has been reported that while the incidence of primary VUR is 1% in the normal population it reaches 30–50 % in recurrent UTI and that 15–60 % of these affected children may have permanent renal scarring after the UTI (8).

Urinalysis is a convenient and readily available diagnostic option. However positive leucocyte esterase (LE) or nitrites have variable and often suboptimal sensitivities (78–88%) and specificities (72–97%) across different studies (9-11). These results are influenced by the different methods of urine collection and laboratory pro-

cedures (12). Inaccuracy of the UA leaves the physician to balance excess antibiotic treatment with potential toxicity and increase of antibiotic resistance risk against lack of action and potential kidney scarring. Therefore, it is difficult to provide a quick diagnostic of UTI in children and thus a reliable marker for the early diagnosis is warranted.

Tc-99m dimercaptosuccinic acid (DMSA) renal scintigraphy is considered the current gold standard in imaging renal parenchymal involvement during acute pyelonephritis (APN) and to detect late renal damage consequent to UTIs (13). Although DMSA scan is highly sensitive method in detecting renal inflammation and scaring, it is invasive to expose young children to radiation. Moreover, to confirm the diagnosis of renal scarring, DMSA must be repeated 4 to 6 months after the APN (13). Since consequences of renal scarring are serious, this aggressive method used to diagnose APN is justified. However, the need for a less invasive diagnostic tool is evident.

Numerous studies have evaluated the usefulness of inflammatory markers for predicting APN, and demonstrated that commonly used laboratory parameters such as white blood count (WBC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) have low sensitivity and specificity for accurately predicting acute renal parenchymal involvement. At present, these tests are not recommended for routine use to differentiate APN from lower UTI in children (14) .

New biomarkers have emerged with promising roles in early diagnosis and prognosis of UTI. Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin family, is an excellent biomarker for predicting and monitoring acute kidney injury (AKI) of diverse etiologies. It is a promising marker for detection of subclinical renal damage due to vesicoureteral reflux (VUR), renal scarring, and obstructive uropathy (15).

NGAL is a member of the lipocalin family that plays an important role in the innate immune response to the bacterial infection. NGAL a protein expressed in neutrophils and other tissues, including the collecting duct of the kidney and released from activated neutrophils, to prevent bacterial iron uptake and growth. It has been linked with acute kidney injury, but urinary NGAL (uNGAL) is significantly elevated with gram-negative UTIs in both adults and pediatric studies (12, 16-18). Different studies found urine NGAL values were increased in both, upper and lower UTI. Urinary NGAL concentrations cut-off values have not yet been established. However, there is indication that uNGAL may decline with age in infants (19). Multiple studies have had controversial results. No current recommendations are made regarding the use of urinary NGAL in pediatric populations (20-25).

To the best of our knowledge, this is the first analysis of uNGAL in pediatric patients with recurrent UTI in northeastern Romania.

Both urinary and blood NGAL (plasma or serum) have been researched for early prediction of UTI and APN in children. A strong argument in favor of measuring uNGAL over pNGAL or sNGAL is the non-invasiveness of the procedure. A meta-analysis by Forster and Devarajan 2017 suggests using pNGAL for pyelonephritis

and renal lesion diagnosis, while uNGAL seems to be superior in diagnosing genitourinary lesions, subclinical scarring and obstructions (26).

In 2009 Ichino *et al*, in an experimental rat UTI model, observed a marked increase of urine NGAL (uNGAL) at the early stage of infection and thereby suggested a potential utility of uNGAL as a marker of APN in humans (27). Several clinical studies have been conducted to assess the diagnostic value of uNGAL for detecting APN, but the results are not clear and, in some cases, pNGAL appears to have higher utility (20-25, 28).

Our cutoff points are close to Kim BH *et al* 2014. They suggested that for a cutoff value of 5.75 ng/mL uNGAL had no significant correlation with UTI (29). While we found that uNGAL is significantly higher in the risk group, there is no statistical correlation between NGAL values in these patients and the presence of UTI at the moment of sample collection. This indicates that uNGAL may be elevated in patients with malformations, but not necessarily related to infection. There are important differences between the two studies, regarding the number of patients and the inclusion criteria.

We also note that values of uNGAL in patients with recurrent UTIs was not significantly smaller than healthy controls, as suggested by Forster *et al* 2017 (30). The result difference could be attributed to the small sample size in both study groups. Median uNGAL values in healthy controls in the Forster group was also higher than both our groups (27.61 vs 13.74 and respectively 3.39 ng/mL). This would indicate that uNGAL does not seem to be a protective factor or predictive diagnostic tool for pediatric UTI.

In comparison with the study of Yilmaz *et al*, 2009, (31) who noted a cutoff value of 20ng/mL for diagnosis of UTI, we would like to note that the median value of non-infected, rITU group patients is smaller than their control group. This may be why in that study the authors noted that higher values would increase the chance of UTI four times. The source of confounding factors could also be related to the age difference (2 months to 12 years compared to 3 months to 17 years), inclusion criteria and sample size.

Yim HE *et al*, 2014 noted that for UTI diagnosis in patients with fever the best cutoff value for uNGAL was 23.95 ng/mL. However, that study only included patients at their first symptomatic febrile UTI. In our study, 3 patients, including one without malformation that had recurrent UTI, presented uNGAL values above 24 ng/mL with a negative urine culture. This suggests that the utility of uNGAL is highly variable and that further research is required to establish the specific situations and related best cutoff-points (32).

There were some limitations of our study. We only evaluated urinary NGAL concentration, but not serum/plasma NGAL concentration. This was a single-center study, with a small cohort of children. In the present study, due to lack of constant test availability, we could not perform DMSA scan for all patients to confirm or differentiate APN from lower UTI. Age can also be considered a limiting factor, in regards to acceptance of introduction into the study. However, in the study we found no correlation between age and uNGAL levels. Patients with early stages of chronic kidney disease have been

shown to present increased levels of uNGAL. No correlation was found between creatinine clearance and uNGAL values in our study, and we have to take into consideration clearance modifications were marginal (lowest value was 82.67 mL / min / 1.73 m^2, and corresponding to a serum creatinine value of 0.43 mg/dL). Finally, reliable reference intervals for uNGAL are lacking for both healthy children and UTI patients.

CONCLUSIONS

Urinary NGAL is significantly higher in patients with a history of recurrent UTI (rUTI), than controls with no history of UTI, with the best cutoff point of 5.27 ng/mL (FPR = 0.3, TPR = 1) with AUC = 0.815.

Further studies that propose uNGAL as a diagnostic method for UTI or non-invasive VUR monitoring should take into consideration the patient's history of UTI regarding cut-off values.

uNGAL does not seem to be a protective factor and low values do not seem to be a predictive diagnostic tool for pediatric UTI.

Further research is required to establish specific situations in which uNGAL may be useful and to identify the best cutoff values. Further research should also focus on differentiating lower UTI from acute pyelonephritis, benefits of evaluating plasma/serum or urinary NGAL values and extending the number of patients included for better statistical value.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of "Gr. T. Popa" University of Medicine and Pharmacy of Iasi no 8753/28 May 2020 and also by the Ethics Committee of "Sf. Maria" Emergency Clinical Hospital for Children no. 5075/17 February 2021.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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PATHOLOGICAL FEATURES IN PATIENTS WITH SEPSIS – AN AUTOPSY STUDY

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PATHOLOGICAL FEATURES IN PATIENTS WITH SEPSIS - AN AUTOPSY STUDY (Abstract) **Background and aim**: Our study aims to evaluate the etiologic profile of lesions and morphologic changes identified in the pathology generating sepsis, and to establish the lesion pattern based on necropsy examination in correlation with relevant clinical and microbiological data. **Materials and methods:** The study group included 100 patients with a clinical diagnosis of sepsis who had died, of whom 41 required autopsies. **Results:** The sepsis was confirmed after completing macroscopic and histological examination of the organs during the autopsy. 18 patients (36.58%) had a severe septic pulmonary involvement, the macroscopic diagnosis of death for 15 of them being bronchopneumonia (83.33%). For 8 patients (19.51%), the septic injury was localized at meningocerebral level, another 4 patients presented intimal valvular injuries, sustaining the diagnosis of infectious endocarditis. The most common non-specific lesions were congestion of red pulp of spleen (85.36%), severe cerebral (78.04%) and pulmonary edema (58.53%), and disseminated intravascular coagulation syndrome (51.21%). **Discussion:** The autopsy confirmed a heterogeneity of specific and non-specific lesions, and no general lesion profile could be drawn. Antibiotics are the mainstay of sepsis therapy. However, antibiotics failed to control the main outbreak of infection, with subsequent sepsis leading to death. **Conclusion**: The identified pathological features delineated the landmarks of the septic lesion within the batch, emphasizing the severity of the single or associated lesions. **Keywords:** SEPSIS, AUTOPSY, PATHOLOGICAL LESIONS.

INTRODUCTION

Sepsis is a syndrome characterized by a generalized exaggerated and unbalanced reaction to infection. The patient's response is determined by the pathogen characteristics: species, pathogenicity, virulence, microbial load, and by the host characteristics: age, gender, genetic predisposition, comorbidities, exposure and immunologic status (1, 2). The septic process is defined as the result of systemic immune reactivity initiated at a primary site of infection, commonly documented as the lungs, then the abdominal cavity, urinary tract, followed by soft tissues and blood (3).

The etiology of septic syndrome is characterized by an evident heterogeneity,

most frequently identified gram-positive infectious agents: *Staphylococcus aureus, Streptococcus pneumoniae* and gram-negative infectious agents: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterococcus sp.*, respectively (2). Lately, fungal species have been identified increasingly frequently (3).

Due to the complexity of the disease, which makes it very difficult to diagnose and apply the most appropriate treatment, sepsis is one of the leading causes of death (1, 3, 4). Sepsis is becoming a public health issue due to the dramatic increase in its incidence year after year (5). The post-mortem diagnosis is also difficult due to the incomplete clinical data available to the pathologist prior to the necropsy, especially due to the short duration of hospitalization. In addition, it is not uncommon for the pathologist to identify new localizations of the infectious process that he adds to the pathogenic mechanism, which further supports the need for a necropsy examination that complements the clinical diagnosis (6, 7).

Our study aims to evaluate the etiologic profile of lesions and morphologic changes identified in the pathology generating sepsis, and to establish the lesion pattern based on necropsy examination of patients to elucidate the mechanism of thanatogenesis as precisely as possible in the sense of the general trend of using post-mortem information in clinical practice.

MATERIAL AND METHODS

Study settings

We conducted a retrospective study (01.01.2018 - 31.12.2023) evaluating medical and autopsy records. A total of 647 deaths in patients with a clinical diagnosis of sepsis were recorded during the 6-year period at "Sfanta Parascheva" Clinical Hospital of Infectious Diseases, Iasi. Deaths caused by sepsis in patients with diabetes mellitus (135 cases), arterial hypertension (258 cases), cancer (147 cases), or those requiring forensic autopsy (7 cases) were excluded from this study. Thus, we selected a group of 100 patients, of whom only 41 required post-mortem examination to clarify the final diagnosis. The medical records, the autopsy protocols, and the histopathological reports were consulted, from which the following data were extracted: age, gender, duration of hospitalization, associated etiologic agent(s), affected organ and/or systems, starting site, and the disease that caused the evolution to sepsis and the patient's death.

Autopsy protocol

Macroscopic examination

It included general macroscopic examination of the body and organs, in situ and outside the body, with evaluation of external and sectional appearance of internal organs, including brain, lungs, heart, liver, spleen, pancreas, small and large intestines, bladder, kidneys, and internal genitalia for visible septic foci. Representative lesions identified at the macroscopic level were collected for the histopathological exam.

Histopathological exam

After fixation in 10% neutral buffered formalin for 24-48 hours, tissue fragments were harvested, primary processed and embedded in paraffin. The paraffin blocks were sectioned (3-4 microns thick), and sections were stained with standardized stain Hematoxylin-Eosin and special stains such as: Periodic acid-Schiff (PAS), Grocott's methenamine silver stain (GMS).

The slides were reanalyzed by two independent pathologists to confirm the histopathologic characteristic lesions of sepsis based on data published in the literature (7, 8, 9).

Ethical Considerations The study was approved by the Institutional Ethics Committee in keeping with the principles of the Declaration of Helsinki. Data was collected anonymously from the medical records without active patient participation.

Statistical Analysis. Statistical data were processed using *SPSS version 29.0* software. Means and standard deviations were calculated for continuous variables, while absolute values and percentages were calculated for categorical variables. The group comparisons were obtained using the Chi-squared test (χ^2) and the Fisher correction if necessary. Comparisons were considered as statistically significant when p values were < 0.05.

RESULTS

General characterization of the study group

In the study group, the age of the patients ranged from 24 days to 98 years with a mean value 67±12 SD years. We noted a more frequent involvement of male patients (57/57%) compared to female patients (43/43%); 51 patients (51%) were from urban areas and 49 patients (49%) from rural areas (tab. I).

TABLE I.
Characteristics of the study group

Baseline characteristics	Units	n (%)
Male sex	n (%)	57 (57)
Age	years	67±12DS
Urban	n (%)	51 (51)
Source of infections	n (%)	
Lungs		40 (40)
Urinary tract		18 (18)
Skin		17 (17)
Abdomen		12 (12)
Central Nervous System		9 (9)
Heart		3 (3)
Pathogens		63 (63)
Gram-positive alone		13 (20.63)
Gram-negative alone		24 (38.09)
Fungal alone		1 (1.58)
Viral alone		3 (4.71)
Mixed organisms		22 (43.92)
Double		17 (26.98)
Triple		4 (6.34)
More than triple (5 agents)		1 (1.58)
Septic shock		8 (8)

Etiopathogenic profile

When assessing the etiology of sepsis, the pathogen was identified in only 63 patients (63%). Therefore, we refined the analysis of the etiopathogenic profile by separately reporting the subgroup of 63

patients in whom the pathogen was confirmed during hospitalization before death.

A total of 18 different etiologic agents were identified, including all major classes of infectious agents, such as gram-positive bacteria (*Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus agalatiae, Enterococcus faecalis* and *Clostridioides difficile*), gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Neisseria meningitidis, Morganella morgani, Enterobacter, Proteus mirabilis, Proteus vulgaris,* and *Acinetobacter baumannii*), viral agents (*Influenza virus, Measles virus*), and fungal agents (*Aspergillus, Candida albicans*).

Forty-one of the patients (65.08%) presented a single pathogen, the other 22 patients (34.92%), presenting multiple associations of germs, respectively two pathogens (17 cases - 26.98%), three pathogens (4 cases - 6.34%) and one case (1.58%) with 5 pathogens simultaneously (tab. I).

Pathological profile

Among the 100 patients included in the study, 41 required the completion of the clinical diagnosis by establishing a final post-mortem diagnosis for the evaluation and refinement of the clinical data (tab. II).

TABLE II.

Distribution of primary septic foci of necropsied cases in relation to the affected system:

System	No. of cases (n)	Percentage (%)
Central Nervous System	8	19.51%
Respiratory System	18	43.90%
Heart	4	9.75%
Renal System	1	2.43%
Gastrointestinal System	1	2.43%
Other (Skin, Soft Tissue)	1	4.87%
Septic Shock/ Multiple organ failure	8	17.07%
Total	**41**	**100%**

The patients had short periods of hospitalization (less than 24 hours - 12 cases, less than 48 hours - 13 cases, between 48 and 96 hours - 16 patients) - meaning a limited time to compile a complete paraclinical picture.

Macroscopic features

Following the macroscopic examination during the autopsy, a diagnosis of death from septic shock which induced a multiple organ failure was established for 8 (19.51%) patients.

In these cases, no obvious macroscopic septic foci to a single organ were identified, instead multiple non-specific systemic lesions associated with sepsis were documented, namely: skin petechiae, pericardial petechiae, medullary-adrenal hemorrhage, acute erosive gastritis, septic muddy spleen and ischemic lesions of kidneys. A particular aspect was observed in two of the patients who, on microscopic examination, had associated focal lesions of myocardial necrosis - acute myocardial infarction.

For eight patients (19.51%), the septic injury was localized the meningocerebral site: a massive diffuse edema with trans

tentorial hernia and purulent deposits at the meningeal level, lesions specific for diagnosis of meningoencephalitis. Three patients had associated well-defined areas of necrosis, an appearance highly suggestive of encephalitis with multiple brain abscesses.

Eighteen patients (36.58%) had a severe septic pulmonary involvement, the macroscopic diagnosis of death for 15 of them (83.33%) being bronchopneumonia. In 7 of these 15 cases, the bronchopneumonia occurred in the context of a lobar pneumonia, 9 of the patients presenting massive pleural effusion (>250 ml of purulent fluid) and pleural lesions of pleurisy. On background, 10 of these patients had specific lesions to a chronic bronchopneumopathy (emphysema and chronic bronchitis) and two of them presented old fibrous cavitary lesions suggestive of sequelae of tuberculosis. Half of the patients had associated micro thrombotic lesions in the small and medium blood vessels, some developing necrotic alveolar lesions around, highly suggestive of areas of recent pulmonary infarction. In the other 3 cases with macroscopic lung injuries, the lesions were viral type, with atypical interstitial localization. In two of these cases, the viral etiology was identified using molecular tests (RT-PCR test positive for type A influenza), the third case had a measles etiology.

Four patients presented intimal valvular injuries, sustaining the diagnosis of infectious endocarditis, with the identification in two cases of septic vegetations adhering to the mitral valve, in one case at the aortic valve level and in another case of perivalvular septic lesions, with the development of an abscess at the level of the metallic prosthetic aortic valve. One patient presented, in the context of *Clostridioides difficile* infection typical lesions of pseudomembranous colitis. Another patient presented macroscopically, in the context of *Staphylococcus aureus* infections, an acute renal abscess that completely affects the lower pole of the right kidney; on microscopic examination, the lesion was purulent.

Only one patient among those hospitalized with the clinical diagnosis of sepsis, who died less than 24 hours after admission, presented macroscopic lesions suggestive of the diagnosis of Baker I aortic dissection; the macroscopic diagnosis was later confirmed microscopically.

The autopsy results indicated a degree of concordance between the clinical and post-mortem diagnosis of 97.56%.

Microscopic features

The microscopic examination allowed the identification and description of the histopathological characteristics, to confirm the macroscopic diagnosis formulated for the establishment of the causes of death. Tables III, IV and V shows the specific and non-specific tissue lesions in the context of the septic process.

The most common non-specific lesions associated with septic sites were congestion of the red pulp of the spleen (85.36%) macroscopically described by a muddy appearance, reduced consistency, followed by moderate to severe cerebral edema (78.04%), with flattening of the outline, deletion of cerebral gyri and reduction the importance of tissue consistency and pulmonary edema (58.53%), renal hypoxia or acute hemorrhagic gastritis. These lesions were associated with other non-specific findings that indicated a process of affecting the coagulation phenomena, namely disseminated intravascular coagulation syndrome (51.21%) (micro thrombosis, skin petechiae, serous petechiae) (tab. V). The images in figures 1-6 illustrates microscopic lesions relevant for substantiating the diagnosis of sepsis.

TABLE III.

Histopathological lesions identified in the central nervous system and lung

Central Nervous System (n = 8)	Lung (n = 18)
Perineural edema (n = 8)	Pneumonia (n = 7)
Perivascular edema (n = 8)	Bronchopneumonia (n = 15)
Neural necrosis (n = 4)	Lung abscess (n = 3)
Septic thrombi (n = 8)	Pulmonary TB (n = 2)
Reactive gliosis (n = 8)	Pulmonary edema (n = 18)
Focal necrosis (n = 3)	Intrapulmonary hemorrhage (n = 7)
Abscess (n = 3)	Leucocytes alveolitis (n = 10)
	Microthrombi (n = 9)
	Pulmonary necrosis (5)
	Interstitial pneumonitis (3)
	Pleurisy (n = 9)
	Diffuse alveolar damages (11)

TABLE IV.

Histopathological lesions identified in heart, renal system and gastrointestinal system

Heart (n = 4)	Renal System (n = 1)	Gastrointestinal System (n = 1)
Cardiomyocytes necrosis (n = 2)	Acute tubular necrosis (n = 1)	Gangrene (n = 1)
Interstitial edema (n = 1)	Septic thrombi (n = 1)	Pseudomembranous colitis (n = 1)
Myocarditis (n = 1)	Renal abscess (n = 1)	Microthrombi (n = 1)
Endocarditis (n = 1)	Pyelonephritis (n = 1)	
Interstitial mononuclear cells (n = 2)	Bacterial colonies (n = 1)	
Septic emboli (n = 2)	Septic thrombi (n = 1)	

TABLE V.

Histopathological non-specific lesions

Non-specific lesions	No. of patients	Percentage (%)
Spleen		
Splenitis	2	4.87%
Red pulp congestion	35	85.36%
White pulp atrophy	7	17.07%
CNS		
Cerebral edema	32	78.04%
Lung		
Pulmonary edema	24	58.53%
Intravenous leukostasis	12	29.26%

Non-specific lesions	No. of patients	Percentage (%)
Heart		
Interstitial edema	5	12.19%
Epicardial hemorrhages	2	4.87%
Pericarditis/myocarditis	4	9.75%
Kidney		
Interstitial inflammation	14	34.14%
Focal necrosis	12	29.26%
Bacterial colony	1	2.43%
Liver		
Intrasinusoidal leukostasis	15	36.58%
Portal inflammation	9	21.95%
Focal necrosis	8	19.51%
Stomach		
Acute necrotizing gastritis	25	60.97%
Diffuse lesions		
Microthrombosis	21	51.21%

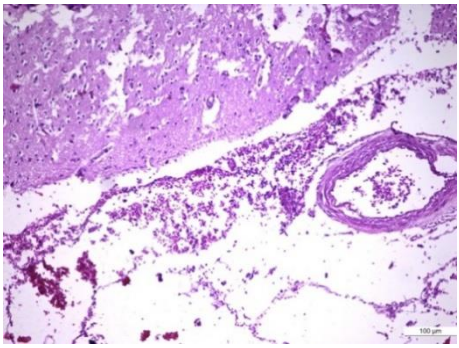

Fig. 1. Purulent meningitis, massive edema, micro thrombosis, HE, x10

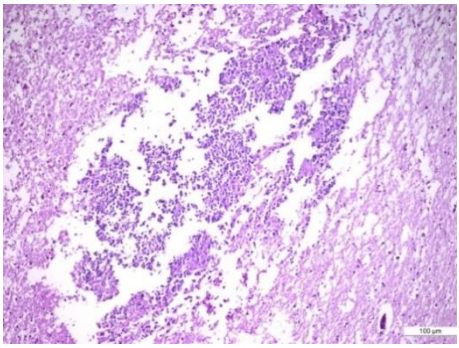

Fig. 2. Septic-pyemic micro abscess in the brain, HE, x10

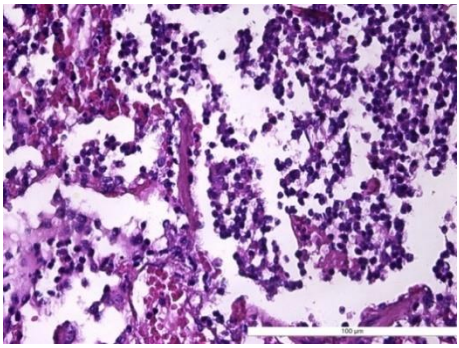

Fig. 3. Hyaline membranes covering the alveolar epithelial layer in a case of septic acute respiratory distress syndrome, HE, x40

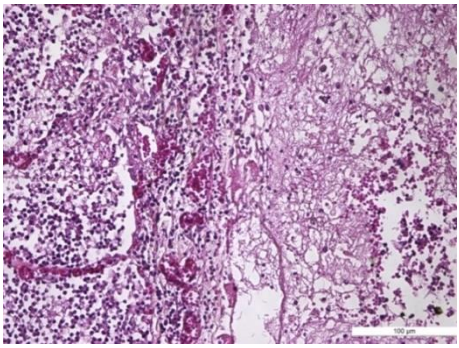

Fig. 4. Detail. Fibrinous pleurisy, HE, x20

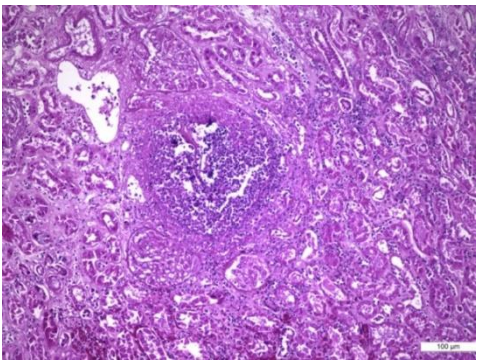

Fig. 5. Renal abscess, HE, x10

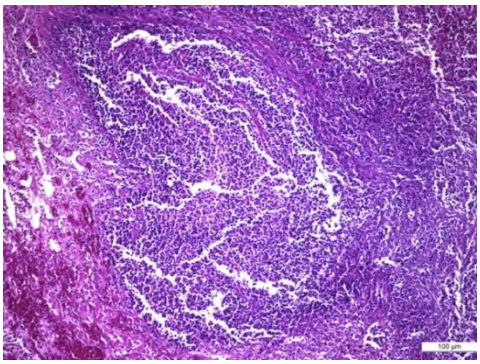

Fig. 6. Renal abscess, HE, x10

The most common microscopic findings that contributed to death were bronchopneumonia (88.33%) (figure 3), acute respiratory distress syndrome (figure 3), pulmonary edema (58.53%) and disseminated intravascular coagulation syndrome - coagulation/microthrombi (51.21%), to which are added edema and brain lesions (78.04%) (figs. 1, 2).

A statistically significant difference was identified between the primary septic site identified clinically versus post-mortem ($p = 0.0059$), with the obvious predominance of pulmonary lesions in general but with a significant contribution of renal and skin lesions.

DISCUSSION

The study made it possible an overview of the etiological profile of sepsis and the morphological changes identified in the pathology generating sepsis, in a group of patients who died of sepsis at "Sfanta Parascheva" Clinical Hospital of Infectious Diseases, Iasi.

According to the literature, sepsis is caused by gram-positive, gram-negative pathogenic bacteria and fungi (10). The main gram-positive pathogens are staphylococci (*Staphylococcus aureus* and *Staphylococcus coagulase-negative*) and streptococci (*Streptococcus pyogenes*, *Streptococcus viridans*, *Streptococcus pneumoniae*), and the main gram-negative pathogens are represented by *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (2). The results of our study are consistent with these data.

Regarding the distribution of sepsis cases by gender, we recorded more male patients (57%). The literature supports male predominance. This finding can be explained by the fact that the concentration of anti-inflammatory mediators is generally higher in females (11).

Following the microscopic examination, it was possible to validate the lesions described macroscopically. The results of the microscopic examination indicated a degree of concordance between the macroscopic and the microscopic diagnosis of 100%, the microscopic one improving the description of the physio pathological substrate of the diseases, a fact that supports the "gold standard" value of the microscopic morphology, in establishing the final post-mortem diagnosis.

The most striking finding of this analysis was that about 80% of all analyzed patients still had a septic focus at autopsy.

It can be argued that the detection of an infectious focus can be expected in patients who die early during sepsis. However, even in patients who were admitted to Intensive Care Unit for sepsis and treated for 7 days, the incidence of a continuous septic focus at autopsy was approximately 90%. Even though targeted antibiotics are the mainstay of sepsis therapy (12, 13), they failed to control the main outbreak of infection, with subsequent sepsis leading to death (14).

The patients included in this study were treated at "Sfanta Parascheva" Clinical Hospital of Infectious Diseases, Iasi - a tertiary healthcare center that receives a high volume of patients with highly complex infectious diseases, especially pulmonary, urinary, cerebral and terminal diseases. Not surprisingly, acute respiratory failure has been a leading cause of morbidity and mortality over the past 6 years (15, 16). Histologically, lung damage was correlated to the increased permeability of the alveolar capillary membrane, which leads to edema, protein exudates, the formation of hyaline membranes, congestion and extensive alveolar hemorrhage, aspects also described in other studies (17, 18). Another interesting finding of this study is that cerebral edema was seen in nearly 80% of patients who died of sepsis. Although only 8 of these patients (19.51%) were hospitalized for a cerebral pathology, with signs of meningeal irritation present, the necropsy examination revealed diffuse cerebral edema present in 32 (78.04%) of the 41 cases, four times more many. It is known that severe arterial hypotension associated with septic shock can cause cerebral hypoperfusion, thus inducing cerebral edema - recent evidence suggesting that the pathophysiological mechanism of septic delirium may include not only functional but also anatomical components (19,20). In the rest, the brain lesions identified were specific to the infectious etiology, complicating even to the formation of brain abscess areas (21). In our study, the most frequent cardiac pathology was the infectious endocarditis macroscopically objectified by the endocardial lesions, and the vegetative formations adhering to the valvular surface. The tropism of the infectious lesion on the prosthetic valve (22) was certified by an aortic perivalvular abscess, present in one of the patients included in the study. In addition, the ubiquitous myocardial ischemia in patients with sepsis, documented in the literature (23), was supported by areas of acute ischemic myocardial necrosis identified in two of the patients comprised in the study group. At the renal level, sepsis caused the installation of non-specific lesions, but it is worth noting the identification of multiple abscessed lesions against the background of acute tubular necrosis and perivascular microhemorrhages. Current concepts about renal failure in sepsis state that the exact onset of renal injury in sepsis remains unknown (24). Furthermore, it was considered that patients presenting with sepsis should be suspected of acute renal failure and, conversely, those presenting with acute renal failure should also be suspected of sepsis (24). Despite the small number of patients analyzed in our study, we should note the polymorphism of the renal microscopic lesions, which leads us to think of a serious, irreversible damage to the renal structure. Fortunately, advances in the understanding of the pathophysiological mechanisms of renal sepsis have offered the prospect of potential new therapies. However, effective and specific interventions for the prevention and treatment of acute renal

failure identified in a septic context are still missing (24).

A statistically significant difference ($p = 0.0059$) between the clinical versus morphological primary septic site was obtained - with the obvious predominance of pulmonary lesions and a significant contribution of renal and skin lesions. We noted a clinical overdiagnosis of renal lesions - indicating, as in the literature, that the paraclinical investigations do not always correlate with the degree of morphological lesions (24). On the other hand, liver damage sets in quite early, remaining subclinical for a longer time, considering the reactive, non-specific lesions identified in approximately 20% of the necropsied cases, without being correlated with the paraclinical data that did not signal any degree of liver failure.

The skin lesions were also frequently considered as the initial clinical site, but the autopsy most often highlights another primary lesional substrate, the skin site being, usually, the result of secondary dissemination.

CONCLUSIONS

The reported pathological features delineate the landmarks of septic lesions within the batch, emphasizing the severity of the single or associated lesions and interfering with the clinicians' efforts to stop the irreversible course of this condition towards death. This idea supports the value of the presented macroscopic and microscopic data, by the heterogeneity of the lesion profile corresponding to sepsis. Given the small number of studies published in the main body of the literature focusing on the pathological changes present at autopsy in deceased patients with sepsis, our data can be integrated into the clinical approach by reference to the etiological agent or therapeutic monitoring, respectively.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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COMPUTED TOMOGRAPHY IMAGING IN POST-SURGICAL SURVEILLANCE OF THORACIC AORTA REPAIR

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COMPUTED TOMOGRAPHY IMAGING IN POST-SURGICAL SURVEILLANCE OF THORACIC AORTA REPAIR (Abstract): Thoracic aorta surgery is essential for managing life-threatening conditions such as acute aortic syndromes and thoracic aortic aneurysms. Postoperative complications are increasingly detected due to advancements in imaging and surgical techniques, necessitating precise imaging protocols to differentiate true complications from mimics. **Materials and methods:** We conducted a retrospective study at the Institute for Cardiovascular Diseases Iași, analyzing 24 patients who underwent thoracic aorta surgery and subsequent CT imaging between November 2023 and December 2024. CT protocols included unenhanced, arterial, and late-phase acquisitions with ECG-gated imaging. Data on demographics, comorbidities, surgical indications, and procedures were statistically analyzed. **Results:** The cohort (79.16% male, mean age 54.83 years) presented aneurysms (38.46%) and dissections (34.6%) as primary surgical indications. Hypertension was the most prevalent comorbidity (66.66%). Common procedures included the Bentall procedure (29.62%) and thoracic endovascular aortic repair (37%). Complications were identified in 54.16% of cases, including pseudoaneurysms, intramural hematomas, and endoleaks. Symptomatic patients had a higher detection rate, but 50% of asymptomatic individuals also demonstrated complications. Reintervention was required in 16.66% of cases. **Conclusions:** CT imaging plays a pivotal role in postoperative surveillance, providing critical insights into complications and mimics. Optimized imaging protocols, including ECG-gated acquisitions and delayed-phase imaging, enhance diagnostic accuracy. Routine follow-up imaging is essential, even for asymptomatic patients, to guide timely interventions and improve outcomes in thoracic aortic surgery. **Keywords:** THORACIC AORTA SURGERY, COMPUTED TOMOGRAPHY IMAGING, THORACIC ENDOVASCULAR AORTIC REPAIR, POSTOPERATIVE COMPLICATIONS.

INTRODUCTION

Thoracic aorta surgery is a cornerstone in managing life-threatening conditions, its main indications being represented by acute aortic syndromes (AAS) and thoracic aortic aneurysms (TAAs) (1, 2). AAS defines a spectrum of life-threatening conditions characterized by damage to the aortic wall induced by similar pathophysiological mechanisms, often sharing overlapping clinical presentations and diagnostic and therapeutic strategies. This spec-

trum encompasses acute aortic dissection (AAD), intramural hematoma (IMH), penetrating aortic ulcer (PAU), aortic pseudoaneurysm, and traumatic aortic injuries (TAI) (3, 4).

While classic open surgical techniques remain standard for ascending aorta repair, aortic arch management has evolved with advanced open and endovascular approaches, often combined to enable more complex and extended anatomical repairs. Surgical ascending aorta repairs associating replacement of the aortic valve (with either a mechanical or biologic valve) can be done with a supracoronary ascending aorta graft (Wheat procedure, not requiring coronary reimplantation) or by employing a composite artificial graft with coronary reimplantation (Bentall and Cabrol procedures). Valve-sparing techniques include the supracoronary replacement of the ascending aorta usually with a synthetic (Dacron) graft, the Yacoub procedure and the David-valve sparing technique and its derivatives. Aortic arch repairs are often performed alongside ascending or descending aorta repairs, utilizing techniques such as hemiarch repair, "island patch" technique, and hybrid aortic arch approaches (5,6). For pathologies of the descending thoracic aorta, thoracic endovascular aortic repair (TEVAR) represents the primary interventional treatment modality, with open surgical repair reserved for patients presenting with anatomies unsuitable for endovascular approaches (7).

Surgical techniques for thoracic aorta repair often result in characteristic computed tomography (CT) imaging appearances that may resemble complications. Accurate interpretation requires awareness of these techniques, understanding the clinical contexts in which they are employed, and familiarity with the typical locations and imaging features of such mimics. For example, structures like coronary buttons, felt buttresses (pledgets and rings), occluded coronary bypass grafts, or debranching grafts may mimic pseudoaneurysms. Similarly, perigraft hematomas, seromas, or hemostatic agents can resemble perigraft abscesses, while graft kinks, elephant trunk or reverse elephant trunk procedures, and the Cabrol procedure may mimic aortic dissection. Recognizing these mimics is crucial to avoid unnecessary surgical consultations, follow-up imaging, or revisions. Optimized imaging protocols play a vital role in distinguishing true complications from their mimics. True complications of thoracic aorta repairs encompass a broad spectrum, including infections (e.g, perigraft abscess, infectious pseudoaneurysm, endocarditis, sternal dehiscence, or osteomyelitis), non-infectious prosthetic material failure (e.g., pseudoaneurysms at anastomosis or cannulation sites), hemorrhage, endograft-related issues (e.g., endoleaks, stent migration, collapse, or false lumen deployment), and pathology involving the adjacent aorta (e.g., aneurysms, aortic dissection, or intramural hematomas) (5, 8, 9, 10).

As the number of patients undergoing thoracic aorta repair increases (due to ageing population and surgical technique improvement) and imaging techniques continue to advance, the detection of complications has become more frequent. These complications, often associated with significant morbidity, may remain clinically silent, underscoring the essential role of CT imaging in patient follow-up protocols. The aim of this study is to evaluate the role of CT imaging in the post-surgical surveillance of thoracic aorta repair, with a focus

on identifying complications, distinguishing between true complications and mimics, and highlighting the clinical implications of these findings in guiding patient management.

MATERIALS AND METHODS

We conducted a retrospective, single-center study on the patients who underwent CT examinations at the Institute for Cardiovascular Diseases Iași between November 2023 and December 2024. The inclusion criterion was the history of thoracic aorta surgery, as mentioned by the CT reports and confirmed by the identification of surgical thoracic aorta prosthetic material on the acquired images. In the aforementioned time frame, from the total of 2,819 CT reports reviewed, we identified 24 patients eligible for this study, who were scanned either as a part of their regular post-operative follow-up or due to the appearance of signs and symptoms suggestive of a potential complication.

The images were acquired using a 256-slice dual-source CT-scanner, using slice thickness reconstructions of 1 mm within a coverage from the thoracic inlet to the femoral arteries. For the optimal assessment of the aortic valve, root and proximal ascending aorta retrospective electrocardiographic (ECG) gating was employed. The standard protocol included unenhanced and arterial phase (employing the "Bolus-tracking" technique) acquisitions, late phase acquisitions being used only in selected cases. The iodinated contrast material was administered intravenously (preferably through a right antecubital vein) at an injection rate of 5 mL/sec, followed by a saline chaser, the total dose being adjusted according to the patient`s body weight.

Data regarding patients' demographics, comorbidities, surgical indication and procedures were collected by consulting the electronic database of the hospital, then introduced into a *Microsoft Excel* database and statistically analyzed.

RESULTS

The study cohort predominantly consisted of male patients, accounting for 79.16% of the total population. Patient ages ranged from 18 to 76 years, with a mean age of 54.83 years. The average age for male patients was 52.79 years, while female patients had a higher mean age of 63 years.

Cardiovascular comorbidities were common in the study group, with essential arterial hypertension observed in 66.66% of patients. Other notable cardiovascular conditions included bicuspid aortic valve (12.5%) and Takayasu arteritis (4.16%). Chronic heart failure was present in 25% of cases, evenly distributed between those with preserved ejection fraction and those with mildly reduced ejection fraction. Additionally, chronic coronary syndrome (12.5%) and atrial fibrillation (20.83%) were frequently identified. The young patients from our study associated systemic disorders such as Marfan syndrome and Turner syndrome, known for their induced predisposition for aortic dilatation and/or dissection. Respiratory comorbidities included obstructive sleep apnea (12.5%) and chronic obstructive pulmonary disease (4.16%). Metabolic disorders were also prevalent, with dyslipidemia identified in 62.5% of patients, chronic kidney disease in 12.5%, and type 2 diabetes mellitus in 8.33%. Three patients presented with oncologic or hematologic conditions: one case each of bronchopulmonary carcinoma, chronic lymphocytic leukaemia, and myel-

odysplastic syndrome (tab. I).

TABLE I.
Associated comorbidities and their prevalence in the study group

Associated comorbidity	Prevalence
Essential arterial hypertension	66.66%
Chronic heart failure	25%
Atrial fibrillation	20.83%
Chronic coronary syndrome	12.5%
Bicuspid aortic valve	12.5%
Marfan syndrome	12.5%
Turner syndrome	4.16%
Takayasu disease	4.16%
Obstructive sleep apnea syndrome	12.5%
Chronic obstructive pulmonary disease	4.16%
Dyslipidemia	62.5%
Chronic kidney disease	12.5%
Type 2 diabetes mellitus	8.33%

The primary indications for thoracic aortic interventions in the study cohort included thoracic aortic dissection in 34.6% of cases, with Stanford A dissections accounting for 23.07% and Stanford B for 11.53%. Pseudoaneurysms were observed in 19.23% of cases, distributed as 7.69% in the ascending aorta, 3.84% in the aortic arch, and 7.69% in the descending aorta. Aneurysms represented the most common indication, affecting 38.46% of patients, with 26.92% involving the ascending aorta and 11.54% the descending aorta. Less frequent indications included infectious endocarditis (3.84%) and aortic coarctation (3.84%). Notably, 71.42% of patients undergoing ascending aortic aneurysm repair presented with severe aortic valve dysfunction necessitating valvular replacement. The surgical techniques employed varied widely, with the Bentall procedure performed in 29.62% of cases and TEVAR in 37%, half of which included debranching procedures. Other approaches included the Wheat procedure (3.7%), Tirone-David procedure (11.11%), and ascending aorta replacement with Dacron grafts (14.8%). Open surgical repair of the thoracic aorta was performed in one patient with anatomy unsuitable for TEVAR (3.7%) (fig. 1). Aorto-coronary bypass for myocardial revascularization was concurrently conducted in two cases.

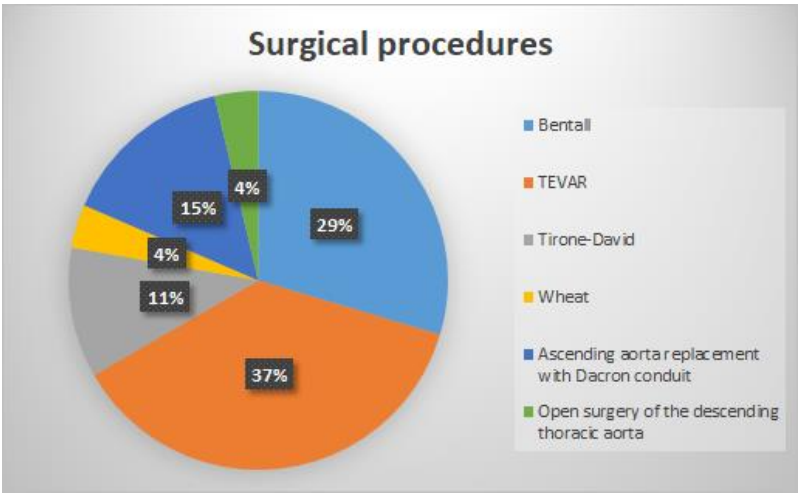

Fig. 1. Graphical representation of the surgical procedures used in the study cohort.

In terms of follow-up imaging, 66.66% of patients were asymptomatic and underwent CT scans as part of routine postoperative monitoring. A further 16.66% were scanned during the immediate postoperative period (within two weeks of surgery) as part of standard post-surgical checks. The remaining 16.66% were scanned due to symptoms suggestive of complications, including fever, chest pain, sudden-onset dyspnea, or syncope. Excluding immediate postoperative checks, the mean interval between surgery and follow-up imaging was 42.6 months.

Complications related to thoracic aortic interventions were identified in 54.16% of cases. These included perianastomotic pseudoaneurysms (12.5%), de novo dissection flaps (8.33%), intramural or periprosthetic hematomas (12.5%), surgical wound dehiscence (8.33%), and issues involving debranching conduits such as thrombosis or anastomotic stenosis (8.33%). Other complications included endoleaks (4.16%), hemopericardium (4.16%), and prosthetic material displacement (4.16%). Half of the asymptomatic patients undergoing routine follow-up imaging, as well as half of the patients scanned as an immediate postoperative check demonstrated surgery-related complications. Among symptomatic patients, CT imaging confirmed the presence of surgical complications in nearly all cases, with the sole exception being a patient in whom acute pulmonary embolism was excluded (fig. 2). 16.66% of the cases included in the study exhibited imaging findings necessitating surgical reintervention (the minor revision of the surgical wound dehiscence and the drainage of the hemopericardium not included). All the required reinterventions were successfully performed, except in one patient with infectious endocarditis complicated by a perivalvular abscess, rupture of the mitro-aortic continuity, and prosthetic material breakdown. This patient succumbed shortly after imaging confirmed the diagnosis.

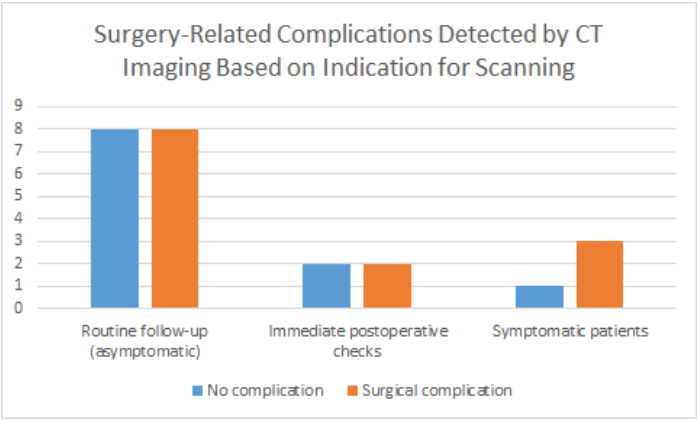

Fig. 2. Postsurgical complications detected by CT imaging based on the indication for scanning.

DISCUSSION

In our cohort, aneurysms and dissections emerged as the predominant indications for thoracic aortic interventions, accounting for over 70% of cases, both of them encountered with a higher prevalence in males. The ascending aorta was the predilect zone for the development of the aneurysmal dilata-

tion, and Stanford A dissections were more frequently encountered than Stanford B, reflecting a distribution consistent with existing literature (11, 12, 13).

Systemic arterial hypertension was the most prevalent comorbidity in our study cohort, consistent with its established role in the literature as the primary risk factor for thoracic aortic aneurysms and the most common contributor to aortic dissection (14). Heritable thoracic aortic conditions (HTAD), bicuspid aortic valve (BAV), Takayasu disease and previous aortic surgeries are more commonly observed in patients under the age of 40 (15, 16). This trend was also observed in our study, where the four patients under 40 years of age undergoing surgery were diagnosed with Marfan syndrome (two cases, one with concomitant BAV), Turner syndrome (with associated BAV), and Takayasu disease. Metabolic comorbidities, especially diabetes mellitus is associated with a higher risk of surgical wound dehiscence, by delaying tissue repair, increasing the risk of infections, and reducing collagen synthesis due to hyperglycemia-induced vascular and immune dysfunction (17). Respiratory comorbidities significantly impact thoracic aortic pathology. Obstructive sleep apnea syndrome (OSAS) is an independent risk factor for aortic dissection and root dilation, while chronic obstructive pulmonary disease (COPD) increases the risk of descending thoracic aortic aneurysm rupture (2). Notably, the study's only COPD patient presented with a complicated descending thoracic aneurysm, manifesting as a pseudoaneurysm from a contained rupture.

In our cohort, the Bentall procedure emerged as a frequent intervention, reflecting its utility in addressing complex pathologies involving the ascending aorta, aortic root and aortic valve, particularly in patients with connective tissue disorders or severe aortic valve dysfunction. Similarly, TEVAR was the predominant approach for descending thoracic aorta pathologies, with half of these cases necessitating debranching to extend anatomical coverage-a testament to the growing reliance on hybrid techniques for managing extensive or anatomically challenging disease. In cases with favorable anatomy and after considering the risk of late dilatation of sinuses when spared, valve-sparing techniques were also employed. In cases of isolated dilation of the ascending tubular (supracoronary) aorta, the diseased ascending aorta was replaced with a tubular Dacron graft, with the distal anastomosis positioned immediately proximal to the aortic arch. Management strategies in AAS aligned with current guidelines, with open surgical repair being the standard for Stanford A lesions due to their proximal location and life-threatening nature, while endovascular approaches such as TEVAR were more commonly employed for Stanford B lesions, highlighting the shift towards less invasive techniques for distal aortic pathologies (18, 19, 20, 21, 22).

Various imaging modalities, including transthoracic echocardiography (TTE), transesophageal echocardiography (TOE), cardiac computed tomography (CCT), and cardiac magnetic resonance (CMR), are used for follow-up after thoracic aortic interventions, with CCT being the most commonly utilized and accessible. However, no standardized imaging protocol exists, as recommendations are primarily based on expert opinion or single-center studies. Follow-up schedules vary by surgical indication and procedure type: imaging after TAA repair occurs less frequently (usually at discharge, one year, and every five years if stable), while AAS repair involves closer monitoring (every six months in the first year, annually until year three, and every two-three

years thereafter). TEVAR requires stricter surveillance, including imaging at one month and annually for at least five years, due to a higher risk of late reinterventions and complications such as retrograde type A dissection (RTAD) (2, 23, 24). Notably, although RTAD usually occurs at the time of the index procedure, up to 31% of the cases may be diagnosed later than 3 months after the endovascular or hybrid repair, as observed in one study patient who developed RTAD one-year post-TEVAR with debranching for a pseudoaneurysm (25, 26).

The imaging protocol at our institution is designed to ensure precise postoperative evaluation by incorporating retrospective ECG-gated acquisition, non-contrast imaging, and delayed-phase imaging (60-90 seconds delay) in selected cases, alongside the standard arterial phase. ECG gating plays a crucial role in reducing motion artifacts and improving visualization of the aortic root and ascending aorta. Retrospective ECG gating, in particular, enables optimal cinematic reconstructions throughout the cardiac cycle, leading to the detection of complications such as small leaks or pseudoaneurysms, which may only be apparent during specific phases of the cardiac cycle. Initial non-contrast images are essential for distinguishing hyperdense surgical materials from pseudoaneurysms or leaks and for identifying hyperdense blood products and postoperative hematomas that may be obscured in contrast-enhanced images (fig. 3). Delayed-phase imaging is crucial for detecting endoleaks, late opacification of the false lumen in residual dissections, and complications such as inflammatory fat stranding or rim enhancement associated with infection (8, 27, 28, 29).

One of the most significant challenges for radiologists is accurately distinguishing normal postoperative findings from potentially life-threatening complications, which necessitates a thorough understanding of surgical procedures and their potential imaging mimics. Synthetic aortic grafts can be identified on noncontrast CT scans by their hyper-attenuating appearance relative to the native aorta, a feature that may be less apparent on postcontrast images due to the adjacent blood pool. Additionally, aortic grafts often display a nonanatomic configuration, being less pliable than the native aorta. Instead of a smooth, curved morphology, they tend to have a straighter shape with associated angulations, appearing more redundant than expected. These grafts may also produce folds or kinks, which can occasionally resemble a short dissection flap on axial imaging but are readily identifiable as such on coronal and sagittal reformats (5, 8). The contrasting aspects of normal aortic graft folds and the presence of a true intimal flap in a patient who developed RTAD after TEVAR associating debranching are depicted in figure 4. Pseudoaneurysms commonly arise at anastomotic suture lines and appear as saccular outpouchings of contrast extending beyond the aorta's expected contours. They may also develop at cardiopulmonary bypass cannulation sites or locations of infection or abscesses. On CT imaging, pseudoaneurysms are characterized by a communication with the aortic lumen, typically contained by the aorta or surrounding tissues. Infectious pseudoaneurysms exhibit irregular borders, may have a saccular shape with a narrow neck, are associated with inflammatory stranding, and tend to grow rapidly. In contrast, noninfected pseudoaneurysms generally lack inflammatory stranding, grow more slowly, and are located at sites of potential structural failure, such as anastomoses or cannulation sites (5). Dense felt pledgets used to reinforce anastomotic suture lines can mimic pseudoaneurysms on CT. Differentiation relies on correlation with noncontrast images and recognition of

typical locations (27, 28). The differences between felt pledgets, infectious and non-infected pseudoaneurysms are depicted in figure 3.

Thrombosis of a bypass graft used as a debranching conduit can also mimic a pseudoaneurysm if a short patent lumen persists at the ostium (8). This distinction proved crucial in a patient imaged 16 months post-TEVAR with debranching, where identifying conduit occlusion with retrograde flow through a secondary bypass graft avoided an unnecessary surgical intervention (had it been misinterpreted as a pseudo-aneurysm). Graft infections may occur at any postoperative stage, initially presenting as abnormal fluid or gas bubbles, which can be challenging to differentiate from perioperative inflammation, particularly in the early postoperative period. Delayed contrast-enhanced images are often useful for identifying rim enhancement indicative of infected peri-graft fluid or abscess formation. Advanced infections may result in pseudoaneurysms or fistulas. In the immediate postoperative setting, complex fluid and gas collections at the surgical site, including mediastinal blood products and hemostatic materials (Gelfoam© or Surgicel©), are common and often benign. Mild rim enhancement may also be normal and not indicative of infection (5, 28, 29). The timing of imaging relative to surgery is crucial for accurate interpretation of these findings.

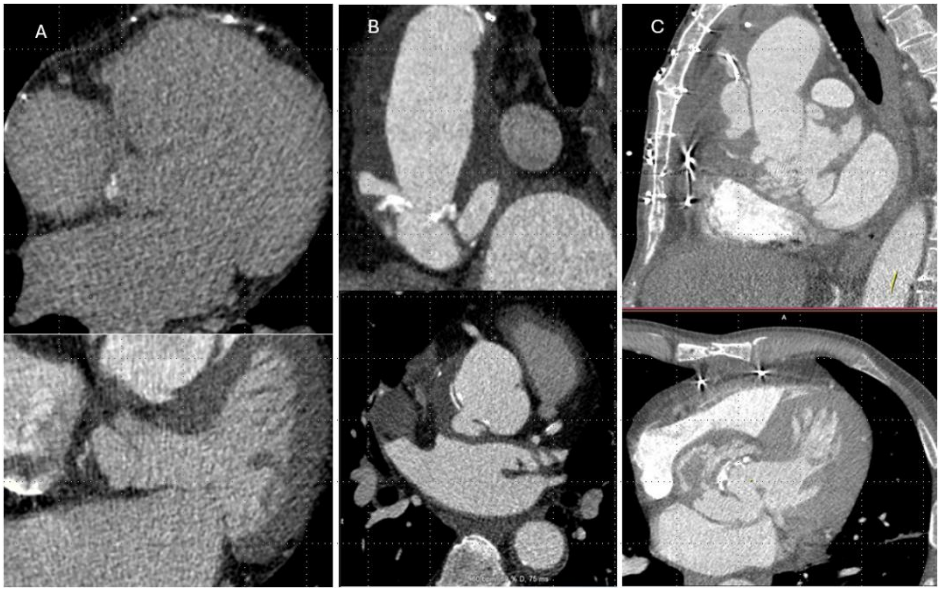

Fig. 3. A. Felt pledget in wall of the aortic root (non-coronary sinus) potentially mimicking a small pseudoaneurysm in the arterial phase (below), the confusion being avoided by the hyperdense aspect present in the non-contrast scan (above). **B.** Noninfected pseudoaneurysm in a typical location, at the proximal anastomosis, without significant stranding in the nearby tissues. Peri-graft fluid collection (seroma) with no mass effect on the ascending aorta graft. **C.** Multiple infectious, communicating pseudoaneurysms surrounding the proximal ascending aorta (with important systolic compression on the ascending aorta graft) in a patient with infective endocarditis on the prosthetic aortic valve, following Bentall procedure.

A breach is demonstrated in the mitro-aortic continuity. An epicardial cardiac stimulation wire courses through one of the pseudoaneurysm.

Fig. 4. A. Normal postoperative aspect after Bentall procedure and TEVAR associating debranching in a 42-year-old female with Takayasu disease - the normal graft folds in the axial plane are not to be confused with an intimal dissection flap (coronal and sagittal reformations are helpful in avoiding a misdiagnosis). **B.** Retrograde type A aortic dissection in a male patient one year after TEVAR associating debranching. Fortunately, the debranching conduit supplying the branches of the aortic arch originates from the true lumen.

In alignment with existing literature, the most common complications observed in the study cohort were pseudoaneurysms and TEVAR-specific complications (RTAD, type II endoleak, debranching conduit occlusion and anastomotic stenosis), particularly in cases performed under emergency conditions (2). The rate of complications necessitating re-intervention was 16.6%, closely approximating previously reported rates of approximately 10% (24). Notably, while the detection rate of complications was higher among symptomatic patients, CT imaging also identified complications in 50% of asymptomatic individuals. The critical role of CT imaging in the postoperative setting is further emphasized by its superior diagnostic capability; transthoracic echocardiography (TTE) raised suspicion of potential complications in only 15% of cases subsequently confirmed by CT.

CONCLUSIONS

This study highlights the critical role of CT imaging in the postoperative surveillance of thoracic aorta repairs, emphasizing its utility in identifying both true complications and their mimics. Complications were observed in over half of the cases, with a significant proportion detected even in asymptomatic patients, underscoring the importance of routine follow-up imaging. Advanced imaging protocols, including ECG-gated acquisitions and delayed-phase imaging, proved essential in distinguishing benign postoperative findings from potentially life-threatening conditions. Furthermore, the findings reiterate the importance of a tailored imaging approach, given the varied surgical techniques and associated imaging appearances. This study reaffirms CT imaging as an indispensable tool in guiding timely interventions, ultimately improving patient outcomes in the complex management of thoracic aortic pathology.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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MEASURING ONCOLOGY PATIENT EXPERIENCE: QUESTIONNAIRE DEVELOPMENT AND VALIDATION

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MEASURING ONCOLOGY PATIENT EXPERIENCE: QUESTIONNAIRE DEVELOPMENT AND VALIDATION (Abstract): The experience of oncology patients has emerged as a critical measure of care quality within the framework of a patient-centered approach to assessing health system performance. The absence of a validated instrument specific to the Romanian context restricts healthcare centers' capacity to effectuate significant enhancements. This research focused on the development and validation of a standardized instrument, the *Oncology Patient's Healthcare Experience Questionnaire* (OPEX), designed to evaluate the experiences of cancer patients. **Materials and methods:** This questionnaire was developed using a conceptual framework, which provided the foundation for creating the questionnaire items. The instrument's initial version underwent content validation with a sample of 50 patients, subsequently being administered to a larger cohort of 200 patients for psychometric analysis. The initial analysis of internal consistency yielded a Cronbach's alpha of 0.604. Following item revision, this value improved to 0.867, demonstrating enhanced reliability and validity. **Results:** The OPEX questionnaire includes 52 items grouped into seven essential dimensions of oncology care: interactions with physicians and nurses, patient information and education, hospital environment and organization, patient safety, relationships with carers, interactions with auxiliary staff, and facility infrastructure. This robust instrument, tailored to Romanian healthcare, captures oncology patients' unique experiences and improves the quality of patient-centered care. **Conclusions:** The OPEX questionnaire may serve as a reference tool for assessing patient satisfaction, providing insights into perceived care quality, identifying systemic gaps, enhancing clinical communication, personalizing interventions, and improving treatment adherence and therapeutic outcomes. **Keywords:** PATIENT EXPERIENCE, ONCOLOGY, DEVELOPMENT, QUESTIONNAIRE, VALIDATION.

INTRODUCTION

In the current landscape of healthcare, evaluating patients' experiences with the national health system has become a key component in assessing its overall performance(1). Patient experience is one of the three core dimensions of healthcare quality included in the OECD Health System Performance Framework (Organization for Economic Co-operation and Development) (2). It is also recognized as a key factor in the WHO's framework for evaluating health system performance (3). Over the past years, amid a growing shift toward patient-centered care, the experience of patients in their interactions with the healthcare system has emerged as a critical indicator of service quality (4).

In the field of oncology, where patients are confronted with complex diagnoses, intensive treatment regimens with substantial psychosocial implications, and an elevated need for multidisciplinary support (5,6), the systematic understanding and monitoring of the patient experience is of critical importance for enhancing clinical outcomes and overall patient satisfaction (7). The assessment of oncology patients' experiences not only provides valuable insights into the perceived quality of care, but also contributes to identifying gaps within the healthcare system, enhancing clinical communication, personalizing interventions, and ultimately improving treatment adherence and therapeutic outcomes (8). Patient experience measurement tools, known as Patient-Reported Experience Measures (PREMs), have been developed to capture patients' perceptions of the quality of care received. A notable example is the PREM-C, a validated instrument that assesses the experience of oncology outpatients, demonstrating high internal consistency as well as appropriate convergent and divergent validity. Additionally, the OUTPATSAT-35RT has been validated in a cohort from India, highlighting strong psychometric properties and relevance in the context of outpatient radiotherapy (9).

Although there are numerous internationally validated instruments available to assess patient satisfaction and experience in general, their adaptation to the specific context of oncology care is frequently inadequate. The duration and intensity of treatment, the ongoing relationship with the medical team, and the psychosocial challenges associated with the disease all significantly influence the needs of oncology patients (10). Therefore, the specific characteristics and priorities of this patient population may not be adequately captured by a generic instrument. Additionally, the necessity of contextualized tools that are tailored to local specificities is emphasised by the cultural and systemic divergences that exist among healthcare systems (11).

Despite the increasing emphasis on the establishment of networks of centers of excellence and the growing interest in enhancing the quality of oncology care, Romania currently lacks a national questionnaire for evaluating patient satisfaction with healthcare services. The capacity of medical providers to implement effective service improvement policies is restricted by this absence.

The aim of this study is to develop and validate a standardized, scientifically rigorous questionnaire designed to assess the experience of patients in oncology care centers across Romania. Our objective was to develop a questionnaire that assesses patients' experiences with oncology care centers. A conceptual framework was established to guide the development of the Oncology Patient's Healthcare Experience

Questionnaire (OPEX).

MATERIALS AND METHODS

Validation of the OPEX Questionnaire

The validation of a questionnaire is an essential process to ensure that the measurement instrument is both reliable and accurately assesses the intended constructs. This process encompasses several stages, from the initial development of the questionnaire to the testing and analysis of the results. The main steps undertaken in the validation of the OPEX questionnaire included:

1. Initial Development of the Questionnaire

a. Definition of Purpose and Objectives:

- The primary purpose of the questionnaire was clearly defined, along with the specific information intended to be obtained.
- Semi-structured interviews were conducted.
- The variables to be measured were identified.

b. Item Construction:

- Items (questions) were formulated to be clear, concise, and aligned with the objectives of the questionnaire.
- A mix of open-ended and closed-ended questions was used, including four-point scales and five-point Likert-type scales.

c. Expert Consultation (Focus Groups):

- Experts reviewed and validated the initial content of the questionnaire to ensure **content validity**.
- Open-ended questions enabled respondents to answer freely, facilitating the collection of qualitative data.
- Closed-ended questions, offering predefined answer options, were useful for quantitative data collection.
- Rating scales (e.g., Likert scales) allowed for measuring the degree of agreement or disagreement with specific statements.

2. Content Validity

a. Expert Evaluation:

- Experts assessed each item for its relevance and ability to accurately measure the intended variables. They also evaluated whether the items adequately covered all relevant aspects of the concept being investigated, and whether the items were clear, precise, and free from ambiguity.
- Techniques such as the **Item Content Validity Index (I-CVI)** were employed to assess the content validity of individual items during the scale development process.

b. Pilot Testing:

- The questionnaire was administered to a small sample from the target population (50 patients).
- Based on the pilot results, the clarity and relevance of the questions were assessed.
- The initial version of the questionnaire was revised, and necessary adjustments were made to improve its quality.

3. Validity and Reliability

a. Construct Validity:

- Statistical analyses, such as **factor analysis**, were used to determine whether items grouped together as theoretically expected.
- This step confirmed that the items measured the theoretical constructs

they were intended to assess.

b. Criterion Validity:

- Correlation analyses were used to evaluate **concurrent and predictive validity**, including intra-item correlations.

c. Reliability (Internal Consistency):

- The **Cronbach's Alpha coefficient** was used to assess the internal consistency of the questionnaire.
- A coefficient of $\alpha \geq 0.70$ was considered acceptable for most research purposes.

4. Administration and Data Analysis

The revised questionnaire was administered to a representative sample of 200 patients from the target population. In this phase, the reliability and validity of the questionnaire were reassessed using the collected data. Internal consistency was re-evaluated using Cronbach's Alpha for the revised version of the instrument.

5. Revision and Finalization

Based on the data analysis results, items that were found to be unreliable or invalid were revised. This process led to the development of the **final version of the OPEX questionnaire**, which demonstrated satisfactory psychometric properties.

RESULTS

The development and validation of the OPEX (Oncology Patient Experience) questionnaire involved a structured, multi-phase process encompassing three overarching stages: the development of a preliminary questionnaire, an initial evaluation of internal consistency, and final validation.

Preliminary Questionnaire Development

The design of the pilot version of the OPEX questionnaire was grounded in the Consumer Quality Index (CQI) methodology. This approach emphasizes co-creation and was implemented through active collaboration with patients, medical oncologists, oncology nurses, and psycho-oncologists.

To inform the development of the preliminary questionnaire, **50 semi-structured interviews** and **2 focus groups** were conducted between January and April 2023. Participants were recruited from the Elias Oncology Clinic (University Emergency Hospital Elias, SUUE). Interviews began with open-ended questions to explore patients' perceptions regarding the importance of various aspects of oncology care. Focus groups, facilitated by two researchers and one psycho-oncologist, involved 6 and 10 patients respectively and encouraged open discussion on improving the efficiency and quality of care at SUUE.

Inclusion criteria for participant selection were as follows:

1. Patients under the care of the clinic for at least three months.
2. Actively undergoing oncological treatment at SUUE.
3. Having a performance status appropriate for outpatient care (patients with ECOG = 4 or those hospitalized under continuous inpatient care were excluded).

Conceptual Framework Development

In the initial stage, a **conceptual framework** for the OPEX questionnaire was established through the following components:

- **A literature review** to identify relevant dimensions of the patient care experience.
- **Semi-structured interviews** aimed at identifying care aspects pertinent

to the patient population at SUUE.

- **Focus groups** that allowed participants to freely express their perspectives on enhancing the quality and effectiveness of oncology care at the center.

The protocol for semi-structured interviews included the following steps:

1. Introduction of the researcher.
2. Explanation of the study's purpose.
3. Informed consent acquisition.
4. Demographic and clinical background questions.

The interview format allowed patients to express their views on one of the **seven key dimensions** of oncology care. These domains also enabled patients to propose additional aspects not previously considered.

During the second part of the interview process, participants were asked targeted follow-up questions:

1. What would improve your experience at this medical center?
2. Which of the aspects discussed is most important to you?
3. On a scale from 0 to 10, how would you rate this aspect?
4. How would you rate the other discussed elements in terms of importance?
5. If you were the hospital director, how would you improve the hospital's conditions/medical team/infrastructure?
6. List three things you appreciate and three things you dislike about this medical center.

Structure of the Pilot Questionnaire

Based on the findings from the interviews and focus groups, the **first version of the OPEX questionnaire** was developed. It included:

- A **general section** collecting demographic and health-related data,
- **Seven sections** corresponding to key domains of the oncology care experience,
- A **final section** capturing the patient's overall evaluation of the received care.

The seven core domains included:

1. Physician-patient communication,
2. Nurse-patient interaction,
3. Patient information and education,
4. Hospital environment and organization,
5. Patient safety,
6. Interaction with family members,
7. Non-clinical staff and infrastructure.

These sections comprised a total of **38 items** (tab. I). The questionnaire concluded with five open-ended items designed to capture personal perspectives on the assessed aspects.

Item Construction Principles

Items were formulated using simple, concise, and culturally appropriate language, ensuring relevance and clarity for the target population. Each item was designed to address a single construct to maintain conceptual consistency throughout the instrument. Complex or double-barreled items (e.g., "My daily activities and mood are disturbed by my pain") were explicitly avoided, as were universally agreeable statements (e.g., "I would like to ease my pain"), which could limit response variability and diminish the questionnaire's discriminative power. This rigorous process ensured that the pilot OPEX questionnaire achieved strong content validity and laid the foundation for subsequent psychometric evaluation and final validation.

TABLE I.
The number of items in the OPEX questionnaire during the internal validation and content development phases

SECTION	OPEX		
	Semi-structured interviews	PHASE II	PHASE III
	ITEMS NUMBER		
I	10	14	14
II	7	8	8
III	7	13	13
IV	5	5	5
V	4	6	6
VI	2	2	2
VII	3	4	4
TOTAL	**38**	**52**	**52**

To ensure that the OPEX questionnaire is psychometrically robust, we applied statistical methods to assess content validity (Phase 2) (tab. II), internal validation, and to evaluate its reliability and validity (Phase 3) (tab. III). In both phases, internal consistency was evaluated, reflecting the degree to which the questionnaire items are inter-correlated and consistently measure the same construct. During the second phase of internal validation, a low Cronbach's alpha coefficient was observed (Cronbach's alpha = 0.604) (tab. III), which may be attributed to weak inter-item correlations. Items demonstrating low correlations with the total score were further analyzed and, where appropriate, were either eliminated or revised.

TABLE II.
Internal consistency analysis of the questionnaire conducted on a sample of 50 patients (Phase 2)

Valid N: 50 (OPEX.sta)					
Summary for scale: Mean=141.280					
Std. Dv. = 6.67631					
Cronbach alpha: 0.604899					
Standardized alpha: .545785					
Average inter-item corr: .031232					
Analysis of Variance (OPEX.sta)					
	Sums of - Squares	df	Mean - Square	F	p
Between Subjects	54.602	49	1.11433		
Within Subjects	3645.350	1950	1.86941		
Between Items	2803.992	39	71.89723	163.3022	0.00
Residual	841.358	1911	0.44027		
Total	3699.952	1999			

Since Cronbach's alpha is a function of questionnaire length, its value tends to increase with the number of items. Additionally, alpha increases when the variability of individual items is higher. Therefore, it is possible to enhance the alpha coeffi-

cient by including more interrelated items or items with greater variability. On the other hand, a very high alpha value ($\alpha \geq 0.90$) may suggest that some items in the questionnaire are redundant. In such cases, it may be appropriate to consider removing items that essentially ask the same question in different forms.

In practice, alpha simply indicates the degree to which the questionnaire is reliable for a specific population. As such, the reliability of the questionnaire was reassessed each time the instrument was administered, and Cronbach's alpha was calculated as part of the validation process. This coefficient ranges from 0 to 1, and a value below 0.7 typically indicates that certain items should be removed to improve internal consistency. The goal is to increase Cronbach's alpha above 0.7 so that the retained items are considered valid and the questionnaire more reliable. The alpha value can thus be improved by removing inappropriate or poorly correlated items. The next phase involves reprocessing the data.

Following this new iteration, items that initially had low but positive correlations with the total scale demonstrated improved correlations. Nevertheless, positively worded items with item-total correlations below 0.3 should not be retained, as they do not contribute meaningfully to the construct being measured and only serve to increase respondent burden.

A low Cronbach's alpha may result from weak inter-item correlations, and items with poor correlation to the total score should be eliminated or revised accordingly.

The **internal consistency** of a questionnaire is a statistical property that reflects the need for its "internal purity." Internal consistency is supported by two essential aspects:

1. Convergence of all items toward the targeted objective of the questionnaire. It is essential that the instrument does not include irrelevant items, as responses to such items may dilute the significance of relevant ones. These nonessential items introduce noise and can distort the overall measurement.
2. Item-level relevance, which requires a detailed examination of each item to determine its significance in relation to the construct being measured. This is typically assessed by the response rate or the proportion of meaningful responses received for each item.

In conclusion, reliability depends not only on the quality of the questionnaire's construction but also on the nature of the traits being measured. It is also crucial to know the reliability coefficient of the questionnaire to assess the degree of confidence that can be placed in its results. Furthermore, the characteristics of the population being assessed must be considered, as reliability may vary and tends to remain constant only within similar populations.

Sensitivity is another statistical quality of the questionnaire and refers to its discriminative precision, i.e., the number of categories it can define within a group. In other words, sensitivity reflects the questionnaire's ability to differentiate between individuals across a wide range. The more finely the instrument can classify respondents-while remaining aligned with real, meaningful differences-the more sensitive it is.

Standardization refers to the degree of uniformity in the administration and interpretation of the questionnaire. By definition, a questionnaire is a standardized in-

strument in three respects: content, application, and interpretation. Adherence to a clearly defined set of norms is essential for ensuring consistency across different uses.

Since all items are expected to reflect a specific attribute, they should demonstrate shared variance and correlate with one another. When each item is relevant, the instrument is said to exhibit internal consistency.

In the initial analysis (Phase II), the Cronbach's alpha coefficient was 0.604-a modest value relative to the accepted threshold of 0.70. This result indicated the need to exclude poorly performing items to improve reliability (tab. II).

The next phase involved reanalyzing and refining the questionnaire, during which items were revised, reformulated, added, or eliminated based on psychometric criteria.

Following this new iteration, items that previously had low but positive correlations with the scale were expected to show increased correlations. Nonetheless, positively worded items with item-total correlations below 0.3 should be removed, as they do not contribute meaningfully to the underlying construct and unnecessarily prolong the administration of the instrument.

After repeating the statistical analysis, a new set of results was obtained (tabs. III and IV), showing a substantially higher Cronbach's alpha. Moreover, inspection of the final column ("Alpha if item deleted") revealed that removing any individual item would not result in an alpha value higher than the one already obtained, confirming the improved internal structure of the final questionnaire.

TABLE III.

Final internal consistency evaluation of the questionnaire conducted on a sample of 200 patients (Phase 3)

Valid N: 200 (OPEX.sta)					
Summary for scale: Mean=5592.18					
Std.Dv. = 14.8269					
Cronbach alpha: 0.867207					
Standardized alpha: 0.886460					
Average inter-item corr: 0.133238					
Analysis of Variance (OPEX.sta)					
	Sums of - Squares	df	Mean - Square	F	p
Between Subjects	487.637	122	3.99703		
Within Subjects	4598.109	6642	0.69228		
Between Items	1101.340	54	20.39519	38.42504	0.00
Residual	3496.769	6588	0.53078		
Total	5085.747	6764			

The greater the number of items included at this phase, the higher the likelihood of retaining items that show stronger correlations with the overall scale, thereby achieving a higher level of internal consistency (12).

The calculated Cronbach's alpha coefficient was 0.867 (tab. III), indicating that the questionnaire is valid and demonstrates high internal consistency.

Two key indicators were analyzed:

- **Item-Total Correlation (Itm-Totl**

Correl.), which reflects the correlation between individual items and the total score. Low values may result from discrepancies in response coding.

- **Alpha if Item Deleted**, which shows the value of Cronbach's alpha if a given item were removed. This metric helps identify items that do not contribute to internal consistency and could be candidates for elimination.

TABLE IV.
Final evaluation of the OPEX questionnaire items

	Mean if deleted	Var. if deleted	Std Dv.If deleted	Itm-Totl Correl.	Alpha if deleted
How would you rate your current state of health?	5489.92	215.67	14.69	0.11	0.87
How would you rate your current emotional state?	5489.98	208.71	14.45	0.45	0.86
How was your treatment plan determined?	5489.65	215.59	14.68	0.13	0.87
I.1. Did your attending physician communicate your cancer diagnosis to you?	5491.09	217.98	14.76	0.00	0.87
I.2. To what extent did you understand the diagnosis communicated by your physician?	5490.59	209.93	14.49	0.52	0.86
I.3. To what extent did your physician explain your treatment plan?	5490.65	208.49	14.44	0.54	0.86
I.4. To what extent did your physician explain the potential side effects of your treatment?	5490.49	207.73	14.41	0.50	0.86
I.5. Were you informed about the possible course of your illness?	5491.04	215.57	14.68	0.23	0.87
I.6. To what extent did you understand how your illness might progress?	5489.38	204.14	14.29	0.31	0.87
I.7. Did you discuss other aspects of your illness with your physician (e.g., its impact on family, work, etc.)?	5490.69	213.94	14.63	0.26	0.87
I.8. What was the waiting time until a complete diagnosis was established?	5490.36	215.38	14.68	0.06	0.87
I.9. How did you perceive this waiting time?	5490.40	220.39	14.85	-0.11	0.87
I.10. What was the waiting time from diagnosis to the start of treatment?	5490.14	205.13	14.32	0.41	0.86
I.11. How did you perceive this waiting time?	5489.94	204.81	14.31	0.39	0.86
I.12. To what extent was your physician available to listen to your concerns about the illness and treatment?	5490.32	201.76	14.20	0.68	0.86
I.13. How would you evaluate the duration of your consultation?	5490.39	212.99	14.59	0.22	0.87
I.14. Were you satisfied with the amount of time allocated to you?	5490.99	210.53	14.51	0.65	0.86
II.1. How would you assess the behavior of the nurses towards you?	5490.76	204.73	14.31	0.69	0.86

	Mean if deleted	Var. if deleted	Std Dv.If deleted	Itm-Totl Correl.	Alpha if deleted
II.2. How would you assess the way nurses fulfilled their duties (medication administration, IV lines, injections, etc.)?	5490.73	202.05	14.21	0.70	0.86
II.3. During hospitalization, were your complaints regarding discomfort (noise, room temperature, symptoms, side effects, roommates, etc.) addressed?	5490.15	209.21	14.46	0.34	0.86
II.4. Do you believe the nurses treated all patients equally and fairly?	5490.76	207.11	14.39	0.58	0.86
II.5. To what extent do you feel that nurses paid attention to you during treatment?	5490.54	204.85	14.31	0.70	0.86
II.6. Did you discuss about your illness with the nurses?	5490.84	214.85	14.66	0.21	0.87
II.7. Did you discuss other aspects of your illness with the nurses (e.g., impact on family, work, etc.)?	5490.57	213.76	14.62	0.28	0.87
II.8. How would you assess the ability of the nursing team to relate to patients?	5490.66	204.57	14.30	0.68	0.86
III.1. How would you evaluate the quality of information provided about your illness by the medical team (physician, nurses)?	5490.34	201.44	14.19	0.58	0.86
III.2. How would you evaluate the information provided about treatment options?	5490.17	201.56	14.20	0.56	0.86
III.3. How would you evaluate the information regarding potential adverse effects?	5490.02	202.42	14.23	0.53	0.86
III.4. How would you evaluate the information about how your illness might evolve under current treatment?	5490.02	203.89	14.28	0.49	0.86
III.5. How would you evaluate the information about how your illness might evolve under current treatment?	5491.13	217.41	14.74	0.09	0.87
III.6. Were you informed by your physician about methods to relieve discomfort?	5491.07	216.70	14.72	0.14	0.87
III.7. In the past three months, did you discuss the progress of your illness with your physician?	5491.03	215.79	14.69	0.15	0.87
III.8. Were the written documents and other information provided regarding investigations or treatment clear?	5490.75	208.24	14.43	0.34	0.86
III.9. Were you informed about the possibility of undergoing a rehabilitation program (e.g., physiotherapy, lymphatic drainage)?	5489.91	213.87	14.62	0.14	0.87
III.10. Were you informed about the possibility of speaking with a psychologist?	5490.88	216.82	14.72	0.08	0.87
III.11. Were you informed about the possibility of receiving spiritual counseling?	5490.85	218.82	14.79	-0.07	0.87

	Mean if deleted	Var. if deleted	Std Dv.If deleted	Itm-Totl Correl.	Alpha if deleted
III.12. Do you know whether your family physician, rehabilitation center, or home care provider was informed about your treatment upon discharge?	5490.00	224.31	14.98	-0.19	0.88
III.13. Were you informed about the availability of home care services?	5490.63	217.58	14.75	0.01	0.87
IV.1. How would you assess the process of scheduling a consultation at this facility?	5490.32	205.19	14.32	0.59	0.86
IV.2. How would you evaluate the waiting time for scheduling a consultation at this facility?	5491.01	213.15	14.60	0.43	0.86
IV.3. How would you assess the cooperation between physicians within the clinic?	5490.65	204.16	14.29	0.73	0.86
IV.4. Based on your experience at this facility, to what extent would you choose to return?	5490.55	205.69	14.34	0.57	0.86
IV.5. To what extent would you recommend this facility to other patients?	5490.53	199.08	14.11	0.75	0.86
V.1. How often do you feel you received contradictory information about your treatment?	5490.55	211.52	14.54	0.17	0.87
V.2. How often do you feel you received contradictory information about your diagnosis?	5490.70	210.34	14.50	0.23	0.87
V.3. How often do you feel you received contradictory information about your prognosis?	5490.54	210.80	14.52	0.18	0.87
V.4. Do you believe information about your illness was ever withheld from you?	5491.07	217.11	14.73	0.08	0.87
V.5. Once you were informed about treatment options, was your right to choose a specific treatment respected?	5491.06	217.37	14.74	0.06	0.87
V.6. Before treatment or clinical examinations, was your identity verified (e.g., were you asked your name or date of birth)?	5490.94	215.01	14.66	0.15	0.87
VI.1. To what extent do you believe that interactions between your family (caregivers) and the medical team improved your illness experience?	5489.85	224.99	15.00	-0.31	0.88
VI.2. To what extent were your caregivers informed about your diagnosis or treatment options?	5490.19	226.20	15.04	-0.30	0.88
VII.1. How would you evaluate the politeness and courtesy of support staff (receptionists, orderlies, porters)?	5490.69	204.31	14.29	0.73	0.86
VII.2. How would you evaluate the promptness of support staff (receptionists, orderlies, porters)?	5490.65	204.39	14.30	0.74	0.86
VII.3. How would you evaluate the waiting conditions at this facility?	5490.32	203.70	14.27	0.57	0.86
VII.4. To what extent do you believe the waiting times met your expectations?	5490.22	207.28	14.40	0.42	0.86

While not the only statistical procedure employed in such contexts, Cronbach's alpha is by far the most widely recognized indicator of measurement precision, internal consistency, and the reliability of a questionnaire. Typically, the value of Cronbach's alpha tends to increase as the number of items increases.

One of the core aims of item analysis is to identify and either eliminate or revise items that are inappropriate or misaligned with the construct being measured. This process is iterative, involving repeated evaluations of inter-item relationships and item selection based on their contribution to the overall consistency of the scale.

The primary criterion for this process is the value of Cronbach's alpha, which ranges from 0 to 1. For a scale to be considered consistent, the coefficient should approach 1, with 0.70 commonly accepted as the minimum acceptable threshold. In practice, values below 0.60 are generally deemed unacceptable.

Two columns in second table are of particular importance:

- Item-Total Correlation (Itm-Totl Correl.), which reflects the degree of correlation between each item and the total score.
- Alpha if Item Deleted, which shows the expected alpha value if the item were removed from the scale.

The analysis reveals that certain items have very low correlations (<0.3 or in the range of [-0.3 to 0.3]). This suggests that they do not contribute meaningful information regarding the measured construct and may, in fact, introduce noise. First, such items should be examined for coding errors. If correctly coded, the only appropriate solution is to exclude or reformulate them.

As shown in the first table, the final version of the OPEX questionnaire includes 52 items across seven sections, representing an increase of 14 items compared to the initial version.

Following the item-level analysis, 14 new questions were added and several others were reformulated, thereby enhancing both the validity and internal consistency of the OPEX questionnaire as an instrument for evaluating the patient experience in oncology care centers.

The outcome of this process is a robust and psychometrically sound tool, capable of generating reliable and comparable data to support oncology centers in their ongoing efforts to continuously improve the quality of care.

DISCUSSION

A positive patient experience is correlated with overall well-being, which in turn is associated with increased adherence to treatment and improved prognosis. Developing a new instrument to quantify the level of satisfaction among oncology patients is a complex endeavor that requires specialized methods of data processing and analysis. Several methodological approaches were considered to support this process, including content analysis, literature review, the critical incident technique, direct observations, expert judgment, and structured guidance. The present study aimed to construct a psychometrically robust instrument that can be used both in clinical settings and academic research. To ensure the validity of the questionnaire, it is essential to demonstrate that the instrument accurately measures the intended construct and consistently produces reliable results. This process involves establishing content validity through expert evaluation, construct validity through factor analysis and theoret-

ical correlations, and criterion validity by comparing the results with those obtained from established measurement tools.

In the validation of the OPEX questionnaire, two major types of validity were taken into consideration: content validity and construct validity. Content validity refers to the extent to which the items included in a questionnaire are representative of the entire theoretical construct the instrument is designed to measure (13). Content validity should not be assessed until the initial version of the questionnaire has been developed, despite the fact that the construct of interest dictates the items that are written and/or selected during the questionnaire development phase. The process of content validation is a critical component of the development of a new questionnaire, as it guarantees that the instrument accurately represents all pertinent aspects of the theoretical construct it is designed to assess (14).

The content validity of the questionnaire should be assessed by a group of experts who have extensive knowledge in the construct it seeks to measure. These experts evaluate the items' capacity to comprehensively measure the targeted domain and whether they are sufficient and appropriate to adequately capture the construct of interest, acting as a focus group (11). Several approaches are also available to quantify expert assessment of content validity, such as the Content Validity Ratio (CVR) and the Content Validity Index (CVI) form (15). However, because the process of content validation largely depends on how well the expert panel can evaluate the extent to which the construct of interest has been operationalized, the selection of appropriate experts is crucial to ensure that content validity is assessed accurately and meaningfully (16).

Construct validity is the most critical concept in evaluating a questionnaire designed to measure a construct that is not directly observable (e.g., pain, quality of recovery). If a questionnaire lacks construct validity, the results become difficult to interpret, and it is not possible to draw meaningful inferences from the responses to broader behavioral domains. Construct validity can be assessed by examining the associations between the questionnaire and other variables (or measures of a similar construct) with which it is theoretically expected to correlate-positively, negatively, or not at all, depending on the nature of the relationship (17). In practice, the questionnaire of interest is administered to the same groups of individuals as existing instruments that measure similar or different constructs (18).

Given the specific needs of oncology patients, the OPEX questionnaire was developed to assess patient satisfaction across key dimensions of cancer care. Patient interactions were conducted through semi-structured interviews, during which the researcher asked a series of open-ended questions aimed at evaluating various aspects of the patient's experience with oncology healthcare services.

The international literature includes several questionnaires similar to the OPEX instrument developed and validated in this study. These include the EORTC IN-PATSAT32 (19), the Cancer Patient Experiences Questionnaire (CPEQ) (20), and The Consumer Assessment of Healthcare Providers and Systems (CAHPS) Cancer Care Survey (21). However, these tools are not adapted to the specific characteristics of the Romanian healthcare system or the socio-cultural values of the local population.

Thus, a comprehensive validation process-outlined in the previous steps-led to the development of a new questionnaire

specifically designed for oncology patients receiving medical care in Romania. This instrument offers the advantage of capturing the care context and the patient-healthcare provider relationship, with significant implications for the quality of life of oncology patients and the overall improvement of healthcare quality.

CONCLUSIONS

To be effective, a questionnaire must be valid, reliable, clear, concise, and engaging. It should be built on a conceptual framework, with each item assessed for relevance, clarity, and analytical approach. For patients' experiences of patient-centered care, such a tool can capture nuanced operational and human aspects of care, such as staff relationships, organization, safety, and infrastructure. The proposed questionnaire complements quality of life assessments and may offer valuable insights into the care context and patient-staff interactions, with significant implications for the overall well-being of oncology patients. This questionnaire also aims to contribute to the development of an organizational culture in which the patient's voice is heard, analyzed, and translated into concrete actions. Ultimately, we hope that this initiative represents a meaningful step toward a holistic and humanistic approach, in which medical performance is accompanied by compassion, respect, and a deep understanding of the needs of those navigating one of life's most challenging experiences.

INFORMED CONSENT

Informed consent was obtained from all study participants.

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DATA AVAILABILITY

The validated Romanian version of the OPEX questionnaire is available and a copy may be obtained by contacting the corresponding author.

CONFLICTS OF INTEREST

The authors of this study declare no conflicts of interest.

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DIGITAL *VERSUS* CONVENTIONAL IMPRESSIONS: A COMPARATIVE STUDY IN SINGLE-CROWN FABRICATION

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DIGITAL *VERSUS* CONVENTIONAL IMPRESSIONS: A COMPARATIVE STUDY IN SINGLE-CROWN FABRICATION (Abstract): **Aim** of the study. This study aimed to assess the dimensional accuracy and time efficiency of digital and conventional impression techniques used in recording complete dental impressions for single-crown fabrication. **Materials and methods:** A standardized typodont model designed for single-crown preparation was used to obtain 45 impressions: 15 with condensation silicone (Zeta Plus with Oranwash), 15 with addition silicone (Elite HD+), and 15 digital impressions using the Primescan AC intraoral scanner. Linear measurements of crown height, cervical widths, and interproximal distances were performed using Dental CAD software. Recording time was documented for each technique. **Results:** Digital impressions exhibited slightly lower mean values at the crown cervical finish line compared with conventional silicones, though all values remained within clinically acceptable limits. Significant differences ($p < 0.001$) were observed for cervical width measurements, while other parameters showed no statistical differences. The digital workflow demonstrated the shortest mean recording time (286 ± 7.39 s), followed by addition silicone (650.8 ± 8.93 s) and condensation silicone (713.67 ± 12.22 s). **Conclusions:** All three impression techniques produced clinically acceptable results. Digital impressions proved to be the most time-efficient and reproducible but tended to slightly underestimate certain dimensions. Although digital systems offer greater efficiency and patient comfort, addition silicone remains the preferred material for many practitioners in cases requiring full-arch or subgingival accuracy. **Keywords:** CONDENSATION SILICONES, ADDITION SILICONES, DIGITAL IMPRESSION.

INTRODUCTION

An impression is defined as a negative replica of the patient's teeth and surrounding tissues, serving as the primary step in the fabrication of indirect restorations such as crowns, bridges, inlays, on lays, orthodontic appliances, and removable prostheses (1-3). To achieve accurate prosthetic design and fabrication, the impression is later converted into a working model using dental stone or other materials. During the laboratory phase, it is converted into a working model (the positive replica) using dental stone or other die materials, allowing for precise prosthetic design and fabrication. Because it plays a crucial role in treatment accuracy, the impression must retain its dimensions, remain stable, and resist distortion. Achieving this requires not only technical skill but also a solid understanding of material science, particularly the behavior

of flow, polymerization, moisture interaction, and tear resistance (4).

Conventional impressions using irreversible hydrocolloids (alginate), polyethers, condensation silicones (polydimethyl siloxanes), and addition silicones (polyvinyl siloxanes) have been the standard in dental practice (5). When properly handled, these materials provide accurate and detailed impressions but are prone to dimensional changes, patient discomfort, and longer processing times due to setting, pouring, and laboratory transfer (6, 7).

The introduction of digital impression systems has revolutionized dental practice. Although the concept appeared in the late 1980s, it became practical only in the early 2000s (8, 9). Early intraoral scanners were oversized, expensive, and limited in performance, but thanks to advancements in optics and software, we now have compact, precise, and user-friendly systems (10). Digital impressions minimize material distortion and operator error, thereby improving precision and patient comfort by eliminating the need for conventional materials (11-13). They also simplify clinical workflows through CAD/CAM integration, allowing faster lab communication and even same-day restorations (14). Despite these benefits, challenges remain, including high equipment costs, the need for training for new users, and difficulties scanning subgingival or edentulous areas (15, 16).

The aim of this study was to evaluate the dimensional accuracy and the recording time of impressions for single-crown fabrication using digital and conventional techniques. To achieve this objective, a null hypothesis was formulated, stating that there are no statistically significant differences between the two methods in terms of dimensional accuracy or the time required to complete an impression.

MATERIALS AND METHODS

The experimental study was conducted at the Faculty of Dentistry, Grigore T. Popa University of Medicine and Pharmacy, Iași. The dental impressions were obtained within the institution's facilities. For the fabrication of the experimental digital models, impressions were taken 15 times from a standardized typodont model designed for single-crown fabrication, using condensation silicone (Zeta Plus with Oranwash, Zhermack SpA, Badia Polesine, Italy) and addition silicone (Elite HD+, Zhermack SpA, Badia Polesine, Italy).

Digital impression. The digital impressions were obtained using a powder-free intraoral scanner, the Primescan AC (Dentsply Sirona, Bensheim, Germany). Prior to scanning, the typodont model was securely positioned and dried to minimize reflective artifacts and ensure optimal surface recognition. The scanning procedure was performed in accordance with the manufacturer’s guidelines, beginning with the occlusal surfaces and then the buccal and oral aspects, to ensure comprehensive data acquisition. Once the full arch scan was completed and verified for precision and completeness, the resulting data were exported in Standard Tessellation Language (STL) format, a widely accepted file type for digital dental workflows and CAD/CAM processing.

For the conventional impression group, digital STL files were generated using a laboratory desktop scanner (Swing DOF, DOF Inc., Seoul, Korea). This device uses a white-light LED source and an L-shaped configuration to capture detailed surface

data, converting the physical impression into a high-resolution digital model.

This protocol enables the generation of digital STL data directly from the impression surface, without the need to pour a conventional gypsum model.

All materials were handled in accordance with the respective manufacturers' instructions, and all impression records were carried out by a single calibrated operator.

Conventional impression. Impressions were obtained using a two-step impression technique using low and high-viscosity silicone materials, a protocol commonly employed in clinical prosthodontics due to its superior ability to capture fine anatomical details with high precision.

To ensure proper retention of the impression materials, medium-sized perforated plastic impression trays (Guangzhou Aurora Health Products Company) were employed. In the first step, an impression was taken using the putty consistency of the selected silicone materials, which were manually loaded into the preselected perforated impression trays. After the putty had set, the light-body silicone material was carefully syringed into the preliminary impression to occupy the negative space corresponding to the dental structures. The tray containing the set putty material was then repositioned onto the typodont model, allowing the low-viscosity material to reproduce fine surface details. The entire assembly was maintained until a complete setting was achieved.

Dimensional accuracy analysis. To evaluate the dimensional accuracy of the impressions, digital linear measurements were performed using Dental CAD 3.0 Galway (exocad GmbH, Darmstadt, Germany) (17). The following parameters were assessed:

- crown height of the maxillary second premolar (tooth 2.5) and first molar (tooth 2.7);
- buccal, distal, oral, and mesial measurements at the cervical preparation of the abutment first molar (tooth 2.6);
- mesio-distal and buccal-palatal dimensions of 2.6 abutment measured at the occlusal level;
- mesial and distal distance between the contact points (fig. 1).

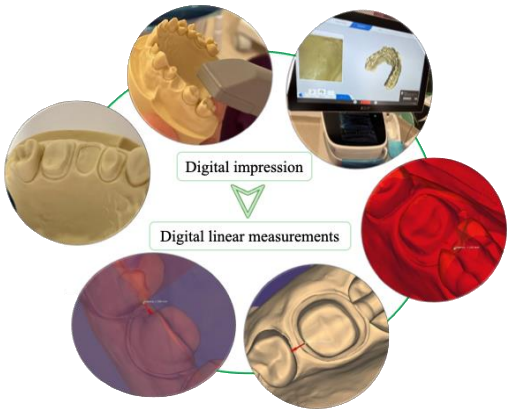

Fig. 1. Schematic representation of the digital impression and linear measurement workflow.

Time efficiency analysis. For the conventional impression procedures, recording time was measured in seconds, from the selection of an appropriately sized impression tray to the removal of the fully set material from the typodont model. Similarly, for the digital impression workflow, the recording time was documented in seconds to allow for direct comparison with the conventional method. In this case, timing began at the moment the intraoral scanner tip was first positioned on the model and concluded upon the completion of the full-arch scan, as indicated by the scanner's software.

Statistical analysis. Statistical analysis was carried out using SPSS software, version 26.0 (SPSS Inc., Chicago, IL, USA). The data were first evaluated for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and descriptive statistics were then applied. For comparisons between groups involving digital measurements obtained from digital models, one-way ANOVA and Kruskal-Wallis tests were used. A significance level of $p < 0.05$ was considered statistically relevant throughout the evaluation.

RESULTS

Dimensional accuracy. A total of 45 samples were analyzed to assess the dimensional accuracy of the three impression techniques, using condensation silicone, addition silicone, and an intraoral scanner. The comparative analysis of the measured landmarks revealed consistent dimensional stability across all parameters, with minor variations among the techniques (tab. I). Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk) indicated no significant deviation from a normal distribution for eight parameters ($p > 0.05$).

TABLE I.

Measured parameters (N=45).

Parameters	Mean ± SD	Min.	Max.	Kolmogorov-Smirnov Sig.	Shapiro-Wilk Sig.
2.5 Crown height	7.530 ± 0.095	7.346	7.733	0.054	0.053
2.7 Crown height	5.844 ± 0.094	5.675	6.022	0.200	0.093
2.6 Mesio-distal dimension	6.882 ± 0.095	6.694	7.088	0.036	0.194
2.6 Buccal-palatal dimension	6.654 ± 0.093	6.471	6.822	0.054	0.033
Mesial contact distance	2.272 ± 0.036	2.201	2.339	0.060	0.054
Distal contact distance	2.026 ± 0.036	1.966	2.104	0.084	0.093
Mesial cervical width	0.885 ± 0.011	0.850	0.910	0.160	0.351
Distal cervical width	0.998 ± 0.020	0.956	1.043	0.200	0.400
Buccal cervical width	0.992 ± 0.023	0.949	1.042	0.200	0.187
Palatal cervical width	1.050 ± 0.027	1.003	1.111	0.039	0.027

SD - standard deviation; Min.- Minimum value; Max.- Maximum value; significant level $p < 0.05$

Material-based comparisons revealed uniform dimensional behavior among the three evaluated impression techniques. Across most parameters, condensation silicone pro-

duced slightly higher mean values, while digital impressions yielded marginally lower values. Differences in crown height, mesio-distal, buccal-palatal, and contact distances were not statistically significant ($p > 0.05$), indicating comparable trueness among the materials for these parameters. In contrast, statistically significant differences ($p < 0.005$) were observed in cervical width measurements, with condensation silicone showing higher mean values than addition silicone and digital techniques (tab. II).

TABLE II.

Dimensional measurements among the study's impression groups.

Parameters	Intraoral scanner	Condensation silicone	Addition silicone
	Mean ± SD	Mean ± SD	Mean ± SD
2.5 Crown height	7.560± 0.096	7.543 ± 0.101	7.488± 0.076
	one-way ANOVA, p = 0.093		
2.7 Crown height	5.817± 0.089	5.855 ± 0.096	5.859 ± 0.098
	one-way ANOVA, p = 0.419		
2.6 Mesio-distal dimension	6.905 ± 0.105	6.883 ± 0.106	6.856 ± 0.0681
	one-way ANOVA, p = 0.381		
2.6 Buccal-palatal dimension	6.647 ± 0.074	6.651 ± 0.090	6.664 ± 0.115
	Kruskal-Wallis Test, p = 0.789		
Mesial contact distance	2.265 ± 0.038	2.282 ± 0.031	2.270 ± 0.038
	one-way ANOVA, p = 0.416		
Distal contact distance	2.040 ± 0.037	2.013 ± 0.034	2.025 ± 0.034
	one-way ANOVA, p = 0.143		
Mesial cervical width	0.877 ± 0.012	0.887 ± 0.009	0.890 ± 0.008
	one-way ANOVA, p = 0.005		
Distal cervical width	0.985 ± 0.016	1.016 ± 0.016	0.993 ± 0.015
	one-way ANOVA, p < 0.001		
Buccal cervical width	0.974 ± 0.011	1.019 ± 0.012	0.983 ± 0.012
	one-way ANOVA, p < 0.001		
Palatal cervical width	1.032 ± 0.015	1.082 ± 0.016	1.034 ± 0.012
	Kruskal-Wallis Test, p < 0.001		

SD - standard deviation; Min.- Minimum value; Max.- Maximum value; significant level $p < 0.05$

Impression recording time. The mean impression recording times differed significantly across the three impression techniques (tab. III and fig 2). The digital impression group recorded the shortest average time (286 ± 7.36 seconds), followed by the addition silicone group (650.80 ± 8.93 seconds), while the condensation silicone group required the longest time (713.67 ± 12.22 seconds).

TABLE III.
Recording time among the study's impression groups.

Impression Recording Time (s)					
Material Type	**N**	**Mean ± SD**	**Min.**	**Max.**	**Kruskal-Wallis Test**
Intraoral scanner (Primescan AC)	15	286.00 ± 7.39	275	299	p < 0.001
Condensation silicone (Zeta Plus + Oranwash, Zhermack SpA)	15	713.67 ± 12.22	694	737	
Addition silicone (Elite HD+, Zhermack SpA)	15	650.80 ± 8.93	637	670	

SD - standard deviation; Min.- Minimum value; Max.- Maximum value; significant level $p < 0.05$

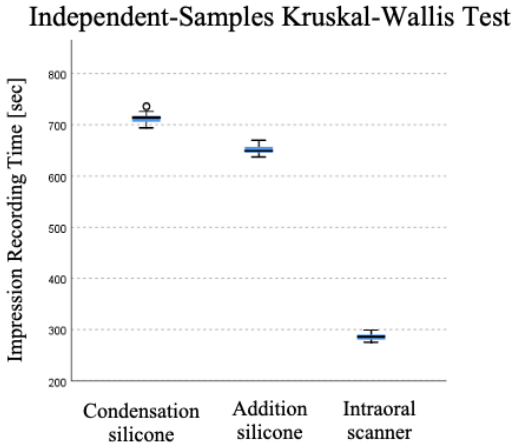

Fig 2. Boxplot analysis of impression recording time across study's impression groups.

DISCUSSION

Proper marginal adaptation is vital for maintaining oral health and ensuring the longevity and structural integrity of tooth-supported fixed prostheses. Accurate margins reduce the risk of restoration fractures, retention loss, postoperative sensitivity, periodontal issues, and recurrent caries. In contrast, poor adaptation facilitates bacterial microleakage and cement dissolution, encouraging secondary caries and plaque-induced periodontal disease (18-22).

Several factors influence marginal fit, including the finish line design, spacer application, cementation protocol, and the type of restorative material used (23-25). According to McLean and von Fraunhofer, a marginal gap of up to 0.12 mm is generally acceptable. Achieving an accurate intraoral impression is essential for producing precisely fitted fixed dental prostheses (26). Conventional techniques can provide reliable results when performed correctly, but they require technical proficiency and careful handling of impression materials to avoid errors such as marginal distortion,

voids, and tissue displacement.

The results of this study showed that digital impressions exhibited slightly lower mean values at the crown cervical finish line. Although these values remained within clinically acceptable limits, they were significantly different from those obtained with conventional condensation silicone impressions. No other statistically significant differences were found between the measurements obtained from digital models generated by intraoral scanning and those derived from conventional silicone impressions. These findings are consistent with the research by Ender *et al*. (27, 28). In their earlier study, the authors compared several conventional impression materials, including polyether (POE), vinylsiloxanether (VSE), and alginate (ALG), along with four digital intraoral scanners (CEREC Bluecam (CER), CEREC Omnicam (OC), Cadent iTero (ITE), and Lava COS (LAV)) using a reference model with a known morphology. The results indicated that VSE and CER achieved the highest trueness and precision across the full dental arch, while POE and ALG exhibited greater deviations. The authors concluded that conventional and digital impression techniques differ in full-arch accuracy, with digital systems showing higher local deviations along the arch (27). One year later, the same authors reported that digital quadrant impressions demonstrated precision comparable to that of conventional methods, though differences persisted in absolute values and deviation patterns (28).

Many studies have evaluated the marginal fit of restorations fabricated using different impression and manufacturing techniques. In the study conducted by Seelbach *et al*. (29), twenty putty-wash impressions (ten single-step and ten two-step) were taken from the same model using a polyvinyl siloxane (PVS) impression material and compared with thirty digital impressions recorded using three different intraoral scanners. The authors reported satisfactory adaptation in restorations fabricated with CEREC Bluecam, Lava COS, iTero, and 3Shape TRIOS scanners, with an accuracy comparable to that of conventional impression techniques.

An S *et al*. (30) found that the iTero system provided marginal accuracy comparable to that of milled polyurethane models. Pedroche *et al*. (31) also observed that intraoral scans yielded better marginal precision than extraoral scans of PVS impressions. Other authors (32-36) confirmed that direct digital impressions often achieve equal or superior fit compared with indirect methods, though results vary by scanner type. In contrast, Shembesh *et al*. (37) reported mixed outcomes, with poorer fit for iTero and improved results for the 3M True Definition Scanner.

In the present research, the digital workflow showed the shortest recording time, 286 ± 7.36 s, compared with 650.80 ± 8.93 s for addition silicones and 713.67 ± 12.22 s for condensation silicones. Similar conclusions were drawn by Yuzbasioglu *et al*. (38), who reported that digital impressions reduced both total treatment and impression times, with average durations of 248.48 ± 23.48 s for digital versus 605.38 ± 23.66 s for conventional impressions. This trend was similarly observed in other in vivo studies, in which total procedure times and impression-specific durations consistently favored digital techniques. The efficiency of intraoral scanning systems likely stems from eliminating the steps required

in traditional methods, such as material mixing, tray insertion, setting time, and removal. Moreover, real-time visualization and immediate error correction reduce the need for retakes, which is not always feasible with conventional materials (32,39).

Based on the findings of this study, the null hypothesis stating that all impression techniques are equally precise and time-efficient must be rejected.

Beyond dimensional accuracy and time efficiency, digital impressions offer additional clinical advantages, including improved patient comfort by avoiding bulky trays and unpleasant materials, particularly beneficial for patients with a gag reflex or anxiety. However, certain limitations remain. Challenges such as the high initial cost of intraoral scanners, the need for training, and occasional difficulty in capturing subgingival margins and reflective surfaces continue to pose barriers to universal adoption.

The *In Vitro* nature of the present study limits its direct clinical applicability, as it does not account for the complex in vivo factors that can influence impression accuracy, including saliva, blood, patient or operator movement, limited access, and soft tissue variability. The use of point-based measurements rather than automated analytical software and the small sample size further constrains the generalizability of the results. Consequently, future in vivo studies employing diverse intraoral scanners are needed to validate the accuracy and reliability of digital and conventional impression systems under real clinical conditions.

CONCLUSIONS

Within the limitations of this *in Vitro* study, the analysis revealed the following findings:

- condensation silicone showed slightly higher mean values than addition silicone and digital impressions.

- digital impressions demonstrated excellent reproducibility, speed, and ease of use but tended to slightly underestimate certain dimensions compared with conventional elastomeric materials;

- all three impression techniques provided measurements within clinically acceptable limits, confirming their suitability for prosthodontic use.

Digital systems represent a reliable alternative for single-unit and quadrant restorations; however, for full-arch impressions or recordings of subgingival margins, conventional addition silicones are still recommended.

CONFLICTS OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding.

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ANTI-INFLAMMATORY EFFICACY OF NSAIDS AND THEIR COMBINATIONS WITH ANTIBIOTICS IN A RAT MODEL OF ACUTE CARRAGEENAN-INDUCED INFLAMMATION

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ANTI-INFLAMMATORY EFFICACY OF NSAIDS AND THEIR COMBINATIONS WITH ANTIBIOTICS IN A RAT MODEL OF ACUTE CARRAGEENAN-INDUCED INFLAMMATION (Abstract): Acute inflammation is a fundamental pathophysiological process involved in numerous diseases. The carrageenan-induced paw edema model in rodents is a well-established method for evaluating anti-inflammatory agents. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac (DCF) and ketoprofen (KET), exert their effects primarily through cyclooxygenase inhibition, while certain antibiotics, including cefuroxime (CFX) and levofloxacin (LFC), may indirectly influence inflammatory pathways. This study assessed the anti-inflammatory effects of DCF and KET, administered alone or combined with CFX or LFC, in rats with carrageenan-induced paw edema. **Materials and methods**: Forty-five Wistar rats were divided into nine groups (n = 5) receiving saline, NSAIDs, antibiotics, or their combinations. Treatments were given intraperitoneally every 12 hours for three days. Acute inflammation was induced by subcutaneous injection of 1% carrageenan into the right hind paw. Paw volume was measured at baseline and at 6, 24, and 72 hours. Edema was expressed as percentage increase in paw volume, and anti-inflammatory activity as percentage of edema inhibition. Statistical analysis was performed using ANOVA method. **Results**: Edema peaked at 6 hours after carrageenan injection. Both NSAIDs significantly reduced paw swelling at all time points, with DCF showing greater and more sustained efficacy than KET. Antibiotics alone had minimal impact, except for a modest reduction with CFX at 24 hours. Combination therapies maintained or slightly enhanced anti-inflammatory effects, particularly KET combined with CFX. **Conclusions:** NSAIDs were the main contributors to the anti-inflammatory response, with DCF demonstrating superior potency. Although antibiotics showed limited direct effects, certain combinations may enhance therapeutic outcomes in acute inflammation. **Keywords:** ACUTE INFLAMMATION, PAW EDEMA, CARRAGEENAN, DICLOFENAC, KETOPROFEN, CEFUROXIME, LEVO-FLOXACIN, RATS.

INTRODUCTION

Acute inflammation is a complex physiological response to tissue injury or chemical irritants, characterized by edema, redness, pain, and increased vascular permeability. It is a critical protective mechanism,

yet uncontrolled or excessive inflammation underlies numerous pathological conditions, including autoimmune diseases, chronic pain, and cardiovascular disorders. Experimental models that reliably reproduce acute inflammation are essential for understanding the underlying mechanisms and evaluating potential anti-inflammatory therapies. Among these, the carrageenan-induced paw edema in rodents is a widely used and well-standardized model, reproducing key features of human acute inflammation, including the exudative phase and the subsequent resolution (1, 2).

NSAIDs act primarily through inhibition of COX enzymes (3), thereby reducing prostaglandin synthesis, which in turn decreases vasodilation, vascular permeability, and pain associated with acute inflammation (4, 5). DCF is widely recognized for its strong and preferential inhibition of the COX-2 enzyme, a key isoform responsible for the synthesis of pro-inflammatory prostaglandins during tissue injury and inflammation. By selectively suppressing COX-2 activity, DCF effectively decreases the production of prostaglandins such as PGE_2, which are directly involved in mediating pain, swelling, and fever (3). This mechanism underlies DCF's ability to produce a rapid onset of analgesic action and a sustained anti-inflammatory response, making it highly effective in both acute and chronic inflammatory disorders, including osteoarthritis, rheumatoid arthritis, and postoperative pain management. Its pharmacological profile is characterized by potent peripheral and central analgesic properties, which contribute to its strong clinical efficacy (6). Moreover, its relatively higher COX-2 selectivity compared to other NSAIDs contributes to a more favorable gastrointestinal safety profile, although risks of hepatotoxicity and cardiovascular effects remain considerations with long-term use (5, 6). In contrast, KET exhibits a more balanced inhibitory action on both COX-1 and COX-2 enzymes, leading to a broader but slightly less selective suppression of prostaglandin synthesis. This dual inhibition contributes to effective reduction of inflammatory edema, pain, and tissue swelling through decreased vascular permeability and modulation of inflammatory mediators. However, because COX-1 plays a role in maintaining gastric mucosal protection and platelet function, KET's balanced inhibition is associated with a slightly increased risk of gastrointestinal irritation and bleeding compared with more COX-2–selective agents like diclofenac (7). Despite its somewhat lower anti-inflammatory potency relative to DCF, KET remains an effective therapeutic option, particularly in conditions where edema reduction and modulation of both central and peripheral inflammatory pathways are desired. Its balanced COX inhibition profile also provides reliable efficacy across a wide range of inflammatory and musculoskeletal disorders, making it a versatile NSAID for pain and inflammation control (8).

CFX and LFC are both broad-spectrum antibacterial agents commonly used in the treatment of a wide range of bacterial infections, but they belong to different pharmacological classes and act through distinct mechanisms. CFX is a second-generation cephalosporin antibiotic that exerts its bactericidal effect by inhibiting bacterial cell wall synthesis. It binds to penicillin-binding proteins located inside the bacterial cell wall, interfering with the final transpeptidation step of peptidoglycan synthesis, which is essential for maintaining cell wall structural integrity (9). As a

result, CFX causes cell lysis and bacterial death. It is particularly suited for infections caused by susceptible Gram-positive cocci and β-lactamase-producing Gram-negative bacilli, being used in respiratory tract infections, skin and soft tissue infections, urinary tract infections, and infections of the ear, nose, and throat. Pharmacokinetically, CFX demonstrates good tissue penetration and is primarily excreted unchanged via the kidneys, making dose adjustment necessary in patients with renal impairment (9).

LFC is a fluoroquinolone antibiotic and the L-isomer of ofloxacin, with potent bactericidal activity mediated by inhibition of bacterial DNA gyrase and topoisomerase IV, two enzymes crucial for bacterial DNA replication, transcription, repair, and recombination. This dual inhibition results in rapid bacterial cell death and prevents the development of resistance mechanisms (10). LFC exhibits broad-spectrum activity against Gram-positive, Gram-negative, and atypical pathogens, making it highly effective in the treatment of community-acquired pneumonia, sinusitis, bronchitis, urinary tract infections, prostatitis, and skin infections. It is primarily eliminated renally and requires dose adjustment in renal dysfunction. However, caution is advised due to potential adverse effects such as tendinopathy, QT interval prolongation, central nervous system effects, and, rarely, dysglycemia (10).

In addition to classical NSAIDs, selected antibiotics such as CEF and LFC have been reported to influence inflammatory pathways indirectly, potentially modulating cytokine release or oxidative stress, though their anti-inflammatory effects are generally secondary to their antimicrobial action (8, 9). Studying these agents alone and in combination with NSAIDs may reveal pharmacological interactions that enhance or modify the anti-inflammatory response.

This study **aimed** to evaluate the dynamics of carrageenan-induced paw edema in Wistar rats and to compare the anti-inflammatory efficacy of DCF and KET, alone and in combination with CFX or LFC. The pharmacological rationale was to investigate how well-characterized NSAIDs exert their effects in this model, and whether co-administration with antibiotics could influence the inflammatory response, providing insight into potential synergistic or modulatory interactions relevant for therapeutic strategies.

Animals. White Wistar rats (200-250 g) from the bio base of Grigore T. Popa University of Medicine and Pharmacy, Iași, were used for the experiments. Animals were acclimated for one week under standard laboratory conditions (temperature 21 ± 2 °C, relative humidity 50–70%, 12-hour light/dark cycle) and tested between 8:00-12:00 a.m. to avoid chronobiological influences. During acclimation, rats received standardized pellet feed and monitored water ad libitum. On the day of the experiment, food and water were withheld.

Protocol of the research

The researches were conducted under controlled laboratory conditions at the CEMEX - Advanced Research and Ostin C. Mungiu Development Center for Experimental Medicine of the Grigore T. Popa University of Medicine and Pharmacy from Iași, ensuring standardized environmental parameters and animal care throughout the study.

For the experiment, nine groups of five animals each were used, receiving intraperitoneal administrations every 12 hours for three days at the same volume as follows: Group 1 (coded C): saline solution; Group

2 (KET): ketoprofen 15 mg/kg; Group 3 (DCF): diclofenac 15 mg/kg; Group 4 (CFX): cefuroxime 20 mg/kg; Group 5 (LFC): levofloxacin 5 mg/kg; Group 6 (KET+CFX): ketoprofen 15 mg/kg + cefuroxime 20 mg/kg; Group 7 (KET+LFC): ketoprofen 15 mg/kg + levofloxacin 5 mg/kg; Group 8 (DCF+CFX): diclofenac 15 mg/kg + cefuroxime 20 mg/kg; Group 9 (DCF+LFC): diclofenac 15 mg/kg + levofloxacin 5 mg/kg.

The carrageenan-induced hind paw inflammation model in rats is recognized as one of the most widely used and well-standardized experimental methods for studying acute inflammation (1, 2, 11), providing valuable insights into inflammatory mechanisms and serving as an effective tool for evaluating substances with potential anti-inflammatory activity. Before administering the irritant, animals were locally anesthetized with 20% benzocaine spray to minimize discomfort. Subsequently, 0.1 mL of 1% carrageenan solution (w/v in saline solution) was injected subcutaneously into the plantar surface of the right hind paw using a fine 27G needle. Paw volume measurements were used as objective indicators of edema, complemented by clinical observation of redness, swelling, and pain-related behaviors. Paw volume was assessed with a plethysmometer (Ugo Basile) to establish baseline values and then at 6, 24, and 72 hours post-inflammation induction. Volume increase was considered an objective indicator of edema intensity. Animals were also directly observed for clinical signs such as redness, local swelling, pain, and behavioral changes. Edema intensity was expressed as a percentage of relative extinction of paw edema from baseline (%EPE), calculated using the following formula (12): %EPE = [(Ve – Vb) / Vb] × 100, where Ve is the paw volume at the time of evaluation and Vb is the initial paw volume (at baseline).

Anti-inflammatory activity was evaluated by measuring the ability to reduce inflammatory paw edema and was expressed as the percentage of edema inhibition (%EI), calculated using the following formula: %EI = [(%EPEc − %EPEt) / %EPEc] × 100, where % EPEc represents the percentage increase in paw volume in the control group, and %EPEt represents the percentage increase in paw volume in the treated group. In this acute inflammation model, a significant reduction in paw volume compared to the control group was interpreted as evidence of an anti-inflammatory effect of the tested substance (13).

Ethical aspects. Our experimental research was conducted in full compliance with both national and international ethical standards governing the use of animals for scientific purposes. The experimental research protocol was reviewed and approved by the Ethics and Scientific Research Committee of the Grigore T. Popa University of Medicine and Pharmacy Iași, receiving a favorable opinion as documented by Certificate No. 553/06.03.2025 and Project Authorization No. 79/15.04.2025. All procedures strictly adhered to the principles outlined in European Directive 2010/63/EU (14) and the Romanian Law No. 43/2014 (15) concerning the protection of animals used for scientific purposes.

Statistical analysis. Experimental data were analyzed using SPSS variant 24 for Windows. Group comparisons were performed mainly with one-way ANOVA, followed by Tukey's post-hoc test when significant differences were found. Data were expressed as means ± standard deviation, with variance and interquartile range calculated.

Independent two-group comparisons used t-tests or, if assumptions were unmet, the non-parametric Kruskal–Wallis test. Pearson and Spearman coefficients assessed linear and rank correlations, respectively. Simple and multiple linear regression evaluated the influence of one or more independent variables on a dependent variable. Statistical significance was set at $p < 0.05$.

RESULTS

The intraplantar injection of 1% carrageenan in C animals induced acute local inflammation, shown by increased paw volume, redness, and paw-licking behavior. Baseline paw volume values were similar across groups ($p>0.05$), ensuring comparability and serving as a reference for %EPE and %EI calculations.

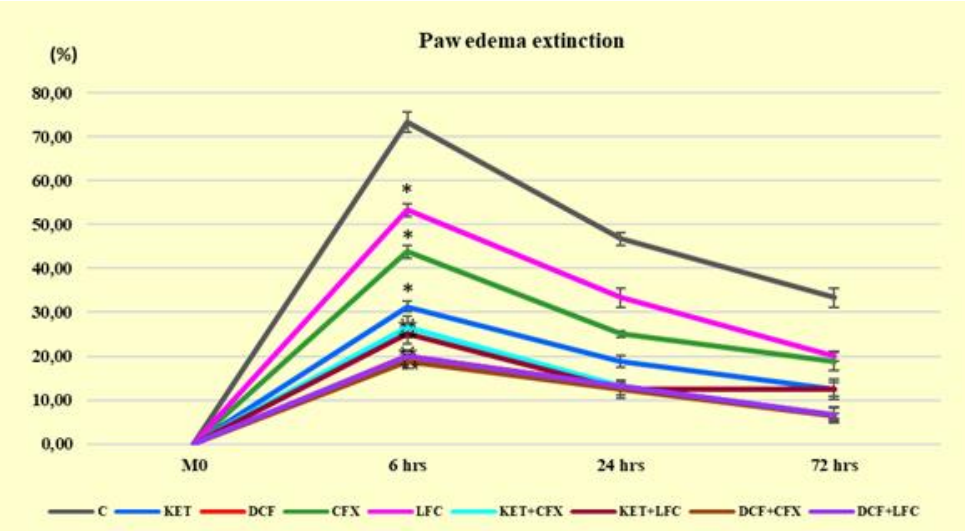

Fig. 1. The paw inflammation test - %EPE.
Each value represents the average ± S.D. (n = 5). *p<0.05, **p<0.01 *vs.* C group.

Six hours after injection, the C group exhibited a marked paw volume increase (0.26±0.04 vs. baseline 0.15±0.01), confirming edema. DCF significantly reduced edema (**p<0.01; 0.18±0.01), indicating effective prostaglandin inhibition. KET *al*so reduced edema (*p<0.05; 0.21±0.01), but less than DCF. CFX and LFC alone had no significant effect (0.23±0.02 and 0.23±0.01). Combinations DCF+CFX and DCF+LFC produced a significant edema reduction (**p<0.01; 0.19±0.01 and 0.18±0.02), similar to DCF alone, showing antibiotics did not impair anti-inflammatory activity. KET+CFX (0.19±0.01) and KET+LFC (0.20±0.01) also reduced edema (*p<0.05), though less than DCF combinations (fig. 1).

At 24 hours post-inflammation, edema in the C group remained elevated but slightly decreased from 6 hours, indicating the start of natural resolution. NSAID-treated groups showed markedly lower edema. DCF significantly reduced paw volume (**p<0.01; 0.17±0.02 vs. 0.22±0.02 in C), confirming its efficacy in the intermediate phase. KET *al*so reduced edema (*p<0.05; 0.19±0.03), though less than DCF (fig. 1). CFX alone caused a modest but significant reduction (*p<0.05; 0.20±0.01), while LFC showed a non-

significant decrease. Combinations KET+CFX and DCF+LFC produced significant edema reduction (**p<0.01; 0.17±0.01), with CFX enhancing KET's effect, while LFC did not influence DCF. KET+LFC (0.18±0.02) and DCF+CFX (0.18±0.01) also significantly reduced edema (*p<0.05), suggesting additional potentiation compared to NSAID monotherapy (fig. 1).

At 72 hours post-carrageenan, edema in the C group decreased compared to 6 and 24 hours but remained above baseline, indicating partial persistence of inflammation. NSAID-treated groups, maintained edema reduction. DCF significantly reduced edema (**p<0.01; 0.18±0.02 vs. 0.20±0.01 in C), showing long-lasting anti-inflammatory efficacy. KET *al*so reduced edema (*p<0.05; 0.18±0.02), though less than DCF (fig. 1).

CFX and LFC alone had no significant effect (0.19±0.01 and 0.19±0.02). Combinations KET+CFX and DCF+LFC significantly decreased edema (**p<0.01; 0.16±0.01), with CFX enhancing KET's effect, while LFC did not clearly affect DCF. DCF+CFX also caused a substantial reduction (**p<0.01; 0.17±0.01), whereas KET+LFC showed a decrease (0.18±0.02) without statistical significance (fig. 1).

The analysis of %CE showed the time course of inflammation at 6, 24, and 72 hours. In the C group, %CE peaked at 6 hours, indicating the acute phase, partially decreased at 24 hours, and further declined by 72 hours, though remaining above baseline.

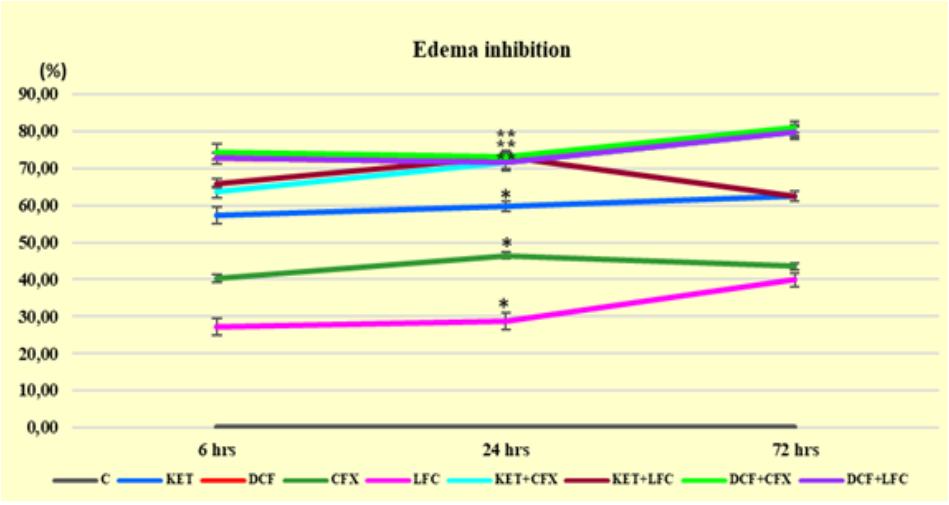

Fig. 2. The paw inflammation test - %EI.
Each value represents the average ± S.D. (n = 5). *p<0.05, **p<0.01 *vs.* C group.

NSAID-treated groups showed significant %CE reductions. KET caused the strongest decrease (**p<0.01 at 6 hours) and maintained significant inhibition at 24 and 72 hours, demonstrating sustained anti-inflammatory activity. DCF also reduced %CE (*p<0.05 at 6 and 24 hours), but less than KET, with a smaller effect at 72 hours.

CFX and LFC alone did not significantly alter %CE, confirming no inherent anti-inflammatory effect. NSAID-antibiotic combinations mirrored NSAID effects:

KET+CFX and KET+LFC matched KET *al*one, while DCF+CFX and DCF+LFC were similar to DCF monotherapy. Overall, KET showed the strongest anti-inflammatory profile, DCF a moderate effect, and antibiotics alone had no impact.

The determination of the %EI allowed a comparative assessment of the anti-inflammatory efficiency of the tested substances relative to C group. The %EI graph provides a direct visualization of the effectiveness of the tested treatments. NSAIDs exhibited the highest inhibition values, with a consistent effect throughout the 72-hour period. DCF stood out through its superior, rapidly established, and sustained inhibition, confirming its strong anti-inflammatory potency. KET *al*so produced a significant inhibitory effect, though slightly lower compared to DCF (fig. 2). Antibiotics administered alone showed low %EI values, close to those of C group, with only a slight influence observed for CFX at 24 hours, suggesting that these substances do not exert a relevant anti-inflammatory effect in this model. The combinations demonstrated inhibition comparable to that achieved with NSAIDs alone, indicating that antibiotics do not diminish anti-inflammatory efficacy (fig. 2). In the case of the KET+CFX combination, an amplification of the inhibitory effect was observed, whereas other associations had a less evident impact. In conclusion, the %EI confirms the major role of NSAIDs, particularly DCF, in limiting inflammation and suggests that some combinations, especially KET+CFX, may enhance the anti-inflammatory response.

DISCUSSION

The present study investigated the temporal profile of carrageenan-induced paw edema in Wistar rats and compared the anti-inflammatory effects of the NSAIDs, KET, and DCF, administered alone or in combination with two antibiotics of distinct pharmacological classes: the fluoroquinolone LFC, and, and the cephalosporin, CFX.

The carrageenan model produced the expected biphasic inflammatory response, an initial exudative phase followed by a proliferative cellular phase, confirming its reliability as a standard model for evaluating anti-inflammatory agents. The inflammation developed progressively, reaching its maximum intensity 6 hours after administration of the chemical irritant, after which it began to gradually subside, though still persisting at 72 hours of the experiment. These results are consistent with data reported in the literature regarding the evolution of carrageenan-induced paw edema in rats (16, 17).

Quantitative paw volume measurements, complemented by clinical observations such as redness, swelling, and pain, enabled a comprehensive assessment of the inflammatory response. Carrageenan administration elicited rapid edema formation, peaking between 3 and 6 hours and progressively declining over 72 hours, in agreement with the classic pattern described in the literature (18, 19). Both NSAIDs significantly reduced paw edema, confirming their capacity to suppress acute inflammation via COX inhibition and the consequent reduction in prostaglandin synthesis (4, 20). DCF demonstrated greater potency and a more sustained anti-inflammatory effect than KET, in line with previous evidence highlighting its higher COX-2 selectivity and prolonged pharmacodynamic action (21).

LFC showed a modest anti-inflammatory influence, particularly during the later stages of the inflammatory process (24-72 hours). This finding supports reports that fluoroquinolones possess secondary immunomodulatory actions independent of their antimicrobial properties. Prior studies have

demonstrated that LFC can downregulate the production of pro-inflammatory cytokines such as TNF-α and IL-6, modulate nitric oxide synthesis, and reduce neutrophil activity (22). These mechanisms may explain the delayed yet perceptible reduction in edema observed in the present study, suggesting that LFC could indirectly contribute to inflammatory resolution through modulation of immune mediators. In contrast, CFX exerted minimal influence on the inflammatory process, consistent with the general pharmacological characteristics of β-lactam antibiotics, which act primarily through inhibition of bacterial cell wall synthesis and lack direct anti-inflammatory or immunomodulatory effects. Importantly, CFX did not interfere with the anti-inflammatory activity of the NSAIDs, indicating pharmacological compatibility and safety in combined administration.

When NSAIDs were administered together with the antibiotics, the overall anti-inflammatory efficacy was maintained or slightly enhanced, suggesting potential additive or complementary effects. The observed trends may reflect the combined modulation of distinct inflammatory pathways, with NSAIDs acting primarily through prostaglandin suppression and fluoroquinolones exerting secondary effects on cytokine and oxidative stress regulation.

Overall, these findings reaffirm the potent and sustained anti-inflammatory efficacy of classical NSAIDs and highlight the potential modulatory role of fluoroquinolones such as LFC in the inflammatory process. CFX did not display intrinsic anti-inflammatory properties but was pharmacologically compatible with NSAID therapy. Collectively, the results support further research into the molecular mechanisms underlying the interactions between anti-inflammatory agents and antimicrobial drugs, particularly in pathological conditions involving both infection and inflammation.

Combination therapy with NSAIDs and antibiotics resulted in variable but generally additive or synergistic anti-inflammatory effects. The enhanced efficacy observed in treatments such as KET+CFX and DCF+LFC may arise from complementary mechanisms, NSAIDs mitigating prostaglandin-mediated vasodilation and nociception, while certain antibiotics modulate cytokine expression or oxidative pathways (22). Notably, antibiotic co-administration did not compromise NSAID efficacy, underscoring their potential compatibility and therapeutic advantage in managing conditions that involve both infection and inflammation. These outcomes are consistent with previous experimental findings demonstrating improved anti-inflammatory responses when fluoroquinolones are combined with NSAIDs (23).

The consistent superiority of DCF over KET throughout the experimental period aligns with pharmacodynamic evidence that DCF achieves higher tissue penetration and sustains COX inhibition for a longer duration (6, 24). Furthermore, the persistence of NSAID activity over 72 hours reinforces the reliability of the carrageenan model in discriminating between drug potency and duration of action.

These results reinforce existing evidence of the robust anti-inflammatory properties of classical NSAIDs while revealing that certain antibiotics, particularly CFX, may exert modulatory effects capable of enhancing or prolonging NSAID-mediated responses. This observation supports growing evidence that fluoroquinolones can influence host inflammatory pathways and highlights the need for further investigation into their potential role as adjuncts in the management of

inflammatory conditions (25).

By integrating the quantitative and qualitative assessments, this research provides a comprehensive evaluation of both classical anti-inflammatory agents and the potential modulatory effects of antibiotics, highlighting the translational significance of these findings for the development of more effective and targeted therapeutic strategies for managing acute inflammation. The results have the potential to inform future clinical applications, optimize drug combinations, and advance our understanding of multimodal approaches to controlling inflammatory responses.

The carrageenan-induced inflammation model proved reliable for studying acute inflammation. NSAIDs, especially DCF, demonstrated consistent and strong anti-inflammatory effects, while antibiotics, particularly CFX, showed possible modulatory influence when combined with NSAIDs. These findings highlight that antibiotics may enhance or modify NSAID-mediated anti-inflammatory responses, opening perspectives for further investigation into their combined therapeutic potential.

CONCLUSIONS

Our research showed that the tested NSAIDs significantly reduced edema throughout the experimental period, with DCF demonstrating greater and more sustained efficacy than KET. Antibiotics alone had minimal impact on inflammation, except for a slight late reduction observed with CFX at 24 hours. DCF maintained consistent anti-inflammatory activity when combined with antibiotics, reflecting a stable pharmacodynamic profile. In contrast, KET showed enhanced efficacy when associated with CFX, while its combination with LFC offered no additional benefit.

Overall, anti-inflammatory effects in the carrageenan-induced model were mainly driven by NSAIDs, with DCF serving as the reference for potency and persistence. Although antibiotics did not exhibit direct anti-inflammatory action, certain combinations, particularly KET with CFX, appeared to optimize therapeutic outcomes, supporting the potential value of multi-drug strategies for improved inflammation management.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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IN VIVO BIOCOMPATIBILITY EVALUATION OF NORFLOXACIN-LOADED CHITOSAN NANOFIBERS AND BORONIC ALDEHYDE-FUNCTIONALIZED DERIVATIVES IN RAT

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IN VIVO BIOCOMPATIBILITY EVALUATION OF NORFLOXACIN-LOADED CHITOSAN NANOFIBERS AND BORONIC ALDEHYDE-FUNCTIONALIZED DERIVATIVES IN RAT (Abstract): Chitosan-based nanofibers can serve as carriers for antimicrobial agents, providing controlled drug release and biocompatibility, which are crucial for tissue integration and healing. This study **aimed** to evaluate the *in vivo* biocompatibility of norfloxacin-loaded chitosan nanofibers (NorC) and norfloxacin-loaded chitosan nanofibers functionalized with boronic aldehyde (NorCB), in comparison with standard wound treatments in rats. **Materials and methods:** NorC fibers were prepared via electrospinning of chitosan (CHIT) solution containing norfloxacin (Nor). NorCB fibers were obtained by imination of NorC fibers with boronic aldehyde. The study included four groups of Wistar rats: (i) a negative control group, which remained intact with no procedures performed, (ii) a positive control group with a simple incision covered by sulfadiazine-impregnated dressing as a clinically established reference for local antimicrobial protection, and (iii and iv) experimental groups with an incision covered by NorC, respectively NorCB nanoporous fibers. Biocompatibility was evaluated through clinical monitoring, as well as hematological, biochemical, and immunological blood analyses. All procedures were performed in compliance with institutional ethical guidelines for animal experimentation. **Results:** Both NorC and NorCB fibers did not impair liver or kidney function, did not induce local or systemic inflammatory responses, and preserved immune defense capacity. Laboratory parameters evaluated were comparable with those of the control groups. Functionalization with boronic aldehyde had no adverse impact on these outcomes. **Conclusions:** Nor-loaded CHIT nanofibers, with or without boronic agent functionalization, demonstrate good *in vivo* biocompatibility, maintaining organ function, immune competence, and absence of inflammatory effects. These findings support their potential as localized antibiotic delivery systems and advanced wound care materials. **Keywords:** CHITOSAN NANOFIBERS, NORFLOXACIN, BIOCOMPATIBILITY, RATS, 2-BORONOBENZALDEHYDE.

INTRODUCTION

Burn injuries remain a significant global health challenge, leading to high morbidity and mortality due to infection, delayed healing, and scar formation (1). Wounds resulting from burns are particularly sus-

ceptible to microbial colonization due to the loss of the protective skin barrier, creating an ideal environment for pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (2). Effective management of burn wounds requires dressings that not only provide a physical barrier but also deliver therapeutic agents to prevent infection and support tissue regeneration (3).

Conventional topical antibiotics have been used widely in burn care, but their effectiveness can be limited by poor penetration into tissue and the development of bacterial resistance (4). Norfloxacin (Nor), a broad-spectrum fluoroquinolone antibiotic, has demonstrated potent antimicrobial activity against Gram-negative and some Gram-positive organisms implicated in burn wound infections (5). Its mechanism of action involves inhibition of bacterial DNA gyrase and topoisomerase IV, which are essential for DNA replication (6). However, systemic use of Nor can be associated with side effects and suboptimal local concentrations, leading to interest in localized delivery strategies (7).

Advanced wound dressings based on biodegradable polymers have been developed to overcome these limitations by enabling sustained local delivery of antibiotics directly at the wound site (8). Among these polymers, CHIT, a deacetylated derivative of chitin, has attracted considerable attention due to its inherent biodegradability, biocompatibility, and intrinsic antimicrobial and hemostatic properties (9). CHIT can also promote cell proliferation and tissue regeneration, making it an attractive matrix for wound healing applications (9).

Electrospun CHIT nanofibers provide a structural architecture that mimics the extracellular matrix, enhancing cell attachment and facilitating gas exchange while also serving as a reservoir for drug loading and sustained release (10). The incorporation of Nor into CHIT nanofibers has been shown to improve localized antimicrobial efficacy and reduce infection in animal models of wound healing (11). Furthermore, CHIT-based nanofiber dressings have demonstrated improved outcomes compared to conventional gauze or hydrocolloid dressings in terms of infection control and accelerated re-epithelialization (12

Despite progress in the development of polymeric wound dressings, there remains a need for further optimization of antibiotic delivery systems that balance controlled drug release with minimal systemic exposure and maximal therapeutic effect at the injury site (13). By integrating advanced polymer science with antimicrobial therapeutics, innovative biomaterials such as Nor-loaded CHIT nanofibers hold promise for improving burn wound management and patient outcomes.

The **aim** of this research was to evaluate biodegradable CHIT-based nanoporous systems loaded with Nor for topical delivery, assessing their *in vivo* biocompatibility, biodegradability, and potential to provide sustained antimicrobial and anti-inflammatory effects.

MATERIALS AND METHODS

Substances. Norfloxacin (≥98%), Chitosan (126 kDa, 97%), poly(ethylene oxide) (1000 kDa), acetic acid (≥99%), 2-boronobenzaldehyde (97%), ethyl alcohol (98.89%), sodium hydroxide (95%) were purchased from Sigma-Aldrich Chemical Co (Steinheim, Germany). 2-boronobenzaldehyde (boronic aldehyde) was purified by column chromatography, and ethanol was dried over molecular sieves prior

to use.

Preparation of composite nanofibers. The composite nanofibers were prepared through a multistep process that included the fabrication of mesoporous CHIT fibers, the incorporation of Nor, and subsequent surface functionalization via imine bond formation with boronic aldehyde.

CHIT was initially subjected to alkaline hydrolysis according to established protocols reported in the literature. Porous CHIT-based fibers were subsequently produced by electrospinning a 2.1% (w/v) chitosan/poly(ethylene oxide) blend (2:1, w/w) prepared in 80% acetic acid, using a NanoSpinner StarterKit. The poly(ethylene oxide) fraction, serving as a sacrificial component, was selectively removed by immersion in 5% sodium hydroxide solution for 2 hours. The resulting fibers were thoroughly rinsed with distilled water until neutral pH was achieved, leading to the formation of a porous fibrous network. Drug incorporation was carried out by equilibrium absorption. In brief, around 400 mg of the prepared fibers were placed in 500 mL of a saturated Nor solution and kept under sealed conditions for 24 hours to allow loading.

Following drug uptake, the fibers were recovered and allowed to dry at ambient conditions, followed by further drying under vacuum. The amount of incorporated antibiotic was determined by UV-Vis spectroscopy, using absorbance readings at 272 nm and quantification based on a previously established calibration curve.

For surface modification, the drug-loaded fibers were treated with a 0.28% ethanolic solution of boronic aldehyde (10 mL per 305 mg of fibers), maintaining a glucosamine-to-aldehyde molar ratio of 10:1. This treatment promoted the formation of dynamic imine bonds on the fiber surface.

FTIR measurements were performed to examine the chemical structure of the nanofibers and to verify the presence of newly formed interactions. Spectra were acquired in ATR mode using a VERTEX 70 FT-IR spectrophotometer (Bruker, Karlsruhe, Germany) over the 4000–600 cm^{-1} range, with 32 scans recorded at a resolution of 4 cm^{-1}. The resulting data were processed using OPUS 6.5 software.

Two distinct nanofibrous systems were thus obtained: NorC, consisting of CHIT fibers loaded with Nor, and NorCB, representing the corresponding fibers additionally modified through imination with the boronic aldehyde.

In vivo **biocompatibility evaluation.** The study was conducted using healthy, pathogen-free, non-genetically modified Wistar rat (300–350 g), obtained from the university bio base and originally sourced from the Cantacuzino National Medico-Military Institute for Research and Development. Prior to experimentation, the animals were allowed a 24-hour acclimatization period and were housed individually under controlled environmental conditions (21±2°C, 50-70% relative humidity, 12 h light/dark cycle), with ad libitum access to standard diet and water. To minimize chronobiological variations, all experimental procedures were consistently performed between 08:00 and 12:00.

The experimental protocol comprised four groups: a negative control group (Negative C), with no intervention; a positive control group (Positive C), in which a standardized incision in the right flank region was treated with a sulfadiazine-

containing dressing as a reference for antimicrobial protection; and two experimental groups in which a similar-sized incision was performed in the same anatomical area (right flank), followed by application of NorC and NorCB nanofibers, respectively, as dressings.

In vivo biocompatibility was evaluated through the analysis of hematological, biochemical, and immunological parameters. Biochemical markers included liver function indicators (alanine aminotransferase – ALT, aspartate aminotransferase – AST, and lactate dehydrogenase – LDH) and renal function markers (urea and creatinine). Hematological assessment comprised leukocyte differentials, including polymorphonuclear neutrophils (PMN), lymphocytes (Ly), monocytes (M), eosinophils (E), and basophils (B). Additionally, immune function was assessed by measuring complement activity and the phagocytic capacity of peripheral blood PMN (NBT%), providing insight into potential systemic toxicity and immune response following treatment.

Blood samples were collected from the lateral tail vein. To minimize stress, animals were gently restrained, and the tail was briefly warmed to promote vasodilation. After local disinfection, approximately 0.3 mL of blood was drawn for analysis. Sampling was performed at two defined time points: 24 hours and 7 days after the surgical procedure and application of the test materials.

Ethical aspects. The experiments were carried out at the CEMEX Laboratory - Advanced Research and Development Center for Experimental Medicine Ostin C Mungiu, Grigore T. Popa University of Medicine and Pharmacy, Iași, following approval from the University Ethics Committee (Certificate No. 160/04.03.2022) and in strict accordance with national regulations (15) and international guidelines on the use of laboratory animals (16).

At the end of the experiments, the animals were humanely euthanized under general inhalation anesthesia with isoflurane (3% induction) using a veterinary open-circuit system (Komesaroff Mark 5, Kruuse, Odense, Denmark).

Statistical analysis. Data were analyzed using SPSS version 17 (IBM Corp., Armonk, NY, USA). Group comparisons were conducted primarily with one-way ANOVA, followed by Tukey's post hoc test when significant differences were observed. Results are reported as mean ± standard deviation (S.D.).

For comparisons between two groups, Student's t-test was applied, or the nonparametric Kruskal-Wallis test when assumptions of normality or homogeneity of variance were not met. Correlations were evaluated using Pearson's coefficient for parametric data and Spearman's rank correlation for nonparametric data. Simple and multiple linear regression analyses were performed to assess the influence of independent variables on dependent outcomes. Statistical significance was defined as $p < 0.05$.

RESULTS

Porous CHIT-based nanofibers incorporating Nor at a concentration of 4.35% were successfully obtained, alongside porous CHIT nanofibers loaded with Nor and subsequently iminated with 10% boronic aldehyde (17). The antibiotic was introduced into the fibers by immersion, ensuring an effective loading process. Further

surface functionalization was achieved through the formation of dynamic imine bonds with a bioactive boronic aldehyde, reaching an imination degree of 10%, which provides a suitable balance between antimicrobial activity and biocompatibility. FTIR analysis confirmed the successful incorporation and good encapsulation of the antibiotic within the nanofibrous matrices (17).

Direct visual inspection of the incision site covered with the investigated dressings did not reveal any visible local changes. No signs of inflammation, redness, edema, or other macroscopic alterations were observed.

TABLE I.

The effects of NOR-loaded nanoporous systems on the percentage values of leukocyte formula components.

	Time points of evaluation	Leukocyte formula components (%)				
		PMN	Ly	E	M	B
Negative C	24 hours	27.8±4.21	68.2±7.67	0.3±0.01	3.5±0.2	0.2±0.01
	7 days	27.2±3.52	68.6±8.13	0.4±0.01	3.6±0.2	0.2±0.03
Positive C	24 hours	27.3±2.67	68.7±7.21	0.3±0.03	3.5±0.1	0.2±0.01
	7 days	27.8±3.55	68.2±7.45	0.4±0.01	3.4±0.1	0.2±0.01
NorC	24 hours	26.8±4.27	69.1±8.33	0.5±0.1	3.6±0.1	0.2±0.03
	7 days	27.1±3.45	68.9±8.17	0.4±0.03	3.4±0.2	0.2±0.01
NorCB	24 hours	26.7±4.17	69.3±8.43	0.3±0.1	3.5±0.1	0.2±0.03
	7 days	27.2±4.25	68.7±7.67	0.3±0.05	3.6±0.1	0.2±0.01

Values are expressed as the arithmetic mean ± S.D. of the mean for five animals per group.

The distribution of PMN and Ly in blood samples from animals treated with NorC and NorCB remained consistent with that observed in both the Negative Control and Positive Control groups across all time points assessed (tab. I).

Likewise, the relative proportions of E, M and B in these treatment groups showed no significant deviations from the values recorded in the control groups throughout the duration of the experiment (tab. I). These results indicate that administration of the Nor-loaded nanoporous systems did not disrupt leukocyte homeostasis or induce detectable alterations in the overall composition of circulating immune cells.

Administration of the Nor-loaded nanoporous systems did not produce statistically significant changes in plasma levels of ALT, AST, or LDH at either 24 hours or 7 days post-treatment (tab. II). The measured enzyme levels in these groups remained comparable to those observed in both the Negative Control and Positive Control groups, indicating that the nanoparticle formulations did not induce detectable hepatocellular injury or general cytotoxicity within the observed timeframe.

No significant changes were observed in plasma urea or creatinine levels in animals treated with NorC and NorCB compared with both the Negative Control and Positive Control groups at any of the evaluated time points (tab. III). These findings suggest that administration of the Nor-loaded nanoporous systems did not impair renal function or induce detectable nephrotoxicity during the course of the experiment.

TABLE II.

The effects of Nor-loaded nanoporous systems on the blood values of ALT, AST, and LDH

	Time points of evaluation	ALT (U/L)	AST (U/L)	LDH (U/L)
Negative C	24 hours	46.82±5.33	122.24±22.29	1289.24±115.17
	7 days	49.11±4.67	123.46±20.17	1291.45±121.21
Positive C	24 hours	48.37±4.55	121.62±21.52	1292.18±122.43
	7 days	48.55±4.41	123.18±23.67	1288.35±120.11
NorC	24 hours	47.26±4.17	122.34±22.21	1290.12±117.07
	7 days	48.38±5.82	124.54±22.45	1294.25±119.55
NorCB	24 hours	47.42±5.21	120.12±21.33	1287.42±122.82
	7 days	48.16±4.63	123.28±22.19	1293.37±118.67

Values are expressed as the arithmetic mean ± S.D. of the mean for five animals per group.

TABLE III.

Effects of Nor-loaded nanoporous systems on serum urea and creatinine levels

	Time points of evaluation	Urea (mg/dL)	Creatinine (mg/dL)
Negative C	24 hours	41.28±5.17	0.15±0.01
	7 days	42.42±4.43	0.16±0.03
Positive C	24 hours	42.64±4.55	0.16±0.01
	7 days	43.36±4.21	0.15±0.01
NorC	24 hours	43.25±4.13	0.16±0.01
	7 days	43.52±5.07	0.17±0.01
NorCB	24 hours	43.16±3.67	0.16±0.01
	7 days	44.38±4.11	0.16±0.03

Values are expressed as the arithmetic mean ± S.D. of the mean for five animals per group.

TABLE IV.

Effects of Nor-loaded nanoporous systems on serum complement activity and the NBT%

	Time points of evaluation	Complement (UCH_{50})	NBT (%)
Negative C	24 hours	51.34±4.45	13.34±1.43
	7 days	52.46±4.21	14.62±1.67
Positive C	24 hours	51.72±5.17	13.74±2.17
	7 days	53.68±4.55	14.26±1.82
NorC	24 hours	52.24±4.43	13.68±2.21
	7 days	53.18±5.15	13.46±1.29
NorCB	24 hours	53.25±4.33	14.24±1.41
	7 days	53.52±4.67	14.12±1.55

Values are expressed as the arithmetic mean ± S.D. of the mean for five animals per group.

In animals treated with NorC and NorCB, serum C levels showed no significant changes compared to the Negative Control or Positive Control groups. Measurements at both 24 hours and 7 days post-treatment were comparable across all groups, indicating that the treatments did not affect C activity (tab. IV). Similarly, the NBT% values, which reflect the oxidative burst capacity of neutrophils, remained similar to those of the control groups at both time points. Overall, these findings suggest that NorC and NorCB treatments did not induce detectable effects on these immune-related blood parameters (tab. IV).

DISCUSSION

Due to its unique biochemical features and natural ability to act against bacteria, chitosan has been widely studied for use as a wound item that brings support against infections. The successful fabrication of porous CHIT-based nanofibers incorporating Nor, as well as fibers further functionalized with boronic aldehyde, highlights the versatility and potential of this system for localized, controlled antibiotic delivery. The combination of efficient Nor loading and surface imination appears to provide an optimal balance between antimicrobial efficacy and biocompatibility, which is critical for wound healing applications.

Scaffolds help keep cells at the wound area and support them in growing, moving, and changing into different types of cells inside their three-dimensional structures. Effective wound-healing scaffolds must exhibit biocompatibility, biodegradability. Among the scaffolds, nanofiber scaffolds have become very popular because they have a large surface area compared to their size, lots of tiny spaces, and the ability to carry different types of substances.

The stable incorporation of Nor within the nanofibrous matrices suggests that the fibers can act as reservoirs for sustained drug release, providing a prolonged therapeutic effect at the wound site. This sustained release is particularly advantageous for minimizing dosing frequency and reducing systemic exposure, thereby lowering the risk of systemic toxicity. The controlled functionalization of the fiber surface with boronic aldehyde may further enhance local bioactivity by promoting favorable interactions with tissue components or modulating the release profile, without triggering adverse inflammatory or cytotoxic responses.

The absence of visible local inflammation, edema, or macroscopic tissue alterations in the treated regions strongly supports the compatibility of these materials with biological environments. This observation indicates that the fibers are well tolerated by surrounding tissues and unlikely to provoke detrimental immune reactions.

In vivo evaluation demonstrated that Nor-loaded nanoporous CHIT systems maintain excellent biocompatibility and biodegradability when applied directly over rat lesions. Importantly, no significant alterations in hematological, biochemical, or immunological parameters were observed, underscoring minimal systemic toxicity and confirming a favorable safety profile for these materials as localized drug delivery platforms (18, 19).

CHIT, a naturally biodegradable and biocompatible polymer, has been extensively employed in wound healing and drug delivery due to its intrinsic non-toxic nature, mucoadhesive properties, and capacity to

support cellular adhesion, proliferation, and tissue regeneration (20). The high surface area, porosity, and interconnectivity of nanofibrous CHIT matrices not only facilitate efficient loading of therapeutic agents such as Nor but also allow for controlled and sustained release, which can be further tuned through surface functionalization.

Previous studies have delineated that Nor-loaded CHIT nanofibers enhance local antimicrobial activity while maintaining compatibility with host tissues (17, 21), consistent with the biological responses observed in our study.

These findings collectively indicate that CHIT-based nanofibers can provide an effective combination of localized antibiotic therapy, tissue regeneration support, and minimal adverse responses, positioning them as promising candidates for advanced wound management and targeted drug delivery applications (22, 23).

The absence of significant systemic inflammation or organ dysfunction in our experimental animals is consistent with previous studies in which electrospun CHIT/Nor scaffolds did not alter liver or kidney function markers in treated rats. This suggests that the embedding of Nor within a CHIT matrix does not interfere with systemic immune homeostasis or provoke adverse organ-specific effects, which is critical when considering potential clinical applications (22, 23). The maintenance of stable hematological and biochemical parameters indicates that the localized release of Nor from the nanofibers provides effective antimicrobial action at the wound site without triggering systemic inflammatory responses or organ stress.

Beyond systemic safety, CHIT nanofibers loaded with Nor have been reported to promote wound healing and tissue regeneration while simultaneously reducing infection and local inflammation (23, 24). These effects are likely mediated by a combination of the intrinsic biocompatibility and mucoadhesive properties of CHIT, which support cellular adhesion, proliferation, and extracellular matrix deposition, together with the sustained local release of Nor, which limits bacterial colonization and inflammatory signaling. The biological responses observed in our study are in strong agreement with those previously reported, reinforcing the concept that CHIT-based antibiotic delivery systems can integrate safely with mammalian tissues and actively contribute to the wound healing process.

A key aspect of this system is the controlled release of Nor from the biodegradable polymer matrices. By modulating the porosity, fiber diameter, and surface chemistry of the CHIT nanofibers, drug release can be sustained over time, maximizing local antimicrobial effects while minimizing systemic exposure and the associated risks of toxicity or dysbiosis. This controlled delivery approach ensures that therapeutic concentrations of Nor are maintained at the wound site for prolonged periods, improving infection control and reducing the need for repeated dosing.

Our observations corroborate previous reports (17, 25), demonstrating that CHIT-based nanofibers can maintain the therapeutic efficacy of Nor without eliciting systemic toxicity, thereby supporting their potential as safe and effective platforms for localized antibiotic therapy in clinical settings.

CONCLUSIONS

The present study demonstrates that NorC and NorCB, exhibit excellent *in vivo*

biocompatibility after administration in rats. Covering the skin lesion with these nanofibers did not impair liver or kidney function, did not trigger local or systemic inflammatory responses, and preserved immune defense capacity. The addition of boronic aldehyde did not negatively affects these outcomes. When compared to standard wound treatments, including a sterile dressing and a sulfadiazine-impregnated dressing as a positive control, NorC and NorCB fibers showed comparable safety and tolerance.

These findings support the potential of CHIT-based nanofibers as localized antibiotic delivery systems for advanced wound care, offering controlled release of Nor while maintaining tissue compatibility and systemic safety. Furthermore, the previously established fabrication and characterization of these fibers by our research team provide a validated foundation for their further preclinical and translational evaluation.

NorC and NorCB nanofibers represent promising candidates for innovative wound management strategies, combining antimicrobial efficacy with biodegradability, biocompatibility, and immune safety.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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DNA EXTRACTION FROM PLAIN SALIVA: ADVANTAGES AND LIMITATIONS

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DNA EXTRACTION FROM PLAIN SALIVA: ADVANTAGES AND LIMITATIONS (Abstract): Sufficient and good quality DNA can be extracted from a variety of biological samples. In most cases, peripheral blood is the preferred source of cells. As blood harvesting is an invasive method that should be performed by trained personnel and in designated facilities, alternative DNA sources are increasingly considered. Saliva represents an important choice, and this is reflected by the growing number of commercially available saliva collecting kits. Besides being extremely easy to use, ship and store, such kits include various stabilizers and hence offer the advantage of remarkable stability of the product. However, the cost of such devices can be prohibitive. Under the circumstances, we have used plain saliva for DNA extractions. Manual extraction using silica columns and magnetic-based automated extraction were both used and compared in terms of DNA yield and purity. The DNA integrity and potential contamination with non-human DNA was also checked in human based primers PCR reactions. Furthermore, the saliva stability was checked by incubating the samples for various periods of time. We were thus able to demonstrate that plain saliva can generate adequate amounts of pure DNA, which can be used successfully in molecular biology techniques. However, in the absence of stabilizers, the samples' stability is limited, hence they should not be stored for more than 24 hours at 4°C and they should be used for extraction within this interval. **Keywords:** PLAIN SALIVA, BLOOD DNA EXTRACTION, PCR, SALIVA STABILITY.

DNA is a biological material required nowadays for an increasing number of investigations. As a vast set of molecular biology techniques require DNA, it is not surprising that we witness a continuous development of various DNA extraction methods, able to ensure not only sufficient amounts, but also able to avoid contamination risks, thus yielding good quality material that will further generate accurate and reliable results.

A constant interest could be noticed in the recent years for the development of various methods of DNA extraction from saliva, reflected by the number of commercial kits offered by various manufacturers. Saliva is considered as an important alternative to the classical invasive approaches targeting peripheral blood or, sometimes, solid tissues. Most of the laboratories involved in molecular biology investigation of human DNA, dealing either with patients

or with healthy persons, as notably is the case for the stem cell registries, or healthy solid organ donors, prefer the peripheral blood and there are solid arguments favoring this choice. The nucleated cells within the blood stream are generally present in sufficient numbers to offer the possibility to extract good quality DNA, in reasonable amounts, that can be further directly used for most regular techniques such as PCR (Polymerase Chain Reaction) amplifications, hybridizations, sequencing or digestions. Furthermore, both blood harvesting and blood DNA extraction are long time standardized, guidelines and standard operating procedures being clearly implemented as part of accreditation procedures (1). However, harvesting peripheral blood is an invasive method, coming with certain risks of hemorrhage and infection, lack of compliance on behalf of certain patients or harvesting difficulties when veins are very small (as is the case for children), difficult to access or fragile. The procedure must be executed by specialized personnel, in adequate facilities. If such a facility is different from the laboratory that performs the DNA extraction, then transportation issues occur. Blood shipment is also strictly regulated and requires, for the biological product stability, temperature and adequate packaging conditions that not all transporters are able to fulfill, especially when samples must travel long distances to the destined center (2).

These are, in fact, some of the major reasons that lead to the pressure of identifying alternative, non-invasive methods of nucleic acids extraction. Saliva was one of the first choices as the harvesting is extremely comfortable for most individuals, and easily accepted especially by children. Furthermore, the procedure not only that it does not require medical assistance, but it can even be performed at home.

It is important to stress that cells are present in abundant amounts in the salivary fluid. Some of these cells are desquamated epithelial cells and it was considered for long time that, since they are considerably numerous (approximately $4x10^5$/mL and they continuously replenished due to the fast turn-over that renews at every 3 hours the surface layer (3), the epithelial cells should also represent the main source of DNA (4).

However, we were able to show that commercial DNA extraction kits dedicated for saliva are in fact targeting leukocytes (5) that are directed by the immune system to the oral cavity to efficiently fight against the remarkable number of pathogens that reside here (6). Our data demonstrated striking similarities between the DNA concentrations generated by peripheral blood and saliva, which allowed us to reinforce the existent parallel between the number of blood and saliva leukocytes (5).

One of the initial major concerns regarding the extraction of nucleic acids from saliva was the potential contamination of the human material especially with bacterial DNA, due to the vast presence of these pathogens in the oral cavity. Numerous studies, including ours, clearly demonstrated that the commercial harvesting kits, regularly including reagents dedicated to removing pathogens and to stabilizing the DNA, are designed such as they can generate pure enough human material, that can be successfully used in large array of techniques, from simple PCR amplifications to the sophisticated High throughout Sequencing (5, 7-10).

Another key issue that manufacturers constantly stress is the remarkable stability of the saliva fluid, and data included in the commercial leaflets show that after an in-

cubation of 187 days, at 50^0C, saliva was still able to generate non-degraded DNA, due to the stabilizers included in the harvesting kits (11).

Despite the many obvious advantages regarding non-invasiveness, DNA yield, less stringent transportation conditions, superior stability of the sample as compared to blood, saliva harvesting is still not a method of choice for most of our laboratories. Besides the lack of trust, or lack of awareness, one possible explanation resides in the relatively high cost of the commercial harvesting devices.

Under these circumstances, we have decided to attempt DNA extraction from plain saliva, harvested in regular 15 mL tubes, without any added buffers, and to characterize the efficiency of this procedure, mainly as the stability of the product concern.

MATERIAL AND METHODS

Saliva harvesting

Saliva and blood were harvested from healthy volunteers. After gently massaging their gums, they were asked to spit in a 15-mL tube a volume of approximately 2 mL. No other substances were further added to the plain saliva. Blood was extracted on anti-clotting agents (Na citrate or EDTA but not heparin, as we wanted to further check the DNA in various PCR reactions).

DNA extraction

Several different approaches and commercial DNA extraction kits are nowadays available. We have considered two such methods, a manual and an automated one.

For the manual method we have used the Invitrogen mini blood kit (Invitrogen, Life Technologies, Carlsbad, CA, USA), based on enzymatic cell lysis, 100% ethanol DNA precipitation, followed by the binding of the released DNA to a silica matrix in the presence of a high salt concentration solution. For both blood and saliva, we have used 200 µL of the respective sample and the DNA was eluted with 200 µL elution buffer.

For the automated method we have used the iPrep™ PureLink™ gDNA Blood Kit (Invitrogen, Life Technologies, Carlsbad, CA, USA). This system is based on 1 µm magnetic beads coated with a silica surface that enables them to bind the DNA. The beads are incubated with the mixture resulted from cell lysis and protein digestion. Further, they are then separated with a magnet, washed and then the DNA is eluted from the bead surface with 200 µL elution buffer. The protocol was optimized for a volume sample of 350 µL, the same procedure being employed for both blood and saliva.

DNA quantification

10 µL of the DNA solution were diluted with 90 µL of water and the DNA concentration was assessed by measuring the absorption at 260 nm with a Beckman Coulter DU800 spectrophotometer. Simultaneously, the potential protein contamination was assessed by measuring the absorption at 280 nm as well. A 260/280 ratio in between 1.8 and 2.1 was considered acceptable and the DNA pure enough to allow its usage for PCR amplification and hybridization.

PCR and hybridization

Some of the DNA samples were checked in PCR reactions as previously described (Cianga et al., 2016). Due to its simplicity, we have considered a commercial single pair primers BAG kit (BAG Health Care GmbH, Lich, Germany), optimized for 10 µL reaction volume. Should a positive reaction occur, besides a 1070 base

pairs (bp) control band, one should visualize a 420/85 bp amplicon as well.

RESULTS

The saliva samples were harvested from ten apparently healthy individuals in sterile tubes, with no additives, according to the procedure previously described. At the same time, blood was also harvested on an appropriate anti-clotting agent and DNA was then extracted simultaneously, immediately after harvesting. The DNA concentration and purity were checked by measuring the 260/280 nm absorption.

The blood DNA was extracted using only the manual method, while the saliva DNA was extracted using both the manual and the automated method. The results presented in Table I show that some of the saliva samples yielded higher DNA concentrations than the blood. The average DNA concentration for blood was 36.44 µg/mL (Standard Deviation SD-5.53) while for saliva was 47.43 µg/mL (SD-21.81, heavily influenced by sample #7 which generated a very small yield). We have also wanted to check the efficiency of the automated DNA extraction as compared to the manual one. As for the automated extraction the method was standardized for 350 µL instead of 200 µL, we have adjusted the values accordingly.

TABLE I.
DNA concentrations yielded by blood and plain saliva

Sample	Blood Manual extraction Concentration (µg/ml)	Saliva Manual extraction Concentration (µg/ml)	Saliva Automated extraction Concentration (µg/ml)	Saliva Automated extraction Adjusted concentration (%57) (µg/ml)
1	45.43	42.19	81.13	46.2441
2	38,.22	41.85	84.4	48.108
3	39.85	45.37	80.02	45.6114
4	38.41	36.62	63.24	36.0468
5	28.38	38,45	65.44	37.3008
6	40.55	62.61	73.28	41.7696
7	29.46	16.1	44.35	25.2795
8	38.57	89.23	102.14	58.2198
9	35.43	64.73	83.32	47.4924
10	30.12	28.18	38.12	21.7284
Average	36.44	47.43	71.54	40.78
SD	5.53	21.81	19.34	11.02

The manual and the automated method of DNA extraction from saliva show comparable efficiency, with a 47.43 µg/mL average yield for the manual method and 40.78 µg/mL average yield (after adjustment) when using the automated one, but sometimes with considerable differences between them. Furthermore, the DNA yields generated by peripheral blood and saliva are as well comparable, showing that

saliva can represent a viable alternative to blood.

The DNA purity was also assessed by measuring the 260/280 nm ratio (tab. II).

TABLE II

DNA purity and protein contamination assessed by the 260nm/280 nm ratio of absorption

Sample	Blood	Saliva Manual extraction	Saliva Automated extraction
1	1.89	1.79	1.91
2	1.95	1.92	1.95
3	1.81	1.9	1.92
4	1.95	1.94	1.96
5	1.92	1.95	2.00
6	2.06	1.96	2.1
7	1.95	1.85	1.96
8	2.04	1.94	1.98
9	1.82	1.89	1.92
10	1.96	1.87	1.91
Average	1.935	1.894	1.961
SD	0.081000686	0.070898989	0.057629082

The ratios shown in Table II demonstrate that extraction from plain saliva generates pure DNA that can be considered for various molecular biology techniques.

The saliva samples were then split in two aliquots which were incubated for a month at 4°C and at room temperature (RT) respectively. After this interval the DNA concentrations were measured again, and the results are shown in Table III.

TABLE III.

DNA concentrations after 30 days incubation

Sample	Saliva Manual extraction Day 0	Saliva Manual extraction 30 days, 4°C	Saliva Manual extraction 30 days, RT
1	42.19	15.69	8.4
2	41.85	15.31	9.31
3	45.37	16.53	9.83
4	36.62	11.96	7.05
5	38,45	10.98	9.6
6	62.61	11.66	7.34
7	16.1	12.4	8.46
8	89.23	23.54	17.88
9	64.73	14.21	8.73
10	28.18	15.47	8.49
Average	47.43	14.77	9,5
SD	21.81	3.64	3.07

The one-month incubation of the plain saliva leads to a remarkable decrease of the recorded concentrations, even if the samples were kept at 4°C. Table IV presents

the effect of the 30 days incubation upon the 260/280 ratio.

TABLE IV

DNA purity and protein contamination assessed by the 260nm/280 nm ratio of absorption after 30 days incubation

Sample	Saliva, Day 0	30 days, 4^{0}C	30 days, RT
1	1.72	1.63	1.66
2	1.92	1.78	1.8
3	1.9	1.81	1.75
4	1.94	1.66	1.66
5	1.95	1.71	1.61
6	1.96	1.7	1.66
7	1.85	1.7	1.7
8	1.94	1.92	1.90
9	1.89	1.65	1.7
10	1.87	1.81	1.71
Average	1.894	1.737	1.724
SD	0.070899	0.090927	0.107414

The 260/280 nm ratio is also dropping after a month incubation. However, somehow surprisingly, these ratios do not decrease as dramatically as the DNA concentration.

Given the considerable decrease in DNA concentration over the one-month incubation interval, we have decided to investigate a new batch of samples, which were further incubated for 72 hours, at 4°C. The extraction was performed automatically, and the concentrations are presented in Table V.

TABLE V

DNA concentrations yielded by plain saliva

	Concentration µg/ml		
Sample	**Saliva Day 0**	**Saliva, 48 h, 4°C**	**Saliva, 72 h, 4°C**
1	47.79	30.35	23.85
2	14.41	13.88	9.03
3	19.22	15.02	8.24
4	46.99	27.07	15.02
5	78	43.28	17.45
6	25.35	19.06	10.36
7	24.44	23.03	18.89
8	23.85	17.61	10.14
9	72.8	71.24	53.38
10	35.99	23.11	15.16
Average	38.84	28.36	18.15
SD	22.2	17.36	13.33

The initial DNA concentrations were again more than satisfactory, with an average yield of 38.84 µg/mL (SD-22.2), but steadily decreasing even when the saliva was incubated at 4°C.

The DNA purity was checked likewise,

and the results are presented in Table VI.

Only minor modifications of the 260/280 nm ration are observed across the 72 hours interval, thus confirming the results generated by the previous batch of samples.

To further verify the quality and integrity of the DNA extracted from plain saliva, we have chosen to use in a PCR reaction the samples harvested in the second batch and which were incubated for 48 hours at 4°C. The DNA volume used in each 10 μL PCR reaction was adjusted to an amount of 30 ng/reaction to meet the manufacturer's indications.

TABLE VI
DNA purity and protein contamination assessed by the 260nm/280 nm ratio of absorption

	260 nm/280 nm ratio		
Sample	**Day 0**	**48 h, 4°C**	**72 h, 4°C**
1	1.97	1.93	1.91
2	1.84	1.81	1.81
3	1.84	1.82	1.8
4	1.94	1.89	1.9
5	1.96	1.95	1.94
6	1.88	1.85	1.86
7	1.88	1.88	1.92
8	1.84	1.83	1.79
9	1.96	1.94	1.96
10	1.86	1.85	1.88
Average	1.897	1.875	1.877
SD	0.054579	0.05126185	0.06019413

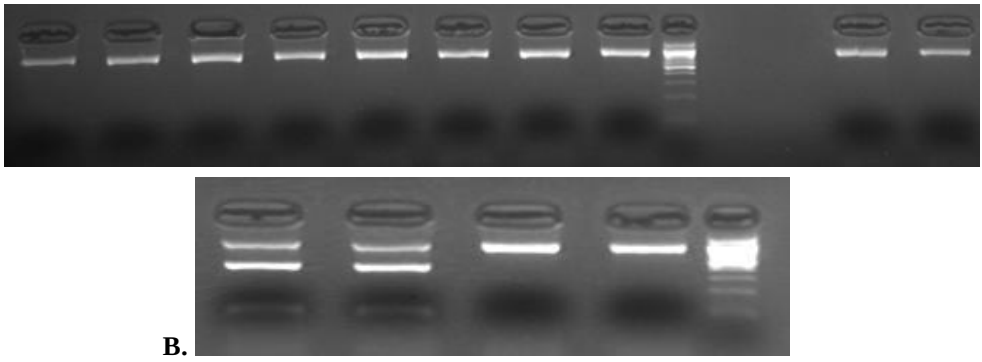

Fig. 1. A. The PCR amplification of DNA extracted from plain saliva of ten healthy volunteers, with primers for HLA-B*27, do not evidence any positive samples. **B.** PCR amplification, under the same conditions, of the DNA extracted from the peripheral blood of two patients suspected of ankylosing spondylitis and two healthy volunteers. The first two samples are positive for the HLA-B*27 allele as shown by the presence of a second amplicon besides the control band. The other two samples are negative for HLA-B*27.

DISCUSSION

DNA extraction from other sources than peripheral blood is no longer a novelty. In forensic medicine, all sorts of tissues are considered, and the extraction methods must consider an adequate digestion of the respective biological sample. For living patients, non-invasive approaches like plucked hair, cytobrush, mouthwash or the use of mucosal swabs are currently used by many molecular biology laboratories (12, 13).

The cytobrushes and the mucosal swabs are acknowledged for the high DNA yields they generate (3), but, in our hands, it proved rather cumbersome to standardize, as we have recorded important differences between the DNA concentrations from one sample to another (data not shown). Furthermore, this harvesting technique rather requires trained personnel and it is not recommended to be performed without supervision by the patients.

The mouthwash method seems more straightforward at first glance, but the washing solution has a high concentration of alcohol and this generates harvesting problems, especially with children (4).

Hence, we have opted for saliva as an alternative source of DNA and we have shown previously that the cells which are in fact targeted by most commercial kits are the leukocytes and not the epithelial cells (5). Two distinctive commercially available saliva harvesting devices were used, but both are based on the same principle of mixing the saliva with a stabilizing agent meant to prevent DNA degradation by various enzymes naturally present in this fluid and the bacterial growth. We were thus able to show that not only a comparable amount of DNA can be extracted from saliva, but also that the DNA is pure, and it can be further used in various molecular biology techniques (5).

Saliva harvesting is a non-invasive method, applicable not only for children or for persons with hardly accessible veins, but also of great use in studies that imply big cohorts of participants and which often require shipping the samples. It is often stressed that transporters are rather reluctant when dealing with blood, regarded as a potential highly infectious substance, requiring packaging; however, it seems they do not consider saliva as posing the same threat (2). Furthermore, the use of various stabilizers in all the commercially available saliva collecting devices leads (according to the manufacturers) to an impressive stability (several months), of this biological fluid, giving it an extra advantage when compared to blood (14).

However, one important downfall of this method has to do with the costs of the harvesting devices that reflect into the costs of the DNA extraction, sometimes to the point when laboratories tend to regard the method as cost ineffective. Hence, we have attempted to extract DNA from plain saliva, without adding any kind additives or stabilizers and we have investigated the stability of these samples.

Sequential DNA extractions were performed at harvesting time and after incubation of plain saliva for 48 hours, 72 hours and 30 days, either at 4oC or at room temperature. Besides confirming once more the correspondence of DNA yields obtained from blood and saliva (tab. I), our data show that the 30 days incubation of the saliva samples at RT led to a dramatic drop of the DNA yield (only 20% as compared to day 0 - tab. III), generating concentrations that would render this DNA useless for most of the commercial molecular biol-

ogy tests. The lack of adequate stabilizers, able to neutralize various enzymes, including DNases, proves to be crucial for the DNA stability. Furthermore, in the absence of stabilizers, the 260/280 nm ratio is also affected by incubation, but, as shown, to a lesser extent than the DNA yield.

As expected, the incubation at 4oC, proved to impact to a lesser extent the yields of DNA extracted under standard conditions, as shown for both batches of saliva, as well as for both DNA extraction methods (tab. III, tab. V).

An important concern addressing saliva as a source of DNA is the presence of countless microorganisms that might also contribute to the overall yield of nucleic acids extracted by one method or another (15). Numerous studies were able to demonstrate that, in fact, excellent quality human DNA can be extracted from saliva harvested with commercial devices and further used in various molecular biology techniques as PCR, hybridization, RT-PCR, PCR-RFLP, or Sanger and NGS sequencing (4, 5, 8-10, 15-17). However, the concerns raised by saliva untreated with any kind of microbicidal agent are still standing.

Nowadays, a variety of fluorochromes able to bind nucleic acids are available. Such dyes are often used to estimate the DNA concentration. However, contaminant nucleic acids can influence the fluorescent signal, either by increasing or decreasing it. This is one of the reasons why we have preferred to use spectrophotometry which is not only faster, but also more accurate. The nucleic acid shows a maximal absorption at a wavelength of 260 nm, while contaminating proteins show the maximum absorption at 280 nm. Thus, spectrophotometry can offer simultaneous information about the DNA concentration as well as about potential contamination with proteins. Furthermore, to double check the quality of the DNA extracted from plain saliva, we have used this DNA in a PCR reactions that can amplify only human DNA, due to the presence of a pair of primers specific to the HLA*B27 alleles. The results shown in Figure 1 demonstrate that the DNA we have managed to extract was not only sufficient, but also was not severely contaminated, so that it could be amplified without any problems in a regular PCR test.

As the DNA we have chosen to use in the PCR reaction was extracted from plain saliva which was incubated for 48 hours at 4oC, this also shows that the samples stored under such conditions and for this amount of time can be relatively safely used for DNA extraction. However, longer incubations at room temperature lead to DNA degradation to the point the samples cannot be used anymore for this purpose, as the yields are below the threshold set by most of commercial kits.

CONCLUSIONS

Plain saliva can thus represent a viable alternative to peripheral blood from several points of view. It is relatively easy to harvest, it does not require qualified personnel for the procedure and it can be performed at home. In other words, patients are not forced to present themselves into a specialized medical facility and can eventually ship the samples by mail. This aspect is of importance when dealing with large cohort studies. Also, given the fact that it is a non-invasive method, the patients' compliance is superior and their acceptance for enrolling in such studies is much higher.

Harvesting plain saliva is also a consid-

erably cheaper method as compared to the usage of commercial saliva harvesting kits, or to drawing peripheral blood.

On the down side, the saliva harvesting can prove sometimes difficult when dealing with children or elderly persons, with patient with psychiatric conditions or with xerostomia generated by an entire set of diseases, infections or treatments.

The DNA yield and quality are of outmost importance for any kind of molecular biology test or study. We were able to show that plain saliva can generate sufficient and pure enough genetic material for PCR test. However, the DNA stability is affected during incubation and temperature is also critical. Should plain saliva be used for DNA extraction, to avoid any risks, our recommendations are to perform the procedure either immediately or within a 24 hours interval; meanwhile the samples should be stored at 4oC.

If longer storage of saliva samples is needed or shipment cannot be performed under refrigerating conditions, then commercial harvesting kits, that include stabilizers, should be considered. The latter method might also represent a viable solution for sample banks as the ones of the Stem Cell Registries which requires HLA-retyping under particular conditions.

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NEWS

NEW COMPOSITE GRAFT USED IN TREATMENT OF CHRONIC PERIODONTITIS

A group of researchers developed a new material composite acid citric based nano-hydroxyapatite (CA-NHA) that they studied it compared with nano-hydroxyapatite alone (NHA). This study was made on 10 patients, 6 women and 4 men with ages between 30-55 years, systemically healthy, diagnosed with chronic generalized periodontitis and contralateral intrabony pockets measuring ≥ 6mm. 33 graftable sites were selected and divided into two sites: 15 control sites were treated with NHA alone and 18 sites with CA-NHA acid formed with NHA powder with POC (Poly 1, 8 octanediol). The clinical exam using plaque index, gingival index, gingival bleeding index, pocket probing depth and clinical attachments level were measured at baseline 3, 6, 9,12 months and radiographic amount of defects fill, percentage of defect fill, change in alveolar crestal level were measured at 6,12 months. The data were analysed by Student`*s t*-test and it showed a significant decrease of clinical and radiographic parameters at the test sites at 12 months. This study demonstrates that CA-NHA can be considered a treatment for periodontal regeneration of intrabony defects. (Clinical and radiographic evaluation of citric acid-based nano hydroxyapatite composite graft in the regeneration of intrabony defects - A randomized controlled trial. *Contemp Clin Dent* 2017; 8: 380-386)

Diana Maria Anton

BLOCKED KNEE EXTENSION DUE TO A CYCLOPS LESION IN A TOTALLY TORN ACL WITH NO PRIOR RECONSTRUCTION. CASE REPORT

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BLOCKED KNEE EXTENSION DUE TO A CYCLOPS LESION IN A TOTALLY TORN ACL WITH NO PRIOR RECONSTRUCTION. CASE REPORT (Abstract): The cyclops lesion, also known as localized anterior arthrofibrosis is a widely recognized complication of anterior cruciate ligament (ACL) reconstruction that may lead to a mechanical block. In this report, we describe a rare case, where the patient was suffering from progressive loss of knee extension without having any prior surgery. An arthroscopy revealed a completely torn ACL along with the presence of a cyclops lesion. Subsequently, the patient was treated by resection of the cyclops lesion and ACL reconstruction using hamstring-tendon graft which enabled the patient to regain stability and full joint range of motion. **Keywords:** CYCLOPS LESION, KNEE ARTHROSCOPY, TORN ACL, BLOCKED EXTENSION.

Cyclops lesion is a common complication of anterior cruciate ligament (ACL) injury and is generally observed in patients with a history of ACL reconstruction. It has a nodular appearance and consists of fibrous granulation tissue. Patients suffering from a cyclops lesion usually present with blocked knee extension which often is accompanied by a click when executing passive full extension. However, we encountered a patient with progressive loss of knee extension that was diagnosed with a cyclops lesion on a completely torn native ACL, the localized anterior arthrofibrosis occurring neither after reconstruction nor on a partially torn ACL.

CASE REPORT

A 22-year-old Caucasian male presented to our hospital with limited right knee extension. His medical history revealed a right knee traumatic episode 9 week earlier while playing soccer. Although the patient experienced a slight sensation of instability, there was no considerable impact on patient's functional abilities which allowed him to return to his routine sporting activities. Subsequently, the patient described pain and "limited stretch" of his right knee which started to worsen 2 weeks prior admission to hospital. The clinical examination revealed a positive Lachman test together with the presence of an audible "clunk" when performing passive knee extension. The active

knee extension executed by the patient was blocked at 15 degrees.

An arthroscopy was carried out which showed a completely torn ACL and a cyclops lesion arising from the anteromedial bundle at its tibial insertion (fig. 1) together with a radial lateral meniscus tear (fig. 2A). The earlier onset of the meniscal damage was confirmed by the identification of another associated longitudinal lateral meniscus lesion that was already scarred (fig. 2B). The cyclops nodule had a typical appearance of chondroid fibro-proliferative tissue together with central neovascularization. A video record was also performed (fig. 3).

The patient came back for follow-up at 3, 6 and 12 months postoperatively. Clinical examination showed good stability with no excessive translation and the preservation of the full knee extension that was achieved postoperatively (0 degrees). An ipsilateral quadriceps atrophy and weaker knee flexion was encountered as expected, for which the patient was attending a rehabilitation program.

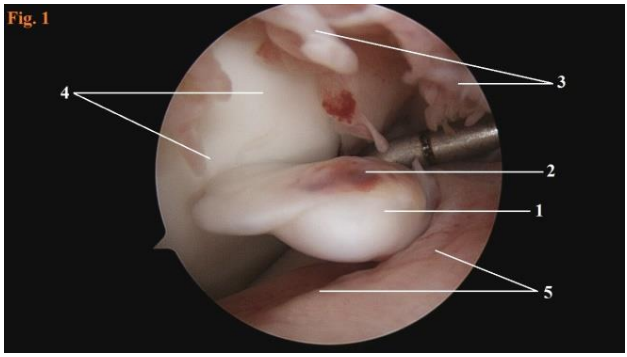

Fig. 1. Cyclops Nodule (patient is placed in supine position, right knee flexed at 60 degrees, view through anterolateral portal) 1. cyclops nodule 2. granulation vessels 3. adhesions to *ligamentum mucosum* remnants 4. external femoral condyle 5. anterior synovium at its tibial insertion

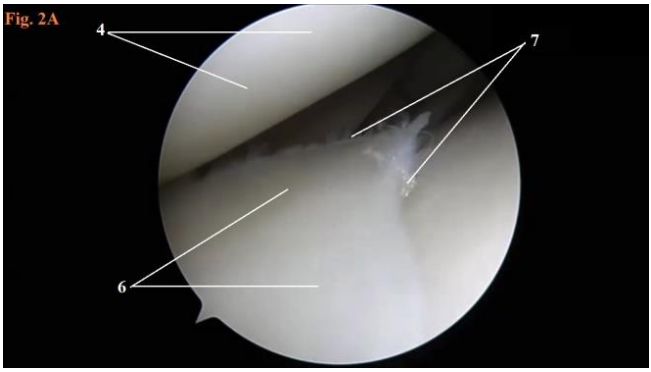

Fig. 2A. Lateral Meniscus Radial Tear (patient is placed in supine position, right knee flexed at 90 degrees, view through antero-lateral portal) 4.external femoral condyle 6. lateral meniscus 7. radial tear

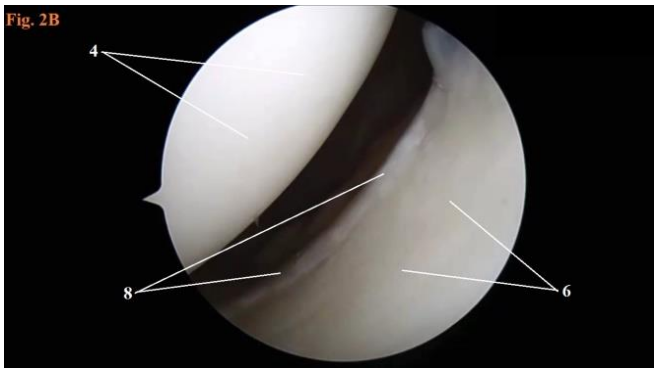

Fig. 2B. Lateral Meniscus Scarred Longitudinal Tear (patient is placed in supine position, right knee flexed at 90 degrees, view through antero-lateral portal)
4. external femoral condyle 6. lateral meniscus 8. scarred longitudinal tear

DISCUSSION

Previously published studies describe a variable incidence of cyclops lesions after ACL reconstruction ranging from 3.61% (1) to 46.8% (2). Conversely, there is limited evidence in the current literature describing cyclops lesions that occur on native ACLs with no history of surgery due to the rareness of this condition. The biggest pool of patients was recorded in 2007 which included a total of 10 patients with cyclops lesions in the absence of ACL reconstruction. (3) When investigating the etiology, recent studies have shown that a small intercondylar notch may contribute to cyclops formation (4), in addition to other already known underlying causes such as: ACL stump remnant, intra-cellular debris from the drilling of the ACL tunnels, non-anatomic tunnel placement that can lead to graft impingement (5-7). Even though measures are taken to prevent the issues, the cyclops lesion can remain clinically silent for an undetermined period and result in progressive loss of knee extension with different times of onset. This demonstrates the presence of other predisposing factors that were not determined yet. A loss of knee extension may be also encountered among the following pathologies: torn meniscus fragments, meniscus cysts or mucoid cysts, villonodular synovitis or synovial hemangioma, synovial osteochondromatosis, Hoffa's disease, osteochondritis dissecans and different tumors. In our patient, we encountered lateral chronic meniscus tears, represented by one radial tear and one scarred longitudinal tear, which possibly may have contributed to the cyclops formation, as they could cause a degree of antalgic knee flexion and predispose to fibrosis. Due to the limited number of published cases regarding this condition (8-14), we believe any addition is crucial.

CONCLUSIONS

The findings of this case report together with previous reports demonstrate that cyclops lesions should be considered among the clinical differential diagnosis in patients with blocked knee extension.

Investigating the predisposing factors might aid in the diagnosis and in minimizing the incidence of cyclops lesions as a potential complication after ACL reconstruction.

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ORAL ANTICOAGULANT TREATMENT IN OBESE PATIENTS

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ORAL ANTICOAGULANT TREATMENT IN OBESE PATIENTS (Abstract): The prevalence of obesity is alarmingly growing worldwide, so managing the oral anticoagulant treatment in this patient category will be a day by day challenge. For warfarin, the appropriate INR monitoring and dose adjustment solve the problem of over- or under-dosing. The non-vitamin K antagonist oral anticoagulants are recommended in fixed dose regimes across different body weights. Given that certain pharmacokinetic parameters are altered in patients with morbid obesity and highlighting the serious consequences of thrombotic events, underdosing these oral anticoagulants is a real concern. We summarized the existing data, drawing attention upon the limited information on the efficacy and safety of non-vitamin K antagonist oral anticoagulant use in patients with morbid obesity and presenting a series of clinical practice guidelines for this special patient category. **Keywords:** ANTICOAGULANTS, OBESITY, ATRIAL FIBRILLATION, VENOUS THROMBOEMBOLISM.

In the last decade, the prevalence of obesity has increased rapidly in both developed and developing countries and is currently one of the most serious health problems worldwide (1). The estimations for the near future are even more alarming: until 2030, 50% of the world population will be obese and 10% will be morbidly obese (2).

The strong association between obesity and cardiovascular disease is well known (3, 4). Ischemic heart disease, stroke and venous thromboembolism (deep vein thrombosis and/or pulmonary embolism) are by far the first three most common cardiovascular diseases and important causes of morbidity and mortality in the obese patient.

In this context, the dosing of oral anticoagulants is one of the challenges physicians will face increasingly often in this continuously growing category of patients.

Some pharmacokinetic parameters are significantly altered in the obese patients. These patients have increased glomerular filtration rate and renal plasma flow by up to 51% and 31%, respectively (5) due to functional and structural renal changes and glomerular hyperfiltration. The absolute drug clearances and volume of distribution are higher in obese than in normal weight patients (6), so in obese patients shouldn't the oral anticoagulant dosage depend on both the patient and the drug? Unfortunate-

ly, many clinical trials excluded or have limited overweight patients enrolled, thus optimal dosing for both safety and efficacy of oral anticoagulants in this population is lacking.

Vitamin K antagonists (VKA)

Warfarin dosing in obese patients is not very difficult because INR directly reflects the level of anticoagulation. Thus, as warfarin is monitored, under- and over-coagulation can be prevented with appropriate INR monitoring and dose adjustment. Still, one must know that obese patients, and particularly the morbidly obese ones, have a decreased initial response to warfarin compared to the normal weight patients. Also, they require higher doses of warfarin and longer times to achieve therapeutic INR values (6 days *vs.* 8 and 10 days, respectively) (7).

Direct-acting oral anticoagulants

In the modern era of the anticoagulant treatment, four direct-acting oral anticoagulants are approved for the treatment of venous thromboembolism (VTE), prevention of VTE after total hip and knee arthroplasty and for the ischemic stroke prevention in patients with non-valvular atrial fibrillation (AF). The non-vitamin K antagonist oral anticoagulants (NOAC) are: apixaban, edoxaban, rivaroxaban (direct factor Xa inhibitors) and dabigatran (direct thrombin inhibitor). Furthermore, rivaroxaban, co-administered with acetylsalicylic acid (ASA) alone or ASA plus clopidogrel or ticlopidine is indicated for the prevention of atherothrombotic events after an acute coronary syndrome with elevated cardiac biomarkers.

NOACs exert their anticoagulant effect closely related to their plasma concentrations. The major determinants of plasma concentration of NOACs are: plasma volume (directly related to body weight), volume of distribution, degree of protein binding and renal clearance. As shown in the table below, the group of the four NOACs is quite heterogeneous (8, 9) (tab. I).

TABLE I

Pharmacokinetic profiles of the newer anticoagulant agents

	Volume of distribution (L)	Protein binding (%)	Clearance
Dabigatran	60-70	35	80% renal
Apixaban	21	87	25% renal; 75% biliary
Edoxaban	107	55	37-50% renal
Rivaroxaban	50	95	66% renal; 33% biliary

Laboratory studies conducted on small numbers of healthy volunteers demonstrate that extreme body weights have modest effects on NOAC exposure and it is unlikely to be of clinical value (10, 11, 12). None of the NOACs phase III clinical trials reported the number of patients enrolled with a body mass index (BMI) of > 40 kg m^{-2} or their clinical outcomes (13), so experience in patients with morbid obesity (> 150 kg) is very limited and there are concerns about their efficacy and safety in this patient category.

The efficacy and safety of NOACs for thromboprophylaxis in orthopedic surgery

The analysis comparing NOACs with

enoxaparin 40 mg daily for prophylaxis in orthopedic surgeries in patients with BMI > 30 kg m^{-2} (RE-MODEL, RE-NOVATE and RE-NOVATE II with dabigatran; ADVANCE-2 and ADVANCE-3 with apixaban; RECORD-1, RECORD-2, RECORD-3 and RECORD-4 with rivaroxaban) found similar rates of VTE and similar or insignificant increase in bleeding rates (13) as compared with enoxaparin. Furthermore, regarding dabigatran, the efficacy and safety were similar in each treatment group across obesity subgroups of BMI 30-35 kg m^{-2}, 35-40 kg m^{-2}, and > 40 kg m^{-2} (14).

The efficacy and safety of NOACs in patients with VTE

The recent Di Minno's meta-analysis (15) of 6 large randomized controlled trials comparing NOACs with VKA in patients with VTE included 27,023 patients, of which 20,1% had high body weight, as specifically defined in each trial (> 90 Kg in rivaroxaban trials and > 100 Kg in dabigatran, apixaban and edoxaban trials).

In terms of efficacy (prevention of VTE recurrence and VTE-related death), NOACs was like VKA in high body weight patients. Considering that the weight may not reflect the patient's actual metabolic status, the authors repeated the analyses using data on BMI and obtained similar results, thus confirming the findings of the principal analysis. These results were also confirmed when the data were stratified by NOAC type (anti-factor Xa or anti-factor IIa).

When comparing obese with normal-weight patients, the NOACs have shown non-inferiority in terms of efficacy, except dabigatran (13) in which the risk of the primary efficacy outcome was significantly higher for patients weighting > 100 kg than for patients of normal weight (50-100 kg).

In terms of safety (major or clinically relevant non-major bleeding) NOACs were comparable to VKA in high body weight patients. Because the original studies on dabigatran did not provide separate safety data according to the body weight, only anti-factor Xa drugs were included in the safety analysis, thus no stratification by NOAC type is available.

The results of this meta-analysis have clinical implications, by allowing the use of fixed doses of NOAC in patients with different body weights. However, due to insufficient data, the use of standard therapy with NOACs needs caution in patients with morbid obesity.

The efficacy and safety of NOACs for the stroke prevention in patients with non-valvular AF

Two of four AF trials have not yet published sub analyses by weight, so the available data are coming from rivaroxaban and dabigatran trials.

In an US Food and Drug Administration analysis regarding the rates of stroke or systemic embolism among patients from ROCKET-AF trial (rivaroxaban) it is stated that there is no statistical relationship between weight category (<50 kg, 50 - ≤ 70 kg, 70 - ≤ 90 kg, 90 - ≤ 110 kg, and >100 kg) and the rate of stroke or systemic embolism (16). The ROCKET-AF trial results demonstrated the efficacy of rivaroxaban 20/15 mg once daily regimen independent on BMI or body weight, so no dose adjustment according to weight category was recommended.

The analysis of pharmacokinetic data of patients enrolled in RE-LY trial pointed out that since the area under the concentration vs. time curve is not affected by body weight (10), no weight-based dose adjust-

ment is required. Another analysis of the RE-LY trial (17) categorized the patients by BMI into three subgroups: a bottom 10% (BMI of $\leq$ 22.5 kg m^{-2}), a middle 80% (BMI of 22.5 to $\leq$ 36 kg m^{-2}) and an upper 10% (BMI of > 36 kg m^{-2}). In the highest BMI group, the 1-year stroke or systemic embolism rate did not differ significantly among the treatment regimens (dabigatran 110 mg bid, dabigatran 150 mg bid or warfarin). Also, one-year bleeding / stroke or systemic embolism rates did not differ significantly between the upper and the middle BMI group (17). Still, due to the very limited clinical experience of dabigatran use in patients weighing more than 110 kg, careful clinical surveillance is recommended.

In the middle of 2016 the Scientific and Standardization Committee of The International Society on Thrombosis and Hemostasis (ISTH) published the following guidance statements regarding the use of the direct oral anticoagulants in obese patients (13):

1. Standard dosing of the NOACs is recommended in patients with a BMI $\leq$ 40 kg m^{-2} or a weight $\leq$ 120 kg for VTE treatment, VTE prevention and prevention of ischemic stroke and systemic arterial embolism in non-valvular AF;

2. NOACs should not be used in patients with a BMI of > 40 kg m^{-2} or a weight of > 120 kg, because there are limited clinical data available for patients at the extreme of weight;

3. If NOACs are used in a patient with a BMI of > 40 kg m^{-2} or weight of > 120 kg, checking the drug-specific peak and trough level is recommended. If the level is within the expected range, continuation of the NOAC seems reasonable. If the drug-specific level is found to be below the expected range, changing to a VKA rather than adjusting the dose of the NOAC is recommended.

CONCLUSIONS

We briefly presented the available data regarding the use of the non-vitamin K antagonist oral anticoagulants in obese patients, noting that neither efficacy nor safety of NOACs in the obese patients has been specifically investigated in large randomized controlled trials. Based on the existing data (from pharmacological and clinical studies), the product labeling (package inserts) of the approved NOACs does not contain a dose adjustment for high weight or BMI in obese categories. Therefore, we emphasize the importance of ISTH recommendations in the current medical practice.

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MULTILOCULAR CYSTIC RENAL CLEAR CELL CARCINOMA - CASE REPORT

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MULTILOCULAR CYSTIC RENAL CLEAR CELL CARCINOMA - CASE REPORT (Abstract): MCR CCC is a rare subtype of renal clear cell carcinoma with specific morphologic and immunohistochemical features. No progression of multilocular cystic renal cell carcinoma is known. We present the case of a 56-year-old male, with an accidentally discovered renal tumor due to its unusual gross appearance and a rare clinical entity. He subsequently underwent a radical right nephrectomy and further histological examination revealed the tumor to be a multilocular cystic renal clear cell carcinoma, Fuhrman grade 2. **Keywords:** CLEAR CELL, KIDNEY, MULTILOCULAR CYSTIC RENAL CELL CARCINOMA.

Multilocular cystic renal clear cell carcinoma (MCR CCC) is a rare cystic renal tumor composed entirely of numerous cysts, the septa of which contain small groups of clear cells resembling grade I clear cell carcinoma (1). Grossly, MCR CCC consists of a well-circumscribed mass of small and large cysts filled with serous fluid and separated from the kidney by a fibrous capsule (2). The tumor has an excellent outcome and a good prognosis, having no known recurrence or metastasis (3). Histologically, the cysts are lined by a single layer of epithelial cells, usually composed of polygonal or cuboidal clear cells. Occasionally, the lining epithelium consists of several layers of cells or few small papillary structures. The nuclei are almost always small, spherical, and have dense chromatin (4). The septa consist of fibrous tissue, sometimes containing a population of epithelial cells with clear cytoplasm, resembling those lining the cysts. The tumoral stroma is also highly vascularized. MCR CCC is usually positive for CD10, vimentin, and epithelial membrane antigen (EMA) (5). We present a case of MRC CCC diagnosed incidentally, during pre-operatory CT investigation, in a 56-year-old male patient, admitted to our Hospital for aortic aneurysm management.

CASE PRESENTATION

We present the case of a 56-year-old male who was admitted to our hospital for abdominal aortic aneurysm management. The patient had a history of abdominal and lumbar pain. A pre-operatory CT scan revealed an abdominal aortic aneurysm, and a

well-defined multiseptated cystic mass in the lower pole of the left kidney. There was no evidence of left renal vein involvement nor was lymphadenopathy seen (fig. 1).

Fig. 1. Post contrast CT scan showed a multiseptated cyst in the lower pole of the left kidney

There was no family history of von Hippel Lindau syndrome or renal tumors of any kind. The biochemical assessment of the renal function was normal. Complete blood count and other preoperative investigations revealed high inflammatory markers (ESR=30mm/hr., fibrinogen= 627mg%, and CRP=17.2 mg %). Since cystic renal cell carcinoma (RCC) could not be excluded, a left-side nephrectomy was performed. The gross examination of the kidney revealed a cystic tumor in the renal lower pole measuring 48 × 40 × 30 mm. The tumor cut surface showed a multiloculated cyst with proteinaceous content, well defined margins, bulging under the renal capsule, without its involvement. No necrotic area and hemorrhagic infiltration was found in the yellowish tumor mass (fig. 2).

On histological examination, the dissected tumor specimen proved to be a multiloculated cyst consisting of cystic cavities separated by septal walls (a). The cyst wall was lined by single or multiple layers of clear cells with well-defined borders, clear cytoplasm and small hyperchromatic nuclei (Furhman nuclear grade 2) (b). No mitotic figures were seen. Focally, few cysts showed small papillary structures (c). The septa between the cysts also contain cords and clusters of similar tumor cells. Few stromal foci exhibited increased vascularity

and hemosiderin laden macrophages. No necrosis or vascular invasion was seen. The tumor did not invade the renal capsule and hilar structures (d) (fig. 3).

Fig. 2. Gross examination of the kidney revealed a multiloculated cyst in the renal lower pole

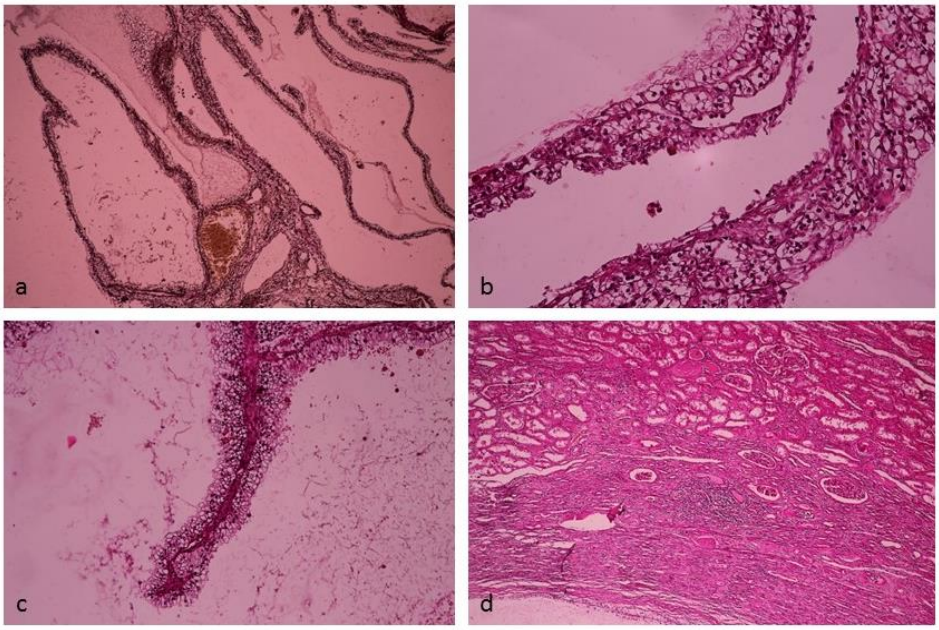

Fig. 3. Histological examination revealed cyst walls lined by one (a: EVG, x10) or multiple layers of clear cells with well-defined cytoplasmic borders and small nuclei (b: HE, x40); few cysts showed small papillary structures (c: EVG, x20); no capsule invasion was seen (d: HE, x10).

The histomorphology was compatible with MCR CCC. TNM staging and Fuhrman nuclear grading were stage T1a and grade 2, respectively.

DISCUSSION

According to the 2004 World Health Organization (WHO) classification of kidney tumors (2), MCR CCC is a rare subtype of renal clear cell carcinoma (1). 90% are discovered incidentally, on radiology for other purposes (6). MCR CCC is also known as multicystic renal clear cell carcinoma (7). The 2016 WHO classification of renal tumors (8) contains significant changes of the 2004 WHO renal tumor classification. The revision of the previous classification was performed under consideration of new knowledge on pathology (and not only), including changes of the existing renal tumor types, new renal tumors and grading system for renal tumors.

The new 2016 WHO classification refers to tumor subtypes named on the basis of predominant cytoplasmic features (e.g. clear cell RCC), architectural features (e.g. cystic RCC), anatomical tumor location (e.g. cortical RCC) and correlation with previous renal disease (e.g. acquired cystic disease-associated RCC) (8, 9).

MCR CCC is used to describe a cystic RCC composed entirely of numerous cysts, the septa of which contain a small mass (less than 25%) with no infiltrative growth (8). Microscopically, per Williamson SR et al (10), MCR CCC is a tumor consisting entirely of cysts with small groups of non-expansive clear cells in the septa. Cysts are lined by polygonal clear cells, with clear cytoplasm and low nuclear grade. Suzigan S et al (3) noted that tumoral cells are also arranged in cords, alveolar structures with small cysts and large cysts covered by a single cell layer; few tumor cells forming small papillary structures are accepted (11). In our case, the tumor histomorphology was suggestive of multicystic clear RCC with no expansive growth. Multiple publications report no recurrence or metastases in patients with MCR CCC (3. 5, 12).

As concerns renal tumors grading, the Fuhrman system was the most frequently used grading system applied to cystic RCC (13). For grade 1 to 3 tumors, the system defines tumor grade based on nuclear prominence. In our case, the tumor was composed of clear cells with low grade nuclei (Fuhrman nuclear grade 2.)

Acquired cystic RCC is a new tumor entity included in the 2016 WHO classification (8). Per Moch H et al (13), acquired cystic disease-associated RCC occurs in patients with acquired cystic renal disease, which was an unknown condition in our case.

Given the presence of cystic and papillary structures covered by clear cells, the differential diagnosis of MCR CCC consists of other cystic lesions of kidney tumors, such as renal cell carcinoma with cystic changes and clear cell papillary RCC.

While cysts are common in clear cell RCC, only rarely is the tumor entirely composed of cysts. Unlike regressing clear cell renal cell carcinoma with cystic degeneration, these cysts are filled with hemorrhage and necrosis. There are areas of expansive growth of neoplastic cells in the septa between the cysts indicating a fully malignant, clear cell carcinoma with cystic changes (12). Clear cell papillary

RCC is usually cystic and the cyst walls are lined by clear cells. However, much of the tumor exhibits papillary architecture, a feature not found in MCR CCC (14).

CONCLUSIONS

We reported this case because of its rarity and for avoiding a misdiagnosis as conventional clear cell RCC.

The limits of the paper are represented by the lack of other exams: no IMH and genetic investigations were made. The criteria for MCR CCC, defined by the 2004-2016 WHO classification of kidney tumors, were enough for diagnosis setting. The elucidation of different mutations on the onset and progression of MRC CCC will be an important future area of research. The identification of new therapeutic agents that are effective on CC RCC will be an important ongoing research topic, as well.

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NEWS

NUMBER OF LYMPH NODES IN PRIMARY NODAL BASIN AND A ''SECOND LOOK'' PROTOCOL AS QUALITY INDICATORS FOR OPTIMAL NODAL STAGING OF COLON CANCER

Regional lymph node (LN) metastasis is a strong negative prognostic factor in colorectal carcinoma. The 5-year disease-free survival of patients with LN metastasis is reduced by up to 48%, depending on the T stage and the number of positive LNs, as compared to patients with nodenegative cancer. Moreover, nodal status is also a predictive factor for the efficacy of adjuvant chemotherapy that confers a definite survival benefit in stage III but not stage II carci noma. The 5-year disease-free survival in stage II colon cancer ranges from 64% to 79% depending on the T stage. Potential causes for relapse in stage II colon cancer include peritoneal involvement, lymphovascular or perineural invasion and understaging of nodal disease status that leads to a patient being inappropriately denied adjuvant chemotherapy. The critical importance of accurate LN staging has led previous studies to address the question of minimum number of LNs that need to be examined for optimal evaluation of nodal status. The recommended minimum number of LNs to be examined varies significantly across publications and ranges anywhere from 6 to 20. Others have suggested that there is no minimum threshold that can accurately stage cancer for all patients and all LNs must be examined to ensure optimal nodal staging of colon cancer resections. There are large population-based studies that do not show an association between increasing numbers of evaluated LNs and the proportion of LN-positive cancers. The current American Joint Committee on Cancer (AJCC) guidelines suggest evaluation of 10 to 14 LNs in colorectal cancer resections as a minimum standard but encourage recovery of more LNs. The Association of Directors of Anatomic and Surgical Pathology recommends that the mean number of LNs in a series of dissections should be approximately 12 to 15 and the current College of American Pathologists colorectal cancer protocol recommends that specimens be reexamined if fewer than 12 LNs are identified. Unfortunately, none of the current guidelines emphasize the importance of the location from where most LNs should be examined and the numerical threshold continues to be the only quality indicator used in current practice. The aim of the study was to evaluate distribution of positive LNs in colon cancer resections in the immediate vicinity and distant from the tumor site and to determine the proportion of cases that could be upstaged by instituting a ''second look'' protocol in all cases that were negative after the first search (Lisovsky M, Schutz SN, Drage MG, et al. Number of Lymph Nodes in Primary Nodal Basin and a ''Second Look'' Protocol as Quality Indicators for Optimal Nodal Staging of Colon Cancer. *Arch Pathol Lab Med* 2017; 141: 125-130).

Doina Butcovan

ATTEMPTED SUICIDE: A CROSS-SECTIONAL SURVEY 2015

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ATTEMPTED SUICIDE: A CROSS-SECTIONAL SURVEY 2015 (Abstract): **Aim:** Suicide is a global health problem with an increasing incidence among young adults and teenagers. The study was aimed at analyzing the profile and identifying the frequency, nature, and contributing factors of suicides. **Material and methods:** This retrospective study included all patients who attempted suicide admitted to the Emergency Department (ED) of 'St Spiridon' Hospital Iasi - Romania, during 2015. They were analyzed by sex, age, residence, suicide method, associated pathology, potential risk factors and interventions. **Results and discussion:** The study included 368 patients (53.81% female and 46.19% male). 63.58% were admitted to ED for drug ingestion, 14.13% for ingestion of organophosphorus compounds, rat poison, herbicide and corrosive substances and 22.28% for traumatic suicide attempts. Drug ingestion (42.11%) was preferred by women while self-inflicted cut injuries (14.94%) and corrosive substance ingestion (8.42%) were more common in males. Benzodiazepines ingestion (20.10%) followed by self-inflicted cut injuries (13.85%) and corrosive substance ingestion (6.52%) were the most frequently used suicide methods. At the time of admission to ED31.52% of patients were on psychiatric treatment and 65.48% were at the first suicide attempt. The admission rate was 57% and mortality rate 0.27%. **Conclusions**: Suicidal behavior can be prevented by psychotherapeutic, pharmacological or neuromodulatory treatments for mental disorders. Also, regular follow-up of people who attempted suicide by mental health services is key to preventing future suicide attempts. **Keywords**: SUICIDE, SUICIDE METHOD, SUICIDE ATEMPT, RISK FACTORS, SELF-INFLICTED CUT WOUNDS.

Suicide is a major health problem worldwide, particularly among teenagers and young adults (1, 2, 3, 4).

Suicide attempt is the main cause of death in young people, accounting for over 50% of violent deaths (5, 6, 7, 8, 9). According to the statistics published by specialized institutions (EUROSTAT, OECD and WHO) for the years 2013 and 2014, Romania ranked ninth among the countries with the highest suicide rates (12.2 per 100,000 population), Lithuania ranked first (27.1 per 100,000 population) followed by Hungary (18 per 100,000) and Estonia (17.3 per 100,000 population) (10, 11, 13).

Per the 2008-2015 annual reports of the "Mina Minovici" Institute of Forensic Medicine, between 2,477 and 3,050 suicides were recorded annually in Romania, accounting for 25% of all violent deaths.

Analyzing the deaths registered in the last 8 years by the mechanisms of death, the most common were: mechanical asphyxia by hanging (78.62%), voluntary intoxication (7.12%) and falling (5.56%) (4, 13, 14).

MATERIAL AND METHODS

A retrospective descriptive study was conducted that included all patients who presented in 2015 (January 1 - December 31, 2015) to the Emergency Department of the Iasi "Sf. Spiridon" Hospital and were diagnosed at presentation with suicide attempt. Our study included 368 patients, 198 (53.81%)women and 170 (46.19%) men. The selection of cases was based on the diagnosis at the time of admission, positive findings on physical examination, results of laboratory tests that confirmed the ingestion of drugs or other toxic substances, patient or witness statements. Toxicological determinations were made by using a Triage Meter Pro that allows fluorescence immunochemical tests. The device is available in the ED and provides quality results fast (~15 minutes), next to the patient.

The study cases were selected per the inclusion criteria from a total of 69,232 patients admitted to the ED of the Iasi "Sf. Spiridon" hospital during the year 2015. Thus, included in the study were 368 patients aged 18 to 87. Excluded from the study were the patients under 18 years of age, patients who died before reaching the hospital, patients with accidental ingestion of toxic substance, and patients with inconclusive history.

The data collected and inserted in databases were statistically analyzed using *Microsoft Excel* and *SPSS 18.00* statistics software. Patients characteristics were expressed as the mean standard deviation or as a number and percentage.

RESULTS

Only 0.53% (368 from 69.232) of all patients presenting to the Emergency Department of the Iasi "Sf. Spiridon", Hospital during 2015 were eligible for the study (368, aged 18 to 86).

Data processing was primarily intended to identify the personal characteristics of the selected patients. It is a first starting point in strategic planning best practices. Most study patients were in the 18-30 age group (38.31%) and gender distribution showed a relatively equal distribution of subjects (53.81% women and 46.19% men, respectively), unlike the national statistics showing that the percentage of men attempting suicide was constantly higher than that of women ((82% *vs.*18%, respectively).

The sociocultural, educational and economic implications lead to an uneven distribution of cases by area of residence, 638% of the study cases being rural patients.

Regarding the suicide mechanism, 63.58% were admitted to the ED for voluntary drug ingestion, 14.13% reported intake of toxic substances (organophosphates, rodenticides, corrosives, herbicides and alcohols) and 22.28% traumatic suicide attempts (cut wounds, stab wounds, self-immolation, mechanical asphyxia by hanging and falls from heights).

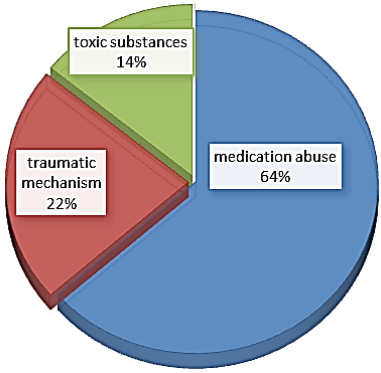

Fig. 1. Method of suicide attempt

The most common suicide method among female patients was voluntary drug ingestion (78.28%), while men preferred traumatic mechanisms (35.29%) and ingestion of toxic substances (18.23%). According to the statistical study conducted, patients in rural areas resorted much easier to ingestion of toxic substances and traumatic mechanisms, while patients in urban areas preferred drug ingestion.

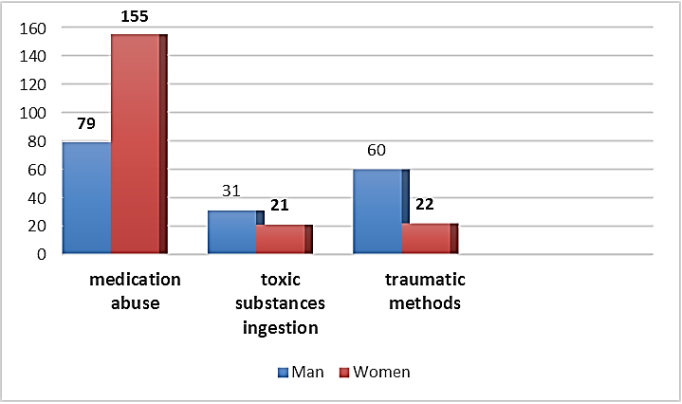

Fig. 2. Suicide method by gender

Of the patients who attempted suicide by drug ingestion, 73.07% were brought to hospital by ambulance from home, being found by the family, the average time interval elapsed between ingestion and hospital arrival being 2 to 6 hours (61.96%). 32.90% of patients used their prescribed medication for suicidal purposes.

Qualitative toxicological determinations in the ED revealed the presence of benzodiazepines in 29.67% of cases, followed by antiepileptics (17.41%) and tricyclic antidepressants (4.27%). 31.19% of patients took drugs in combination with alcohol.

In patients who ingested toxic substances, the most common were: corrosive substances (48.07%), organophosphates (21.15%), rodenticides (11.53%), toxic alcohols (7.69%) and herbicides (1.92%).

Traumatic suicide attempt was mostly preferred by male rural patients and the most common mechanisms were self-inflicted wounds (70.83%), followed by foreign body ingestion (12.5%), mechanical asphyxia by hanging (9.72%), self-immolation (2.77%) and falls from heights (2.77%). Six patients (7.31%)were imprisoned with a history of multiple suicide attempts, and sought medical care for foreign body ingestion (66.66%) and self-inflicted wounds. Per the Suicide Prevention Alliance, every year in Romania there are around 13 deaths by suicide per 100,000 inhabitants, thus ranking third in the hierarchy of violent death circumstances.

Of all the study patients 83.69% said that it was the first attempted suicide, triggered by a family conflict or pre-existing psychiatric diseases.

The average length of stay in ED was about 6 hours, during which the patients received therapy to stabilize the vital signs. Early diagnosis and initiation of specific therapy is the best way to reduce the complications that may occur.

Importance was given to medical history and physical examination. Blood and urine toxicology tests had an important

place among the biological parameters analyzed in all study patients. Throughout the ED stay, all the patients were monitored in terms of Glasgow Coma score, blood pressure, heart rate, respiratory rate, peripheral blood oxygen saturation (SatO2), temperature and diuresis. 92.94% of patients were hemodynamically stable, however 7.06% of patients required supportive care (orotracheal intubation, mechanical ventilation and positive inotropic and chronotropic agents). Also, depending on the associated pathology, patients received specialist advice (toxicology, cardiology, surgery, gastroenterology, gynecology, etc.). Simultaneously with the symptomatic treatment and detoxification the supportive therapy was initiated, by which the patients were helped to understand how and why they resorted to these methods and what they can do in the future to prevent relapse.

All patients presenting to the emergency department were stabilized. Subsequently,57% of the patients required hospital admission because of significant medical complications that required further treatment and follow-up. The remaining 43% of patients were hemodynamically stable and were referred to other hospitals, returned home or left the ED against medical advice.

Two cases (3.84%) of voluntary methyl alcohol ingestion referred to the Nephrology Clinic required hemodialysis. One death was recorded (0.27%) because of mechanical asphyxiation by hanging in a patient with pre-existing psychiatric disorder.

DISCUSSION

Comparing the rate of suicide attempts in our study with the national statistics for Bucharest, the capital municipality of Romania, reported by the "Mina Minovici" Institute of Forensic Medicine we found an equal incidence, in both cases with an average of 10% (13, 14).

Nationally, mechanical asphyxia by hanging is the most frequently used suicide method followed by voluntary drug ingestion (14) and fall from heights while in the Iasi County 63.58% reported voluntary drug ingestion and 14.13% ingestion of toxic and non-pharmacological substances for suicidal purposes and only 22.28% had attempted suicide by traumatic mechanism. As suicide mechanism women prefer drug ingestion, while men resort to more painful methods: traumatic mechanisms or ingestion of toxic substances, which can lead to a much higher death rate among men.

The time elapsed between ingestion of toxic substance and arrival at the hospital is extremely important in determining patient prognosis.

Even if worldwide suicide attempt is the main cause of death in young people, accounting for over 50% of violent deaths, in our case study one death was recorded (0.27%) because of mechanical asphyxiation by hanging in a patient with pre-existing psychiatric disorder (4, 5, 6, 7, 8, 9).

Mental disorders and physical illness may lead persons to commit suicide. In a retrospective study of suicide notes, medical and police charts of persons who committed suicide, the patients were examined at the Department of Forensic Medicine of Ludwig-Maximilians-University Munich between 2009 and 2011. The study included 1069 cases,18.9 % gave a physical disorder as reason for committing suicide and 32.7 % a medical disorder. Regarding to method of suicide, patients with mental disorders had more often previous suicidal attempts and jumping in front of a car was the most frequent method of suicide while persons with physical diseases most often

shoot themselves. Comparing to literature, in our case, 83. 69% said that it was the first attempted suicide, triggered by a family conflict or pre-existing psychiatric diseases (15).

CONCLUSION

Patients included in the study were in most of cases women, with ages less than 30 years old, from urban area, who chose as a suicide method the voluntary drug ingestion.

Suicidal acts are rarely an act of free will. In most cases, suicidal attempts are the result of untreated psychiatric disorders.

Suicidal behavior can be prevented by psychotherapeutic, pharmacological or neuromodulatory treatments for mental disorders. Also, regular follow-up of people who attempted suicide by mental health services is key to preventing future suicide attempts.

Reduced access to toxic substances (organophosphates, methyl alcohol) fighting alcoholism, early detection, treatment and medical care given to people with mental disorders and monitoring people who have experienced a suicide attempt are some steps that should be taken to prevent and reduce the size of this phenomenon.

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FECAL MICROBIOTA TRANSPLANTATION IN RECURRENT NAP1/B1/027 CLOSTRIDIUM DIFFICILE INFECTION (CDI) RESISTANT TO VANCOMYCIN AND METRONIDAZOLE IN A PATIENT WITH ULCERATIVE COLITIS (UC): A CASE REPORT

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FECAL MICROBIOTA TRANSPLANTATION IN RECURRENT NAP1/B1/027 CLOSTRIDIUM DIFFICILE INFECTION (CDI) RESISTANT TO VANCOMYCIN AND METRONIDAZOLE IN A PATIENT WITH ULCERATIVE COLITIS (UC): A CASE REPORT (Abstract): Most of the studies showed that IBD patients inflammatory bowel diseases (IBD) with CDI have more of the whole range of short- and long-term worst outcomes than those without CDI. Initial infection with the BI/NAP1/027 epidemic clone was found to be a significant risk factor for relapse. However, current literature is suggesting increasingly that for patients with infections that fail to resolve with traditional antibiotic regimens, FMT's average cure rate of > 90%. We report a case of a 40-year-old man, diagnosed with ulcerative colitis (UC) in 2012 who presented in our clinic for 20 watery stools per day with mucus and blood, hypogastric pain, pyrexia and chills. Rectosigmoidoscopy and histopathological examination diagnosed active lesions of ulcerative colitis with *Clostridium difficile* toxins A/B enzyme immunoassays (EIA) testing initially negative. The patient was non-responder at day 10 of intravenous (iv) corticotherapy and received induction therapy with Infliximab 5 mg/kg. EIA testing for *Clostridium difficile* was repeated at day 12 of hospitalization with positive results for toxins A/B, and associated oral therapy with Vancomycin and Metronidazole was initiated without clinical response in day 7, reasons for what intravenously therapy with Tigecycline was started with good response. Patient was discharged after 10 days of Tigecycline, but came back twice for two relapses of *Clostridium difficile* colitis treated successfully with Tigecycline, reasons for what fecal transplantation was performed in Matei Bals Institute, which induced remission of both CDI and UC. **Keywords:** FECAL MICROBIOTA, TRANSPLANTATION, CLOSTRIDIUM DIFFICILE, INFECTION, ULCERATIVE COLITIS.

Most of the studies showed that IBD patients with CDI have more of the whole range of short- and long-term worst outcomes than those without CDI. Patients with ulcerative colitis have more severe outcomes than those with Crohn's disease (1).

Rates and severity of *Clostridium difficile infection* (CDI) in hospitals in North America and Europe have increased since 2000 and correlate with dissemination of an epidemic strain characterized by higher than usual toxin A and B production, the presence of a third toxin, binary toxin, and

high-level resistance to antibiotics. The strain, which is restriction endonuclease analysis group BI, pulse-field gel electrophoresis type NAP1, and polymerase chain reaction ribotype 027, is designated BI/NAP1/027 (2, 3, 4).

CASE PRESENTATION

A 40-year-old man, was admitted in our department in May 2014, for 20 watery stools per day with mucus and blood, hypogastric pain, pyrexia and chills.

His medical history includes chronic hepatitis B infection in treatment with entecavir 0.5 mg per day and UC diagnosed in 2012, in treatment with azathioprine at a dose of 150 mg per day (2,5 mg/kg). He confessed cessation of smoking for one year, previously being a chronic smoker of 20 cigarettes per day.

Should be mentioned in his medical history multiple flares of UC since diagnosis due to low-compliance to medication, also self-discontinuation of azathioprine 2 weeks before hospital admission because of lack of money.

At clinical examination the patient was apyretic, underweight with a BMI of 17,5 kg/m^2 with skin and mucosal pallor, tachycardia and with tenderness in lower abdomen with palpation.

Laboratory tests showed moderate leukocytosis, important increase in C-reactive protein levels and hypochromic microcytic anemia.

Stool test were negative for infections. *Clostridium difficile* toxins A/B enzyme immunoassays (EIA) tested negative.

Rectosigmoidoscopy revealed: absent vascular pattern, edema, erythema, friability of the mucosa and erosions. Continuous lesions on 60 cm explored (fig.1).

Histopathological examination showed: Diffuse chronic mucosal inflammation, cryptitis, regenerating epithelium with erosion and superficial fibrino-leukocytic exudate, HE, 200 x (fig. 2)

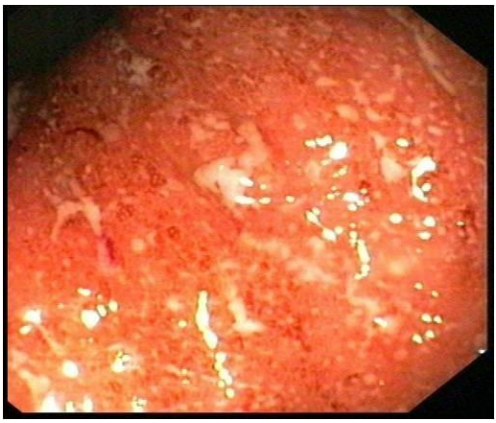

Fig. 1. Rectosigmoidoscopy absent vascular pattern, edema, erythema, friability of the mucosa and erosions.

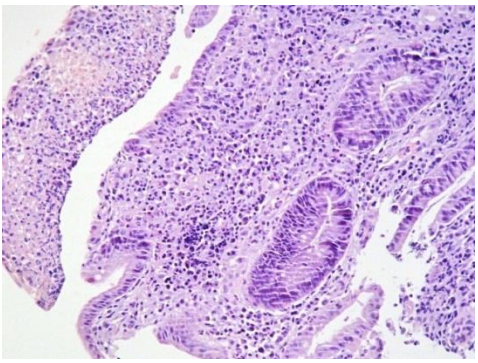

Fig. 2. Histopathological examination diffuse chronic mucosal inflammation, cryptitis, regenerating epithelium with erosion and superficial fibrino-leukocytic exudate, HE, 200x

The severity of UC flare was framed in a Mayo score of 12 points.

The patient received intravenous (iv) corticotherapy with lack of response at day 10.

First induction dose of infliximab 5

mg/kg was administered with the maintenance of oral corticotherapy. EIA testing for *Clostridium difficile* was repeated at day 12 of hospitalization with positive results for toxins A/B.

We decided to stop further administration of infliximab and start tapering corticotherapy until *Clostridium difficile* eradication. We initiated oral Vancomycin 1000 mg per day in association with oral Metronidazole 1500 mg per day and *Saccharomyces boulardii* capsules 500 mg per day. After three days the patient developed digestive intolerance with vomiting and the regimen was changed to i.v. Metronidazole 1,500 mg/day.

At day ten of treatment the patient had a decrease in the number of stools (9 per day, with no blood but with the presence of pus), still with persistent abdominal pain. Blood tests showed no modification regarding inflammatory panel with increased values in serological inflammatory markers, severe hypochromic microcytic anemia that required blood transfusions and severe hypoalbuminemia.

Fecal cytology showed the presence of leukocytes.

Nucleic acid amplification test (NAAT) for *Clostridium difficile* toxin genes was positive for hipervirulent NAP1/B1/027 strain.

Oral vancomycin was continued with increased dosage 2,000 mg per day (500 mg every 6 hours) in association with vancomycin enemas 500 mg four times per day.

After ten days of previous regimen due to lack of response we added tigecycline 50 mg every 12 hours intra-venously for 16 days with clinical response consisted in normalization in the frequency and consistency of stools.

The patient was discharged from hospital with the recommendation to continue vancomycin in a tapering regimen 250 mg four times daily with halving doses weekly until 125 mg every other day for 7 days in association with *Saccharomyces boulardii* capsules 500 mg per day. Oral corticotherapy tapering was continued.

After one month the patient was readmitted to the hospital for the recurrence of watery bloody stools and severe abdominal pain.

We performed EIA retesting for *Clostridium difficile* which was positive for both toxins A and B and fecal cytology which showed the presence of leukocytes.

Patient received a second course of intravenous tigecycline 50 mg every 12 hours with favorable evolution and the normalization of stools at day 7.

At two weeks follow up the patient presented with the second relapse of *Clostridium difficile* colitis and again was treated with tygecicline in association with oral metronidazole, oral vancomycin and *Saccharomices boulardii* capsules with favorable evolution.

After this third recurrence of Clostridium difficile infection patient received fecal microbiota transplantation (FMT) in Matei Bals Institute with a donor from the personal of the Hospital, through a nasogastric tube.

One month after, the patient had a fourth recurrence of *Clostridium difficile* colitis meeting the same pattern as the previous and we performed a second procedure of FMT (changing the donor with one person from the patient's village).

At further follow-ups for a period a 2 years the patient was in clinical remission of inflammatory bowel disease with the normalization of clinical examination, weight

gain and with normal range for serological inflammatory markers, on maintenance therapy with oral 5-ASA 3 g/day.

DISCUSSION

The endoscopic finding of pseudo membranes found in 50% of *Clostridium difficile* infected patients (according to data from last decade) is less common (13%) in IBD patients with CDI (7) and our case had also an endoscopic aspect of ulcerative colitis flare.

A strong pre-test suspicion for CDI (as in our case watery diarrhea, severe hypoalbuminemia, severe anemia, severe acute inflammatory syndrome), empiric therapy for CDI should be considered regardless of the laboratory testing result, as the negative predictive values for CDI are insufficiently high to exclude disease in these patients (8). In our patient the clinical presentation of Clostridium difficile colitis mimics an underlying IBD flare and the first test for toxins in the stool was negative, and that made us to escalate the immunosuppressive therapy that lead to a very severe evolution of this patient.

Toxins A+B EIA testing commonly used as first diagnosis test for *Clostridium difficile* infection has a sensitivity of single sample testing low (72%) and may increase by 10% with second stool testing (84%). Three sample testing increase sensitivity to 86%(8).

Currently, standard recommendations for treatment of mild CDI include metronidazole or vancomycin, with data suggesting that vancomycin is more efficacious than metronidazole in severe CDI (1).

This is the first Romanian report of a case of *Clostridium difficile* colitis with the BI/NAP1/027 strain in a patient with ulcerative colitis, community acquired. Rafila et al report in their study that PCR ribotype 027 was found to be the most dominant type in CDI in Bucharest, accounting for 68% of the Romanian isolates during 2011-2012, 84% healthcare associated and 16% community acquired (9). Now we report resistance of this strain to metronidazole or vancomycin and a good response to tigecycline therapy, which practically saved the life of this patient.

We think that Infliximab worsened the evolution of C difficile infection in our patient, our point of view differs from Seicean A et al (10). FMT was indicated because of repeated relapses of C difficile colitis, according to current data from the literature (5). FMT practically cured both diseases, with the condition of a good quality donor.

ACKNOWLEDGEMENTS

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NEWS

SUCCESSFUL DIETARY INTERVENTION IN PATIENTS WITH SYSTEMIC SCLEROSIS AND FRUCTOSE MALABSORPTION

Gastrointestinal involvement is frequent in systemic sclerosis (SSc) and leads to an important decrease in patients' quality of life. A recent study aimed to evaluate the prevalence of fructose malabsorption in 80 patients with systemic sclerosis recruited between 2011 and 2014. The study group was composed of 23 patients with diffuse cutaneous SSc (dcSSc) and 57 patients with limited cutaneous SSc (lcSSc). Gastrointestinal symptom severity was quantified using the Global Symptomatic Score (GSS). A respiratory test was performed according to strict protocols to estimate fructose absorption following the ingestion of 250 ml 10% fructose solution. Fructose malabsorption was shown in 40% of SSc patients with a higher prevalence in patients with lcSSc (78.1% in lcSSc vs 21.9% dcSSc patients). Fructose malabsorption was strongly correlated with higher GSS scores (p=0.000004). However, BMI values did not differ significantly between individuals with or without fructose intolerance. Patients that exhibited fructose malabsorption were placed on a low fermentable oligosaccharides, disaccharides, monosaccharides and polyol diet (low-FODMAP) for a period of 5 weeks. Symptoms like nausea, vomiting, diarrhea, constipation and abdominal pain were reduced significantly (up to 0%) by the low-FODMAP diet. The study emphasizes the importance of personalized dietary interventions in SSc patients. (Marie I. et al. Fructose Malabsorption in Systemic Sclerosis. *Medicine* 09.2015, doi: 10.1097/MD.0000000000001601. 94 : 39 : e1601).

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VENOUS ULCER - A NEW THERAPEUTIC APPROACH

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VENOUS ULCER-A NEW THERAPEUTIC APPROACH (Abstract): Trophic leg ulcer is a major health problem affecting approximately 1-2% of the population, the incidence being higher in the elderly (70-80 years). It is a multifactorial condition, but the most common cause is chronic venous insufficiency. This can be attributed to reflux in the saphenous system and calf perforator vein incompetence. These were first described by Linton, the first intervention designed to correct perforator vein incompetence bearing his name. Today Linton's operation has been abandoned due to the large unaesthetic incision and great postoperative pain. Also, ulcer healing time is long (2 months) and recurrence rate is high. Currently a series of minimally invasive procedures are used to close these perforator veins, such as ultrasound-guided sclerotherapy. The advantages of these techniques are less discomfort to the patients, low rate of complications, short hospital stay. **Keywords:** LEG ULCER, PERFORATOR VEINS, SUBFASCIAL ENDOSCOPIC PERFORATOR SURGERY, ULTRASOUND GUIDED SCLEROTHERAPY, MINIMAL INVASIVE.

Trophic leg ulcer (TLU) is a major health problem affecting approximately 1-2% of the population at some point in time, the incidence being higher in the elderly population (70 to 80 years) (1). It is also associated with high financial costs, the treatment usually being of long duration. TLU is associated with reduced quality of life and high morbidity (2). Venous ulcers are known to have the highest rate of recurrence, 25% of cases relapsing within the first year (1). This condition is multifactorial, but the most common cause is venous insufficiency accounting for 70% of all leg ulcers. It was estimated that 4% of patients with chronic venous insufficiency develop venous stasis ulcers (1). Beside greater saphenous vein incompetence it is also associated with the presence of incompetent calf perforators, thus the treatment would be incomplete if it deals only with the greater saphenous vein neglecting the incompetent perforator veins, ulcer recurrence being therefore high (1,3). Not too long ago, the incompetent perforator veins were surgically corrected with the Linton operation (which requires a large unaesthetic incision and causes great postoperative pains) or sub fascial endoscopic perforator vein *s*urgery. Currently, minimally invasive endovascular percutaneous methods are used. As these methods do not require anesthesia or sedation, patients experience little discomfort, the complication rate is low, and a hospital stay short (3).

Venous insufficiency of the great saphenous vein correlates with the number of incompetent perforator veins per leg as

well as the diameter of the perforator veins (4). This implies that the presence of venous reflux through the perforators is an important factor in developing venous reflux in the greater saphenous vein. Chronic venous insufficiency is associated with perforator vein insufficiency in approximately 77% of patients with venous stasis leg ulcers and 57% of patients with trophic changes in the lower extremities. Interruption of the perforator veins with significant reflux (shown by Doppler ultrasound) must be done especially in cases associated with such trophic skin changes as dermatitis, hyperpigmentation, lipodermatosclerosis, and most importantly active stasis ulcers. Patients with recurrent varicose veins have a higher prevalence and a greater number of incompetent perforator veins (5,6).

The perforator veins go through the fascia connecting the superficial and deep venous systems. Most have bicuspid valves (1-3) which assure the one-way flow of blood from the superficial to the deep venous system. This is also favored by the oblique trajectory of the perforator veins.

The first classification of perforator veins was (fig. 1):

- Cockett perforators which connect the greater saphenous vein with the posterior tibial vein;
- Boyd perforators situated immediately under the knee, connecting the greater saphenous vein with the tibial and popliteal veins;
- Bassi perforators between the lesser saphenous veins and peroneus veins;
- Dodd (Hunterian) perforators between the greater saphenous vein and the popliteal and femoral veins.

The International Interdisciplinary Consensus Committee on Venous Anatomical Terminology recommends the classification of perforators into 6 groups depending on the anatomical position in the lower extremities: perforators of the foot, ankle, calf, knee, thigh, gluteus. One of the objectives of this classification is to allow topographic description of the perforator veins without relying on person's names which are quite often historically inaccurate (7).

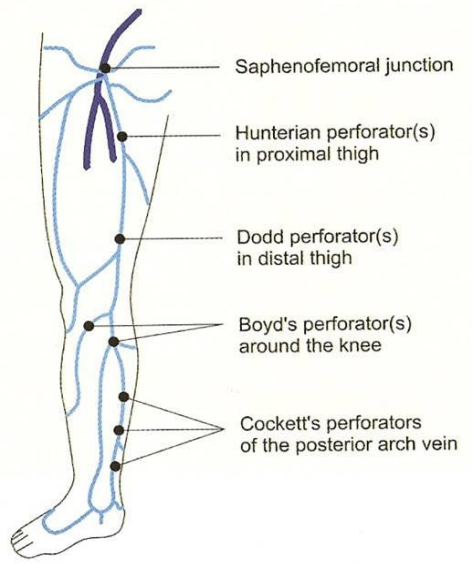

Fig. 1. Perforator veins of the lower leg (7)

The incompetent perforator vein is identified as follows: depression in the muscular fascia, area in which at little digital pressure the reflux can be felt during the Valsalva maneuver or cough. On Doppler ultrasound these perforators have a diameter greater than 4mm and a reflux higher than 0.35 seconds (8).

Precise indications for perforator vein interruption are necessary in clinical practice in order to offer a basis for evaluating treatment outcome. These indications are:

- ulcer healing in extremities with Clinical-Etiology-Anatomy-Pathophysiology (CEAP) class C6;
- prevention of ulcer recurrence in ex-

tremities with classes C5 and C6;

- lowering of severity of chronic venous insufficiency and resolution of symptoms in stages C2 to C5;

- prevention of progression to more advanced stages in C2 to C4 extremities (9).

This first technique to close the perforator veins was the Linton operation (1938) which consists of a longitudinal incision followed by the dissection and subfascial ligation of the perforator veins (10).

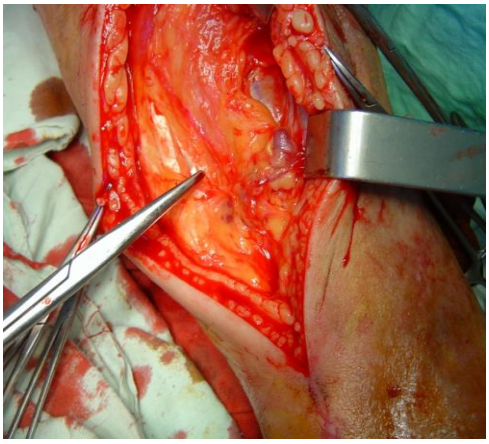

Fig. 2. Linton operation

The second method is sub fascial endoscopic perforator surgery (SEPS). It became the most accepted surgical method for advanced stages of chronic venous insufficiency. The results offered by the North American SEPS Registry show a rate of ulcer healing of 88% at 1 year following the procedure and a higher ulcer recurrence rate in the patients with postthrombotic syndrome compared to those with primary chronic venous insufficiency (11).

The new therapeutic standards is a minimally invasive procedure which does not require anesthesia or sedation (patients are usually old and have a history of other medical illnesses), causes little discomfort to the patients, low complicationrate, and short hospital stay. This standard can be achieved through the ultrasound-guided sclerotherapy of perforator veins.

Sclerotherapy is used for the treatment of perforators with a diameter of 4-7 mm, the ones less than 4 mm in diameter being rarely incontinent and those greater than 7 mm requiring a larger amount of sclerosing substance and thus are at higher risk for complications. Under ultrasound guidance the vein is punctured with a 25 gauge needle, venous blood is aspirated to check needle placement into the vein, injection of 1-2 ml polidocamol foam, needle retraction, compression for a few minutes, and ultrasound control for persistent flow (fig. 3). Postoperatively, elastic compression is instituted for 24-48h followed by 5 days of daytime elastic compression (minimal period).

Post procedural ultrasound examination shows thickened venous wall, change in vein diameter, absent flow, and higher echogenicity. A significant decrease in echogenicity signifies the presence of thrombosis and it is associated with a high risk of thrombus propagation into the deep venous system or recanalization (9,12).

Immediate success rate is >98% and at 6 month > 90% perforators remained closed. Post procedurally, the venous ulcers heal quickly and if they are of great size a skin graft should be used. There is no evidence that ulcer recurrence is caused by the same perforator which underwent recanalization or by a new incompetent venous perforator (13,14) (fig 4).

Complications of ultrasound-guided sclerotherapy: multiple punctures can lead to vasospasm and hematoma which will make the procedure difficult, accidental injection of the substance into the artery, cutaneous necrosis, and anaphylactic shock (13).

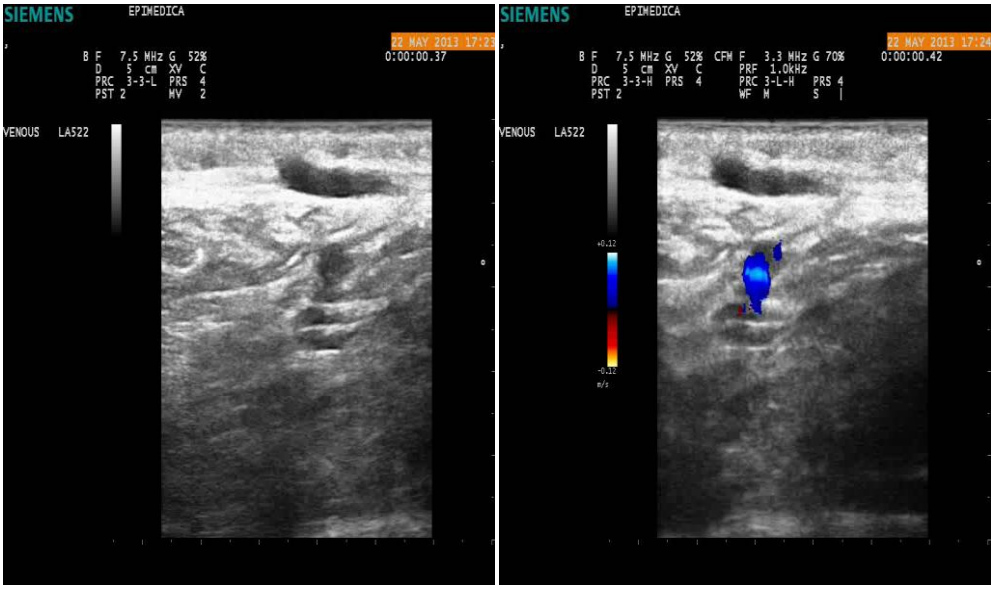

Fig. 3. Sclerozing substance injection into the perforator vein site

Fig. 4. Venous ulcer healing after the ligation of the saphenous-femoral junction = ultrasound-guided sclerotherapy and skin grafting.

CONCLUSIONS

Our experience with patients with stasis ulcer and trophic changes associating chronic venous insufficiency with perforator vein reflux should undergo perforator vein besides classical surgical treatment of venous insufficiency, otherwise ulcer healing time is longer and recurrence rate is

high.

In our clinic approximately 150 patients with venous leg ulcers and incompetent perforator veins were treated by sclerotherapy. The postoperative results showed that ultrasound-guided sclerotherapy of incompetent perforators is a minimally invasive method effective in the treatment of venous ulcers. The immediate favorable results in a high proportion of cases, low rate of complications, accessibility, and the fact that patients can resume their normal activities immediately after the procedure highly recommend it. Ulcer healing time is short and the incidence of leg ulcer recurrence is low.

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PROLONGED FEVER WITHOUT OBVIOUS ETIOLOGY FINALLY DIAGNOSED AS HIGH-GRADE SOFT TISSUE SARCOMA

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PROLONGED FEVER WITHOUT OBVIOUS ETIOLOGY FINALLY DIAGNOSED AS HIGH-GRADE SOFT TISSUE SARCOMA (Abstract): Sarcomas represent a challenge to clinicians as they are rare and diagnosis is often delayed. Soft tissue sarcomas are relatively uncommon cancers, the incidence is estimated about 1% from all malignant tumors. Due to aggressive biological behavior of pleomorphic undifferentiated sarcoma diagnosis must be made as quickly as possible. MRI is the standard imaging modality for investigating possibly malignant masses. Excisional biopsy appears to be necessary for differentiating soft tissue sarcoma from expanding hematoma. FDG PET scans may be helpful in specific circumstances (as was the situation in our case, because the lesion is composed from two components: an area without precise edges and a necrotic-cystic area). **Keywords:** SOFT TISSUE SARCOMA, FDG PET SCAN, PROLONGED FEVER, EXCISIONAL BIOPSY.

The large majority of soft tissue tumors are benign, with a very high cure rate after surgical excision. Soft tissue sarcoma (STS) incidence is estimated about 1% of malignant tumors, life-threatening being the main problem, the diagnosis and treatment representing a significant challenge due of more than 50 histological subtypes of soft tissue sarcomas which often have specific characteristics in clinical presentation, prognosis and treatment (1).

Undifferentiated high grade sarcomas are usually present in patients aged over 40 years, predominance beings around the age of 60-70 years, rare examples may be encountered in adolescents and young adults. Preponderance it's a little bit higher in men versus women, approximately 1.2:1(2). STS distribution according to the anatomic area as follows: head and neck 8.9%, trunk 17.9%, upper extremities 13.1%, lower extremities 46.4%, retroperitoneum 12.5%, mediastinum 1.3% (3). In the extremities, STS it's presenting most often as a painless lump or swelling, usually 5-10 cm in diameter, located in muscle (4). In order to be able to differentiate between various types of sarcomas and discriminating through the newly diagnosed low-grade and high-grade sarcomas, a high sensitivity it's required and this may be achieved by combining metabolic and morphological information of FDG PET/CT imaging (5). These combined images have been already used for multiple purposes: biopsy guidance, response assessment , grading, and follow up in STS (6).

Among the most often used systems for classification we notice: French grading and the National Cancer Institute grading,

which are based on histologic type and subtype, tumor necrosis, and mitotic activity. Further evolution to metastatic disease is given by histological grading which is also an important prognostic factor (7).

CASE REPORT

A 67 years old Caucasian female, without significant medical history, was admitted with prolonged fever (up to 39°C), chills, sweats, diffuse myalgia, fatigue, persistent inflammatory syndrome, weight loss (10 kg in last 5 months) and a recent appearance of a pain in left thigh without swelling. The medical investigations performed before admission: thorax scanner, bone scintigraphy, abdominal ultrasound and transthoracic cardiac echography were negative. The patient was treated during 15 days with corticosteroids (Medrol 12 mg/d), but without symptomatic improvement.

Clinical examination reveals pain on deep palpation in the left trochanter without local swelling. Vital parameters were normal except the fever.

Laboratory investigations show the following results: CRP 148mg/L, hemoglobin 10,2g/dL, ferritinemia 840,4µg/L(normal value between 30 to 400), serum iron 16µg/dL, hypoalbuminemia 20,9g/L. Bacteriological analyzes of urine and blood were negative.

Due to the fact that the patient began to invoke a pain in left thigh we chose to do some investigations to discover the etiology. A pelvis and femur radiography reveals no bone lesions. A bone scintigraphy shows on the precocious images an intake at the soft tissue of the left thigh. We continued investigations with a hip ultrasound which revealed a heterogeneous formation of 112 mm on 40mm which occurs vascularized. We followed with an MRI exams which detected a lesion of 10 cm containing two components: a fleshy area without precise edges and a necrotic-cystic area, suspicious of malignancy (rhabdomyosarcoma), as illustrated in the following two figures:

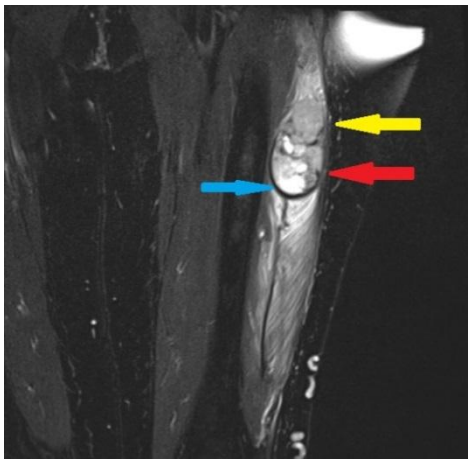

Fig. 1. MRI of the thigh: coronal cut, T2-weighted. The lesion at the vastus lateralis muscle occupying a major part of this muscle, that seems not to cross the vast intermediate aponeurosis, showing a double signal: the cranial part appears fleshy (yellow arrow) and caudal part appears necrotic-cystic (red arrow) well delimited by a fibrous membrane (blue arrow).

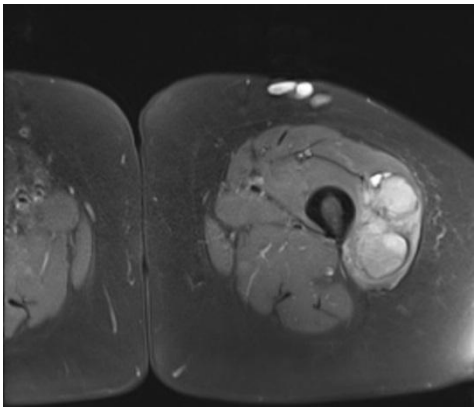

Fig. 2. MRI of the thigh: axial cut, T2-weighted. The lesion in the vastus lateralis muscle occupying a major part of this muscle.

We have continued the investigations with a FDG PET scan which revealed us a voluminous lesion very hypermetabolic. We proceeded to the surgical excision of the mass which led us to the diagnosis of soft tissue sarcoma.

DISCUSSION

Unexplained prolonged fever is characterized by a higher temperature than 38,3°C(101°F) lasting more than 3 weeks for no apparent reason after clinical examination and routine tests.

The main causes of prolonged fever (except patients with a known HIV positive serology) are: infections (20 to 35% of cases), malignancies (10 to 15% of cases), autoimmune conditions (20 to 30% of cases) and miscellaneous (5 to 15% of cases) (8). In rare cases, soft tissue sarcoma can cause general symptoms such as: fever, chills, sweats, weight loss, fatigue. Soft tissue sarcoma in its early stages may grow without any signs or symptoms, pain or soreness appear when it presses against nearby nerves and muscles.

The first imaging test is a X-ray, followed by an MRI which is the most helpful test to image soft tissue tumors as it provides very important information about the mass such as the size, location, and proximity to neurovascular structures but the diagnosis of a tumor cannot be made by MRI alone. MRI cannot be done to patients with metal implants or pacemakers, in those cases a CT scan may be an option.

Rhabdomyosarcoma is part of tumors of the musculoskeletal tissue for which the performing of a 18F-fluorodeoxyglucose (FDG) PET-CT is indicated in order to stage the high-grade sarcomas (9). Considering the suspicion of rhabdomyosarcoma and that the lesion has several components, to avoid the risk of false negative result at biopsy we opted to continue investigations with a FDG PET scan due its direct relationship to tissue metabolism. The highest FDG uptake areas in a tumor reflect the most biologically aggressive areas with the probability of malignant behavior.

Below we present PETAC axial and axial CT images which have been fused to obtain a third image which shows a voluminous lesion very hypermetabolic.

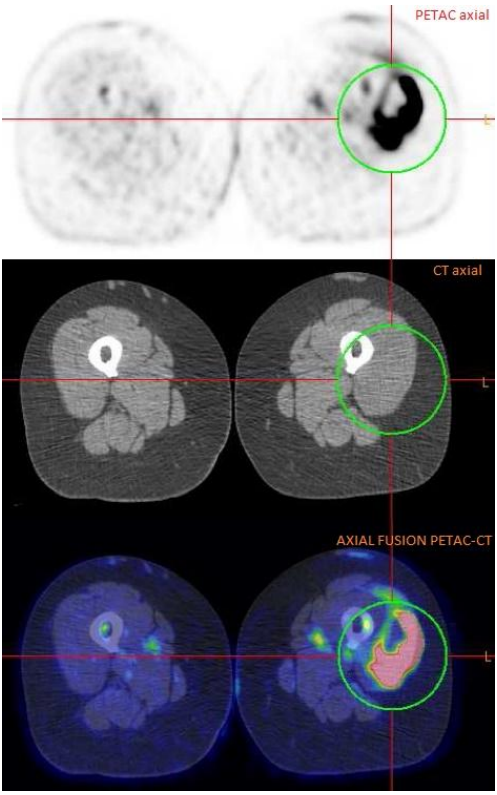

Fig. 3. Fusion between Positron Emission Tomography with Carbon-11 Acetate axial and CT axial images.

The standard uptake value(SUV) used in Flourodeoxyglucose (FDG) imaging, has the capability in helping to distinguish between benign and malignant lesions, high SUV uptake may predict for an aggressive

histology. Because the lesion was intense hypermetabolic (SUVmax=49,6) we decided a total excision of the mass.

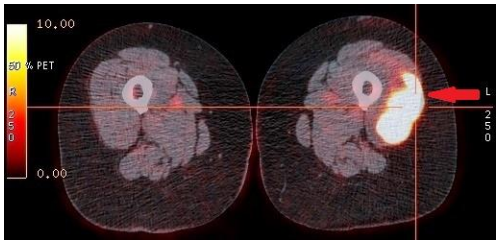

Fig. 4. Fused axial PET/CT images showed tracer uptake in a bulky soft-tissue mass in the left vastus lateralis muscle as indicated by red arrow

In case of mass suspicious of malignancy, the FDG PET image used in conjunction with other imaging can guide the surgeon to the most appropriate area to get biopsy tissue for diagnosis because the most biologically aggressive areas of a tumor have the highest FDG uptake (10).

Only the biopsy can provide a specific histological diagnosis of pleomorphic sarcoma which cannot be obtained by radiologic imaging. There are three procedures to obtain biopsy: fine needle aspiration (FNA) is not recommended as a primary diagnostic modality, core needle biopsy but several cores should be taken to maximize diagnostic yield and excisional biopsy.

After analyzing of surgical fragments, the anatomopathological result concludes the following diagnostic: Soft Tissue Sarcoma undifferentiated High-Grade 3 in the classification FNCLCC (Federation Nationale des Centres de Lutte le Cancer).

When the diagnosis of sarcoma is made, it is important to determine if a tumor is isolated (localized) or has spread (metastatic) by way of PET scan, which nowadays has become a routine exam in the extension evaluation of STS. Sarcomas can develop distant metastases and the most common site is the lung with the observation that if a pulmonary nodule is discovered after the patient has been diagnosed with a sarcoma, this one may be a metastasis of sarcoma. Rarely metastases may occur in the skin, soft tissues, liver, and lymph nodes (11).

Once the diagnosis of Soft Tissue Sarcoma has been confirmed, an individual treatment plan is made for each patient. Sarcoma treatment requires a multimodality approach and hence a team of physicians will participate in a patient's care. There are essentially three main types of treatment: surgery, radiation, chemotherapy.

Adjuvant chemotherapy is among the treatments that can reduce the risk of developing subsequent systemic metastasis in addition to achieving a good local control in case of high grade sarcomas, greater than 5cm (12).

In our case, after surgical excision of the tumor (with healthy margins of resection) were not found metastases in tests performed. Due of large size of the tumor (10 centimeters) and because there were no metastases found corroborated with a good health condition of the patient, we had considered appropriate to administrate an adjuvant chemotherapy by Adriamycin 3 sessions of intravenous continuous infusion over 3 days every 3 weeks followed by a complement of radiotherapy.

CONCLUSIONS

In prolonged fever of unknown origin we can expect sometimes an infrequent diagnosis, so the presumptive diagnosis should not remain obtuse, but must keep a wide vision to avoid missing the rare diagnosis and particularly aggressive as in our case. Diagnostic steps must be well structured in order to reach the diagnostic as quickly as possible to discover the disease in its earliest stages

to improve the survival prognosis.

While some imaging scans such as CT and MRI isolate organic anatomic changes in the body, PET is capable of detecting areas of molecular biology detail (even prior to anatomic change).

The high diagnostic accuracy of FDG-PET and PET/CT has been well documented for several neoplasms, with a consequent rapid increase in the utilization and acceptance of PET imaging in the clinical setting.

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INFLAMMATORY ABDOMINAL AORTIC ANEURYSM – A FORM OF CHRONIC PERIAORTITIS

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INFLAMMATORY ABDOMINAL AORTIC ANEURYSM - A FORM OF CHRONIC PERIAORTITIS (Abstract). Chronic periaortitis represents a unique pathogenic concept for three entities: Inflammatory Abdominal Aortic Aneurysm, Idiopathic Retroperitoneal Fibrosis and Perianeurysmal Retroperitoneal Fibrosis. The fundamental meaning of an inflammatory reaction to advanced atherosclerosis has been developed on the bottom of clinical and histological features. The triad of abdominal pain, weight loss and elevated inflammatory markers: erythrocyte sedimentation rate /C-reactive protein in patients with abdominal aortic aneurysms revealed on contrast-enhanced computer tomography is highly suggestive for inflammatory aneurysm. We report a case of a heavy-smoker adult male presented with suddenly abdominal symptoms suggestive for mesenteric ischemia which have proved to be due to inflammatory abdominal aortic aneurysm. The most favorable management of patients with inflammatory aneurysm is ambiguous. Surgical approach seems reasonable even supposing inflammatory aneurysm emerges less likely to rupture than the atherosclerotic variant. Corticosteroids are used in inoperable inflammatory aneurysm, even if is well known that this treatment does not change the long-term outcome of the disease. Surgical-open or Endovascular Repair of the aneurysm is the elective treatment. **Key words:** INFLAMMATORY ABDOMINAL AORTIC ANEURYSM, CHRONIC PERIAORTITIS, RETROPERITONEAL FIBROSIS.

Inflammatory abdominal aortic aneurysm (IAA) represents 10 % of all cases of abdominal aortic aneurysm (1) and is a form of chronic periaortitis (CP) which includes idiopathic retroperitoneal fibrosis and perianeurysmal retroperitoneal fibrosis (2, 3). Unlike atherosclerotic aortic aneurysm, the inflammatory type is characterized by remarkable thickening of the aneurysm wall and fibrosis of the adjacent retroperitoneum (4). The classical presentation consists in the triad of abdominal /back pain weight loss and raised inflammatory markers (2, 5, 6). It is important to recognize inflammatory abdominal aortic aneurysm in order to prevent the severe compli-

cations and the potentially fatal course (2, 6). Contrast-enhanced computed tomography and magnetic resonance imaging (MRI) is a reliable diagnostic modality (7, 8, 9). Knowing the features of inflammatory abdominal aortic aneurysm would allow doctors to distinguish it from atherosclerotic aneurysm and to treat it with the suitable combination of surgical and medical therapies (6, 10).

CASE REPORT

A 61–year-old man, presented with a short history of one week of recurrent diffuse abdominal pain, nausea and vomiting. In the last 24 hours the symptoms got worsened, the abdominal pain increased and localized in the left lower quadrant and low-grade fever appeared. The patient is a heavy smoker, overweight, known with dyslipidemia and has a family history of myocardial infarction, and stroke. In the last two months the patient have had a dull intermittent low back pain which worsen at night and weight loss of 3 kg. He denied intestinal transit alteration or presence of blood in stools.

Clinical examination in the emergency room showed an obese patient with bilateral xanthelasma, abdominal distension, and important pain on palpation on left iliac fossa with no rebound tenderness accompanied by discomfort with palpation in the whole left lower quadrant. Blood pressure 180/110mmHg, heart rate 89/min with extrasystoles.

Investigations showed raised C-reactive protein (CRP) 33 mg/dl and erythrocyte sedimentation rate (ESR) 65 mm/h, mild leukocytosis 12200/mm^3, lactate dehydrogenase (LDH) and creatine kinase MB (CK-MB) slightly increased, low-density lipoprotein cholesterol (LDL-cholesterol) 150 mg/dl, urinalysis were in normal range. Plain abdominal x-ray showed few hydroaeric levels without pneumoperitoneum; abdominal ultrasound revealed aortic dilatation suggestive of abdominal aneurysmal aortic appearance with 40 mm diameter. Chest X-ray was normal. Abdominal computed tomography revealed the presence of a 5 cm aneurysm of the infrarenal abdominal aorta on a length of 7 cm and aneurismal dilatation of both common iliac arteries and also, a soft-tissue density mass enveloping the aorta from the renal arteries down to the aortic bifurcation, that caused left mild hydronephrosis and dilatation of the ipsilateral ureter; the left kidney appeared enlarged, with important densification of perirenal fat extended up to the ureter (fig. 1).

Fig. 1. Abdominal computed tomography

These appearances were consistent with inflammatory abdominal aortic aneurysm, in agreement with cardiologist and vascular surgeon, we decided to perform an angiography which confirmed aneurismal aortic dilatation, normal mesenteric and renal arteries. Additional investigation showed atherosclerotic lesions in the carotid artery, mitral regurgitation and aortic valve stenosis. Serologic testing for autoimmune diseases was negative.

During hospitalization the clinical picture worsened with the appearance of signs suggestive for mesenteric ischemia: intense abdominal distension with ileus, important abdominal pain with vomiting and peritoneal irritation, hemodynamic instability. The medical team (composed of Internal Medicine Specialist and Vascular Surgeon) consultation considered appropriate the surgical treatment for the inflammatory abdominal aortic aneurysm. Transperitoneal standard median open laparotomy was performed, the aneurysmal segment was replaced with an appropriately sized Dacron tube graft and an aorto-bifemoral bypass operation was performed by using another Dacron tube graft. The retroperitoneal fibrosis involved the ureters therefore ureteral dissection was necessary. Intraoperative diagnosis of inflammatory aneurysm was based on the characteristic appearance of an aorta encased in a thick white fibrotic tissue. Specimens were obtained and were sent to the laboratory for histological examination.

The main finding was fibrosis of the adventitia with dense chronic inflammatory infiltrates with lymphoid follicle formation, presence of lymphocyte and other plasma cell proliferation.

As a result of the extensive retroperitoneal fibrosis technical difficulties with increasing bleeding appeared, thus patient had to be transfused in the postoperative period. Postoperative outcome has been favorable.

The first control visit was performed at one month after the operation. Patient underwent clinical examination by vascular surgeons and computer tomography examination at 6 months after the operation and annually thereafter.

One-year follow-up of the outcome contrast-enhanced abdominal computer tomography reveals functional aortic bifemoural prosthesis; mild delay in opacification of both iliac arteries which appear dilated and tortuous. Periaortitis fibrosis tissular mass is diminished compared to previous examination. The kidneys have normal dimensions with regular excretion; there is no hydronephrosis (fig. 2).

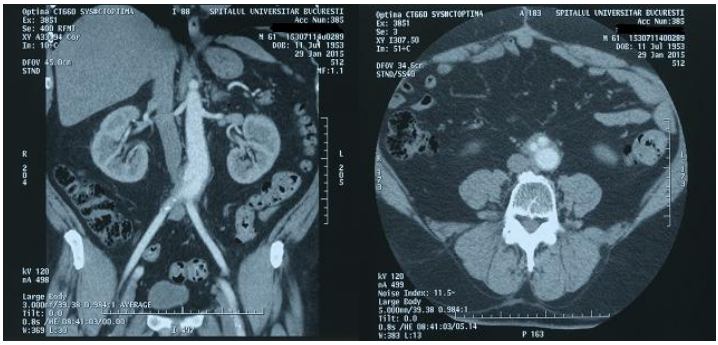

Fig. 2. Abdominal computed tomography at one year follow-up

DISSCUSIONS

Dr. James reported a case of inflammatory abdominal aortic aneurysm in 1935 for the first time. However, barely in 1972 Dr. Walker described histopathological features of inflammatory abdominal aortic aneurysm and formulated a definition in the British Journal of Surgery (4, 11).

Chronic periaortitis represents a rare condition (0, 2 -0, 5/100000/ year) that affect middle - aged/elderly men (12). It includes three diseases that have clinical and pathological related features, and thus being various manifestations of the same disease: inflammatory abdominal aortic aneurysm, idiopathic retroperitoneal fibrosis and perianeurysmal retroperitoneal fibrosis (2, 3, 4). Male sex and smoking are the main risk factors for inflammatory abdominal aortic aneurysm (13). Chronic periaortitis is defined by atherosclerosis of the abdominal aorta, medial thinning, periadventitial inflammation and fibrotic reaction enclosing the abdominal aorta correlated with the retroperitoneal inflammatory process (8). Inflammation and autoimmunity have been discussed as the main pathogenic process leading to periaortitis (2, 14, 15). Admitting the pathogenesis of chronic periaortitis appears to associate an immune response localized to a vessel wall, the etiology of the inflammatory reaction is still unknown (4). Inflammatory abdominal aortic aneurysm is not at all correlated with inflammation of other arteries (4, 8).

In the absence of any autoimmune conditions, the pathogenesis of our case of inflammatory abdominal aortic aneurysm could be the result of a local inflammatory reaction to progressive atherosclerosis process, as Mitchinson's concept proposed about pathogenesis of chronic periaortitis (8). Histological findings include chronic inflammatory infiltrates, comprised B-lymphocytes, even T- lymphocytes and plasma cells in the adventitia distinct to those of atheroma itself (14, 16).

In contrast with non- inflammatory abdominal aortic aneurysm, patients with inflammatory abdominal aortic aneurysm become symptomatic more frequently. The onset of inflammatory abdominal aortic aneurysm is in most cases of nonspecific nature (17). The clinical picture is dominated by abdominal pain, connected to the retroperitoneal mass, which represents the most frequent symptom accompanied by one of these unspecific manifestations of a systemic disease: weight loss, low-grade fever, fatigue, anorexia, vomiting (12). Back or flank pain and colicky pain are suggestive of ureter entrapping (12, 15, 17). Ureteral obstruction is cause of unilateral or worse, bilateral hydronephrosis that leads to renal failure (12, 17).

Diagnosis is made by clinical picture and the imagistic investigations along with nonspecific serologic features. Atheromatous determinations in other territory, cerebral, carotid arteries, ischemic heart disease, complete the clinical picture of inflammatory abdominal aortic aneurysm (12). Inflammation markers, C-reactive protein and erythrocyte sedimentation rate, are invariably raised together with mild normocytic, normochromic anemia and abnormal renal function markers (12, 17).

Contrast-enhanced computed tomography and magnetic resonance imaging are especially important not only for positive diagnosis but for differential diagnosis of a clinical picture centered by abdominal pain and weight loss (5, 18). Computer tomography is the most reliable diagnosis procedure, hence computer tomography images has to be checked cautiously before surgery

decision to recognize evidence like mantle sign, characteristic inflammatory abdominal aortic aneurysm (3, 5, 17). Retroperitoneal fibrosis may also occur secondary to oncologic diseases such as lymphoma, sarcoma or metastasis and not least related with some drugs - for instance methysergide, methyldopa, beta-blockers (12, 15, 19). Therefore, obtaining biopsy specimen of the fibrotic tissue (intraoperative) is mandatory (17).

CONCLUSIONS

The most favorable management of patients with inflammatory aneurysm is ambiguous. Medical treatment of inflammatory abdominal aortic aneurysm exclusively with corticosteroids is controversial (20, 21). Even if some authors reported initial regression of inflammation, recurrence after stopping steroids has been observed. Corticosteroids are used in inoperable inflammatory aneurysm, even though is well known this treatment do not alter the long-term outcome of the disease (22). Surgical approach seems reasonable even supposing inflammatory aneurysm emerges less likely to rupture than atherosclerotic variant (8) and, in comparison with non-inflammatory aneurysms, inflammatory abdominal aortic aneurysm is related to a four-times higher perioperative morbidity and mortality rate (17). Surgical-open or Endovascular Repair of the aneurysm is the elective treatment particularly when the diameter of the aneurysm exceeds 5 cm (8, 23).

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NEWS

BIOFILM-INHIBITORY COMPOUNDS AGAINST *PSEUDOMONAS AERUGINOSA* FROM BURDOCK LEAF

In a study by Lou Z *et al,* basic anti-biofilm profile data for the compounds in burdock leaf were analyzed and a convenient method for fast screening of anti-biofilm compounds from natural plants was provided. According to this study, 34% ethanol elution fraction of burdock leaf could completely inhibit biofilm formation of *Pseudomonas aeruginosa* at 1 mg·mL(-1). Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) was used to determine the chemical composition of burdock leaf fraction and to obtain the metabolic fingerprints of burdock leaf fractions before and after inhibiting *P. aeruginosa* biofilm. The analysis identified 11 active compounds: chlorogenic acid, caffeic acid, p-coumaric acid, quercetin, ursolic acid, rutin, cynarin, luteolin, crocin, benzoic acid, and Tenacissoside I. Partial least squares discriminant analysis (PLS-DA) of the metabolic fingerprints showed that, among 81 compounds that were screened as potential anti-biofilm ingredients, rutin, ursolic acid, caffeic acid, p-coumaric acid and quercetin are the main anti-biofilm compounds in burdock leaf (Lou Z, Tang Y, Song X, Wang H. Metabolomics-Based Screening of Biofilm-Inhibitory Compounds against Pseudomonas aeruginosa from Burdock Leaf. *Molecules*. 2015;20(9):16266-77. doi: 10.3390/molecules200916266).

Teodora Vremera

DESOGESTREL IN THE PREOPERATIVE TREATMENT OF ENDOMETRIOTIC CYSTS

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DESOGESTREL IN THE PREOPERATIVE TREATMENT OF ENDOMETRIOTIC CYSTS (Abstract): Endometriosis is an estrogen dependent disease characterized by the presence of endometrial tissue outside the uterus. It affects women of reproductive age and is associated with infertility and pelvic pain, with impact on their mental, physical and social well-being. **Aim:** To evaluate the effects of continuous desogestrel treatment on cyst size and the risk of intraoperatively bleeding. **Material and methods:** Our study included two hundred fifty-five patients with infertility and endometriotic cysts operated at the Iasi "Cuza Voda" University Hospital in the interval 2006-2014. One hundred fifty-five patients received 6-month preoperative treatment with 75 micrograms of desogestrel daily. The patients were monitored every three months by vaginal ultrasound associated with Doppler for assessing the cysts size,homogeneity, shape, vascularization, position relative to the uterus and the contralateral ovary, presence of sludge and horizontal levels. **Results:**There was asignificant decrease in cyst diameter and vascularization in most cases with sludge and low echogenicity. Intraoperatively we noticed less bleeding during cyst dissection and a better cleavage plan between the cyst capsule and the normal ovarian tissue. **Conclusions:** Treatment with desogestrel for at least 6 months preoperatively has a shrinking effect on the endometriotic cyst, reduces intraoperative bleeding and improves the dissection and operative conditions. **Keywords:** ENDOMETRIOSIS, INFERTILITY, DESOGESTREL, PELVIC PAIN.

Endometriosis is an estrogen-dependent inflammatory disease defined by the growth of endometrial stroma and glands outside of the uterus, especially on the ovaries and in the peritoneum. Affected women often present with chronic pelvic pain, dysmenorrhea, dyspareunia and infertility (1, 2).

The prevalence of endometriosis increases dramatically to as high as 25% to 50% in women with infertility, and 30% to 50% of women with endometriosis are infertile (3).

It is often cited that retrograde menstruation is the cause of this condition; however retrograde menstruation occurs in nearly all women, yet not all women are afflicted with this condition. Therefore, it has been suggested that women with endometriosis are likely to have underlying molecular abnormalities that allow the continued growth of this endometrial tissue outside the uterus

and that ectopic implantation is possible as a consequence of altered immunity or preexisting peritoneal lesions (4,5).

Ovarian endometriotic cysts are usually detected ultrasonographically but peritoneal lesions and small ovarian cysts are more difficult to detect, so laparoscopy and histopathological diagnosis remain the gold standard. Endometriomas are particularly difficult to excise as the cyst wall is very adherent and well vascularized, thus surgery may reduce the ovarian reserve. The surgical treatment of endometriosis is effective in the short-term, but if the patient does not get pregnant very soon after surgery, it is most probably because endometriosis has relapsed. Recurrence rates after surgical intervention can be decreased with the use of menstrual suppression, such as hormonal contraceptives (6).

Hormone therapy is frequently effective in reducing or even eliminating endometriosis-related pain. The success of various hormonal therapies depends on the location and type of the endometriotic lesions. Superficial peritoneal and ovarian implants seem to respond better to hormone therapy than deep ovarian, peritoneal or lesions within organs. Moreover, hormone treatment has no effect on adhesion of endometriotic cells and cannot improve fertility. Nonetheless, a number of hormonal agents remain the mainstay of endometriosis therapy (7,8).

Progesterone is a potent antagonist of estrogen-induced proliferation in the endometrium and may play a pivotal role in the pathogenesis of endometriosis (9).

Desogestrel is a progestogenic 19-nortestoterone derivative, which is widely used for oral contraception both combined with ethinyl estradiol and alone in progestin-only pills (10).

Our primary goal was to assess the results of treatment with desogestrel regarding improvement of clinical symptoms, ultrasonographic findings and intraoperative bleeding, adhesions and ease of cyst dissection.

MATERIAL AND METHODS

Our study included two hundred fifty-five patients with infertility and endometriotic cysts (diameter less than 20 mm) operated laparoscopically at the Iasi "Cuza Voda" University Hospital Iasi between the years 2006-2014. One hundred fifty-five patients (60.78%) - group A, received a 6-month preoperative treatment with 75 micrograms of desogestrel per day and one hundred patients (39.22%) – group B, received preoperative placebo treatment.

Women with body mass index > 30, neoplasias, autoimmune diseases, diabetes mellitus, infectious diseases, pregnant, under anti-inflammatory or hormonal treatment were excluded from the study.

A written informed consent was obtained from all patients enrolled in the study. The consent form was approved by the Medical Ethics Committee of the Iasi „Grigore T. Popa" University of Medicine and Pharmacy.

The patients were monitored monthly both clinically and ultrasonographically by evaluating the cyst size, homogeneity, shape, vascularization, position relative to the uterus and contralateral ovary, presence of sludge and horizontal levels. All patients were examined by vaginal ultrasound associated with Doppler at the moment of diagnosis and then every 3 months.

In all cases the diagnosis was confirmed postoperatively by histological examination, which demonstrated the presence of histopathologic features similar to endometrium, namely endometrial stroma, endo-

metrial epithelium, glands that respond to hormonal stimuli and hemosiderin deposits.

Age of the patients ranged from 20 to 49 years (mean age 32 years), all of them still menstruating.

The parameters followed intraoperatively were the ease of dissection, amount of bleeding, possibility of conservative treatment, extension of adhesions, and disease staging.

Postoperatively the patients were reevaluated three months later by clinical examination and vaginal ultrasound.

RESULTS AND DISCUSSION

In group A patients (on desogestrel treatment) we found a significant decrease in cyst diameter and vascularization associated with sludge and decrease in echogenicity (tab. I).

TABLE I

Preoperative ultrasonographic changes

	Group A	Group B
Cyst diameter	↓	↑/-
Vascularization	↓↓	↑/-
Sludge and hyperechogenicity	↓↓	↑/-

During the follow-up we noticed in the patients treated desorgestrel (group A) an improvement in clinical symptoms with impact on their mental, physical and social well-being.

In the same group A patients we noticed intraoperatively less bleeding during cyst dissection and a better cleavage plan between the cyst capsule and the normal ovarian tissue (tab. II).

Desorgestrel was well tolerated and no patient had to stop the administration because of side effects. Eighty women became amenorrheic, 58 had irregular vaginal bleeding and 17 women had regular periods. The bleeding amount was described as much lower than a normal period. Fifty-one patients complained of weight gain, the maximum being 5 kg. Twenty-eight patients complained of vaginal dryness and sexual discomfort. Fifty-five patients had decreased libido (fig.1).

TABLE II

Surgical parameters

	Group A	Group B
Easy dissection and capsule cleavage	+++	+/-
Heavy bleeding	+/-	+++
Thick adhesions	+	+++
Operative time	+	++

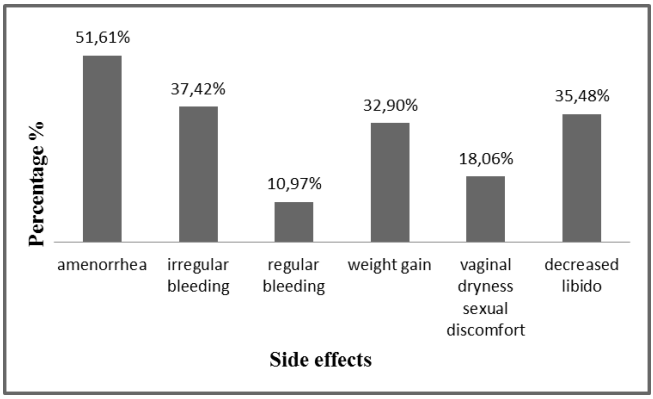

Fig.1. Side effects of Desogestrel treatment

Endometriosis is a major public health problem, due to the number of cases that continues to increase and the risk of consecutive ovarian and endometrial tumors (10% of endometrioid carcinomas of the ovary being associated with uterine endometrioid carcinomas), fact that motivates many researches focused on the mechanisms of endometriosis development and progression (3). Although endometriosis is considered a chronic benign condition, it can present invasive characters, inducing severe symptoms (13).

The study of endometriosis is not fortuitous since this gynecologic disease has an increased incidence, accounting for 2% of the general population, and is the third cause of gynecological checkup for infertility, chronic pelvic pain, dyspareunia and dysmenorrhea. Endometriosis affects 7-15% of women of childbearing age, accounting for 25-30% of cases of sterility, with important social impact (3).

In endometriosis research there are numerous scientific data, with big names in the scientific world who say the same things about its incidence, mechanism of production and social repercussions.

Laparoscopic intervention is the "gold standard" approach in the treatment of endometriosis, and the results may be enhanced with an appropriate technique and reasonable adjuvant therapies (14).

Of the adjuvant therapies for endometriosis, desogestrel may be one of them.

This study showed that treatment with desogestrel improves the operative conditions mainly by decreasing the bleeding and increasing the laxity of the connective tissue between the cyst capsule and the normal ovarian tissue.

However its side effects, such as amenorrhea, irregular bleeding, weight gain and decreased libido as well as its contraceptive action might reduce patient compliance to this treatment.

CONCLUSIONS

In our study, progestative treatment improved the operative conditions and decreased the recurrence risks of ovarian endometriotic cysts.

Treatment with desogestrel for at least 6 months preoperatively has a shrinking effect on the endometriotic cyst, decreases intraoperative bleeding and makes dissection and surgery easier.

In progress researches will demonstrate the action of preoperative treatment with desogestrel on the factors involved in the progression of endometriotic lesions.

ACKNOWLEDGEMENS

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NEWS

TEA DRINKING AND TUBERCULOSIS

Numerous studies showed that tea drinking helps to prevent obesity, cardiovascular diseases, autoimmune diseases, neurodegenerative diseases and tumors. Chinese researchers discovered the negative association between tea drinking and tuberculosis (TB). Tea leaves contain epigallocatechin-3-gallate, a flavonoid which inhibit the bacterial growth and the bacillus survival in macrophages. The increasing tea consumption is associated with a decreased risk of TB. Subjects who consumed 151-300 g tea / month had a lower risk to develop TB than those with a lower intake of tea. The green tea led to the most evident protection against TB, compared to oolong and black tea. Future studies are needed to determine whether drinking tea can be used in the prevention and control of TB (Mengshi Chen; Jing Deng; Wufei Li; Dan Lin; Congxu Su; Mian Wang; Xun Li; Benjamin Kwaku Abuaku; Hongzhuan Tan; Shi Wu Wen. Impact of tea drinking upon tuberculosis: a neglected issue. *BMC Public Health* 2015;15(515)).

Cătălina Luncă

LIVER METASTASIS OF A GASTROINTESTINAL STROMAL TUMOR OF LARGE BOWEL: CASE REPORT

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LIVER METASTASIS OF A GASTROINTESTINAL STROMAL TUMOR OF LARGE BOWEL: CASE REPORT (Abstract): Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal malignancies of the digestive tract. We present the case of a 61-year-old male patient liver metastasis of a GIST with primary location at the level of sigmoid colon. Like in the majority of cases, the symptomatology in this patient has long been faint and when it became manifest, it was nonspecific. Imagery wise, the computer tomography (CT) scan was the most efficient, showing the origin of the tumor from large bowel, its dimensions, as well as the relations with the other abdominal viscera and the liver metastasis. Surgery in this patient was en-bloc, according to the principles of GIST and for the metastasis he followed treatment with Imatinib. The histological aspect is characterized by a proliferation of spindle cells positive for CD117 and CD34. Despite complete microscopic resection, the exhibit of liver metastasis remains an important relapse factor. **Keywords:** GASTROINTESTINAL STROMAL TUMOR, LARGE BOWEL, LIVER METASTASIS.

Initially believed to arise from smooth muscle cells, it is now known that gastrointestinal stromal tumors (GISTs) are malignancies that arise from the interstitial cells of Cajal, which are pacemaker cells found in the bowel wall (1).

They are defined pathologically as c-Kit-positive mesenchymal spindle cell or epithelioid neoplasms (2). The most common location of GISTs are stomach (60-70%), followed by the small intestine (20-30%), the rectum (5%) and the esophagus (<5%). Patients with GISTs are 70% symptomatic but mostly nonspecific, while 20% are asymptomatic and the tumors are detected incidentally and a part of cases are detected during autopsy. The most common symptoms of GISTs are nausea, vomiting, abdominal discomfort, bleeding from the gastrointestinal tract and weight loss. The most common

metastatic sites are the liver and peritoneum, whereas GISTs are rarely found to metastasize intracranially or to the lymph nodes, lungs or subcutneous tissue (3, 4, 5). That being said, surgical resection is still the most important component in the treatment of resectable non-metastatic GISTs (6). Also, with the aforementioned advances made in the medical treatment of these tumors, there is the possibility of a future broadening of surgical indications to include patients with metastatic disease, but this currently remains investigational (7). Imatinib mesylate, a tyrosine kinase inhibitor, shows high clinical efficacy in advanced GIST (8, 9) and is now the standard treatment for unresectable and metastatic GISTs (10). The response to imatinib is known to depend on the mutation sites of the c-kit gene or the platelet-derived growth factor receptor alpha gene (11). The present article describes one case of liver metastasis in patient with GISTs of large bowel and reviews the relevant literature.

CASE REPORT

We report the case of a 61-year-old male patient. He presents pain in lower left abdomen what subside after defecation, accompanied by a quick sensation of fullness after meals and pollakiuria. These symptoms appeared in last three months, worsening progressively. Physical examination reveals lipomas in the abdominal region, chest, neck and limbs, painful left hypogastric region, a palpable tumor in the left iliac fossa, fixed to the deep anatomic plans, with a diameter of 15 cm and hepatomegaly.

We didn't found any pathological laboratory tests except LDL-cholesterol 300 mg/dl and triglycerides 180mg/dl.

The patient's medical history includes lipomatosis, dyslipidemia, and heart attack in 2005 with percutaneous transluminal angioplasty with stent, hepatic steatosis, and liver hemangiomas.

A week before admission in our hospital he was diagnosed in another medical service with left kidney stones and liver hemangiomas.

In our service he made an abdominal ultrasonography which shows a tumor with a maximum diameter of 15 cm, heterogeneous, with interior transonic images, which occupies the hypogastrium and left iliac fossa without being able to specify the origin (fig. 1) .

The right hepatic lobe (segment V, VI, VIII) contains tree lesions hypoechoic, with peripheral halo, size 25mm, 35mm, 54mm and multiple lesions hyperechoic (segment III, IV, VII, VIII), well defined, size 5 to 18 mm without Doppler signal (fig. 2).

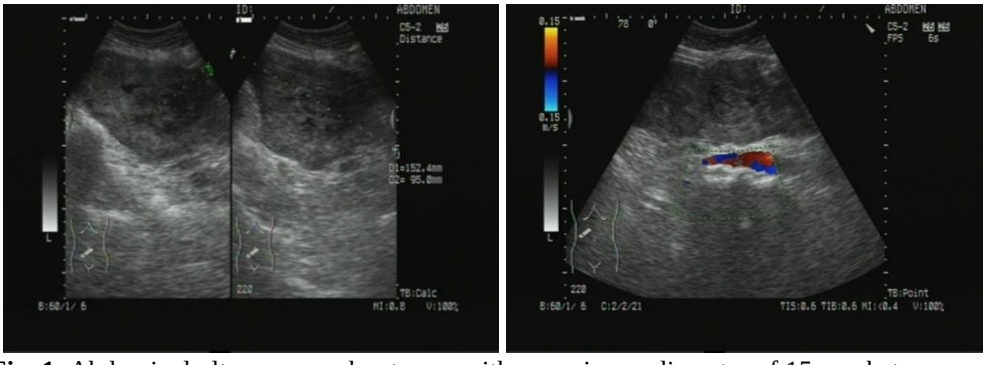

Fig. 1. Abdominal ultrasonography: tumor with a maximum diameter of 15 cm, heterogeneous, with interior transonic images, which occupies the hypogastrium and left iliac fossa.

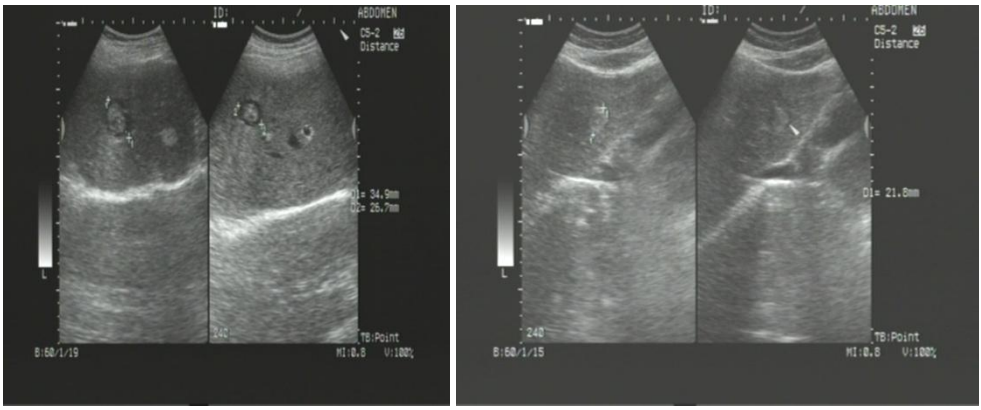

Fig. 2. Abdominal ultrasonography**:** Liver metastasis and hemangiomas

Colonoscopy exam revels between 40 and 45 cm from the anus segmental venous dilatation with no other pathological lesions (fig. 3).

Computerized tomography shows a heterogeneous tumor size117/110/207 mm, situated intraperitoneally in left iliac fossa, located between ileum and sigmoid colon with intimate contact with them, well defined, with numerous necrosis areas inside. No intra-abdominal adenopathies noted (fig. 4).

In the liver describe the same benign lesions from the ultrasound exam with follow sizes: 17mm, 8mm and 15mm. At the VIII liver segment we detected a 9 mm lesion, between V and VI segments we founded a 29mm lesion and in the VI segment we detected two lesions by 20mm and 23mm suggestive of hepatic metastasis (fig. 5).

Surgery is approached with a median laparotomy. Exploration highlights the origin of the tumor in the sigmoid colon, with invasion to the ileum. The tumor is extremely hemorrhagic at the maneuvering. The tumor was en-bloc resected, with resection of a part of ileum and achievement of an enteroanastomosys and a limited resection of the sigmoid colon because the tumor was started from the serous of the sigmoid colon and also achievement of a coloanastomosys. Surgical recovery was favorable.

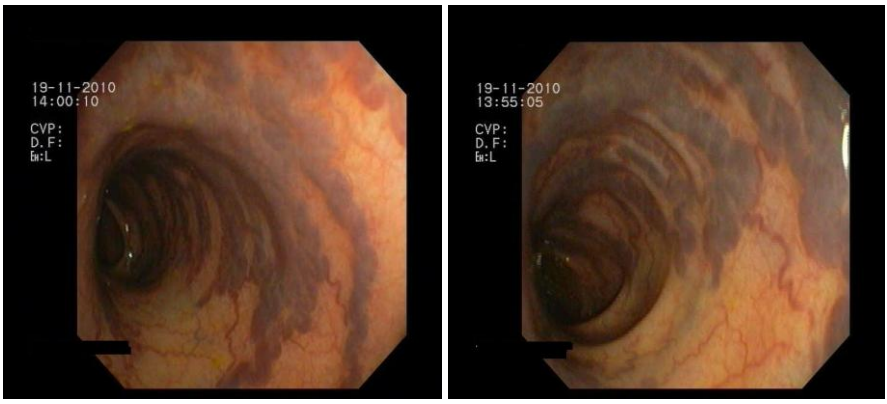

Fig. 3. Colonoscopy exam

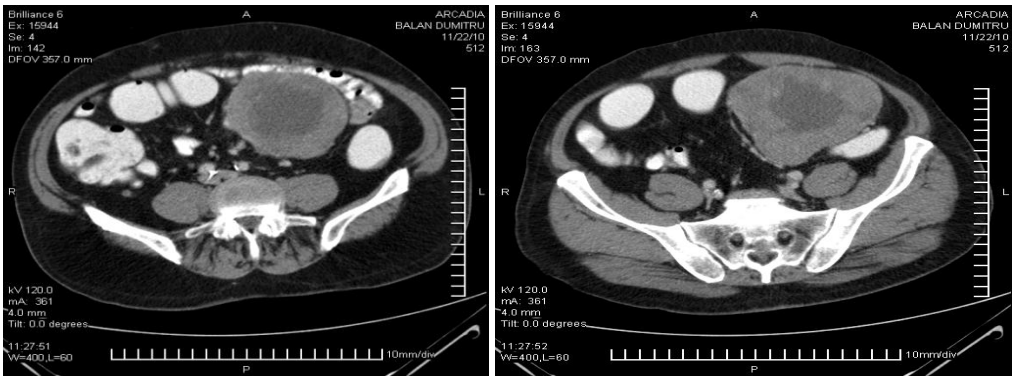

Fig. 4. Computed tomography exam: heterogeneous tumor in left iliac fossa, well defined, with numerous necrosis areas inside

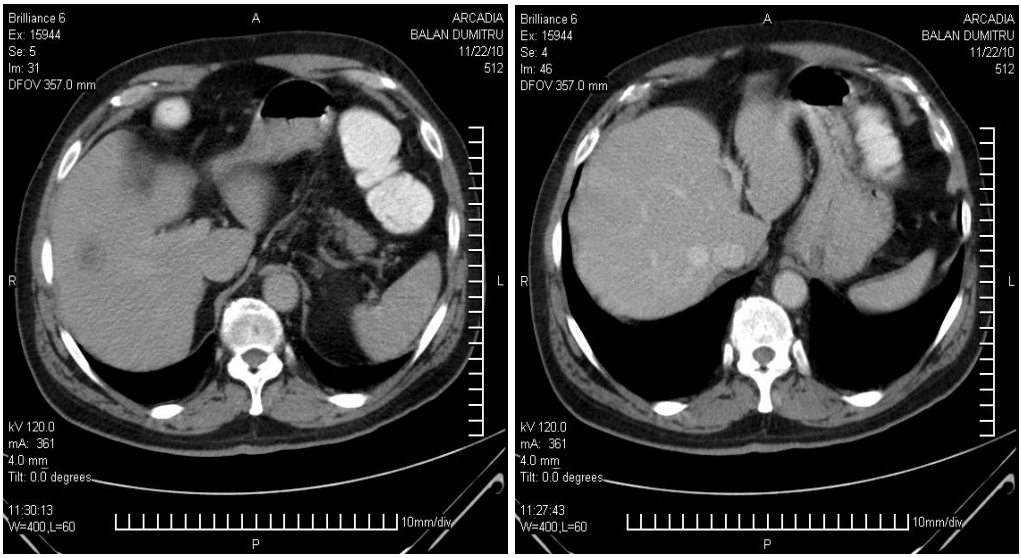

Fig. 5. Computed tomography exam: hemangiomas and liver metastasis

Histopathological appearance of the surgical specimen in HE staining, we used the CD117 antibody (c-kit), as we know that this is a specific marker for GIST (fig. 6).

Another significant marker in the diagnosis of GIST is CD34 antigen. CD34 is a glycoprotein that facilitates the intercellular adhesion or the cellular adhesion to the stroma and it is also positive in the endothelium of blood vessels (fig.7).

For the liver lesions there was no surgical treatment indication and the patient started treatment with Imatinib after 3 weeks from surgical intervention. After one month of treatment liver metastases were transformed into sharply circumscribed, non enhanced homogeneous lesions. After 6 months the aspect of the metastasis was the same.

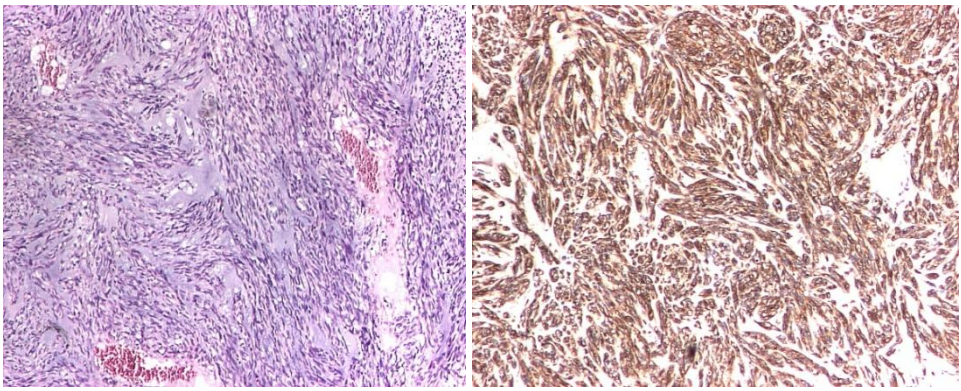

Fig.6. Intense reaction of tumoral cells to this antibody CD117 in colon tissue.

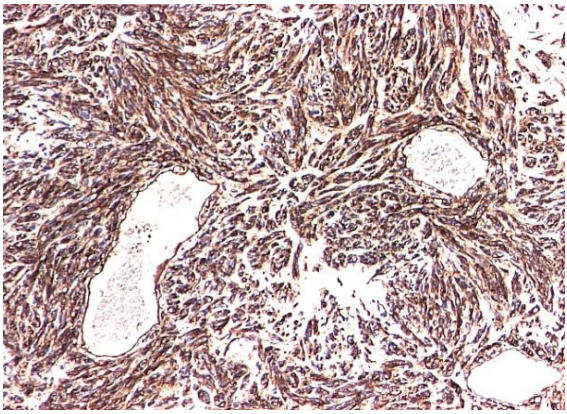

Fig. 7. CD34 positive in the endothelium of blood vessels of colon.

DISCUSSION

Asymptomatic tumors diagnosed at a late stage, which is often the case, can be large on presentation. Prognosis for patients diagnosed with GIST depends on tumor size, mitotic rate, histopathological subtype and tumor location. That is why early diagnosis and R0 resection are the key factors for further treatment and good prognosis (12).

Colonic GIST is even rarer because many of the large bowel GISTs arise in the rectum. Owing to the lack of data on this rare malignancy, the clinical and pathological features of colonic GIST are still unclear (13).

Imatinib mesylate, a tyrosine kinase inhibitor, shows high clinical efficacy in advanced GIST (8, 9) and is now the standard treatment for unresectable and metastatic GISTs (10, 14).

CONCLUSIONS

We reported this case because gastrointestinal stromal tumors (GISTs) developing in the colon are rare. Our patients was symptomatic but mostly nonspecific, most common symptoms were nausea, vomiting, abdominal discomfort, bleeding from the gastrointestinal tract and weight loss and because of that he was diagnosed in the metastatic stage.

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LYMPHOMA IN AN HIV-POSITIVE PATIENT

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LYMPHOMA IN AN HIV-POSITIVE PATIENT (Abstract): HIV/AIDS is considered to be revealing of oncological diseases, of which most frequent are lymphomas. The main causes for this type of disease are non-adherence and non-compliance to antiretroviral therapy (ART). We are hereby presenting a clinical case of lymphoma in an HIV-infected adult, with non-adherence for 30 months. In such a case the interdisciplinary collaboration with the hematologist was essential for the patient's survival. **Keywords**: HIV/AIDS, LYMPHOMA, ANTIRETROVIRAL THERAPY, ADHERENCE.

The HIV pandemic is a reality that is increasingly frequent in Romania. Studies have observed that the median age of HIV-positive patients has slowly shifted from the "pediatric cohort" (born in '87-'89) to adults and even patients over 60 years old (1).

Frequent cases of cancer are observed in patients with severe immunosuppression. Lymphomas are dominant in literature and they require a combined therapy and monitoring by both the infectious diseases doctor and the oncologist (2).

CASE PRESENTATION

We aim to present the case of a patient born in 1979, diagnosed in 2010 (31 years old) with pulmonary tuberculosis. We used two specific tests to screen for HIV: the enzyme-linked immunosorbent assay (ELISA) and antigent-anticorp confirmation test Western Blott. They both were positive. At the very beginning, CD4 cell count was 120/mmc and viral load (VL) 742000 copies/mmc (fig. 1). We applied therapeutic protocol, initiating therapy with tuberculostatics for 10 days. Next we started antiretroviral therapy with zidovudine and lamivudine (AZT+3TC) and efavirenz (EFV). To avoid the immune reconstruction syndrome we administered dexametazone.

Serology for hepatitis B and C were negative, as well as syphilis. Acute phase reactants for citomegalovirus and toxoplasma are were also negative. The resistance report for antiretroviral drugs was negative for all antiretroviral (ART) classes. However, we identified 2 minor mutations: L10V for protease inhibitors and K103R for non-nucleotidic revers-transcriptase inhibitors. We also started prophylaxis for pneumocistosis.

The patient's evolution was favorable. In February 2011 (6 months after diagnosis), CD4 had increased to 414 cells/mmc and the viral load decreased to 160 cop-

ies/mmc.

The patient ended treatment with tuberculostatics after one year, but adherence to ART decreased, up until VL=99900 copies/mmc and CD4=251 cells/mmc in Nov 2012 (fig. 1).

We lost track of the patient for 30 months. In June 2014 the patient presents herself to the Infectious Diseases Hospital with a massive clinical deterioration, with wasting syndrome, loosing over 15 kg in body mass. Also important generalized edema, hypoproteinemia 41.6 g/l, hemoglobin 2.7 g/l, white blood cells 2800/mmc and mononucleosic syndrome present; CD4=58 cells/mmc and VL=54300 copies/mmc (fig. 1). Pulmonary radiological imaging revealed a small amount of pleural liquid, arising the suspicion for pulmonary tuberculosis. This is why our option for antiretroviral therapy consisted of raltegravir (RAL) with abacavir+ lamivudine (KVX) (RAL+KVX), avoiding protease inhibitors. Sputum samples were negative for Koch bacillus (BK) bacterioscopy.

We started support therapy with blood derivates, intravenous aminoacids, albumin, filgastrinum, cotrimoxazol for pneumocystosis prophylaxis and fluconazol for oral candidiasis.

The patient is released from the hospital after 33 days of hospitalization; biological parameters were much emproved – white blood cells 3000/mmc, a normal proteinemia 64.5 g/l, and hemoglobin 9.1 g/l. The patient also gained 4 kg in weight. Renal and hepatic functions were normal.

For the whole duration of the hospital stay, the patient had psychological evaluation and regular counseling sessions. This improved her self-esteem and self-worth, which motivated her to decide for a better adherence to antiretroviral therapy. Through specific techniques (3), the psychologist encouraged the patient to take responsibility for her health-related behavior, thus changing her lifestyle as well.

Two months later, the patient is admitted again with fever and swallowing of latero-cervical lymph nodes. BK cultures were negative, which allowed a switch to last generation antiretroviral molecules – boosted darunavir (DRV/r), etravirine (ETV), abacavir+lamivudine (KVX).

The node biopsy showed mixed cellularity Hodgkins lymphoma; CD4=82 cells/mmc, VL=80 copies/mmc (very close to non-detectable) (fig. 1).

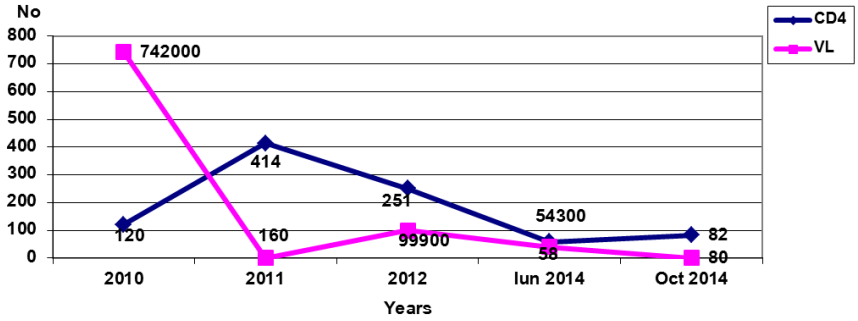

Fig. 1. CD4 cell count and viral load

The clinical and biological aspect of this patient allowed chemotherapy in repeated rounds, but in the presence of substitutive support (blood derivates, filgastrinum, intravenous aminoacids, and albumin).

Latero-cervical lymph nodes decreased

in size. Antiretroviral therapy was continued until present day, keeping this ART scheme for the rest of her life.

DISCUSSION

Immunosuppression with values of CD4<200/mmc is manifested in patients that are non-adherent, non-compliant to ART, in cases of therapeutic fatigue and acquired resistance to highly active antiretroviral therapy (HAART). The Romanian cohort has an extended experience with antiretroviral therapy; patients are multi-experimented and have a history of antiretroviral regimens from the first PIs to last generation molecules (4). The cancer incidence in HIV-positive patients decreased dramatically after 1990, along with the HAART era (5).

From an epidemiological point of view, the risk factors for lymphoma in the United States of America are sex, age and socio-economical status; 80% of people with non-Hodgekin lymphoma are men who have sex with men (MSM) and bisexuals. The high incidence in the East Coast is a reflection of HIV incidence (6).

One of the most significant differences in lymphomas occurring among HIV-infected individuals is a high association with oncogenic herpes viruses. There is a molecular heterogeneity of HIV-related lymphomas. From a pathogenic point of view, this includes mono- or polyclonal subtypes, the presence or absence of the Epstein-Barr virus (EBV) genome and herpes virus 8 genome, which is integrated in the genome of the host cell. The roles of cytokines and tumor suppression genes, as well as the uncontrolled growth of B lymphocytes, are to be further researched. The main hypothesis is that, in the context of HIV immunosuppression, the EBV infection leads to a polyclonal stimulation and the immortalization of B cell clones (7).

Specific antiretroviral therapy is one of the pillars in sustaining the clinical and biological status of the patient, alongside support therapy. A high level of CD4 and a low viral load, as well as balanced biological parameters, assure a better response to specific chemotherapy in lymphomas (8).

CONCLUSIONS

The present case has revealed the importance that adherence to antiretroviral therapy has in the control of the HIV infection. This allowed the possibility to administrate full-dose-intensive chemotherapy regimens with improved patient outcomes.

We found that it is essential that the infectious diseases doctor and the hematologist work together to ensure the best therapeutic solutions to support the HIV patient with lymphoma.

Psychological support and motivational psychotherapy have also been essential in sustaining this patient's desire to go through with therapy. It improved adherence to therapy and generated a change in the patient's lifestyle, as well as a sustained self-esteem and self-worth.

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NEWS

A MIXTURE OF CHIA SEED AND POMEGRANATE FRUIT EXTRACTS-SYNERGISTIC EFFECT ON INHIBITION OF MELANIN BIOSYNTHESIS

Fatty acids (especially omega-3 and omega-6) have not only nutritional benefits, but also cosmetic ones. They are involved in many biological functions in the skin (prevention of transepidermal water loss, maintenance of the *stratum corneum* epidermal barrier and disruption of melanogenesis in epidermal melanocytes). *Salvia hispanica* L. (commonly known as chia) seeds are used especially as dietary supplements due to their content in fatty acids and phenolic compounds. The consumption of the seeds decreases the triglyceride and blood glucose levels, thus reducing the risk of heart disease and obesity. *Punica granatum* L. (pomegranate) fruits, rich in polyphenols, have the capacity to reduce melanin content in the skin. Because chia seed oil (rich in α-linolenic and linoleic acids) has high viscosity, low solubility and poor cellular permeability, Diwakar *et al*. have tested a mixture of chia seed and pomegranate extracts (1:1) to reduce melanin content in Melan-a cell culture. The results demonstrated that a mixture containing 25 μL of each extract reduced melanin production by 80% as compared to chia seed extract (10%) and pomegranate fruit extract (40%). The down-regulation of melanogenesis-related genes (Tyr, Tyrp1 and MC1r) could be a mechanism involved in melanin production inhibition, since the mixture did not inhibit tyrosinase activity. The study proved that the mixture of chia seed and pomegranate fruit extracts (1:1) had synergistic effect on melanin inhibition in Melan-a culture cells (Diwakar G, Rana J, Saito L, Vredeveld D *et al*. Inhibitory effect of a novel combination of *Salvia hispanica* (chia) seed and *Punica granatum* (pomegranate) fruit extracts on melanin production. *Fitoterapia* 2014; 97:164-171).

Cristina Lungu

ENDOSCOPIC MANAGEMENT OF CHOLEDOCOLITHIASIS RELATED TO PERIAMPULLARY DUODENAL DIVERTICULA

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ENDOSCOPIC MANAGEMENT OF CHOLEDOCOLITHIASIS RELATED TO PERIAMPULLARY DUODENAL DIVERTICULA (Abstract): **Aims**: Periampullary duodenal diverticula is a subject still widely debated in the literature. Our study aimed to describe the characteristics and the clinical outcomes of a case series of subjects with periampullary duodenal diverticula and bile duct stones, receiving therapeutic endoscopic retrograde cholangiopancreatography (ERCP), by comparison to patients having only bile duct stones. **Material and methods**: 639 consecutive patients with bile duct stones have been investigated through ERCP during 2011 – 2016 in a single specialized center of gastrointestinal surgery. Periampullary duodenal diverticula (PADD)'s prevalence reached at 16.9% (n=108 cases). The patients with PADD have been compared to those without PADD, by personal characteristics, pathophysiological mechanisms incriminated in producing symptoms and therapeutic outcomes. **Results**: Patients with PADD showed a predominance of males, and a significantly older age. Most frequent abnormality was angulation of the terminal CBD group and jaundice occurred in a significantly higher proportion compared to non PADD group. The percentages of successful selective cannulation and sphincterotomy were significantly lower in the PADD group, even comparable to the literature; meanwhile mechanical lithotripsy was required in a higher proportion of cases. Main cause of failure of cannulation was the changed distal track of the duct. The frequency of postoperative complications was like patients without periampullary duodenal diverticula. **Conclusions**: Therapeutic endoscopic retrograde cholangiopancreatography is obviously a more difficult procedure in the presence of periampullary duodenal diverticula, due to the difficult access to the papilla and the common bile duct, but the success rate remains high even in such cases. **Keywords**: PERIAMPULLARY DUODENAL DIVERTICULA, CHOLEDOCHOLITHIASIS, ENDOSCOPIC RETRO-GRADE CHOLANGIO-PANCREATOGRAPHY (ERCP).

Duodenal diverticula (DD) are known for more than three centuries, the first descriptions belonging to Chomel (1710), Morgagni (1762) and JT Case (1913) (1). Their implications in biliary pathology, especially in syndromes associated with cholestasis (with or without bile duct stones) are known and studied systematically only after introduction into practice of Endoscopic Retrograde Cholangio-Pancreatography (ERCP) in mid-1980. It is also a still widely debated topic in litera-

ture, which is why we present our experience in this regard. Reporting their occurrence depends on the method of diagnosis: 1-6% in patients undergoing a barium transit, 8-12% during an upper gastrointestinal endoscopy, in 15-17% of patients treated by ERCP, about 20-23% after autopsy studies (1).Obviously, the differences between statistics is due to several factors: specific diagnostic accuracy of the method, the type of pathology to which it is addressed, and not least upon the age groups most frequently investigated. Most DD are clinically latent. They rarely produce symptoms, which are usually the consequences of their complications. Of these, the most common are the biliary-pancreatic and more rarely the inflammatory and drilling complications.

Our study aimed to describe the characteristics and the clinical outcomes of a case series of 108 subjects with periampullary DD (PADD) and bile duct stones, receiving therapeutic ERCP in a single specialized center of gastrointestinal surgery, by comparison to patients having only bile duct stones.

MATERIAL AND METHODS

In our clinic, we investigated through ERCP 639 consecutive patients with bile duct stones during 2010 –2015and we found a prevalence of PADD of 16.9% (n=108 cases). We compared the group of patients with PADD to those without PADD, following the personal characteristics (age and gender), the pathophysiological mechanisms incriminated in producing symptoms and the therapeutic outcomes.

As regard the pathophysiological mechanisms, few abnormalities could induce symptoms in DD: 1) angulation of the terminal common bile duct (CBD) with changes in local anatomy and disturbed excretion dynamics of bile and pancreatic juice; 2) compression and stenosis of the terminal portions of the common bile duct and Wirsung duct, with impaired evacuation and dilated CBD; 3) impaired duodenal peristalsis and 4) incomplete evacuation of the diverticular bag, exacerbating the local microbial flora (2). These abnormalities usually induce biliary-pancreatic symptoms like: jaundice, cholangitis, recurrent acute pancreatitis or diverticulitis and perforation. We considered the presence of all these symptoms in the study, as binary variables.

The therapeutic outcomes included the ERCP performance (with focus on the technical and tactical problems found during sphincterotomy), the occurrence of complications and the length of hospitalization. For measuring the ERCP performance we followed the proportion of patient receiving selective cannulation and sphincterotomy in each group and the proportion of cases which required mechanical lithotripsy. As complications, we followed the acute pancreatitis (classified as mild, medium and severe) and post ERCP hemorrhage.

At admission, all patients were investigated following a protocol that included clinical exam, biochemical tests (blood counts, liver and pancreatic tests, coagulation and renal function), electrocardiogram and thoracic-pulmonary radiography. After ERCP, at 24-48 hours, blood count and serum biochemistry tests were repeated to identify possible post-procedural complications. Antibiotic therapy was indicated in patients with vascular prostheses, heart valves or immunosuppressed status. Piperacillin-tazobactam was administered 12 hours before and 24 hours after ERCP. The endoscopic procedure was performed under sedation with propofol and midazolam (3).

If during the same session laparoscopic cholecystectomy for gallstones was performed, the patients received anesthesia with endotracheal intubation.

The data were analyzed using descriptive statistic techniques. The continuous variables were assessed for normality using the Kolmogorov-Smirnov test. The scale variables were discussed as mean ± standard deviation (SD) and as medians (25th, 75th percentile). T Student-test was used for comparison. A p-value <0.05 was considered for statistical significance (two tailed test). Categorical data were presented as proportions with one decimal. Proportions were compared by using Chi square or Fisher exact tests. Statistical analysis was performed with the SPSS 23.0 and Open Epi.

RESULTS

The personal characteristics: The subjects with PADD tended to have a predominance of males and to be significantly older compared to the subjects without PADD (males 65% in PADD group *vs.* 35% in non PADD group, p<0.001).In the PADD group, females tended to be significantly younger compared to males (females median age 54 vs. males median age 64, p<0.001). Generally, it is considered that the clinical manifestations of the presence of DD occur after the age of 60, being correlated with the duration of the biliary disease and the PADD diameter. In our PADD group, only 52.8% of the subjects were older than 60 years, with a clear predominance of the females in younger ages (ratio males/females 0.5-1.9).

PADD had been diagnosed before the endoscopic procedure in only 50 patients, among which 23, 17, 5 after axial upper gastrointestinal endoscopy, computed tomography, barium transit and MRI cholangiography respectively. Of these, five were less than 40 years old. Colonoscopy has been performed in 21 of the subjects due to some digestive symptoms and colon diverticulosis has been identified 17 patients (among which 14 older than 60 years).

The pathophysiological mechanisms incriminated in producing symptoms: We found in our study significantly higher frequency of abnormalities in the PADD group compared to non-PADD subjects (4). Most frequent abnormality in the PADD group was angulation of the terminal common bile duct (CBD), followed by compression and stenosis of the CBD and Wirsung duct, impaired duodenal peristalsis and incomplete evaluation of the diverticular bag.

In the non-PADD group, most common was compression, followed by angulation and impaired peristalsis, but, as mentioned, all abnormalities occurred in a significantly lower proportion (5). Among the symptoms induced by the pathophysiological mechanisms (jaundice, cholangitis and recurrent acute pancreatitis), only jaundice occurred in significantly higher proportion in the PADD group. According to the literature, jaundice is usually moderate (with a bilirubinemia of 4-8 mg/dl), but it can be persistent or wavy, being produced by disturbed bile elimination, compression of CBD, ampulla dysfunction, concomitant bile stones and cholangitis (6). In our study, 77.8% of the PADD subjects had jaundice (moderate in most cases), compared to 42% of patients without PADD. Cholangitis was diagnosed based on clinical symptoms (Charcot-triad: pain, fever, jaundice), clinical investigations and laboratory examinations. It occurred in similar frequency in both groups. Most of the cases with cholangitis had prolonged episodes of low grade fever, occasional vesperal chills – all of them signs of torpid, subacute, slow-

evolving cholangitis. Also, similar frequency was found for recurrent acute pancreatitis. Diverticulitis and perforation - in general, inflammation, ulceration and even perforation of the diverticular wall can occur (commonly retroperitoneal, less intraperitoneal), due to food stasis. We recorded 7 cases of diverticula with intense mucosal congestion, crumbly and easy bleeding. At those specific maneuvers of ERCP these cases had a high risk of bleeding and perforation (7).

TABLE I.
Pathophysiological mechanisms and complication involved in PADD and non-PADD groups

	PADD Group		Non-PADD Group		p-value*
	No	%	No	%	
Pathophysiological mechanisms					
Angulation of the terminal common bile duct	84	77.8%	90	16.9%	<0.001
Compression and stenosis of the terminal portions of the common bile duct and Wirsung duct	62	57.4%	128	24.1%	<0.001
Impaired duodenal peristalsis	42	38.9%	54	10.2%	<0.001
Incomplete evacuation of the diverticular bag	17	15.7%	0	0%	NA
Symptoms					
Jaundice	79	73.2%	223	42.0%	<0.001
Cholangitis	37	34.3%	181	34.1%	0.972
Recurrent acute pancreatitis	9	8.3%	57	10.7%	0.455
Diverticulitis	7	6.5%	NA	NA	NA

*Chi^2 test

***Acute biliary symptoms in pregnant women*:** Beside the above-discussed symptoms, we noticed that five patients who required ERCP for biliary pain induced by PADD were women aged between 30 and 43 years. Three of these women were pregnant - one in the first and the other two in the second trimester of pregnancy. For these patients, emergency ERCP was motivated by the presence of CBD stones complicated by repeated episodes of acute cholangitis (two cases) or acute biliary pancreatitis due to intrapapillar impacted stone (one case). Sphincterotomy, clearance of CBD and prosthesis were made without fluoroscopic control. In all three cases, the pregnancy was carried to term without obstetric complications. After birth, the patients were again investigated (biochemical and imaging) and no residual CBD lithiasis was found. Laparoscopic cholecystectomy was performed in the same operating time.

***ERCP performance*:** Usually, the first step of ERCP is CBP selective cannulation associated or not with "pre-cut" papillotomy (8, 9). In our study, this step was conducted with a standard pull-type sphincterotomy, which was later replaced by a guide wire. We succeeded to perform selective cannulation in 94.4% and 98.9% of PADD and non-PADD patients respectively. No pancreatic sphincterotomy was been done. After performing the cholangiography, most patients require papilo-sphincterotomy for the extraction of stone, especially in patients with

PADD inducing biliopancreatic suffering (10). In our study, papilo-sphincterotomy has been performed in 88.9% and 97% of PADD and non-PADD patients. Normally, this should be followed by appropriate extraction of stones, papilla and terminal CBD prosthesis. Mechanical lithotripsy could be required. As seen in table II, both selective cannulation and papilo-sphincterotomy were performed in slightly lower proportion in the PADD group, meanwhile mechanical lithotripsy was required in a higher proportion of cases.

In four cases from the PADD group we were not able to perform sphincterotomy due to abnormalities in the profound position of the papilla (11). In other two patients, we performed only autostatic balloon dilatation (younger patients, less dilated common bile duct, reduced diameter calculi). Extraction of stones was performed using probes with basket-type slumber. Mechanical lithotripsy was required for calculi over 12 to 15 mm diameter or even less if sphincterotomy was limited by local anatomical changes (12, 13).

TABLE II.
ERCP performance in PADD and non-PADD groups

	PADD Group (N=108)		Non-PADD Group (N=531)		p-value
	No	%	No	%	
Selective cannulation	102	94.4%	525	98.9%	0.016*
Papilo-sphincterotomy	96	88.9%	515	97.0%	0.001*
Mechanical lithotripsy	17	15.7%	42	7.8%	0.010**

* Fisher exact test; ** Chi^2 test

Complications. The frequency of acute pancreatitis was slightly higher in PADD group, but without statistical significance: 24 (22.2%) and 97 (18.3%) cases in PADD and non-PADD groups respectively, p=0.339, Chi^2 test. They appeared to be attributable to accidental clouding of the Wirsung (12 patients) and to sphincterotomy (12 cases). In both groups (PADD and non-PADD) most cases of acute pancreatitis were mild, limited to biochemical expression and with quick resolution in 24-48 hours (19 and 74 in PADD and non-PADD groups respectively). All patients received indomethacin postoperatively. The medium or severe forms, requiring specific treatment, occurred in similar proportion in both groups (p=0.99, Fisher exact test). Post ERCP hemorrhage occurred in 4 and 13 cases respectively in PADD and non-PADD group (p=0.64, Fisher exact test). Neither other complications specific to ERCP, nor deaths related to ERCP procedure were registered in our study. Length of hospitalization was also similar between groups with a median of 3 days (min 1, max 14 days, p=0.34, Mann Whitney U test).

DISCUSSION

In our study, the patients with PADD were cured using ERCP in slightly lower proportion compared to those without PADD, the results being still comparable to the literature. Few factors could contribute to this lower performance of ERCP.

Firstly, we should consider the position of the diverticula. In terms of relations with the papilla of Vater the diverticula may be juxta-ampullary - when the papilla is adjacent 1 to 2 cm to the diverticulum, or peri-

ampullary - containing both the papilla and bile duct intramural. The chance of generating biliary diseases is more reduced in juxta-ampullary diverticulum, but the periampullary one is most common variety and correlated directly with extrahepatic biliary pathology. Papilla can be located anywhere in the diverticulum, but most commonly it is on the floor. More rarely, we can find one of the side walls of DD. In our case series, all DD were located periampullary (14).

The first step of ERCP, the selective cannulation of CBD, is potentially more difficult in patients with PADD (15). In our study, CBD catheterization average time was 6.6±3.14 minutes compared to only 4.0±2.12 minutes in patients without PADD. Sometimes the papilla of Vater is "pulled" into the DD, its pore is often hidden, diverted down or sideways. The direction of the sphincterotomy is essential to be properly judged in relation to the common bile duct intramural axis, and its length to be correlated with the projection of the papilla. The terminal juxta-ampullar CBD is often strongly angled, making selective cannulation difficult. In our study, this was observed in 48 patients diagnosed with PADD (44%), compared to only 14% in the overall group of non-PADD patients. The changed path of the distal CBD represented the main cause of cannulation failure (16).

We faced technical and tactical problems during sphincterotomy: The correct orientation of sphincterotomy in a distorted papilla, with deeply modified anatomical ratios – 81 (75.0%) versus 59 (11.1%) PADD and non-PADD groups respectively ($p<0.001$, Chi^2 test). The hardest part was the approach of a deeply located papilla (in the cavity of a DD) - 39 patients, (36.1%). The room for maneuver in this case is very narrow. The second difficult selective catheterization was when the papilla was located on one of the side walls of the PADD - 42 patients, (38.9%). The depth of the sphincterotomy was difficult to assess, due to the thin DD wall. The landmarks are the ridge of the actual papilla and the cholangiographic appearance of the juxta-ampullary region. The classical landmark for the limit of incision is the mucosal suprapapillary. The depth of sphincterotomy is generally limited, which explains the lower rates of stones extraction. The average time for sphincterotomy was 4.9±2.17 minutes in the PADD group versus 3.2±1.56 minutes in non-PADD group.

CONCLUSIONS

In our study PADD were associated with an obvious predominance in males but seemed also to become symptomatic in younger age for females.

The mechanisms implied most frequently in the biliary-pancreatic pathology were angulation, compression and stenosis of the terminal portions of the common bile duct and Wirsung duct, with impairment of bile drainage and pancreatic secretion. By this, favorable conditions for lithogenesis, cholangitis, jaundice and chronic pancreatitis are created. Incomplete evacuation of the diverticular bag exacerbates local microbial flora, becoming a possible source of colonization upward the CBD.

Performing ERCP was obviously more difficult in the presence of PADD. The percentages of successful selective cannulation and sphincterotomy were slightly lower in the PADD group compared to patients having only CBD stones, even comparable to the literature. Proportion of post ERCP complications was similar in both groups. These results can be reached only by accuracy, experience and meticulous execution of maneuvers. Our experience with the pregnant women showed that,

when necessary, ERCP can be performed safely during pregnancy and without important risk for mother or child.

Our experience proved that therapeutic endoscopic retrograde cholangio-pancreatography obviously a more difficult procedure in the presence of periampullary duodenal diverticula, due to the difficult access to the papilla and the common bile duct, but the success rate remains high even in such cases.

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FIBROSIS ASSESSMENT IN CHRONIC HEPATITIS C – IS THE LIVER BIOPSY STILL NECESSARY? THE PATHOLOGIST POINT OF VIEW

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FIBROSIS ASSESSMENT IN CHRONIC HEPATITIS C – IS THE LIVER BIOPSY STILL NECESSARY? THE PATHOLOGIST POINT OF VIEW (Abstract): **Aim**. The aim of this study was to compare the histological stage of fibrosis determined by liver biopsy with the stage of fibrosis assessed by *Fibroscan*, to analyze the correspondences and inconsistencies between obtained values and to discuss the role of the microscopic exam, from the pathologist point of view. **Material and methods.** The study group consisted of 185 patients diagnosed with chronic hepatitis. Serological tests diagnosed chronic hepatitis C in 183 patients, and chronic hepatitis B and C for 2 patients. The patients were evaluated to determine the stage of fibrosis using two methods: liver biopsy and elastography (*Fibroscan*). **Results.** Based on the pathologic evaluation, 124 cases were diagnosed as moderate chronic hepatitis (score 6 – 8), and the remaining 60 cases as severe hepatitis (score 9 – 12). Comparison of data from examination of liver biopsy with that obtained by *Fibroscan* examination revealed overlapping and divergent aspects. The fibrosis stage established through liver biopsy did not always coincide with the one assigned by liver stiffness measurement, particularly for intermediate stages F2 and F3. The best overlap was noted for F0–F1 and F4 stages, which indicates the evident ability of transient elastography to separate patients with minimal or no fibrosis from patients with extensive fibrosis. **Conclusions**. Our data concurs with the literature, which confirms presence of differences between *Fibroscan* and biopsy. From the point of view of the pathologist, liver biopsy still remains a valuable instrument, offering a relevant image of liver changes – as it is regarded more rather a selective than routine technique.
Keywords: LIVER BIOPSY, CHRONIC HEPATITIS, FIBROSIS

A common feature of all chronic viral hepatitides caused by hepatotropic viruses is formation of a glycoprotein network instead of normal glycolipoprotein one (1). As liver function is impaired, the assessment of fibrosis is essential in the decisions taken for the initiation of treatment, in the appraisal of treatment response and screening for liver cancer (2-7).

Liver biopsy classically represents the gold standard in the evaluation of the pathological lesions specific for chronic hepatitis – including the staging of fibrosis by the application of various existing scoring systems (8-13). However, the procedure has a series of disadvantages: it is invasive, expensive and the results may be influenced by sampling errors and intra– and interobserver variability (14).

Therefore, through concerted concerns of hepatologists, non–invasive methods based on physical approach have been de-

veloped in the past 15 years, in order to provide similar assessment of the lesions. These methods measure liver stiffness by ultrasound or magnetic resonance elastography and they were immediately implemented in diagnosis practice due to their non–invasiveness (14, 15). Consequently, several research reports have examined in correlation the advantages and limitations of the pathological and elastographic evaluation. Although many papers show positive correlation between the liver stiffness established by transient elastography and fibrosis stage determined by biopsy (16-19), the correspondence of results is not absolute. Consequently, it cannot be firmly stated that elastography could represent a surrogate for biopsy (20-23). Most experts believe that non–invasive techniques may not replace completely liver biopsy, but they may contribute to the decrease of the number of required biopsies (24, 25, 26).

The aim of this study was to compare the histological stage of fibrosis determined by liver biopsy with the stage of fibrosis assessed by *Fibroscan*, to analyze the correspondences and inconsistencies between obtained values and to discuss the role of the microscopic exam, from the pathologist point of view.

MATERIAL AND METHODS

The study group consisted of 185 patients diagnosed with chronic hepatitis at The Institute of Gastroenterology and Hepatology Iaşi, between 2007 and 2010. Serological tests diagnosed chronic hepatitis C in 183 patients, and chronic hepatitis B and C for 2 patients.

The patients were evaluated to determine the stage of fibrosis using two methods: liver biopsy and elastography (*Fibroscan*). Liver biopsies were processed in Pathology Department, University of Medicine and Pharmacy "GrigoreT.Popa" Iaşi. Histological sections were stained with Haematoxylin–Eosin and Van Gieson colorations in order to reveal and stage fibrosis according to METAVIR score.

The elastography with *Fibroscan* was performed at the Institute of Gastroenterology and Hepatology, Iaşi. In the determination of tissue elasticity, threshold valuesare 7.1 kPa for F≥2, 9.5 kPa for F≥3 and 13 kPa for F = 4 (fibrosis stage determined according to METAVIR score) (13, 22, 27).

RESULTS

Based on the pathologic evaluation, 124 cases were diagnosed as moderate chronic hepatitis (score 6–8), and the remaining 60 cases as severe hepatitis (score 9–12).

Comparison of data from examination of liver biopsy with that obtained by *Fibroscan* examination revealed overlapping and divergent aspects.

In the 3 cases with chronic moderate hepatitis with necro-inflammatory activity score 6, the diagnosis of fibrosis stage coincided between pathological and *Fibroscan* exam.

In the 28 cases with chronic moderate hepatitis, necro-inflammatory activity score 7, the fibrosis stage was consistent only in 4 of the 15 cases assigned F0 – F1, the other 11 cases being evaluated by *Fibroscan* with more severe fibrosis stages (F2, F3, F4); none of the 11 cases assessed as F2 and the 2 cases evaluated as F3 by the pathological investigation received a similar fibrosis stage by *Fibroscan* exam, which was less or higher.

In the 94 cases with chronic moderate hepatitis, necro-inflammatory activity score 8, fibrosis stage was consistent in only 27 of the 51 cases assessed as F0 – F1, the other 24 cases evaluated by *Fibroscan*

receiving more severe fibrosis stages (F2 – 13 cases, F3 – 7 cases, F4 – 4 cases). Diagnosis overlapping was recorded in 8 of the 35 cases classified as F2, the other 27 presenting after evaluation by *Fibroscan* fibrosis stage F0 – F1 – 9cases, F3 – 6 cases and F4 – 9 cases; for 3 cases, the comparative evaluation was not possible. Also, fibrosis stage F3 was consistent in 2 of 8 cases, the other 6 presenting after the evaluation by *Fibroscan* fibrosis stage F0 – F1 – 3 cases, F2 – 1 case and F4 – 2 cases.

TABLE I

Parallel assessment of the stage of fibrosis by liver biopsy and elastography

Diagnosis / necro-inflammatory activity score	**Total number of cases**	**Liver biopsy**		***Fibroscan***	
		Fibrosis stage	**Number of cases**	**Fibrosis stage**	**Number of cases**
MODERATE CHRONIC HEPATITIS					
6	3	**F0**	**1**	**F0 – F1**	**2**
		F1	**1**		
		F2	**1**	**F2**	**1**
7	28	**F0**	**8**	**F0 – F1**	**2**
				F2	4
				F3	2
		F1	**7**	**F0 – F1**	**2**
				F2	3
				F3	0
				F4	2
		F2	**11**	F0 – F1	8
				F2	**0**
				F3	2
				F4	1
		F3	2	F2	1
				F4	1
8	94	**F0**	**18**	**F0 – F1**	**10**
				F2	4
				F3	2
				F4	2
		F1	**33**	**F0 – F1**	**17**
				F2	9
				F3	5
				F4	2
		F2	**35**	F0 – F1	9
				F2	**8**
				F3	6
				F4	9
				unmanageable evaluation	3
		F3	**8**	F0 – F1	3
				F2	1
				F3	**2**
				F4	2

Diagnosis / necro-inflammatory activity score	Total number of cases	Liver biopsy		*Fibroscan*	
		Fibrosis stage	Number of cases	Fibrosis stage	Number of cases
SEVERE CHRONIC HEPATITIS					
9	32	**F1**	**4**	**F0 – F1**	**2**
				F2	1
				F4	1
		F2	**13**	F0 – F1	4
				F2	**5**
				F3	2
				F4	2
		F3	**15**	F0 – F1	2
				F2	2
				F3	**3**
				F4	7
				unmanageable evaluation	1
10	16	F0	1	F3	1
		F1	1	F2	1
		F2	**5**	**F2**	**1**
				F3	2
				F4	2
		F3	**9**	F0 – F1	2
				F3	**2**
				F4	5
11	9	F1	1	F3	1
		F2	1	F0 – F1	1
		F3	**5**	**F3**	**2**
				F4	2
				unmanageable evaluation	1
		F4	2	F3	1
				F4	1
12	3	F3	3	F0 – F1	1
				F4	2

In the 32 cases with chronic severe hepatitis, necro-inflammatory activity score 9, fibrosis stage diagnosis was consistent only in 2 of the 4 cases assessed as F0–F1, the other 2 cases being evaluated by *Fibroscan* with more severe fibrosis stages (F2 – 1 case, F4 – 1 case); also, it was consistent in 5 of the 13 cases classified as F2, the other 8 being assessed by *Fibroscan* as fibrosis stage F0 – F1 – 4 cases, F3 – 2 cases, and F4 – 2 cases; finally,diagnosis was consistent for 3 of the 15 cases assigned as F3, the other 12 cases presenting according to assessment by *Fibroscan*, fibrosis stage F0–F1 – 2 cases, F2 – 2 cases and F4 – 7 cases; in 1 case, the comparative evaluation was not possible.

In the 16 cases with chronic severe hepatitis, necro-inflammatory activity score 10, there was no concurrence for stage F0 –

F1. The fibrosis stage was consistent only in 1 case of the 5 cases assessed as F2, the other 4 cases presenting fibrosis stage F3 – 2 cases and F4 – 2 cases when assessed by *Fibroscan*. Fibrosis stage diagnosis was consistent only in 2 of the 9 cases classified as F3, the other 7 presenting fibrosis stage F0 – F1 – 2 cases and F4 – 5 cases in the evaluation by *Fibroscan*.

In the 9 cases with chronic severe hepatitis, necro-inflammatory activity score 11, there was no overlap for F0, F1 and F2 stage. Fibrosis stage diagnosis was consistent only in 2 cases of the 5 cases assessed as F3, 2 cases presenting after *Fibroscan* evaluation fibrosis stage F4 – 2 cases, while for 1 case the evaluation was impossible. Fibrosis stage diagnosis was consistent only in 1 case of the 2 assigned as F4, the other case being evaluated by *Fibroscan* as F3.

In the 3 cases with chronic severe hepatitis, necro-inflammatory activity score 12, there was no overlap between histological stage F3 and the stages assesed by *Fibroscan* (F0 – F1 – 1 case, F4 – 2 cases) (tab. I).

DISCUSSION

The Pathology Department of University of Medicine and Pharmacy „Grigore T. Popa" Iaşi has an old tradition in the field of liver biopsy diagnosis, as a result of the close relationship with the Institute of Gastroenterology and Hepatology Iaşi. Pathologic evaluation was joined, after the year 2000 by the investigation through specific imagistic methods (transient elastography) performed by the gastroenterologists in the Institute (28-31).

By the assessment of the stiffness in liver tissue the imagistic noninvasive methods allow for the identification and quantification of fibrosis degree in hepatitis and cirrhosis (14,15,29-31). As they are non–invasive, easily accepted by patients, quick and reproducible, non–invasive methods replaced gradually liver biopsy – because they are regarded as alternatives for diagnosis, on the basis of their relatively similar efficiency (14,29,30).

The implementation of these methods in current diagnosis practice is based on reliable evidence which certify the presence of a close correlation between elasticity of liver parenchyma and stage of fibrosis. This evidence represents the result of wide studies that compared the fibrosis stage provided by non–invasive imagistic methods with the stage determined by pathology (16-19, 33, 34). Moreover, there are other clinical data that challenge the necessity of liver biopsy for staging fibrosis prior to antiviral therapy in chronic hepatitis B (31, 32).

Hence, liver biopsy lost its traditional status as gold standard.

The question to be asked is whether it can be completely replaced by the new technologies. The answer is negative.

The analysis of our results reveals that the fibrosis stage established through liver biopsy does not always coincide with the one assigned by liver stiffness measurement (*Fibroscan*), particularly for intermediate stages F2 and F3. The best overlap was noted for F0–F1 and F4 stages, which indicates the evident ability of transient elastography to separate patients with minimal or no fibrosis from patients with extensive fibrosis. Our data concurs with the literature, which confirms the presence of differences between *Fibroscan* and biopsy (19). Although numerous studies focused on the efficiency of non–invasive tests (sensitivity, specificity, accuracy and prediction) (17-19, 33-35) support their diag-

nostic value, liver biopsy remains a reliable method (36).

Even if the role of liver biopsy as a diagnostic test is fading, the role in establishing prognosis is unquestionable (37). Microscopic aspects supply a relevant image for the general characterization of liver changes. The context of liver damage is interpreted through the correlation with the necro–inflammatoy activity that leads to fibrosis and by association with alterations in hepatocytes, other cell population or biliary canalicules (38). From the point of view of the pathologist, liver biopsy represents a valuable instrument (36), as it is regarded as rather a selective than routine technique (39).

CONCLUSIONS

Liver biopsy is no longer considered the gold standard in the assessment of fibrosis from the hepatitis context, but the morphologic information which reveals the underlying background of lesions associated with chronic hepatitis cannot be ignored. The development of new technologies reflects directly in the selectivity of recommendations for liver biopsy.

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NEWS

THE UTILITY OF LIPOPOLYSACCHARIDE-BINDING PROTEIN IN THE DIFFERENTIATION BETWEEN PERIPROSTHETIC JOINT INFECTION AND ASEPTIC LOOSENING?

The pre-operative differentiation between periprosthetic joint infection (PJI) and aseptic loosening after total hip (THA) or knee (TKA) arthroplasty is essential for successful therapy and relies in part on the use of molecular markers. A recent prospective, controlled, clinical trial study conducted by Friedrich MJ and collaborators assessed serum levels of lipopolysaccharide-binding protein (LBP) from one hundred and twenty patients presenting with a painful TKA or TKA with indication for surgical revision. Pre-operative blood and serum samples were collected and analysed also for white blood cell (WBC) count and C-reactive protein (CRP). The definite diagnosis of periprosthetic joint infection was determined on the basis of clinical, microbiological and histopathological examination. LBP showed significantly higher values in PJI compared with aseptic loosening ($p < 0.001$) and control ($p < 0.001$), with a specificity of 66 % and a sensitivity of 71 % at a cutoff value of >7 ng/ml. In combination with CRP, the positive predictive value for PJI was at 0.67; nega-tive predictive value with both negative was at 0.77. the authors concluded that the use of LBP in serum appears not to be a more accurate marker than CRP level in serum for detect-ing PJ, and on the basis of these results, they cannot recommend the sole use of LBP for dif-ferentiating PJI and aseptic loosening following THA and TKA. (Friedrich MJ, Randau TM, Wimmer MD et al. Lipopolysaccharide-binding protein: A valuable biomarker in the differ-entiation between periprosthetic joint infection and aseptic loosening? *Int Orthop*. 2014 May 15. [Epub ahead of print]. PMID:24827968).

Simona Tucaliuc

MANAGEMENT DILEMMAS OF RETROPERITONEAL PARAGANGLIOMA: REPORT OF A CASE

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MANAGEMENT DILEMMAS OF RETROPERITONEAL PARAGANGLIOMA: REPORT OF A CASE (Abstract): A 17-year-old boy presented to emergency department with abdominal pain in the left upper quadrant along with a palpable mass. A CT scan revealed a 10 x 8.5 cm mass in the retroperitoneal area, suggestive of a sarcoma. The patient underwent surgical resection of the lesion, the histological examination of which confirmed a paraganglioma. An ^{131}I-Metaiodobenzylguanidine (MIBG) scan excluded distant metastasis. Paragangliomas are rare tumours of the neuroendocrine system and certain considerations are pivotal in the management of these tumours. Urinary and plasma catecholamines measurement along with CT, MRI, and MIBG scanning facilitate diagnosis, localisation and characterisation of these tumours. Surgical excision is the treatment of choice for both primary and recurrent tumours. Unfortunately, histological characterisation for malignancy is limited and these tumours are considered malignant upon development of metastasis or local recurrence. Inexistence of histological criteria for malignancy means a long-term surveillance is the norm in these patients. **Keywords:** ADOLESCENT, PARAGANGLIOMA, RETROPERITONEAL

Paragangliomas are the rare tumours arising from the extra-adrenal neuroendocrine cells (1). Most paragangliomas occur in head and neck region and occurrence at other sites including the retroperitoneal area is relatively uncommon (1,2). Although a familial predisposition has been described, these tumours are seldom considered in healthy teenagers (3). We report a case of an adolescent boy who presented with severe abdominal pain and a palpable mass. The diagnosis was established on histological examination of the tumour. This report is aimed at discussing certain diagnostic and management challenges, and providing the clinicians with a concise account of how to address those.

CASE REPORT

A previously healthy 17-year-old boy attended the emergency department with severe abdominal pain of a day's duration. A physical examination revealed a palpable mass in the left upper quadrant of the abdomen. The patient was apyrexial and full blood count, urea and electrolytes

were unremarkable. On a CT scan, the mass appeared to be a heterogeneous tumour in the retroperitoneal area in close proximity to pancreatic tail and descending colon, measuring 10 x 8.5 cm. The tumour appeared to be a retroperitoneal sarcoma containing areas of calcification and with internal haemorrhage. Angiographic embolisation of the rich vascular arcade derived from the inferior mesenteric, subcostal, and lumbar arteries was achieved preoperatively to minimise the blood loss. At operation, the mass was found to be an encapsulated, highly vascular tumour occupying the left half of retroperitoneal area and adherent to the upper descending colon (fig. 1). Complete removal of the tumour was performed along with segmental resection of the involved descending colon. The histological examination of the tumour showed the features consistent with a paraganglioma.

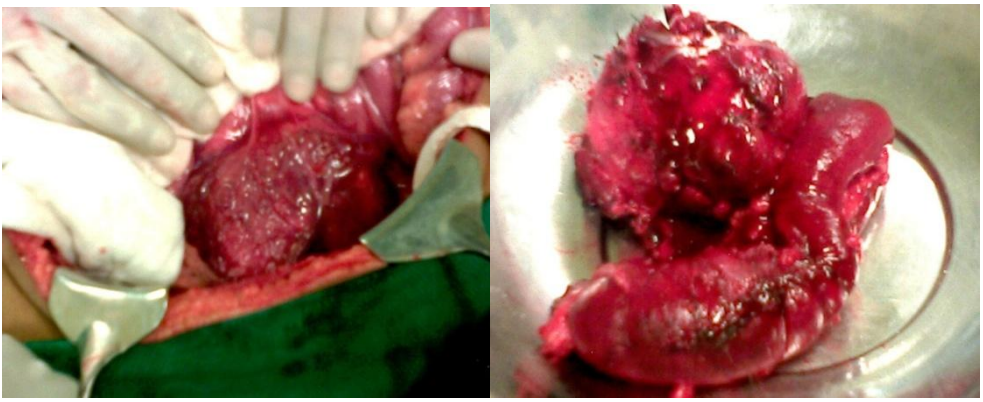

Fig. 1. Retroperitoneal extra-adrenal paraganglioma.
Left: In-situ tumour appears adherent to descending colon. **Right:** The resected specimen consisting of the involved segment of descending colon along with paraganglioma

The patient made an un-eventful recovery in the postoperative period and was discharged home on the 5th day after operation. A subsequent ^{131}I- Metaiodobenzylguanidine (MIBG) scan in the early postoperative period excluded distant metastasis. Clinically, the tumour was considered benign in view of the non-metastatic disease, as discussed below. Nevertheless, the patient will require a long clinical follow-up due to inexistence of definable histological criteria for malignancy in paragangliomas.

DISCUSSION

The paragangliomas arise from the neural crest cells lying outside adrenal medulla. These tumours originate both in the hereditary as well as the sporadic form and are most commonly diagnosed in the fourth or fifth decade of life (3, 4). Anatomically, these tumours occur anywhere from upper neck to pelvic floor, however, head and neck remain the commonest regions affected (4, 5). Retroperitoneal paragangliomas usually develop in sympathetic paraganglia and at least half of these secrete catecholamines. Such tumours are called functional paragangliomas. It has been reported that up to half of the abdominal paragangliomas are malignant (5). The common presenting features include abdominal mass, pain, palpitations, headache, sweating, hyperten-

sion, and genitourinary symptoms (2, 5, 6).

The diagnosis is established by measurement of the urinary and plasma catecholamines, radiological findings of tumour on CT or MRI scan, and ^{123}I- or ^{131}I-MIBG scanning for functional paragangliomas (5, 7). Tumour localisation has improved remarkably owing to the use of different imaging modalities. Unfortunately, the histological examination is limited by its inability to distinguish between benign and malignant tumours, and tumour recurrence or metastasis are generally accepted as features of the malignant paragnagliomas (8, 9). Therefore, these patients usually require a long clinical follow-up. Annual biochemical testing, CT or MRI scan, and MIBG scintigraphy are essential in the follow-up process to diagnose recurrence, delayed appearance of multiple tumours, or metastasis (4, 7, 8).

Surgical excision remains the mainstay of treatment for both primary and recurrent tumours (1, 4, 5, 7). Feasibility of the laparoscopic approach has been reported albeit open resection remains the commonest operative strategy (4, 10). The functional tumours require reversal of circulating catecholamines prior to the operation. Both alpha and beta antagonistic drugs are used preoperatively to control blood pressure and to prevent intraoperative hypertensive crisis (4, 7). Radiotherapy or chemotherapy may be used for preoperative debulking of tumour. Ablation of the tumour with high dose I-131 MIBG has shown promising results and more evidence is awaited (5, 11).

The abdominal paraganglioma is an uncommon condition in adolescents (6, 12). Therefore, it remains a rare clinical consideration in previously healthy teenagers presenting with abdominal pain. In managing such cases, a degree of suspicion should be maintained for uncommon pathologies like neuroendocrine tumours. A careful clinical assessment cannot be overemphasized which would help detect an associated mass. Absence of acute inflammatory markers should trigger instigation of further investigations, the most appropriate being a CT or an MRI scan. It is vital that such cases are assessed and managed on an individual basis given the implications of misdiagnosis.

CONCLUSIONS

Extra-adrenal retroperitoneal paraganglioma presents diagnostic and management challenges and, once suspected, certain management considerations are pivotal. A careful clinical assessment differentiates it from common causes of pain in this age group. The urinary and plasma catecholamines measurements along with CT, MRI, and MIBG scanning should be employed to diagnose, localise and characterise these tumours. Surgical excision is the treatment of choice for both the primary and the recurrent tumours. Inexistence of histological criteria for malignancy means a lifelong surveillance is the norm in these patients.

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NEWS

NEW APPROACH IN TREATMENT OF MULTIDRUG-RESISTANT TUBERCULOSIS

Multidrug-resistant tuberculosis (MDR-TB) is a major problem health concern. According World Health Organization (WHO), in 2012, MDR-TB accounted for 3.6% of new cases and 20% of previously treated cases of TB (1). Therapy for MDR-TB is long, hard and expensive with antiquated drugs. After 40 years, a new antituberculosis drug – bedaquiline - has become available for use. Bedaquiline is an inhibitor of mycobacterial ATP synthase with bactericidal activity. A recent clinical trial, which included 160 new cases of MDR-TB treated with bedaquiline in combination with background treatment, showed that the patients who received bedaquiline have had a median time to sputum-culture conversion by 83 days, with 42 days shorter than placebo group. Also, bedaquiline used in therapeutic regimen for MDR-TB determined an increase of rate of culture conversion at 24 weeks (79%) and at 120 weeks (62%) compared with placebo (58% respectively 44%). The same study showed that the risk of acquiring new resistance to anti-TB drugs was lower in patients treated with bedaquiline (2). These findings support the use of bedaquiline in MDR-TB treatment (World Health Organization. Global tuberculosis report 2013. www.who.int/tb/publications/global_report/en/; Diacon AH, Pym A, Grobusch MP et al. Multidrug-resistant tuberculosis and culture conversion with bedaquiline. *N Engl J Med*. 2014;371:689-691, 723-732).*Cătălina Luncă*

THE IMPORTANCE OF RENAL BIOPSY IN THE MANAGEMENT OF RENAL TRANSPLANTATION

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THE IMPORTANCE OF RENAL BIOPSY IN THE MANAGEMENT OF RENAL TRANSPLANTATION (Abstract): Renal biopsy remains the"gold standard'' for the diagnosis of renal allograft dysfunction. The aim of our study was to highlight the importance of renal biopsy in the management of allograft dysfunction (early diagnosis and therapeutic approach). Our study included 23 renal allograft biopsies from 20 patients. This retrospective study analyzed the demographic and clinical data, histological results and the evolution of renal function under the immunosuppressive regimens. Conclusions: the most frequently encountered histopathological patterns were humoral rejection, interstitial fibrosis and chronic cellular rejection. Patients under continuous treatment with cyclosporine developed chronic cellular reject more frequently; on the opposite, patients under continuous treatment with tacrolimus developed chronic humoral reject more frequently. **Keywords**: RENAL BIOPSY, ALLOGRAFT DYSFUNCTION, IMMUNOSUPPRESSIVE TREATMENT

The mechanisms responsible for the appearance of lesions in renal allograft are complex and include numerous multifactorial processes that are influenced by a variety of factors, both donor and recipient related, but also secondary to post-transplant events: ischemia-reperfusion injury, cellular and humoral immunity, viral and bacterian infections, or calcineurin inhibitors toxicity (1). For the diagnosis of allograft injury, clinical and paraclinical tests had been used, but the renal biopsy still remains „the golden standard" method (2).

The allograft biopsy is usually performed when cellular or humoral rejection is suspected. The main indicator for allograft dysfunction is represented by a rise in serum creatinine levels above the baseline values. One single abnormal value is not generally an indication, but on the other hand a rise of at least 25% above the baseline values represents an indication to perform such a biopsy (3).

Other signs of rejection, like inexplicable fever, edema, hypertension, eosinophilia, oliguria or anuria and proteinuria, are unable to predict this diagnosis in 30-50% of cases; as such histological confirmation is frequently necessary (4, 5, 6).

The aim of this study was to underline the importance of renal allograft biopsy (RAB) in the management of transplant pa-

tients from a single Renal Transplant Center.

MATERIAL AND METHODS

We analyzed all biopsies performed between in „Dr. C.I. Parhon" Dialysis and Transplantation Center, from 01.01.2007 to01.07.2012. A TRU-CUT 14 G needle and a BARD INSTRUMENTS biopsy pistol were used to perform RAB. All biopsy pieces were fixated in formaldehyde and later in paraffin. All sections had between 3 and 4 μm in thickness. We used HE, PAS and Masson Trichrome stain. For the interpretation of RAB the 2009 Banff Classification was used (7).

We also included in our analysis baseline demographic, clinical and therapy data of the patients, but also the evolution in renal function.

RESULTS

We included 23 biopsies from 20 patients that were from patients evaluated in our Transplant Centre.

A. General results

1. Age and sex – the age of patients was between 19 and 58 years (media 38.5 years);

2. The majority of biopsies were from young male patients (tab. I);

3. From the total of 20 patients, 17 had received the renal allograft from a living relative, while the rest from a cadaveric donor.

4. The median duration from the moment of transplantation till that of the biopsy was 7.05 years (between 1 and 15 years) (fig.1). The RAB was performed more frequently in patients with a transplant between 1 and 4 years.

5. Patients' co morbidities are presented in table II. Most patients had associated infectious and cardiovascular pathologies.

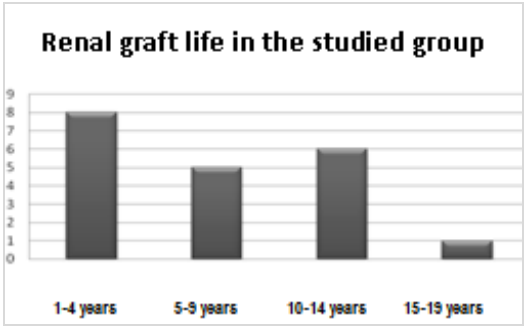

Fig. 1.The duration of renal transplant allografts.

TABLE I

The distribution of patients in regard with age and sex

		Number of patients	Number of biopsies
Sex	M	15 (75%)	18 (78.26%)
	F	5 (25%)	5 (21.73%)
Age intervals	15-24	3 (15%)	3 (15%)
	25-34	8 (40%)	10 (50%)
	35-44	6 (30%)	7 (35%)
	45-54	2 (10%)	2 (10%)
	55-64	1 (5%)	1 (5%)

TABLE II
Associated co morbidities

Associated pathology	Number of patients
HCV	**4**
VHB	1
CMV	13
PBKV	4
Hypertension	16

HCV – Hepatits C virus, HBV – Hepatits B virus, CMV - Citomegalovirus, PKBV – Polyoma BK virus.

4. **Pretransplant chronic kidney disease etiology –** in our study population the most frequent cause of CKD was chronic glomerulonephritis (12 patients); 3 patients had congenital renal abnormalities, while in the rest of patients the etiology was unknown.

5. **RAB indication –** six patients had a rapid rise in serum creatinine levels, the rest associating a more gradual decline in renal function with proteinuria > 1g/day.

B. Histopathological results

The most frequently histopathologies observed in our population were antibody mediated rejection (4 cases), interstitial fibrosis with tubular atrophy (3 cases) and T cell mediated rejection (3 cases) – see Table 3.

C. Post biopsy complications

Two patients had complications post RAB, and only case it was severe (subcapsular hematoma that later spontaneously remitted).

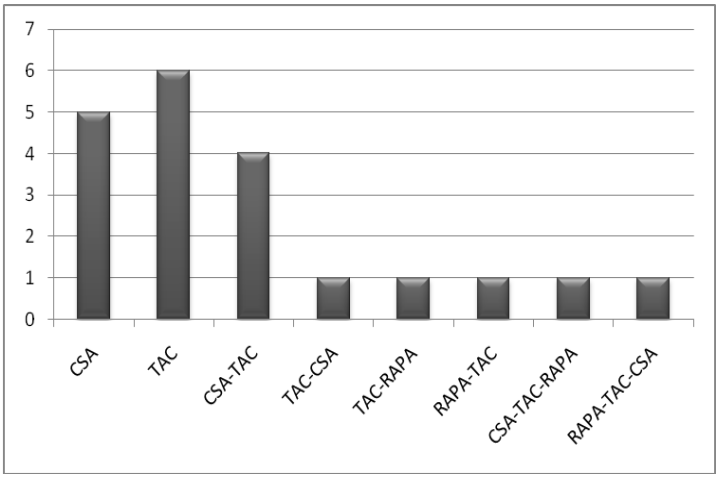

Fig. 2. Immunosuppression schemes in the study group:
CSA – Continuous therapy with Cyclosporine A. **TAC** – Continuous therapy with Tacrolimus. **CSA – TAC**: Cyclosporine – Tacrolimus switch. **TAC-CSA**: Tacrolimus – Cyclosporine switch. **TAC-RAPA**: Tacrolimus – Rapamycine switch.

D. Immunosupressive regimens

All regimens were based on three immunosupressives: prednison, myco-phenolate and either a calcineurin inhibitor (cyclosporine A (CSA) or tacrolimus (TAC)) or rapamycin (RAPA). The distribution of immunospressive regimens is presented in figure 2. In the observation period, most

patients remained on a calcineurin inhibitor (CSA or TAC) with a good evolution.

The modification of therapy was most frequently performed for chronic degradation of renal function (in the cases with CSA), diabetes (in the cases with TAC) or proteinuria (in the cases with RAPA).

Patients who had a CSA based regimen were more likely to develop chronic cellular rejection, while those with a TAC based regimen were more likely to develop chronic humoral rejection (tab. IV).

TABLE III
Histopathological results

Diagnostic	Number	Percentage (%)
Antibody mediated rejection	4	17.39 %
T cell mediated rejection	3	13.04 %
Interstitial fibrosis with tubular atrophy	3	13.04%
Non-diagnostic	3	13.04 %
Chronic allograft glomerulopathy	2	8.69 %
Borderline lesions	2	8.69 %
Virus infection	2	8.69 %
Chronic interstitial nephritis	2	8.69 %
Glomerulosclerosis	1	4.34 %
Calcineurin inhibitor toxicity	1	4.34 %

TABLE IV
Histopathological modifications according to the immunosupressive regimen

	CSA-TAC	TAC-CSA	TAC-RAPA	RAPA-TAC	CSA-TAC-RAPA	RAPA-TAC-CSA
T cell mediated rejection	**1**					
Interstitial fibrosis with tubular atrophy	**2**					
Borderline			**1**		**1**	
Calcineurin inhibitor toxicity	**1**					
Antibody mediated rejection	**1**					
Virus infection		**2**				
Chronic interstitial nephritis				**1**		**1**

DISCUSSION

The prevalence of end-stage chronic kidney disease is rising worldwide. At this moment there are 3 renal replacement therapies: peritoneal dialysis, hemodialysis and renal the quality of life and survival with the lowest long-term costs.

RAB represents the most sensitive instrument for detecting the cause of renal allograft dysfunction.

This paper underlines the significance of RAB for the management of a renal transplant patient. We retrospectively evaluated the histopathological results of all RAB performed in „Dr. C.I. Parhon" Dialysis and Transplantation Center in a five years period (01.01.2007 - 01.07.2012). There were only 23 biopsies from 20 patients.

The 4 cases with humoral rejection were diagnosticated at 1, 2, 7 months and 13 years after renal transplantation. After the treatment (all cases received 3 plasmapheresis, 2 patients also i.v. Imunoglobuline) there was amelioration in 3 cases. In the other case (the patient transplanted 13 years ago) there was an initial stabilization of serum creatinine levels, but he later returned to hemodyalisis.

In the three cases with cellular rejection, the evolution after methylprednisolone pulse therapy was favorable in all cases.

In the observation period, there was a 100% patient survival rate and a 95% renal allograft survival rate.

CONCLUSIONS

1. This study highlights the importance of RAB in the evaluation and management of renal transplant recipients.

2. RAB is of utmost importance in the clinical and therapeutical decisions, allowing medical practitioners to:

- identify and differentiate chronic or acute lesions in the allograft;
- initiate appropriate measures to stop or delay the progression to evident dysfunction and subsequent loss of the renal allograft.

3 . In our study the most frequent histopathological findings were antibody mediated rejection, followed by interstitial fibrosis with tubular atrophy and chronic cellular rejection;

4. Patients on continuous CSA treatment had a higher incidence of chronic cellular rejection, while those on continuous TAC treatment had a higher incidence of humoral rejection.

5. Post RAB complications have been rare, making this procedure a safe method of investigation kidney allograft dysfunction.

6. During the 5 years of the study, there was a 100% patient survival rate and a 95% renal allograft survival rate.

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LEISHMANIASIS – AN UNUSUAL CAUSE OF SPLENOMEGALY IN ROMANIA

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LEISHMANIASIS–AN UNUSUAL CAUSE OF SPLENOMEGALY IN ROMANIA (Abstract): Leishmaniasis is a parasitic infection caused by protozoans classified as *Leishmania* species. Romania is not considered an endemic country and there are only few reports of sporadic cases in the last 100 years. However, studies suggest that the disease is spreading north. We present the case of a 44 year old female that presented with asthenia, perspirations, vertigo, weight loss and menometrorhagias in small to medium quantity. Clinical exam revealed the presence of splenomegaly and her blood tests indicated she had pancitopenia; differential diagnosis included myeloproliferative or lymphoproliferative disorders, infections that evolve with spleen enlargement, autoimmune-related splenomegaly and hepatic - all tests were negative. She refused the bone marrow aspiration. Three months later, her condition worsened and the menometrorragias became more severe. Bone marrow aspiration revealed the presence of numerous intra and extracellular *Leishmania spp*. amastigotes. A detailed anamnesis showed that she had worked for six months in Italy as a care-giver nine months ago. She was transferred to Bucharest where she received optimal treatment. However, due to the continuous bleeding, the evolution was unfavourable. This is an alarm sign for physicians that should take into account the fact that, due to population migration and global warming, tropical infectious diseases are becoming more and more common. The signs and symptoms, as well as the treatment in leishmaniasis are reviewed, as well as a brief history of leishmaniasis in Romania. **Keywords**: VISCERAL LEISHMANIASIS, SPLENOMEGALY, TROPICAL DISEASES

Leishmaniasis is a parasitic infection caused by protozoans classified as *Leishmania* species, of the trypanosomatidae family. Transmission can occur after the bite of a phlebotomine sand fly (1) whose hosts are either animals (dogs or rodents), either humans, depending on geographical area (humans are more often hosts in hyperendemic areas).

The World Health Organization recognizes four forms of leishmaniasis, the occurrence of which depends on parasite

species and the reactivity of the immune system of the patient: cutaneous leishmaniasis (often self-healing and the most common form), diffuse cutaneous leishmaniasis (especially in immunocompromised patients), mucocutaneous leishmaniasis and visceral leishmaniasis (VL) (also known as "kala azar") - the most severe form, fatal if left untreated (2).

Over 90% of confirmed leishmaniasis infections are from India, Bangladesh, southern Sudan, Nepal and North-East Brazil (3); some Mediterranean countries such as Italy, Greece, Cyprus, France are considered endemic, as well as other European countries such as Portugal. Romania is not considered an endemic country and there are only few reports of sporadic cases in the last 100 years. However, studies suggest that the disease is spreading north because of several risk factors such as climate, population migration, (4) pet import (dogs as reservoir for the disease - leishmaniasis has up to 25% seroprevalence in domestic dogs in endemic countries) and adaptability of the vector to new environmental conditions (1, 2).

CASE REPORT

We present the case of a 44 year old female that was admitted to the Haematology department for asthenia, perspirations, vertigo, weight loss (7-8 kg in the last three months) and menometrorhagias in small to medium quantity. The bleeding had appeared one week prior to admission, whereas all the other symptoms had appeared three months ago. She had a history of uterine fibroma, diagnosed 9 years ago, and treated conservatively. She was a stay-at-home mother, did not smoke or drink, and she lived in a spacious rural household.

Clinical examination revealed only a massive splenomegaly (10 cm below the costal margin). She was pale and dehydrated but had no fever. Rectal examination showed no signs of active bleeding.

Differential diagnosis was centred on splenomegaly and included: 1. myeloproliferative or lymphoproliferative disorders (syndrome?); 2. infections that evolve with spleen enlargement (such as hepatitis, HIV, mononucleosis); 3. autoimmune-related splenomegaly (encountered in lupus and rheumatoid arthritis); 4. hepatic disorders (cirrhosis);

5. idiopathic splenomegaly (exclusion diagnosis).

Blood tests revealed the presence of pancitopenia, with hipochrome normocitary anaemia (Hb = 5,1 g/dl); her platelets were 50.000/mmc and white blood cell count was 1790/mmc, with a normal distribution and mature polymorphous lymphocytes that had morphological signs of activation (possibly due to a viral infection), thus eliminating myeloproliferative or lymphoproliferative disorders.

Echographic imaging confirmed the clinical suspicion of splenomegaly, with a 135 mm - spleen, but revealed no other pathological findings (echographically normal liver); hepatic tests (ALAT, ASAT) were within normal range, thus eliminating cirrhosis as a possible aetiology.

Markers for autoimmune diseases - rheumatoid factor, anti-cyclic citrullinated peptide antibodies, antinuclear antibodies and anti double-stranded DNA antibodies were negative, thus eliminating autoimmune-related splenomegaly. HIV testing, HbS antigen, anti-HCV antibodies, Cytomegalovirus antibodies and anti-toxoplasmosis antibodies were absent as well, with IgM, IgA and IgG values within normal limits, thus eliminating the most

common infections that evolve with spleen enlargement.

In order to further investigate the pancitopenia, other blood tests were performed: bilirubin (both fractions were only slightly increased) and reticulocyte count (increased); these results, correlated with the fact that the anaemia was mycrocitic, lead to the conclusion that the main mechanism by which it had appeared had been blood loss. Gynaecological consult confirmed the presence of a fibroma with no active bleeding at the time of the consult. Although the patients' stool tested positive for occult gastrointestinal bleed, both endoscopy and colonoscopy were negative for signs of active bleeding. Coagulation tests were performed - all within normal range. A bone marrow aspiration was suggested, but the patient refused the investigation.

The splenomegaly was considered to be reactive due to the presence of the activated lymphocytes in the formula. Leucopoenia and thrombocytopenia were interpreted in the context of a medullar aplasia. Anaemia was interpreted as having a mixed etiology - medullar aplasia, hypersplenism, menometrorhagias. The patient received blood transfusions and dietary supplements for the correction of anaemia. She was discharged with haematological parameters that had much improved, and she was prescribed dietary supplements (iron, folic acid, vitamin C).

Three-months follow-up examination revealed an increase in the dimensions of the spleen (175 mm), hepatomegaly and important gynaecological bleeding. Blood tests revealed the persistence of pancitopenia with a neutrophil count of 380 elements/mmc and elevated ferritin level. Due to her important bleeding, the patient was transferred to the Gynaecology department, where haemostatic uterine curettage was performed.

After surgery, she returned to the Haematology department. A bone marrow aspiration was suggested once more and this time the patient consented. The results showed the presence of numerous intra and extracellular *Leishmania spp.* amastigotes (fig. 1). A detailed anamnesis showed that she had worked for six months in Italy as a care-giver nine months ago.

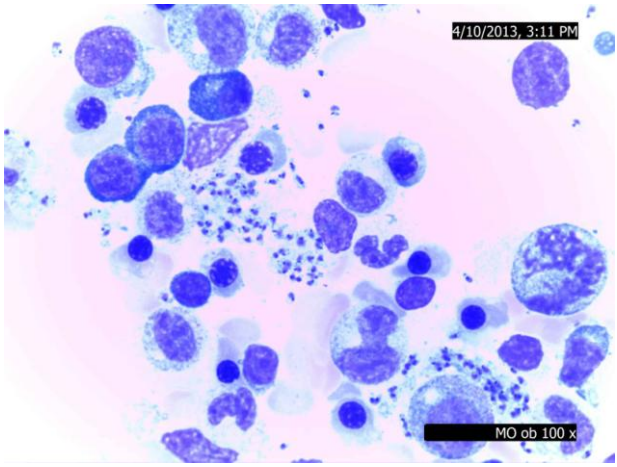

Fig. 1. Microscopic aspect of *Leishmania spp.* amastigotes from bone marrow aspiration

The patient was transferred to the "Victor Babes" Hospital of Infectious Diseases, where treatment with liposomal Amphotericin B was initiated. Metrorrhagia persisted (due to her uterine fibroma) and a second surgery was performed after which the bleeding continued; although best supportive care was given, the patient had an unfavourable outcome.

DISCUSSION

The purpose of this case presentation is to attract attention to the necessity of including tropical diseases in the differential diagnosis of difficult cases that do not respond to standard therapy. Considering the marked increase in population migration, the clinician must take into account imported infectious diseases. A meticulous anamnesis is essential in such situations, and it may help narrow the spectrum of etiological microorganisms and parasites, thus limiting the amount of tests required for a positive diagnosis.

Leishmania infection in Romania

The first report of a leishmaniasis case dates to 1912. No other cases were reported until 1954, when an outbreak of visceral leishmaniasis was reported in the Oltenia region. One other case was reported in 1991 (5). However, in the last 25 years, more and more leishmaniasis cases have been diagnosed in Romania - 14 cases of visceral leishmaniasis reported between 2000 and 2010. All patients had recently travelled to Mediterranean countries (6-8), as did our patient, who had travelled one year prior to her first hospital admission to Italy.

Signs and symptoms in VL

A descriptive study on 71 VL patients diagnosed in an African hospital indicated that the most frequent symptoms were fever, abdominal pain, loss of appetite, weight loss, epistaxis, diarrhoea, cough and joint pains (10). Similar rates are reported on an Italian population - all children and 90% of the adults were febrile at presentation (1).

Most common signs in VL include splenomegaly (60-97%), hepatomegaly (50-60%), lymphadenopathy (20%), ascites and lower limb oedema (around 10%) (1, 3, 10-11). The most common clinical presentation consists in fever associated with hepatosplenomegaly (1); some authors have identified a "classical triad" of signs, symptoms and laboratory findings in VL that consist in fever, splenomegaly and pancitopenia (9).

At admission, this classical triad was NOT present; the fact that our patient was afebrile at her first presentation augmented the difficulty of including leishmaniasis in the differential diagnosis; initially, there was no hepatomegaly (although it appeared at follow-up), no enlarged lymph nodes, no oedema and no bleeding other than the genital one interpreted in the context of the uterine fibroma. Laboratory findings were similar to other reported VL cases (12) - anaemia, leucopoenia with relative lymphocitosis and thrombocytopenia.

Delay in diagnosis in countries that are not endemic

VL is frequently misdiagnosed, especially in non-endemic countries and by physicians that have not had a lot of experience with tropical diseases. Diagnosis is difficult due to the unspecific early phase and the fact that the parasite is largely sequestered in the spleen, liver and bone marrow - traumatic interventions are required for a positive diagnosis (9).

For instance, Brazil is one of the coun-

tries that have experienced an increase in VL cases over the last two decades, with more and more areas becoming endemic for this disease. In the 1980's, in urban Brazil, there was an increase in VL mortality rates due to the fact that physicians underestimated and failed to diagnose VL - it was encountered only in certain rural areas at the time and they had little to no experience with this disease (13).

Our patient was correctly diagnosed approximately 14 weeks after her first hospital admission. This delay lead to an unfavourable prognosis and was a consequence of several factors: incomplete anamnesis, impossibility to perform bone marrow aspiration and the concomitant fibroma that complicated the case and aggravated the prognosis.

Particularities of the clinical case: young immunocompetent female (HIV negative, no immunosupressant medication); first anamnesis did not stress on her recent travels, so differential diagnosis did not include tropical diseases; first hospital admittance showed isolated splenomegaly (no associated hepatomegaly) and pancitopenia that could not be thoroughly investigated; associated fibroma as a source of continuous bleeding and confounder that lead to an unfavourable outcome.

CONCLUSIONS

This case report presents the management of a VL patient, with unfavourable outcome due not only to associated comorbidities, but also to delay in diagnosis and, consequently, treatment. Its purpose is to attract attention to the increase in incidence of this disease in particular and exotic diseases in general in non-endemic countries due to globalization and increasing migration; thus, they can no longer be excluded by Romanian physicians from the differential diagnosis. Such aetiologies should be suspected in patients that do not respond well to initial treatment, or that have travelled in the last three years to other countries.

ACKNOWLEDGEMENTS

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NEWS

PERIODONTAL CHANGES IN PATIENTS UNDERGOING RADIOTHERAPY FOR HEAD AND NECK MALIGNANCY

The most common modalities used in head and neck cancer treatment are represented by surgical resection, radiotherapy and chemotherapy, used singly or in combination. Direct damage to the oral soft and hard tissue structures frequently occurs from radiotherapy and chemotherapy, and indirect damage may also arise from systemic toxicity. The prospective study conducted by a group of researchers from the department of Periodontics - Government Dental College, Calicut, India was designed to analyze the effect of radiotherapy on periodontal parameters in oncology patients prior to irradiation in the head and neck area, compared with 6 months following treatment. The statistical analysis was done using SPSS. The study sample consisted of 29 patients of head and neck malignancy (17 male and 12 female), of mean age 45.66 years. Only 22% of patients had various habits (eg. smoking, tobacco chewing, and alcohol consumption) and the majority of patients (27.59%) had tongue malignancy. All periodontal parameters represented by OHIS (the oral hygiene index Siller), CAL (clinical attachment level), GR (gingival recession), except PPD (probing pocket depth) were significantly increased after radiotherapy. When comparison was made between doses and fractions, only in mandible statistically significant difference were observed in gingival recession.Although there was no significant change probing pocket depth, there was increased gingival recession, clinical attachment level, and plaque index associated with radiotherapy. In this study, 61.5% cases of mandibular teeth and 34.4% cases of maxillary teeth showed attachment loss greater than 0.2 mm. In conclusion, all patients with head and neck malignancy should undergo an oral examination before the initiation of cancer therapy with adequate prophylaxis. (Ammajan RR, Joseph R, Rajeev R, Choudhary K, Vidhyadharan K. Assessment of periodontal changes in patients undergoing radiotherapy for head and neck malignancy: A hospital-based study. *J Cancer Res Ther*, 2013 ; 9 (4) : 630-637).

Irina Grădinaru

THE EFFECT OF PIOGLITAZONE ON NON-PREGNANT FEMALE GENITAL TRACT IN EXPERIMENTAL DIABETES MELLITUS

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2. Discipline of Biochemistry
3. Discipline of Pharmacology

THE EFFECT OF PIOGLITAZONE ON NON-PREGNANT FEMALE GENITAL TRACT IN EXPERIMENTAL DIABETES MELLITUS (Abstract) **Aim**: The effects of pioglitazone, a very used drug in the treatment of non-insulin dependent diabetes mellitus, were tested at the level of ovary of non-pregnant female rats. **Material and methods**: The experiment was performed on three groups of adult non-pregnant female rats. **group 1** was a control group (and did not receive any substance), **group 2** received streptozotocin 60mg/kg i.p. (single administration), and **group 3** received streptozotocin 60 mg/kg i.p. (single administration) and pioglitazone 5 mg/kg/day p.o., daily for 8 weeks. The plasma glucose, cholesterol and triglyceride levels were determined before drugs administration and during the experiment. After 8 weeks the animals were anesthetized and sacrificed. The ovaries were examined by optical microscopy. A morphometric evaluation was performed. The obtained data were statistically interpreted by ANOVA test. **Results**: After 8 weeks of treatment the plasma glucose and triglyceride levels were significantly lower in the pioglitazone treated group compared to the streptozotocin only group. In the pioglitazone group the number of primordial and primary ovarian follicles was significantly higher than in streptozotocin only group. **Conclusions**: The results showed a partial protective action of pioglitazone on ovary in non-pregnant diabetic female rats. **Keywords**: DIABETES MELLITUS, STREPTOZOTOCIN, PIOGLITAZONE, OVARY

Diabetes mellitus (DM) is one of the most important diseases in human clinic. It affects all body tissues but unequally. In the therapy of non-insulin dependent diabetes mellitus (NIDDM) a significant number of oral antidiabetic agents with different chemical structures and mechanisms of action are used. One of the major classes of antidiabetic drugs is the thiazolidinedione group.

Thiazolidinediones (such as rosiglitazone and pioglitazone) are peroxisome proliferator-activated receptor-γ (PPARγ) agonists and are used to improve insulin sensitivity in DM patients. Thiazolidinediones are drugs that reduce the plasma glucose level, haemoglobin A_1c level and enhance tissue insulin sensitivity (1). There are many data on how diabetes mellitus affects the female reproductive organs but much less information on DM influence on non-pregnant female genital tract. The aim of this study was to highlight the action of pioglitazone on ovaries in non-pregnant female rats with experimentally induced diabetes.

MATERIAL AND METHODS

We worked on three groups of eight adult non-pregnant female Wistar rats each (eight weeks old, weighing 170-250 g) bred in normal laboratory conditions. The animals were housed in polycarbonate cages, at a temperature of 22±2°C. During the study the animals were fed with a commercial granular diet and water *ad libidum*. DM was induced in overnight fasted rats by a single intraperitoneal administration of 60 mg/kg streptozotocin (STZ) (Sigma®) dissolved in citrate buffer.

Group 1 of female rats was the control group and did not receive any substance.

Group 2 of female rats received STZ 60 mg/kg i.p.

Group 3 of female rats received STZ 60 mg/kg i.p. and pioglitazone 5 mg/kg/day p.o., daily for 8 weeks.

After 8 weeks all animals were anaesthetized and killed by decapitation and the ovaries were removed and examined by optic microscopy. Plasma glucose, cholesterol and triglyceride levels were determined at baseline (before STZ administration) and after 8 weeks of treatment in each group. The obtained data were statisticaly interpreted by ANOVA test. Morphometric analysis was performed on scanned images of histologic samples, using Zeiss Observer Z1, Tissue Gnostics 9, Tissue Faks system. The aquisited images were analysed in order to determine the most representative fields; all the investigated parameters were identified in selected fields of view (FOV) from each distinct region of the resulted images.

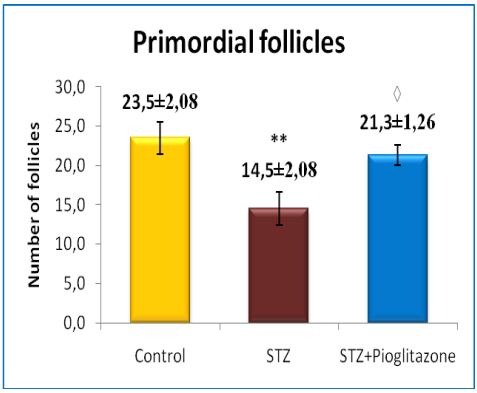
The study protocol was approved by the Ethics Committee of the Iasi "Grigore. T Popa" University of Medicine and Pharmacy. All animal procedures were performed according to the European Union law on the "Care and Use of Animals for Scientific Purposes" and in accordance with the "Recommendations of the Declaration of Helsinki".

RESULTS

After 8 weeks of treatment the plasma glucose and triglyceride levels were significantly lower in the pioglitazone group compared to STZ only group (mean plasma glucose levels of 203.83±14.61 mg/dl in pioglitazone group vs. 352.17±38.79 mg/dl in STZ group, and mean triglyceride levels of 191.3±12.04 mg/dl in pioglitazone group vs. 261.2±9.52 mg/dl in STZ group, $p<0.05$). The mean cholesterol level was 93.83±7 mg/dl in STZ group compared to 80±7.51 in pioglitazone treated female rats. In pioglitazone group the number of primordial and primary ovarian follicles was significantly higher than in STZ only group ($p<0.05$).

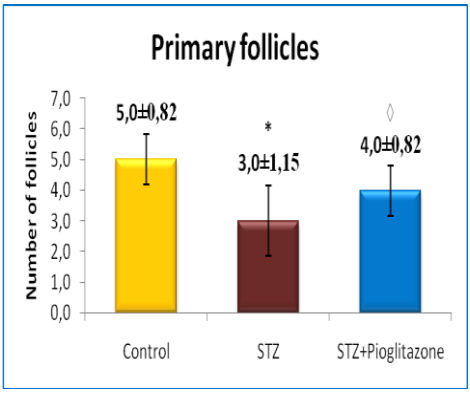
DISCUSSION

DM is associated with diabetic impairment of female reproductive function and the reduction of fertility (2). The STZ-induced diabetes mellitus is a classical model for experimental diabetes. Streptozotocin (60 mg/kg) is a substance known to destroy only a part of beta pancreatic cells (about 60% of all beta pancreatic cells). The plasma insulin level significantly decreased but with the preservation of an important mass of functionally beta cells (3, 4). After STZ administration the rat pancreas produces about 25-30% of the initial insulin secretion. The STZ-induced diabetes is characterized by the increase in the levels of fasting plasma glucose, lipid peroxidative products and a significant decrease of antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase and non-enzymatic antioxidants (reduced glutathione) (5).



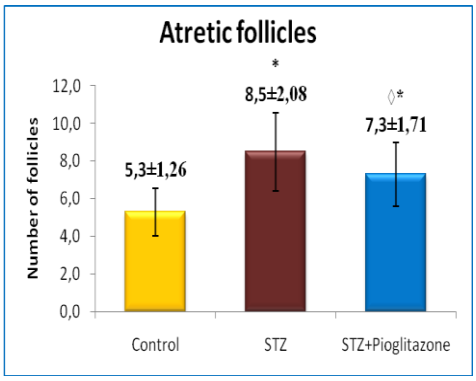
** p<0,01 vs. Control group;
◊ p<0,05 vs. STZ group.

Fig. 1 The influence of pioglitazone on primordial follicles in STZ- induced DM



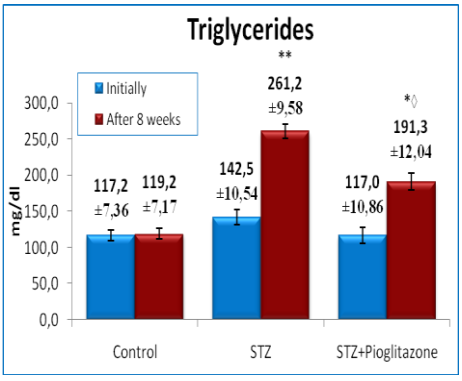
* p<0,05 vs. Control group;
◊ p<0,05 vs. STZ group.

Fig. 2 The influence of pioglitazone on primary follicles in STZ- induced DM



* p<0,05 vs. Control group;
◊ p<0,05 vs. STZ group.

Fig. 3 The influence of pioglitazone on atretic follicles in STZ- induced DM



* p<0,05 vs. Initially; ** p<0,01 vs. Initially
◊ p<0,05 vs. STZ group.

Fig. 4 The influence of pioglitazone on triglycerides in STZ- induced DM

Thiazolidinediones have been extensively used as insulin-sensitizers in the treatment of NIDDM. Activation of PPARγ by this group of drugs leads to a lot of metabolic and anti-inflammatory effects (6).

PPAR-γ is a member of the nuclear hormone receptor super-family. Pioglitazone, a specific agonist for PPAR-γ, was implicated in the control of inflammatory processes and the modulation of various cytokines expression. PPAR-*γ* agonists inhibit the synthesis of pro-inflammatory cytokines, such as TNF-*a* and IL-*6 (*involved in the failure of female reproductive activity) (7), diminish the oxidative stress (8) and inhibit or prevent cell apoptosis. Pioglitazone also reduces the pro-inflammatory cytokines IL-6 and IL-8 synthesis in endometrial stromal cells by a PPAR-γ-independent mechanism (9) and has a favourable effect in NIDDM, decreasing the incidence and severity of some complications. This drug also showed

a protective effect in retinal ischemia/reperfusion injury in rats (10).

Suppression of IL-1β could be another mechanism in pioglitazone-induced protective effect on ovary and uterus in STZ diabetic rats (11).

In our study, the effects of pioglitazone on non-pregnant rat ovary were different in different follicle types. The protective effect was stronger in the primordial follicles. The results were obtained with pioglitazone doses usually used in rat experimental tests (3-20 mg/kg/day) (12, 13). There were not observed influences on genital female system and fertility in normal animals after long time (8 months) of pioglitazone administration (14)

The obtained data are in agreement with the results of other authors which observed a partial protective effect of pioglitazone in experimentally-induced gastric ulcer (11), streptozotocin-induced diabetic nephropathy (13, 15) and experimentally-induced liver fibrosis in rat (16). Besides increasing sensitivity to insulin, pioglitazone increases tissue glucose utilization and decreases the synthesis of glucose from the liver (17). Pioglitazone also exerts a protective effect on β-cell function. Thiazolidinediones promote beta-cell survival and regranulation as well as maintenance of beta-cell mass (18). In addition to its beneficial effects on glucose homeostasis, pioglitazone exerts a number of other pleiotropic effects. This drug reduces blood pressure, improves endothelial dysfunction, corrects diabetic dyslipidemia and decreases the circulating levels of inflammatory cytokines and others (19). PPAR γ agonist pioglitazone used to treat NIDDM has also been demonstrated to be an effective antioxidant substance in oxidative stress in the animal models (20). Pioglitazone improves adipose tissue and liver insulin sensitivity and increases insulin-stimulated inhibition of lipid oxidation (21).

The obtained data show that pioglitazone significantly reduces the number of atretic ovarian follicles in STZ diabetes and increases survival of the ovarian primordial follicles.

Our results are in agreement with data indicating that pioglitazone treatment in polycystic ovary syndrome is associated with improvements in insulin action and glucose homeostasis and ameliorated the hyperandrogenic ovarian response. The granulosa cell responsiveness to FSH was enhanced by insulin after improved insulin sensitivity induced by pioglitazone (22). This drug ameliorated endothelial dysfunction of NIDDM patients. Increased nitric oxide production could be associated with a protective action and with the improvement of endothelial dysfunction.

CONCLUSIONS

This study showed that in STZ-induced DM in non-pregnant rats, the treatment with pioglitazone increased the number of primordial and primary ovarian follicles and reduced the number of atretic follicles.

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METABOLIC SYNDROME: PARTICULARITIES IN OVERWEIGHT WOMEN WITH POLYCYSTIC OVARY SYNDROME AS COMPARED WITH OBESE WOMEN WITHOUT OVARIAN DYSFUNCTION?

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METABOLIC SYNDROME: PARTICULARITIES IN OVERWEIGHT WOMEN WITH POLYCYSTIC OVARY SYNDROME AS COMPARED WITH OBESE WOMEN WITHOUT OVARIAN DYSFUNCTION? (Abstract): **Objective.** The study assesses the frequency of metabolic changes in overweight patients with or without polycystic ovary syndrome. **Material and methods**: The study group was made up by 148 patients of whom 99 patients without polycystic ovary syndrome (group A, control group) and 49 with polycystic ovary syndrome (group B), that came in our endocrine unit for a weight loss program, in the September 2008 - March 2009 period. Morphometric parameters (height, weight, body mass index), biological parameters (cholesterol, triglycerides, blood glucose, glycated hemoglobin) and body composition analysis by measuring the electrical bioimpedance, were evaluated. **Results:** Patients with polycystic ovary syndrome have a higher percentage of total fat (38.22±7.2) than patients without polycystic ovary syndrome (36.316±5.65) ($p<0.05$), for the same characteristics. Glycated hemoglobin, blood glucose and triglycerides were found higher in group B patients. Furthermore, the amount of free testosterone is higher in group B patients compared to those in group A. The prevalence of metabolic syndrome was higher in polycystic ovary syndrome cases (26.13%) comparative with overweight cases (16.16%, $p<0.01$). **Conclusions:** In the group of overweight patients, the group of patients with polycystic ovary syndrome is a particular group showing more severe metabolic changes. **Keywords**: OVERWEIGHT, POLYCYSTIC OVARY SYNDROME, METABOLIC SYNDROME.

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder that affects the female population of reproductive age. Its incidence is variable, ranging from 4% (1), 7.1% (2) to 33% (3), depending on the working group, ethnic group (4,5) or definition criteria for metabolic syndrome. (3). There are many diagnostic criteria; generally the presence of oligo-ovulation or anovulation, the clinical and/or biochemical signs of hyperandrogenism are considered, excluding other diseases: Cushing's syndrome, 21 hydroxylase deficiency, other forms of congenital adrenal hyperplasia, hypothyroidism, androgen secreting tumors (4, 5). Highlighting the morphology of the polycystic type is not compulsory; on the contrary, 8-25% of healthy women and 14%

of those under contraceptive therapy show the typical ultrasound appearance (6). Overweight and peripheral insulin resistance is common issues encountered in this syndrome (2). The metabolic syndrome (MeTS) can be considered as part of the PCOS, in the vast majority of cases (7). Obesity and insulin resistance are closely linked to the initiation and maintenance of hyperandrogenemia (2, 8). Obesity, especially the abdominal one, may play a pathogenetic role in the development of PCOS (9) favoring excess androgen synthesis.

Hyperandrogenemia *per se* favors visceral obesity (10) in its turn. However, not all overweight women have changes in the ovaries; respectively not all the patients having PCOS are obese.

The core of PCOS is a MeTS. This is the difference between simple ovarian cysts and PCOS, respectively the metabolic implication, cardiovascular and diabetic long-term risk, for this particular group of young females. Moreover, if not only the presence of MeTS, but also the severity of the metabolic impairment, as considered the number of altered metabolic parameters is considered, differs in overweight females with PCOS, the implications regarding cardiovascular risk are more important than we think.

Data on potential metabolic-hormonal differences between obese patients with or without PCOS are relatively few.

This study aims to evaluate mainly the metabolic and hormonal profile of obese patients, with or without the diagnosis of PCOS in order to develop an innovative method and integrating support in diagnosis assistance.

MATERIAL AND METHODS

The study group was recruited from patients who resorted of the Clinic of Obstetric Gynecology I and III, Timisoara, in the endocrine ambulatory, between September 2008 and March 2009. All the cases were self-addressed for establishing a weight loss program by our endocrinologist, because her results in the field of weight loss and life style changes. They were with no pre-diagnosed ovarian dysfunction or other endocrine diseases. All the patients entitled themselves as "healthy but overweight".

Out of the total of 227 women who came for assessment in order to establish a nutrition program, 20 cases were of normal weight, 59 subjects met one of the exclusion criteria, and 148 patients remained to form the actual study group. Their age was between 16 and 40, the mean age being 29.35±6.225, their weight was between 54 and 124.9 kg, the mean weight being 90.76±15.24 kg, and their body mass index (BMI) was 31.50±5.18 kg/m^2, ranging between 25.4 and 46.9 kg/m^2. Of the total of 148 overweight women, 99 women were considered as the control group (group A) and 49 women had the general characteristics of the PCOS (group B). The general characteristics of the two study groups are presented in table I.

- **Inclusion criteria:** Young females address by themselves to the endocrine unit of our Clinic, starting September 2008 to March 2009, with overweight weight exceeding the ideal weight for one's age (BMI>25 kg/m^2), age below 40, with not known endocrine disease.

- **Exclusion criteria:** male patients, hypothyroidism (37), 21-hydroxylase deficiency (5 non-classical form/late-onset), primary hypercortisolemia (1 case), functional hyperprolactinemia (15 cases), precocious ovarian failure –premature menopause (0 cases), diabetes mellitus, hyper-

tension, normal weight (BMI < 25 kg/m^2) and morbid obesity (BMI>45 kg/m^2).

All patients completed a comprehensive assessment protocol:

- **Anthropometric assessment:** height (without shoes) by means of a stadimeter (Tanita), weight (without clothes), and BMI calculation. Abdominal circumference was measured with a tape 1 cm wide, as the minimum circumference between the iliac crest and rib side edges, respectively hip circumference as the maximum circumference of the hips.

Body composition was determined by means of an analyzer of electrical bioimpedance, Tanita BC-418, Tanita Corp. Japan, with evaluation of composition of each segment and determining the basal metabolic rate. The general and segmental composition of trunk, upper and lower limbs was assessed for each patient;

- **Assessment of menstrual disorders:** cycles longer than 45 days, or less than 8 cycles per year, respectively secondary amenorrhea, inter-menstrual bleeding, menstrual flow changes: longer than six days or less than 2 days;

- **Hirsutism:** Ferriman Gallwey score values greater than 6;

- **Metabolic Assessment:** determining blood glucose, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (Tg), glycated hemoglobin, uric acid;

- **Hormonal profile:** free testosterone, 5-dehydroepiandrosterone sulphate, androstenedione, luteinizing hormone/ follicle stimulating hormone ratio, 17 hydroxyprogesterone, thyroide stimulating hormone, prolactin, plasma cortisol;

- **Diagnostic criteria used:**

1. hypothyroidism: thyroid stimulating hormone greater than 4 IU/L, with or without symptoms or obvious clinical signs;

2. primary hypercorticism: elevated plasma cortisol values, insuppressible with nocturnal inhibition with 1 mg dexamethasone;

3. 21-hydroxylase deficiency: increased levels of 17-hydroxyprogesterone, positive response to stimulation with 100 mg adrenocorticotropic hormone;

4. PCOS (Rotterdam 2003 criteria): anovulation/menstrual disorders, hyperandrogenemia: Ferriman-Gallwey score over 8, elevated free testosterone values >0.011 nM/L, 5-dehydro-epiandrosterone sulphate above the age appropriate limit, polycystic ovary morphology in ultrasound images;

5. MeTS (at least three criteria): waist circumference ≥80 cm, hypertension ≥130/85 mmHg or antihypertensive therapy, hypertriglyceridaemia, Tg ≥150 mg/dL, hypercholesterolemia, HDL-C <50 mg/dL;

STATISTICAL ANALYSIS

T-student test was performed with the NCSS 2007 software.

RESULTS

a. Mean weight, BMI and total body fat in obese patients with or without PCOS

The mean weight of patients who have PCOS is higher as compared to that of patients without ovarian involvement, but does not reach the threshold of statistical significance. In contrast, BMI is significantly higher in women with PCOS ($p<0.015$) (fig. 1). The difference is emphasized when body composition is assessed, the adipose tissue being significantly better represented in the case of PCOS. The differences are not due to differences in size, weight or fluid com-

partment (tab. I).

The percent of total body fat is significantly higher in B group (38.22±7.20 %) than in control (36.31±5.65 %), p<0.06. When we evaluated each section, the statistical significance was observed only in the upper half of the body (values measured in % of fat):

- Right hand: 40.64±9.27 vs. 38.43±7.85, p<0.05;
- Left hand: 40.64±8.77 vs. 37.98±7.47, p<0.04;
- Trunk: 35.102±8.53 vs. 33.63±6.8, p<0.008.

TABLE I

General characteristics of the two study groups

	Group A (without PCOS)			**Group B (with PCOS)**			**p**
Parameter	Mean±SD	Min	Max	Average SD	Min	Max	
Age (years)	29.9±6.40	18	40	28.41±5.55	16	40	0.916
Height (cm)	165.79±5.94	144.4	180	164.02±5.96	154	182	0.092
Weight (kg)	79.41±1.41	75.5	124.9	82.57±1.73	54	124.3	0.060
BMI (kg/m^2)	28.84±4.97	27.5	46.9	30.93±5.398	25.4	44	0.011

Even the fat mass was higher at the inferior limbs, the difference was not statistically significant (40.31±6.77 vs. 39.23± 5.56, p=0.1658 right side, 40.91±9.27 vs. 39.23±5.56, p=0.1561 left side).

The same bioimpedance evaluation is measuring the basal metabolic rate (BMR). When considering that besides genetic type, age, sex, height and weight are the principal determinants of BMR, we could expect the PCOS group should have higher BMR. But this was not the case: 1544.57±178.284 (group A) vs. 1574.67± 201.603 (group B), p=0.1798.

b. The degreee of weight excess in overweight patients with or without PCOS

Because overweight was an inclusion criteria, we could observe differences only in the prevalence of obesity between the two groups. When we looked at the pool of patients, we observed that the presence of obesity *per se* (67.35% vs. 51.52%) but also the severity of the disease was higher in PCOS cases: moderate obesity 29.40 vs. 12.12%, severe obesity 12.24% vs. 9.09% respectively (tab. II).

The same significant difference was observed also at the level of circumferences, as a surrogate marker for overweight (tab. III).

TABLE II

Severity of overweight in absolute values and percentages in the two study groups.

Severity of overweight	**Overweight**	**Degree of obesity**		
		I	**II**	**III**
Group B (with PCOS)	16 (32.65%)	17 (34.69%)	10 (29.40%)	6 (12.24%)
Group A (without PCOS)	48 (48.48%)	30 (30.30%)	12 (12.12%)	9 (9.09%)

TABLE III
Circumferences specific for assessment of metabolic involvement in the two study groups.

Circumference	Testifier (Group A)	PCOS (Group B)	Significance threshold
Abdominal (cm)	98±5	99±6	p<0.05
Hips (cm)	116±6	118±7	p<0.05
Waist/hips ratio	0.84±0.06	0.87±0.009	p<0.02

c. Metabolic imbalances in overweigt females with or without PCOS

Differences between the two groups are apparent not only in morphometry, body composition and degree of obesity but also from the metabolic point of view.

Patients with PCOS have significant higher values of glycated hemoglobin (p=0.035 vs. p<0.04), glicemia (p=0.009 vs. p<0.001) and Tg (p=0.029 vs. p<0.02), compared with cases without PCOS. The other metabolic parameters, HDL-C, LDL-C, and uric acid were higher in PCOS group, even though the difference did not met a level of significance (p=0.661 vs. p=0.546).

The metabolic assessment of the two groups of patients shows a high incidence of metabolic complications. Each metabolic component is modified to a greater extent in case of combination with the MeTS: HgA1c increased in 18/49 cases, HDL-C decreased in 28/49 cases, LDL-C increased in 9/49 cases and Tg in 20/49 cases (fig. 1).

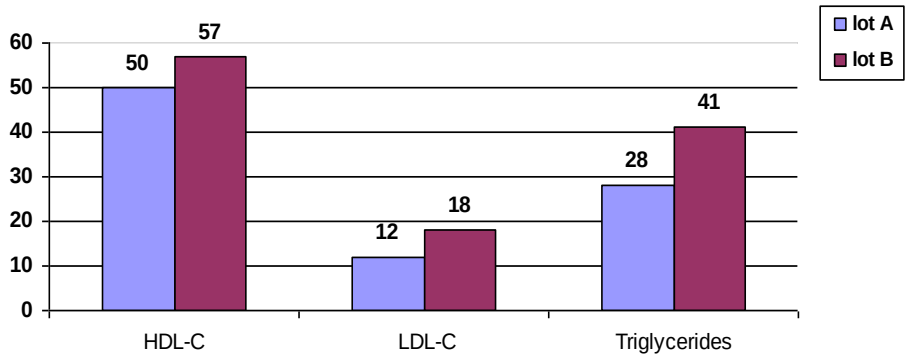

Fig. 1. Prevalence of isolated metabolic changes in the two groups with PCOS (group B) and without PCOS (group A).

The ordinate represents the percentage of patients with abnormal metabolic parameters.

d. The presence of metabolic changes, defined as the presence of increased 2, 3 or 4 metabolic components besides increased waist circumference, as a minimum criteria of MeTS in obese patients with or without PCOS

The overall prevalence of MeTS is

19.59%. There are significant differences between the prevalence of MeTS in the group of the overweight patients without PCOS (16.16%) (Group A) and in combination with this syndrome (26.53%, $p<0.01$) (group B).

The differences are great, no matter if considering the presence of two, three or even four criteria defining MeTS (fig. 2). If we consider the number of metabolic changes as a severity of the metabolic disbalance

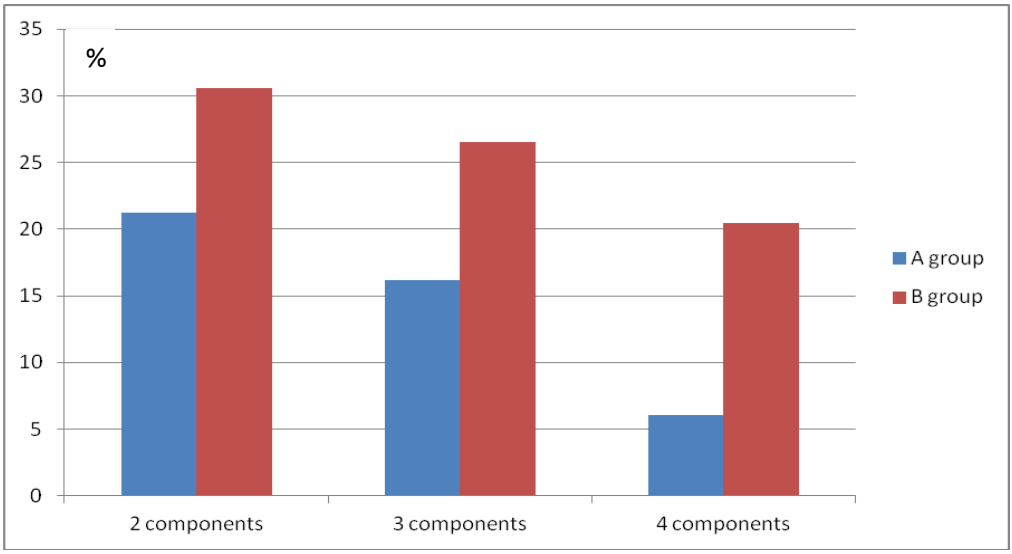

Fig. 2. Prevalence of MeTS in the groups of patients with PCOS (group B) and without PCOS (group A).

The ordinate represents the percentage of cases presenting 2, 3 and respectively 4 metabolic components defining MeTS besides increased waist circumference

e. Hiperandrogenemia in cases with or without PCOS

Clearly, not only overweight determines the presence of MeTS, but also the typical hormonal constellation, hyperandrogenemia. Of course, the mean values of all androgenic hormones were higher in cases of group B, as compared with control group, because hyperadrogenemia is a criteria for PCOS diagnostic. In the same overweight group, the hyperandrogenic patients have significantly higher metabolic complications. The estimated relative risk (ERR) of having MeTS in the presence of PCOS is 1.873, nearly four times higher compared with the ERR in the absence of PCOS. In case of overweight combined with hyperandrogenemia, the relative risk (RR) of developing MeTS is threefold as compared to the one generated by isolated overweight (RR 0.3265 vs. 0.1313, $p<0.02$) (tab. IV).

The coefficient of correlation between the severity of hyperandrogenemia (expressed by the values of free testosterone) and metabolic parameters is poor (tab. V).

Analyzing the prevalence of MeTS in the group of patients with PCOS (group B) we have identified differences between the level of hyperandrogenemia in the presence or absence of the MeTS (tab. VI).

TABLE IV

Metabolic parameters assessed in the two groups.

Parameter		Average	DS	ES	95% LCL	95% UCL	T	p
Glycated Hg	A	5.436	3.1383	0.313	4.814	6.059	-1.8184	0.035
	B	7.1537	8.310	1.199	4.740	9.566		
Glucose mg%	A	90.054	12.48	1.248	87.576	92.53	-2.3678	0.009
	B	95.089	11.27	1.628	91.81	98.36		
HDL-C mg%	A	50.365	8.914	0.891	48.59	52.13	0.4171	0.661
	B	49.60	13.023	1.879	45.82	53.38		
LDL-C mg%	A	128.84	24.716	2.471	123.94	133.75	-0.6037	0.546
	B	131.60	28.52	4.116	123.32	139.885		
TG mg%	A	119.53	42.70	4.314	110.97	128.09	-1.9033	0.029
	B	135.08	53.15	7.672	119.649	150.517		
Uric acid mg%	A	4.243	1.293	0.129	3.986	4.500	-0.9663	0.133
	B	4.473	1.384	0.199	4.07	4.877		

TABLE V

Indices of correlation and significance between the hyperandrogenemia (free testosterone) and values of metabolic parameters (HDL-cholesterol, LDL-cholesterol, Tg, glycated hemoglobin) in the PCOS group (group B).

	Free testosterone		DHEAS	
Parameter	R	p	R	p
HDL-cholesterol	-0.167	0.272	0274	0.059
LDL-cholesterol	0.2853	0.095	0.095	0.517
Triglycerides	0.229	0.123	0.2544	0.519
Glycated Hb	0.041	0.787	-0.054	0.713

TABLE VI

Different levels of hyperandrogenemia in patients of group B (with PCOS) with or without MeTS

	without MeTS	with MeTS	T	p
Free testosterone nM/L	0.014±0.00419	0.0193±0.0018	-1.529	0.05
DHEAS µg/dL	414.29±63.37	444±58.116	0.5530	0.291

DISCUSSION

The purpose of this study was to evaluate the presence and severity of MeTS in young, overweight women with PCOS. We defined a control group made up by patients whose weight was over the ideal weight for their age, who did not meet the diagnostic criteria of ovarian involvement of the hyperandrogenic type. Under the same conditions of height, weight, age and BMI, the prevalence of metabolic involvement is much higher in subjects with PCOS.

The relatively high prevalence (25.6%) of MeTS falls within the limits described in literature (11, 12). But there are studies that

show a lower incidence of only 5 to 10% (13). Yet diagnostic criteria of MeTS often vary, low-prevalence studies (13) have the same incidence of about 20% (22.7%) when there are 2 metabolic changes and also an increased waist circumference. Even without the criteria for MeTS, the prevalence of metabolic problems was high (50-55%), as described in the literature (4). Also the use of adult, respectively adolescent criteria generates differences (11). We can consider that the presence of PCOS increases the prevalence of MeTS (11, 12, 14). In our case the estimated RR of MeTS in the presence of PCOS was four times higher, similarly to other studies (12). The most frequent components of MeTS were increased waist circumference and LDL-C associated with peripheral insulin resistance (11, 12, 14).

As we saw from the data of Table II, not only the prevalence but also the severity of metabolic imbalance are higher in cases of associated PCOS: basic hyperglycemia as well as hypertriglyceridemia are significantly higher (12, 13). Even if hyperandrogenemia is considered the key to this syndrome (8, 9) we have not noticed the described important direct correlations with free testosterone, total or free fraction and the degree of imbalanced metabolic parameters (13, 15). However, the PCOS cases with MeTS had higher androgen levels as compared with non-MeTS PCOS cases (tab.VI) (15, 16). Levels of correlations were low and did not reach the statistical significance threshold (tab.III). However there are significant differences between the levels of hyperandrogenemia in patients with PCOS with or without metabolic complications (tab.V).

Generally hyperandrogenemia and increased waist circumference have the most significant associations with the incidence of MeTS, respectively peripheral resistance to insulin.

The present paper brings the metabolic component of the PCOS forward. Considering that in daily medical practice there is a large overlap between polycystic ovary morphology, secondary to anovulatory cycles, and PCOS diagnosed without assessing hyperandrogenemia and/or metabolic implications, it is imperative for us to warn our colleagues regarding the differences in pathology as well, besides the differences in nomenclature.

To our knowledge, this is the first study in our country, which overweight assessment includes measuring of the presence and distribution of adipose tissue by determining electrical bioimpedance of tissues. Higher abdominal fat content are associated with the presence of PCOS and MeTS as compared with control group, as abdominal fat and obesity related hyperinsulinemia favors hyperandrogenism (9).

Developing an innovative method and integrating support in diagnosis assistance for all the patients from the study would facilitate collaboration on a multicenter and multispecialty level, thus allowing the assessment of a greater number of cases and the participation of more physicians in the diagnosis and therapy procedure.

CONCLUSIONS

1. PCOS is associated with high incidence of MeTS as compared with aged matched controls.

2. The severity of metabolic imbalance (assessed by percent of changes and also degree of variance from the upper normal range) is more important in overweight PCOS cases, as compared with overweight age matched controls.

2. In a case of excess weight and multicystic ovarian morphology on ultrasound, investigation of hyperandrogenemia and metabolic components is important for identifying the moderate and long-term risk group for cardiometabolic disease.

3. Excess adipose tissue in cases of PCOS association with overweight is higher than in cases of overweight without ovarian complications, both in general and in the abdominal perivisceral area in particular.

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OSTEOPOROSIS IN RHEUMATOID ARTHRITIS

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OSTEOPOROSIS IN RHEUMATOID ARTHRITIS (Abstract): Osteoporosis (OP) is a disease of great medical and social importance and it is reported frequently and precociously in rheumatoid arthritis (RA) patients. **Material and methods**: A retrospective study was conducted on a random sample of 180 patients admitted in the period 2011-2012 to the Rheumatology Clinic of the Iasi Rehabilitation Hospital. The patients were diagnosed with RA and osteoporosis and some clinical and biological parameters were analyzed. Patients were divided into two groups for a comparative study: group I – seropositive = 115 cases (63.89%); group II – seronegative = 65 cases (36.11%). **Results and discussion**: The 180 patients diagnosed with RA and osteoporoses were randomized into other two groups. The serum rheumatoid factor (RF) in RA and osteoporosis patients was determined by Latex and Waaler Rose reaction. The radiological examination showed that 23.47% of patients in group I and 9.23% of patients in group II have experienced at least one fracture. DXA (*Dual X-Ray Absorptiometry*) testing showed a value of <-3.5 in group I (13,04%) and in group II (9.23%), these results indicating a high risk of fracture, particularly in group I (seropositive). **Conclusions**: The results confirm that RA patients are at increased risk of osteoporosis, and the additional risk of disability and serious socioeconomic implications negatively affect the prevention and development of the disease. **Keywords**: RHEUMATOID ARTHRITIS, OSTEOPOROSIS, CHRONIC INFLAMMATION, BONE MINERAL DENSITY.

Rheumatoid arthritis (RA) is a disease with high risk of osteoporosis, resulting from the association of several factors: bone mass loss (characteristic to RA development) by the risk conferred by the disease itself (inflammatory mechanisms with activation of various cytokines and alteration in the RANKL/OPG balance, prolonged immobilization, effects of corticosteroid therapy and other therapeutic agents), the predominance of female population (which implies an increased risk of bone mass loss caused by the occurrence of postmenopausal osteoporosis), and the increased life expectancy, that leads to an increase in the number of elderly persons who are also at increased risk of decline in bone mass (1,3,4).

The study aims to assess the prevalence of osteoporosis (OP) in RA patients, to establish the relationship between OP and clinical, biological and therapeutic variables, and to identify the risk factors that contribute to the decrease in bone mineral density in order to assess the bone tissue affected by disease and treatment.

MATERIAL AND METHODS

One hundred and eighty patients were surveyed for RA and OP in the period 2011-2012 in the Rheumatology Clinic of the Iasi Rehabilitation Hospital. The patients were divided into two groups for a comparative study: group I included seropositive patients (115:63.89%) and group II included seronegative patients (65:36.11%). The patients in the two groups did not differ significantly in age, average age at onset, sex distribution, and disease severity. There have been two studies:

A. *Epidemiological* study – aimed to investigate the effects of the disease and to determine if correlations can be made with environmental factors involved in the onset and development of the disease;
B. *Clinical* study - aimed to measure bone mineral density in patients diagnosed with RA in order to assess bone tissue.

ACR criteria (1987) were used in the diagnosis of RA, and the diagnosis of OP was made by osteodensitometry with dual beam X-ray using Hologic densitometer.

RESULTS AND DISCUSSION

A. Epidemiological study – the sex ratio was consistent across the two groups (χ^2=0.02; GL=1; p=0.889), with odds ratio of 1.05 (OR=1.05; IC95%: 0.52÷2.11).

The *age* and *sex* distribution of patients showed the predominance of the female sex, which accounted for 95.65% in the first group and 100% in the second group.

Age distribution revealed that the incidence of disease increases from the fourth decade of life onwards, especially in group II (χ^2=24.62; GL=5; p=0.0002) (fig.1, 2).

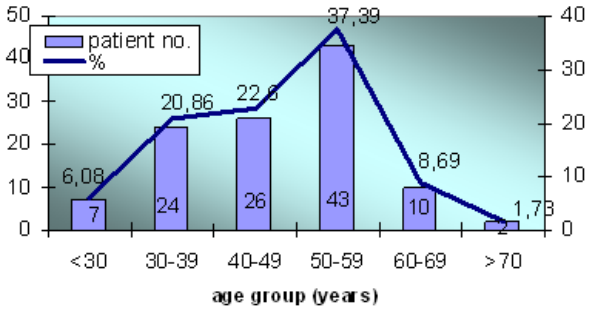

Fig. 1. Age distribution in group I of seropositive patients

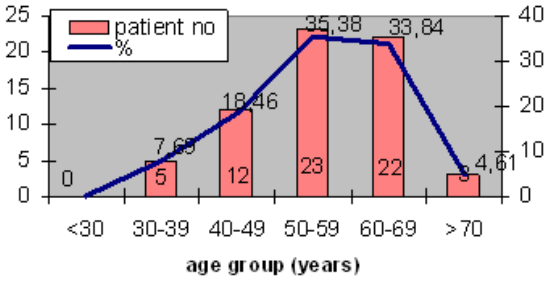

Fig. 2. Age distribution in group II of seronegative patients

The investigation of rural-urban differences showed that most patients lived in urban areas, 62% urban and 38% rural patients.

The analysis of educational level showed that 72% of the patients had primary education, 26% secondary education and 2% higher education. The average time between disease onset and diagnosis was 2.2 years, with significant differences. This also correlated with the severity of disability, showing that early diagnosis and appropriate treatment during the onset stage lead to good outcomes and maintenance of work ability (tab. I).

TABLE I
Duration of rheumatoid arthritis

CASES	NUMBER OF PATIENTS	AGE AT DISEASE ONSET	DURATION OF RHEUMATOID ARTHRITIS
Group 1	115	40± 15(years)	12 ± 5(years)
Group 2	65	40 ± 5	5 ± 5
Statistical significance		p>0.05	p<0.001

In the study groups the onset of RA was earlier in the seropositive as compared to the seronegative patients, with a peak in the fourth decade of life.

A psychological examination was conducted in 50 patients for a psychological profile of RA patients. One of the dominant negative affective states was pain, expressed as a particular, unpleasant feeling, accompanied by a number of reflex somatic and vegetative phenomena with a negative psychological connotation, because pain was caused by the body but affected the psyche, and it was more or less accepted. The onset of pain leads to a series of psychological changes that provide a certain experience of the disease. This is especially the case for chronic diseases, such as RA (4,8). Failure of treatment or the natural course of the disease associated with painful symptoms can lead to anxiety and depression with a poor prognosis. Knowing the individual psychological characteristics of RA patients plays an important role in the therapeutic approach because a good therapy includes also the emotional and affective aspects (2, 7).

B. Clinical Study

DXA osteodensitometry allows the early assessment of the degree of decreased bone density and the early diagnosis of osteopenia or osteoporosis, based on scores that suggest a different therapeutic approach (5, 6, 7).

The T score expresses the difference between the measured value and the reference value in control population. The Z score compares bone density in the patient with that of an age-matched person in order to assess the degree of bone loss. A negative Z score means that the patient has brittle bones, and a positive Z score means normal ranges. According to test results, the doctor may prescribe a medication that maintains or restores the skeletal system and recommend regular exercise and an adequate intake of calcium and vitamin D. DXA technology is also effective in monitoring the effects of OP treatment and in diagnosing other diseases that cause bone loss (2, 3, 6).

Morning stiffness was one of the main symptoms that made patients to see a doctor. It lasted significantly longer in group II

(χ^2=9,79; GL=1; p=0,007). In group I patients, 43.5% compared with 23.1% in group II, had morning stiffness lasting 15-30 minutes, while a duration of 30-60 minutes was observed in 41.7% of the patients in group I and 55.4% in group II. Morning stiffness lasting over 60 minutes was recorded in 21.5% of patients in group II and only in 10.4% of patients in group I.

TABLE II

Type of onset articular damage

Groups	Damage: No total	No articular damage	Monoarticular Nr.	Monoarticular %	Oligoarticular Nr.	Oligoarticular %	Polyarticular Nr.	Polyarticular %	NPJ*	NSJ*
I	115	15	23	20%	60	52.17%	17	14.78%	11±5	8 ±3
II	65	0	2	3.07%	50	76.92%	13	20%	6 ± 3	4 ±3

**NPJ=number of painful joints*

**NSJ=number of swollen joints*

Rheumatoid arthritis is characterized by symmetrical polyarticular damage. Group I (seropositive patients) showed a higher number of affected joints and a more severely compromised quality of life: the average number of painful joints – 11 in group I *vs.* 6 in group II (t-Student=8.38; GL=178; p<0.001), mean number of swollen joints – 8 in group I *vs.* 4 in group II(t-Student=8.59; GL=178; p<0.001)(tab. II, fig. 3).

Fracture risk assessment plays an important role in the treatment of OP. FRAX, a recently developed algorithm of fracture risk assessment does not change the principle of defining OP as a decrease in T-score below -2.5 SD measured by DXA (tab.III, IV).

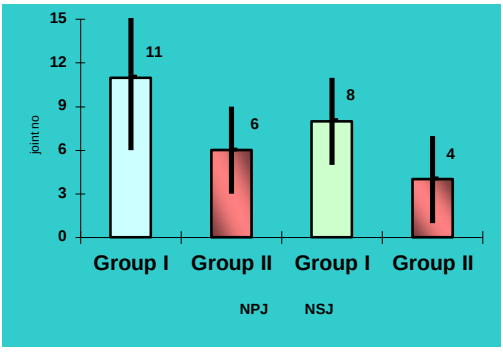

Fig. 3. Average number of affected joints in each study group

TABLE III

Fracture risk assessment

Gr.	Patient no.	Sex female %	Weight (60-75kg) %	Height (155-165cm) %	Fracture occurrence %	Parents with femoral neck fracture	Smoker %	GCS %	Secondary OP %	Alcohol use >3U/day %	**DXA 3-3,5 %**
I	115	100	73.9	69.5	23.47	11.20	4.35	92.17	15	5.74	34.78
II	65	95.65	68.7	83.7	9.23	6.4	2.45	80	3	1.3	16.92

TABLE IV
Fracture risk assessment

Groups	Risk	PATIENTS			
		WITH AT LEAST ONE FRACTURE		WITH NO FRACTURE	
		DXA-2.5	DXA-3.5	DXA-2.5	DXA-3.5
I		Risk 7.3	Risk 7.8	Risk 1.4	Risk 2.8
II		Risk 5.6	Risc 6	Risk 1.1	Risk 2.1

Using FRAX index, patients in group I with a T score of -2.5 measured by DXA had a 7.3% risk of fracture, and for a T score of -3.5 the risk was 7.8%. Patients in group II with a T-score of 2.5 had a risk of fracture of 5.6%, and for a T score of -3.5 the risk was 6%.

Severity criteria were comparatively assessed in the two study groups: NPJ > 6, significantly more common in group I patients (87%) compared to group II (χ^2=42.29; GL=1; p<0.001). NSJ > 6, significantly more common in group I patients (63%) as compared with group II (18%) (χ^2=40.17; GL=1; p<0.001). ESR > 28 mm/h, significantly more common in group I patients (58%) compared to group II (25%) (χ^2=21.09; GL=1; p<0.001). CRP > 20 mg/l, significantly more common in group I patients (82%) as compared with group II (28%) (χ^2=56.75; GL=1; p<0.001). ESR: group II: median = 25 +1 / 2 = 13; group I: median = 29 +30 / 2 = 29.5, CRP: group II: median = 14 + 15/2 = 14.5; group I median = 41 + 42/2 = 41.5; NPJ: group II: median = 21 +22 / 2 = 21.5, group I: median = 87 +1 / 2 = 44; NSJ: group II: median = 9 +10 / 2 = 9.5; Group I: median = 63 +1 / 2 = 32 (fig. 4).

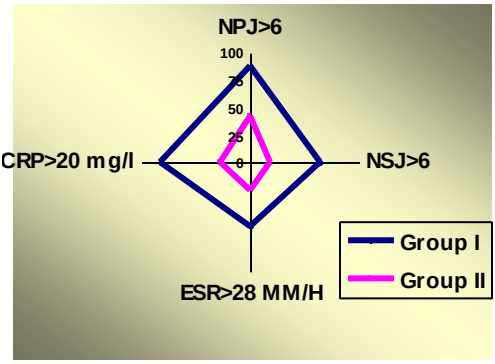

Fig. 4. Severity criteria in the two study groups

The *median* was used in assessing the severity criteria. The median is the middle value of a data set. It is the measure of center because to its left and to its right 50% of all data are found (fig. 4).

The biological factors of inflammation were most influenced in the seropositive form of disease, proving that this form is more severe.

Age distribution analysis showed that OP is common in the fifth decade of life as it is influenced by the presence of menopause (χ^2=23.60; GL=5; p=0.0003)(fig. 5, 6).

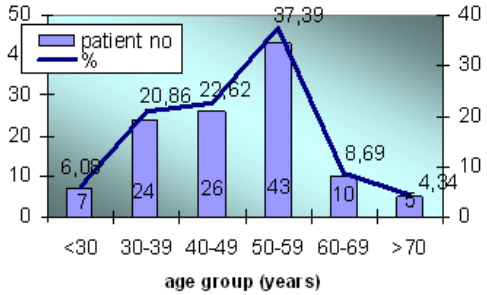

Fig. 5. Age distribution of osteoporosis patients in group I

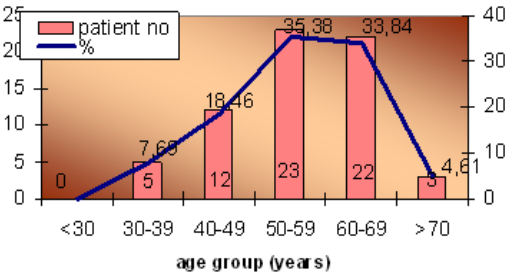

Fig. 6. Age distribution of osteoporosis patients in group II

Menopause is an important risk factor for OP due to hormonal and metabolic changes occurring after the menopause (2, 5, 6). Menopause was present in 89.56% of group I and 89.23% of group II patients, with a statistically insignificant frequency distribution (χ^2=0.56; GL=1; p=0.453). ESR and CRP correlated with disease activity, assessment of therapeutic outcome and prognosis. ESR reflects disease activity of the past few weeks, while CRP reflects short-term changes in disease activity. Case distribution in relation to ESR level showed no statistically significant differences between the study groups (χ^2=4.47; GL=1; p=0.346), but CRP was more common in group I patients (63.5%) as compared with group II (24.6%) (χ^2=23.56; GL=1; p<0.001).

Integument rheumatoid nodules were present in group I (p = 0.0003), but the distribution of cases with pulmonary fibrosis or vasculitis did not differ significantly between the two study groups.

TABLE V

Systemic effects in the study groups

GROUPS	PATIENT NO.	INTEGUMENT RHEUMATOID NODULES		PULMONARY FIBROSIS		VASCULITIS	
		No. %		No. %		No. %	
I	115	23 20	χ^2=13.16 GL=1 p=0.0003	25 21.73	χ^2=2.71 GL=1 p=0.0997	7 6.08	χ^2=0.29 GL=1 p=0.593
II	65	0 0		7 10.76		2 3.07	

Rheumatoid factor measured by Latex and Waaler Rose (4) is one of the diagnostic criteria and it differentiates seropositive from seronegative forms having a high negative prognostic value, correlated with the highly erosive characteristic of the disease, marked functional impairment, articular index (radiological), and presence of systemic manifestations (fever, Raynaud's syndrome) (4). Rheumatoid nodules were found only in patients with seropositive forms of disease, who showed an increased occurrence of systemic effects. Radiological changes showed significantly more erosions in group I (p = 0.003). Distribution of cases

with geodes, impingement of the joint or juxtaarticular osteoporosis showed no significant differences between the two study groups (tab. V, VI).

TABLE VI

Radiological changes

GROUPS	GEODES		EROSIONS		IMPINGEMENT OF THE JOINT		JUXTAARTICULAR OSTEOPOROSIS	
I	63	χ^2=0.01 GL=1 p=0.904	98	χ^2=9.04 GL=1 p=0.003	75	χ^2=0.01 GL=1 p=0.935	96	χ^2=0.70 GL=1 p=0.404
II	35		47		42		58	

Group I, with marked impairment in a great number of joints, had more advanced OP. The radiologic examination showed that the seropositive form of RA had a severe evolution(χ^2=8.21; GL=2; p=0.017). *Corticosteroid* (CS) therapy had a negative impact on bone metabolism, increasing bone resorption and decreasing bone formation, resulting in reduced bone mass and increased fracture risk. It is thus an important risk factor for OP, especially in group I. 92.17% of patients in group I and 80% of patients in group II received this therapy (p = 0.031).

Reduced bone formation is caused by the direct effects of CS on osteoblastic cell line and by indirect mechanisms, such as inhibition of gonadotropin release and of sex steroid formation. CS causes a degradation of type I collagen (the major constituent of bone matrix), which explains the inhibitory action of CS on bone matrix and bone mass.

A direct relationship between CS treatment, duration and dose of PDN was shown. Patients who received CS treatment more than a year had osteoporosis. The PDN dose of 5-7.5 mg / day showed the lowest risk of OP (tab. VII).

TABLE VII

Patients receiving corticoterapy

GROUPS	PATIENT NO.	PATIENTS RECEIVING CS TREATMENT	STATISTICAL DIFFERENCE
		Nr. %	
I	115	106 92.17	χ^2=4.66 GL=1 p=0.031
II	65	52 80.00	

Anti-osteoporotic therapy considered the severity of bone loss and fracture risk, the individual contraindications, and the availability of a specific product used by the study group (3.7). All OP drug therapies influence bone metabolism (*turnover*). Anti-osteoporotic therapy showed a good outcome in our study groups and was administered at approximately the same doses in both study groups (p = 0.729) (tab. VIII).

TABLE VIII

Patients receiving anti-osteoporotic therapy

GROUPS	TOTAL NO.	PATIENTS RECEIVING ANTI-OSTEOPOROTIC THERAPY	STATISTICAL DIFFERENCE
		Nr. %	
I	115	61 53.04	χ^2=0.12 GL=1 p=0.729
II	65	37 56.92	

Biological therapy - clinical and biological improvement occurred early in all patients treated with MTX at a dose of 7.5 mg/week in association with biological therapy with infliximab (Remicade) 3 mg/kg/infusion (mean dose provided by international algorithms for the use of this product).This way, a decrease in disease activity score level was achieved, which correlated with improvement in the other quantifiable parameters. Biological therapy was used in about the same proportions in both study groups (p=0.518); it has remarkable results in rheumatoid arthritis therapy, as it can lead to RA remission (tab. IX).

TABLE IX

Patients receiving biological therapy

GROUPS	PATIENT NO.	PATIENTS RECEIVING BIOLOGICAL THERAPY	STATISTICAL DIFFERENCE
		Nr. %	
I	115	69 60	χ^2=0,42 GL=1 p=0.518
II	65	35 53.84	

Kinesiotherapy was a treatment method used in both study groups (p = 0.257). The physiotherapy program included the same exercises for patients in the two groups. The physical therapy program was designed to meet the general principles of kinesiology: specificity, progressiveness and reversibility (tab. X).

TABLE X

Patients receiving kinesiotherapy

GROUPS	PATIENT NO.	PATIENTS RECEIVING KINESIOTHERAPY	STATISTICAL DIFFERENCE
		Nr. %	
I	115	41 35.65	χ^2=1.29 GL=1 p=0.257
II	65	23 35.38	

Balneo-physical therapy must be given with great caution in patients with rheumatoid arthritis and it may relieve pain, inflammation, strengthen muscles and prevent osteoporosis.

Monitoring the development of disease

Using the ROC curve to show the comparative effectiveness of treatment in the two study groups revealed that the anti-osteoporotic treatment was effective, with

an accuracy of 63.9%, followed by the biological treatment, with an accuracy of 63.2% (tab. XI, fig. 7).

EULAR response - the number of patients with a good or moderate response was significantly higher in group I as compared with group II (χ^2=10.19; GL=2; p=0.006) (tab. XII).

TABLE XI

Comparative analysis of the results of therapy methods

Therapy	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)	p
CS	67.1	40.9	89.1	14.8	51.9	0.031
Anti-osteoporotic	62.2	59.3	68.5	59.3	63.9	0.729
Biological	67.0	60.5	69.7	56.8	63.2	0.518
Kinesitherapy	64.0	63.8	49.4	63.7	56.5	0.899

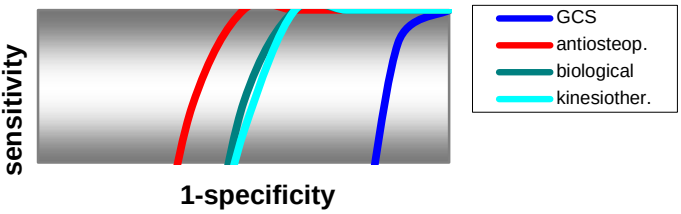

Fig. 7. TheROC curve for the effectiveness of treatment

TABLE XII

Monitoring the development of disease

GROUPS	PATIENT NO.	DEGREE OF THERAPEUTIC RESPONSE		
		PATIENTS WITH A GOOD RESPONSE (>1.2)	PATIENTS WITH A MODERATE RESPONSE (0.6-1.2)	PATIENTS WITH NO RESPONSE (≤0.6)
		Nr. %	Nr. %	Nr. %
I	115	28 24.34	39 33.91	48 41.73
II	65	21 32.30	32 49.23	12 18.46

Age distribution of the study groups shows an increasing incidence of the disease from the fourth decade of life onwards. The seropositive form of the disease has an earlier onset than the seronegative one, and this is reflected in a higher number of affected joints, the Steinbroker functional classrevealing a more severely compromised quality of life in group I patients.

The biological factors of inflammation - ESR and CRP - correlate with the evaluation of disease activity (DAS28), with the therapeutic response and prognosis evaluation. These parameters of inflammation were high in group I, showing that the seropositive form of the disease progresses faster and more

severely. C-reactive protein value was independent of the disease duration. Pain assessment (pain is the main complaint in RA patients) was made using a visual analogue scale (VAS) and showed an increased frequency of pain in the patients with a longer disease suration. Therefore, there is a direct correlation between pain and disease duration. A direct correlation between HAQ scores and duration of disease was also noted, and HAQ was higher in seropositive patients.

The age distribution of osteoporosis patients showed a peak in the fifth decade of life and menopause contributed to this peak, as menopause was present in 84.61% of the patients in group I and 89.23% in group II. DXA showed important pathological features in the seropositive group I, indicating a more severe form of disease. The radiological examination revealed a risk of fracture: 23.47% of group I patients had at least one fracture and only 9.23% of group II patients. Correlating with DXA assessment, a T score of <-3.5 in 13.04% of group I and 9.23 of group II patients, a high risk of fracture was observed, it being higher in the seropositive group I.

CONCLUSIONS

Osteoporosis in RA patients has a severe course, inducing disability and affecting furthermore the their quality of life. However, the quality of life may be improved by early treatment. Osteoporosis and "rheumatoid" osteopenia appear to be independent of individual indicators of disease evolution, but they are directly related to the low values of composite indices. The low functional capacity favors bone loss in RA patients.

Research has provided evidence that the pathogenesis of osteoporosis involves a complex interaction of genetic and environmental factors, affecting bone growth, peak bone mass, calcium homeostasis and bone loss and it is influenced by age, physical inactivity, hormonal deficiency and vitamin D metabolism.

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MANGANESE INTERACTIONS WITH DOPAMINERGIC SYSTEM

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MANGANESE INTERACTIONS WITH DOPAMINERGIC SYSTEM (Abstract): Manganese is a trace element with important involvement in the central nervous system functioning. The effects of manganese excess on central dopaminergic system in case of both basal nuclei and reward system are linked to important pathologic states such as Parkinsonism and cognitive impairment. Our data show that Mn^{2+} reduces the intensity of experimental morphine addiction in rats (manganese chloride unequally decreases several symptoms of opioid withdrawal syndrome – signs such as compulsive mastication, grooming and teeth chattering being particularly affected). Manganese chloride, administered during conditioning acquisition phase in rats, significantly reduces the intensity of morphine conditioned place preference. We believe that manganese interaction with dopaminergic circuits in the brain reward system represents one of the most important aspects of the action of bivalent cations at central nervous system level. **Keywords**: MANGANESE, DOPAMINE, BRAIN REWARD SYSTEM, MORPHINE-DEPENDENCE, PLACE PREFERENCE.

Bivalent cations, either macro- or trace elements, are present in different concentrations in the central nervous system (CNS). Most of them play complex roles in the functioning of CNS, neurons, glial tissue or blood vessels. There is increasing evidence referring to the complex regulatory action determined by the bivalent cations on CNS. This regulatory action has three different essential items: modulation of enzymatic activity (most enzymes contain a bivalent cation as co-factor or allosteric modulator); modulation of synthesis and activity of different neurotransmitters (presynaptic release, re-uptake etc); modulation of neurotransmitter action at receptor site.

Manganese is an essential trace element with important catalytic and structural roles. As in the case of other bivalent cations deficiency and excess, in case of Mn^{2+} misbalances dysfunctions in the activity of numerous organs and systems (including CNS) were identified. Mn^{2+} excess is more frequently encountered and has more severe consequences than Mn^{2+} deficiency, determining mainly neurological and psychiatric disturbances.

Human body contains approximately 10 – 20 mg of manganese. It is considered that serum Mn^{2+} level should be lower than 1.4 μg/100 mL. The most important Mn^{2+}-dependent enzymes include: glycosyltransferase, phosphoglucomutase, different kinases, hydrolases (including phosphatases), decarboxylases, pyruvate carboxylase, peptidases (arginase), cholinesterase, and mitochondrial superoxide dismutase (1).

The highest CNS Mn^{2+} concentrations are in nucleus pallidus, as well as in other regions with predominant dopaminergic neurotransmission. Also, brain dopaminergic sites are concentrating most of the Mn^{2+} excess, both in case of chronic exposure or hepatoportal encephalopathy (2). There is no correspondence relationship between serum and brain Mn^{2+} concentrations: while increased Mn^{2+} serum concentration may revert to normal after exposure is stopped, brain Mn^{2+} levels continue to be increased. Little is known about Mn^{2+} passage through brain-blood barrier. Aschner et al., 1999 (3), showed that manganese, as a citric acid salt, is better captured by the cerebral tissue compared to free Mn^{2+}cations. In the lateral choroid plexus, there are mechanisms protecting against Mn^{2+} ions passage into the cerebral-spinal fluid in case of high blood Mn^{2+} concentrations (4). Sloot et al., 2004 (5), have shown that Mn^{2+} is captured by dopaminergic and GABAergic neurons in substantianigra, and that there are mechanisms for the axonal transport of the cation. Also, chronic Mn^{2+} exposure determines its accumulation into astrocytes (6). The most characteristic aspect regarding Mn^{2+} excess in the CNS is represented by the occurrence of Parkinson-like disease syndrome. Chronic Mn^{2+} administration and intoxication are used as experimental models of induced Parkinson disease in animals (7). Extrapyramidal phenomena can be reproduced, for example, by the administration of 5mg/kg/day Mn^{2+}, for 2 to 8 weeks in rats.

I. Mechanisms of manganese-induced central nervous system toxicity

CNS is the main target of Mn^{2+} toxicity. Even though data on the mechanisms by which Mn^{2+} determines its toxic effect on the CNS are not very clear, the hypothesis of increased oxidative stress, followed by a reduction in mitochondrial anti-oxidant protectionsystem, is ever more considered (8):

1. Fitsanakis et al., 2006 (9), have demonstrated that intra-cellular Mn^{2+} is sequestrated by the calcium-importer system in the mitochondria, and that neuronal mitochondria are particularly sensitive to Mn^{2+} toxic activity (Mn^{2+} inhibits oxidative phosphorylation);

2. A possible explanation for the long term Mn^{2+} neurotoxic effect related to chronic exposure may be the following: excess brain Mn^{2+}cation levels are oxidized to superior valence forms, and therefore catecholamine, and particularly dopamine oxidation is enhanced (10; 11);

3. Manganese also determines an increase in H_2O_2 neuronal generation, probably as a consequence of reduced catalase activity (12);

4. Also, the increased oxidative stress hypothesis to explain Mn^{2+}-induced neurotoxicity was suggested by Prabhakaran et al., 2008 (13), who incriminated NF-κB induction and the activation of nitric oxide synthesis through NOS (nitric oxide synthase) as a probable mechanism for Mn^{2+} CNS toxic effects;

5. Manganese induces caspase activation and DNA fragmentation;

6. Another possible hypothesis for Mn^{2+} brain cytotoxic effects is linked to metabolism impairment. Glucidic metabolism is particularly affected. Especially in astrocytes, glycolytic activity inhibition may result in the accumulation of toxic glutamine.

Central dopaminergic systems are particularly affected by Mn^{2+} excess. A significant and long-lasting dopaminergic depletion (tens of years after exposure is stopped) is recognized as a consequence of

Mn^{2+} exposure. Mn^{2+} excess is considered dopaminergic neurotoxin, and Hamai et al., 2001 (14), show that Mn^{2+} is highly effective in blocking dopaminergic neurotransmission. Chronic exposure to Mn^{2+} is associated with lesions in the basal ganglia neurons. Intrathecal Mn^{2+} administration in rats is followed by dopaminergic depletion, especially in the caudate-putamen regions. Manganese phosphate ($MnPO_4$) is more potent than manganese sulphate ($MnSO_4$) and manganese chloride ($MnCl_2$) in producing dopaminergic depletion (15). The selectivity of Mn^{2+} toxicity for the regions rich in dopamine does not have a clearly elucidated cause, but there are some partial explanations. For example, Anderson et al., 2007 (16), proved that Mn^{2+} accumulation in dopaminergic neurons in globuspallidus is at least partially dependent on dopamine transporter (DAT), as by inhibiting DAT with GBR12909 substance (1 mg/kg, 3 times a week, for 4 weeks) Mn^{2+} accumulation is stopped and Mn^{2+} levels show a significant decrease in that region. A study by Hirata, 2008 (17), contradicts the idea that toxicity determined by Mn^{2+} in the PC12 cell line is DAT-dependent, as Mn^{2+}-induced DNA fragmentation is present in both PC12 cells over-expressing DAT and in PC12 control cells (although the latter are affected at slightly higher doses); however DAT inhibitors (such as mazindol, nomifensine, or GBR12909), inhibit MPP+-induced DNA fragmentation.

As dopaminergic systems are particularly sensitive to the influence of Mn^{2+} ions, Mn^{2+} causing, as shown above, dopaminergic depletion, several studies were focused on different aspects of the interactions between Mn^{2+} ions and dopaminergic neurotransmission, including dopamine synthesis, release, action at specific receptor site, re-uptake, and metabolism. Different Mn^{2+}-induced effects on the above-mentioned aspects of dopaminergic neurotransmission were evidenced. Some of them are quite contradictory and some of the studies do not support the idea that Mn^{2+} impairs dopamine functions at any of its levels.

a. ***Dopamine synthesis***. Bonilla et al., 1980 (18) reported that following Mn^{2+} chronic treatment in rats the enzymatic activity of L-tyrosine hydroxylase (an enzyme involved in catecholamine synthesis) is increased. The augmented enzymatic activity persisted in neostriatum, midbrain and hypothalamus on the third month and remained elevated only in neostriatum on the sixth month. After eight months a significant decrease in the activity of the enzyme was found in neostriatum with no changes in the remaining studied regions. Also, in male albino rats exposed to $MnCl_2 \cdot 4H_2O$ (1 mg/ml) through drinking water there is an initial increase in the stream dopamine level, followed by a period when concentration is almost normal (from 120 to 240 days) (19). Thereafter, the contents of dopamine declined significantly at 300 and 360 days of treatment. Mn^{2+}, 125-520 μM in cell culture increases intracellular concentrations of L-Dopa (catecholamine precursor) (20). These findings are in agreement with the fact that human Mn^{2+} intoxication starts with a psychiatric phase bearing similarities to schizophrenia in which the primary disturbance has been suggested to be an over-activity of dopamine neurons. A totally different situation is in newborns compared to adults. Neonatal rats, intubated daily with distilled water, 25 or 50 μg $MnCl_2$/g/day for 14 or 21 days (starting from the day of birth) did not show significant changes in norepineph-

rine, dopamine, serotonin and their metabolites in the striatum, hypothalamus, and nucleus accumbens, though Mn^{2+} brain levels were significantly higher than in controls. The developing CNS may be able to counteract neurochemical changes caused by Mn^{2+} exposure.

b. ***Dopamine release***. Semama et al., 1995 (21) have demonstrated partial Mn^{2+} blocking of calcium channels in renal blood vessels. If this action also takes place in the cerebral tissue, this may explain a possible Mn^{2+}-induced alteration of glutamate ability to stimulate dopamine release. On the other hand, chronic Mn^{2+} exposure results in a marked decrease of in vivo dopamine release, in the absence of a change in markers of dopamine terminal integrity or dopamine receptors in the striatum (22).

c. ***Dopamine – specific receptors binding***. Butterworth et al., 1995 (23) showed that Mn^{2+} potentiates dopamine binding to specific receptors. On the other hand, Mn^{2+} seems to determine a reduction in dopamine receptors antagonists binding to their specific sites. In monkeys with Mn^{2+} intoxication, the specific D1 receptors antagonist SCH 23.390 expresses a lower binding to specific receptors compared to controls (24). Chronic manganism is associated with markedly decreased D2 receptor density, especially in the striatum (25).

d. ***Dopamine re-uptake***. Chen et al., 2006 (26) proved in a study on primates (baboons) that acute manganese sulphate (MnSO4) administration determines a decrease in dopamine binding to DAT (dopamine transporter), but a significant increase in DAT level (probably compensatory to the decrease in dopamine binding capacity), while dopamine re-uptake is inhibited.

Our data show a reduction in the intensity of both morphine physical dependence and morphine-induced conditioned place preference by Mn^{2+}. We have found a significant reduction in morphine dependence intensity by the administration of $MnCl_2$ (0.1 mEq/kg/day, 10 days) (27, 28). Compulsive mastication, teeth chattering and grooming are particularly reduced by manganese administration ($p<0.01$ vs. morphine-only treated group), while locomotor activity, weight loss, aggressive postures are moderately reduced ($p<0.05$). Global withdrawal score GellertHolzmann is also reduced ($p<0.01$). The influence on withdrawal symptoms is therefore unequal and dose-dependent ($MnCl_2$ administration, 0.05 mEq/kg/day, has no influence on morphine global withdrawal score, reducing only teeth chattering and grooming, $0.01<p<0.05$). As $MnCl_2$ was administered during morphine dependence induction phase and not during withdrawal, we consider this not to be a direct effect of manganese on morphine withdrawal, but on the intensity of morphine-dependence. The intensity of morphine induced conditioned place preference in rats is reduced by $MnCl_2$, (0.1mmol/administration, administered 2 hours before morphine, in the conditioning acquisition phase; 0.01mmol/kg/day, 10 days before preconditioning) (29). We believe that decreases in both morphine dependence intensity and morphine behavioral effects represent important proofs of the consequences of Mn^{2+} excess and support that their mechanism is related to dopamine neurotransmission impairment. Glutamatergic system is particularly sensitive to manganese actions, glutamate being one of the neurotransmitters mainly involved in the addictive processes. A subacute manganese exposure (7 to 21 days) in mice is sufficient to alter presynaptic do-

pamine release in the striatum (30).

II. Clinical consequences of the interactions between manganese and dopaminergic neurotransmission and of manganese excess

Chronic Mn^{2+} intoxication is characterized by weakness, headache, muscle cramps, loss of appetite, apathy, insomnia, diminished libido, which are associated with psychotic reactions known as *"locura manganica".* This "manganese madness" is characterized by aggressive manifestations and emotional lability. Characteristic clinical signs often include extrapyramidal symptoms such as bradykinezia, tardive dyskinesia (myoclonic movements), akathisia, dystonia (31). Other symptoms are less common and include behavioral impairment. As a consequence of manganese toxicity, symptoms such as confusion, monotone voice, un-expressive face, spastic movements and disturbed gait, hallucinations, renal failure may also appear. Mn^{2+} excess also impairs cognitive capacity (learning and memorization skills). There is a very clear relationship between manganese excess and dopamine central circuits which explain the behavioral disorders and cognition impairment associated with Mn^{2+}; taking this into consideration, new perspectives are opened for human clinical studies.

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SIDE EFFECTS OF ANTIVIRAL THERAPY IN HEPATITIS C VIRUS INFECTION–SARCOIDOSIS - CASE REPORT

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SIDE EFFECTS OF ANTIVIRAL THERAPY IN HEPATITIS C VIRUS INFECTION – SARCOIDOSIS (CASE REPORT) (Abstract): Standard therapy in chronic hepatitis C virus infection is still a combination of peginterferon alfa2a/2b and ribavirin for 48 weeks. As of side effects, there are organic side effects, such as hematologic disorders, and functional side effects, reflected in the quality of life of hepatitis C patients. Up to 30% of the patients develop specific side effects such as headache, fever, fatigue. Sarcoidosis, known as a granulomatous disease of uncertain cause, is an uncommon finding in this category of patients. This cause-effect relation is accounted for by the convergent action of peginterferon and ribavirin of stimulating type 1 T helper cells and reducing type 2 helper T cells activation. We present the case of male patient known with chronic hepatitis C who developed pulmonary sarcoidosis following antiviral therapy**.** The first manifestation of the disease was unexplained fever accompanied by pulmonary tract disease. The diagnosis was established by immunophenotyping in bronchial aspirate **Keywords**: HEPATITIS C, INTERFERON ALPHA, SARCOIDOSIS.

Combination therapy using peginterferon alpha2 a/2b and ribavirin for 48 weeks is still the gold standard of treatment for chronic hepatitis C. Nearly 30% of patients develop nonspecific side effects (headache, fever, fatigue) during this treatment. Sarcoidosis associated with this therapy is rare, being described in less than 1% of hepatitis C patients. The association between sarcoidosis and antiviral therapy is explained by the immunomodulatory action of both substances expressed in the stimulation of type 1 T helper (Th1) cells activity and decrease in type 2 T helper lymphocytes (Th2) activity. We present a case of sarcoidosis diagnosed in a patient with chronic hepatitis C treated with peginterferon alpha-2a and ribavirin.

CASE REPORT

MD, 43-year-old male, was diagnosed in 2009 with chronic HCV infection (HCV RNA - 907,000 IU/ml; pathology findings: chronic hepatitis of moderate activity Ishak ANI score = 7, fibrosis = 3, metavir A_2F_2). Based on liver function testing, antiviral therapy with peginterferon alpha-2a (180 mcg/week) and ribavirin (1000 mg/day) was initiated. As the 4-month assessment revealed that viral load became undetecta-

ble, the patient continued the initially recommended therapy. Also, during this period no notable side effects were reported. Starting with the fifth month of antiviral treatment the patient complained of fever, chills, sweating, fatigue, chest pain, and headache. Physical examination revealed fever 38°C, submandibular adenopathy, and sonorous and subcrepitant rales in the right lung area. Laboratory findings: severe thrombocytopenia without radiological pulmonary changes (tab. I).

TABLE I
Laboratory findings during febrile syndrome

Z 1	Z 7	Z 14
Hb – 12,4 g%	Hb – 11,4 g%	Hb – 12.2 g%
GA – 5.080/mm^3	GA – 2540/mm^3	GA – 2800/mm^3
Tr – 48.000/mm^3	Tr – 101.000/mm^3	Tr – 100.000/mm^3

Discontinuation of antiviral therapy for 7 days and administration of single-drug antibacterial therapy for 2 days (ampicillin), and then, because of febrile syndrome persistence, administration of dual antibacterial therapy (Ampicillin - Ciprofloxacin) were decided. Radiological reassessment after 7 days of antibacterial therapy detected a focus of right infrahilar condensation. Clinically, the respiratory manifestations gradually faded within 14 days, and biologically an improvement of thrombocytopenia was found. Radiological examination at 21 days revealed a decrease in size of the initial inflammatory focus. Clinical and biological monitoring during the remaining seven months of antiviral therapy showed fluctuations in platelet and leukocyte count, reason why no temporary reductions in antiviral drug dosage were necessary. Clinically, the patient continued to complain of fatigue, intermittent fever, and dry cough. As these manifestations have not outlined any ongoing clinical syndrome, they were classified as clinical adverse reactions of antiviral therapy and treated symptomatically.

After 48 weeks of antiviral medication, the patient complained again of respiratory manifestations: progressive dyspnea, and cough with purulent expectoration. Radiological examination revealed bilateral hilar lymphadenopathy (fig. 1). Additional tests detected significant pulmonary and cardiac changes (tab. II).

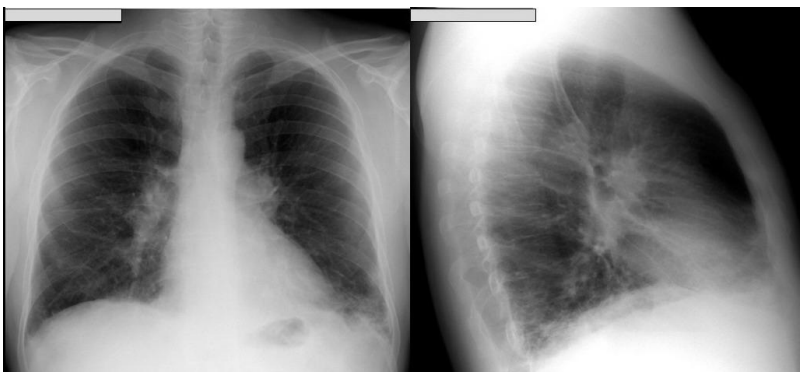

Fig. 1. Bilateral hilar lymphadeopathy

TABLE II

Additional examination 50 weeks after the initiation of antiviral therapy

- IDR 2u PPD – negative
- Fibrobronchoscopy: friable mucosa, abundant secretion
- Bronchial aspirate – BAAR negative
- Anatomopathological examination of bronchial aspirate - inflammatory smear with metaplasia and moderate dysplasia
- Bronchial aspirate immunophenotyping - 21% Ly, 20% - LT, TCD4/CD8 ratio = 3.3
- Pulmonary function tests: FEV1 - 77.7%, FVC - 77.4%, PEF - 90.1%, MEF50 - 64.1%. Conclusion: mild restrictive dysfunction
- ECG - sinus rhythm, supraventricular extrasystoles with aberrant conduction
- Chest CT (fig. 2):
  - mediastinal lymph nodes: enlarged, confluent, bilateral and relatively symmetric, noncalcifed, without necrosis, uncompressed
  - parenchymal changes: round, small, perilymphatic nodules, nodular or irregular areas of increased „ground glass" attenuation, and peripheral, subpleural peribronhovascular condensations
  - retroperitoneal lymph nodes, interaorticocaval ~ 2cm, celiomesenteric < 1cm

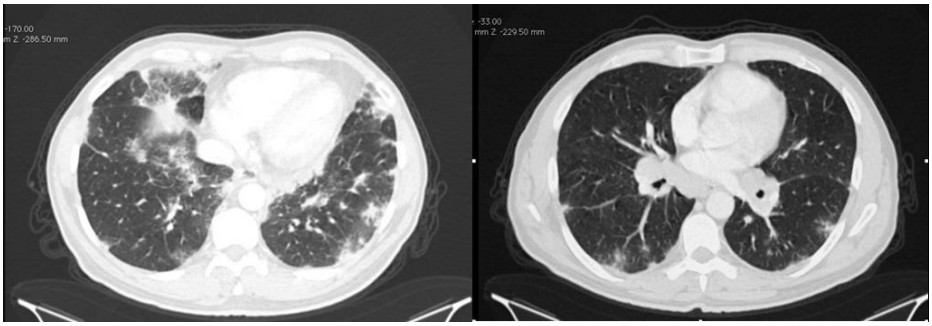

Fig. 2. Mediastinal lymphadenopathy

As the patient did not present peripheral adenopathy and the risk of a mediastinal biopsy was higher that its benefit (immunosuppressed background), this test (pathology) required for differential diagnosis (tuberculosis, lymphoma, sarcoidosis) was not performed. Phenotypic analysis of bronchoalveolar lavage sample (Tab. II) showed criteria suggestive of sarcoidosis. In the absence of other consistent evidence for a diagnosis of lymphoma or tuberculosis, we interpreted these findings as stage II sarcoidosis. Prednisone therapy was instituted; fever disappeared after a week, the patient being monitored by an infectious disease specialist. Viremia after 18 months of therapy remained undetectable.

DISCUSSION

The first case of sarcoidosis associated with interferon therapy was published in 1978. Since then, similar cases have been reported in the literature, but not being a widespread phenomenon it did not lead to

the cessation of the above mentioned therapy (3)

Chronic hepatitis C infection currently affects over 170 million people worldwide. Current standard therapy of this disease is the association of two antiviral drugs (peginterferon alpha2a/2b - ribavirin) with immunomodulatory role, amplifying the immune system response on infected cells. (2). In 40-50% of cases, subjective side effects (fatigue, myalgia, headache, depression) and others representing well-defined organ dysfunctions (e.g. autoimmune thyroiditis) are reported.

Sarcoidosis diagnosed in patients on antiviral treatment for hepatitis C is recognized as a side effect of interferon therapy. Interferon-induced sarcoidosis is known to be a chronic multisystem inflammatory disease of unknown cause manifest by the appearance of non-caseous granulomas in various organs/systems: lung (90%), lymphatic system (33%), liver (50/80%), eyeball (11-83%), and skin (25%).(1) As to its pathogenesis, it requires the intervention of one or more factors (infectious, tumor antigens/autoantigens) that prime immune mechanisms through which a prolonged stimulation of T lymphocytes with the appearance of an exaggerated T-helper cell response is produced: predominance of Th1 cells over Th2 cells. Interferon-alpha stimulates Th1 cell differentiation and decrease in Th2 lymphocytes activity leading to the formation of granulomas (6, 8). Compared to its predecessor, peginterferon-alpha has a higher influence on Th1 response, and therefore an increased risk for sarcoidosis. (4, 5, 9) Ribavirin, guanosine analogue, also stimulates Th1 lymphocytes and decreases Th2 lymphocyte activity. Thus, this association accounts for the increased predisposition to sarcoidosis of hepatitis C patients on antiviral therapy. In this category of patients, sarcoidosis can occur during treatment or after its discontinuation (7).

We presented a case of pulmonary sarcoidosis induced by peginterferon-alpha2a - ribavirin antiviral therapy in a 43-year-old patient. In this patient the onset of prolonged febrile syndrome associated with respiratory symptoms occurred after the first four months of therapy. In the absence of clear evidence (absence of superficial or deep lymph nodes), the clinical picture was initially interpreted as a right lower lobe pneumonia favored by drug induced leukopenia. Fever, fatigue, and cough are frequent adverse reactions in patients on long-term antiviral therapy. Our interpretation of patient's symptoms was revised only when after antiviral treatment discontinuation at 48 weeks radiological examination showed a bilateral hilar lymphadenopathy. Pulmonary examinations completed this information puzzle leading to a correct interpretation of the picture. Bronchoalveolar fluid immunophenotyping is the key diagnostic test on which the disease was diagnosed in the absence of other criteria.

CONCLUSIONS

Antiviral therapy with peginterferon alfa2a/2b and ribavirine in patients with hepatitis C has many functional and organic side effects. Before therapy, these reactions, which can be temporary, have to be divulged while obtaining consent. During the long-term antiviral therapy the physician has to pay attention to all side effects that can appear and take measure for preventing or diminishing their actions. Sarcoidosis is an uncommon occurrence, and in our case we misinterpreted the first disease manifestations: fever of unknown origin and pneumonia. Following the detection of mediastinal adenopathy, the addi-

tional investigations made us realize that all manifestations could be interpreted as pulmonary sarcoidosis. Therapy with prednisone was initiated and fever disappeared within a week. So it is important to keep in mind that respiratory disorders occurring in hepatitis C patients on antiviral therapy can be early signs of sarcoidosis.

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NEWS

EXPOSURE TO CIGARETTE SMOKE AND THE PATHOGENESIS OF RESPIRATORY INFECTION

Staphylococcus aureus, conditioning pathogenic bacteria, can colonize the upper respiratory tract and can cause severe infections under favorable conditions such as exposure to cigarette smoke (CS). CS has immunosuppressive and irritant effects on human cells. A recent study released by Kulkarni R and colleagues show that CS also affect the microorganisms by normal microbiota and potentiate their virulence. CS increased *S. aureus* ability to form biofilm and to adhere to the host cell. Oxygen free radicals present in CS inhibit Accessory Gene Regulator (agr) system responsible for dispersing bacterial biofilms. Also these compounds determine transcriptional induction of bacterial oxidoreductases. By inducing transcription of fnbA (encoding fibronectin binding protein A), increases the ability of *S. aureus* to bind fibronectin and thus CS can favor bacterial adherence to the host cell. This study concluded that the bioactive effects of CS may extend to the resident microbiota with implications for the pathogenesis of infection (Kulkarni R, Antala S, Wang A et al. Cigarette smoke increases Staphylococcus aureus biofilm formation via oxidative stress. *Infect Immun*, 2012; 80 (11): 3804-11).

Cătălina Luncă

QUALITY OF LIFE IN PATIENTS WITH ULCERATIVE COLITIS

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QUALITY OF LIFE IN PATIENTS WITH ULCERATIVE COLITIS (Abstract) : Patients with inflammatory bowel disease (IBD) have to live with the perspective of a potential disability, which may lead in time to impaired quality of life (QoL). In order to fully understand the impact of IBD on patients and their careers we need to quantify the QoL**. Aim**: To evaluate HRQol in ulcerative colitis patients as opposed to the general population and to identify the significant changes of HRQoL in accordance with the specific progression of UC. **Methods**: The prospective study (January 2010 - December 2012), included 105 patients with ulcerative colitis (UC). Disease activity was quantified by means of the Truelove and Witts score (mild, moderate and severe). All patients were administered a self-completed IBD questionnaire (IBDQ). Lower scores were related to a worse QoL. Statistical analysis was performed and differences were considered significant when $p \leq 0.05$. **Results**: Ninety-one percent (91%) were admitted with a flare, while they were asked to fill in the IBDQ. Proctitis was met in 2.94% of the cases, left-sided colitis in 71.1% and pancolitis in 26%. There were 32 patients with mild activity, 62 with moderate activity and only 3 with severe activity. The range score for QoL was 90-180. In proctitis: 80- 190, in left-side colitis: 120- 200, in pancolitis: 70- 160 ($p>0.05$). The patients with severe form had a lower score (50-140) than patients in remission (130-180) ($p<0.01$). **Conclusion**: Patients with UC have significantly influenced the QoL as compared to the general population. We found no significant differences regarding the extension of the lesions. The severity of the flare had a significant impact on the QoL. **Key words**: QUALITY OF LIFE, ULCERATIVE COLITIS, STATISTICAL ANALYSIS.

The "quality of life" (QoL) concept has been taken into consideration and studied ever since 1946, when World Health Organization defined health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (1).

Quality of life represents a "holistic self-assessment of satisfaction, with reference to the individual", being a highly controversial multidimensional concept, which is difficult to define, although it seems quite easy to grasp its meaning (2, 3, 4).

The term "health-related quality of life" (HRQoL) was defined as being the functional effect of a disease and the treatment of the patient, as it is perceived by the patient. The patient's perception of his health

is vital for understanding the way in which he/she organizes his/her activity. HRQoL may be influenced by a number of factors such as: financial status, habitat, job, spirituality, social support, and, last but not least, the health of the individual.

Although they represent different concepts, functional status and HRQol are used together in clinical practice. Functional status reflects the ability to fulfill daily activities (physical, social, and emotional), while HRQoL takes into account the subjective, personal aspects of the impact, the health status on the quality of life of the individual (5).

Ulcerative colitis (UC) is a chronic disease with an unpredictable evolution, marked by episodes of both activity and remission. Patients with UC are affected not only by the physical effects of the disease, but also by the impact it can have on the QoL.

The instruments of assessing HRQoL are represented by general questionnaires, and disease-specific questionnaires. These questionnaires assess HQoL by calculating a score on account of the patient's perception on HRQoL. The HRQol score reflects the evaluation of the life lived in a specific state of health, having the patient himself as the primary source of information.

The aim of this study is to evaluate HRQol in ulcerative colitis patients as opposed to the general population and to identify the significant changes of HRQoL in accordance with the specific progression of UC.

MATERIAL AND METHODS

A prospective case-control analytic study has been conducted inside the Gastroenterology and Hepatology Center in Iaşi over a period of 50 months (January 2010 - February 2012). The study included 189 patients, assigned to 2 groups: Group A, containing 105 patients with UC, and control Group B, containing 84 patients without UC.

The UC diagnostic was supported by complete hematological and biochemical tests and colonoscopy and, also, histopathologically. The activity of the disease was quantified by means of the Truelove - Witts score.

Patients were evaluated based on questionnaire IBDQ-32. This is one of the most widely used HRQoL evaluation questionnaires. It contains 32 questions grouped into four areas: gastrointestinal symptoms (diarrheic stools, abdominal pains, rectal bleeding, tenesmus), systemic symptoms (fatigue, sleep disorder), emotional functions (depression, irritability, anger), and social functions (absenteeism, affected social status). The answers range from 1 (the worst) to 7 (the best). Total score ranges from 32 to 224. The lower the score, the more HRQoL is affected (5).

Patients with UC were asked to send the IBDQ-32 questionnaire to someone they know, without UC, of the same age and sex, in order to make up the control group.

In order to process and interpret the data, *MS Excel*, *EpiInfo*, and *SPSSS* computer software were used. The interpretation also contained a descriptive analysis and the following tests: χ-square, t-Student. The differences were considered significant when $p \leq 0.05$.

RESULTS

Structure of the studied group

The group of 105 patients diagnosed with UC consisted of 42 men (40%) and 63 women (60%). The average age was 42.67 (+/- 13.74), the ages of 22 and 63 being the extremes. Most of the patients were living in an urban area - 70 (66%), with only 35

(33%) of them living in a rural area. The non-smoking status was predominant, 60 patients being non-smokers (57.14%), while 45 patients were smokers (42.85). The disease duration had an average of 29.3 months (+/- 30.47), while the number of previous relapses was 3.74 (+/- 3.88).

Ninety-one percent of the patients with UC completed the IBDQ-32 during the active period of the disease. Proctitis was present in 2.94% of the cases (3 patients), left-colitis in 71.1% (74 patients), and pancolitis in 26% (27 patients) of the cases. A mild activity of the disease was present in 32 patients; 62 presented a moderate activity, while only 3 presented a severe activity.

The IBDQ-32 Score

The QoL score ranged between 90-180. For proctitis: 80-190, for left-colitis: 120-200, and pancolitis 70-160 ($p<0.05$). Patients with severe forms had a lower score (50-140) as compared to the patients in remission (130-180) ($p<0.01$) (tab. I). The number of previous relapses was significantly influenced QoL ($p<0.05$); patients with previous active disease had a lower QoL. An important factor which negatively influenced QoL was also the long course of the disease, the results showing an average of 29.3 months ($p<0.05$) of disease evolution.

Age, sex distribution, smoker/non-smoker status and the living area (urban/rural) have not shown significant statistical differences. Also, the extension of the lesions has not influenced significantly the statistics regarding the QoL (tab. II).

TABLE I

HRQoL in accordance with the specific progression of UC

	Group A (UC) Activity period	**Group B (control)**	**Group A (UC) Remission period**	**Group B (control)**
HRQoL	145.23 ±30.27	201.34 ± 20.16	165.23 ±20.27	201.34 ± 20.16
p	p<0.05		p>0.05	

TABLE II

Clinical and evolutive characteristics and HRQoL

	UC	**HRQoL**	**p**
Sex: M **F**	42 63	117.35± 32.5 104.74 ± 42.26	p>0.05(NS)
Age	42.67 ±13.74	112.45±51.5	p<0.05 (r =0.230)
Living area: urban/rural	70 35	114.56 ±52.14 123.35±71.15	p>0.05 (NS)
Status: smoker **non-smoker**	45 60	144.53 ±53.27 131.37±30.43	p>0.05 (NS)
Disease duration (months)	29.3 ±30.47	165.23 ±20.27	***p<.05 (r =0.475)***
Number of previous relapses	3.74±3.88	142.34±25.26	***p<0.05(r = 0.521)***
Extension: proctitis **Left-colitis** **pancolitis**	3 74 30	112.33±40 132.27±20.46 70-160	p>0,05 (NS)
Activity: remission **mild** **moderate** **severe**	8 32 62 3	165.23 ±20.27 143.65±40.2 123.15± 23.21 80.33±30.67	***p<0,01(remission/ severe)***

DISCUSSION

The QoL evaluation in inflammatory bowel disease has become a primary concern and a major objective in recent studies. It serves to appreciate the efficiency of the treatment, helps describe the nature and gravity of the disease and also to assess the prognostic.

IBD has a negative impact on all aspects concerning QoL. The emotional behaviour is mainly disturbed, triggering the development of depression and anxiety during and after the active disease periods (6, 7).

The present study identifies a group with an altered QoL: a long period evolving disease, with an early age debut, with frequent, severe and extended activity periods that would gain benefit after psychiatric counseling.

The importance of the HRQoL is thus confirmed in IBD patients, especially in those with UC. Our results are similar to other studies in which the same test or different HRQoL tests were applied.

An USA study established that the main concern in patients with IBD is related to the doubtful disease progression, the drugs side effects, the impact of the disease on the energy level, the risk of surgical correction, the possibility of a colostomy, the fear of being a "burden" for others and the risk of possible neoplasia. The group of women, also preoccupied with the physical aspects, and younger people were interested in how the disease will influence their sex life (8). In general, women manifested concerns and fears during the active period of the disease (9).

For patients in the active phase, the main attention is directed towards the improvement of the overall status. In order to achieve it, suitable medical and surgical treatment can be used but also through professional psychological and psychiatric support.

Some studies reveal that even patients in remission state may have negatively influenced QoL (10). The main concerns include the side effects of the medication, self esteem and the risk of an evolving neoplasia.

The symptoms frequency is and will remain the main factor to influence QoL. The first diagnostic age doesn't seem to influence HRQoL, but significant inversely proportional correlations with the overall duration of the disease were observed (the longer the progression was, the lower the HRQoL indexes) (11).

Moreover, it has been proven that IBD patients are more likely to develop depression and anxiety as compared with the general population. These findings are associated mostly with the symptoms frequency, the undergoing invalidity and the poor nutritional status (12). The intensity of the psychic disturbance seems to be linked to the severity of the illness but the psychic factors do not seem to have a major influence on the progression of the disease (11).

The overall perception regarding the health status in UC patients is more pessimistic as opposed to the general population as the patients feel more susceptible to the probability of getting sick. They must live with a potentially handicap perspective, the "burden" sensation for others, which may affect in time the QoL and even help develop major psychic illness. They are confronted with everyday stigmatization, fear and repulsion, frequently describing feelings of discomfort and disability. There are also studies proving a decrease in the sexual activity.

CONCLUSIONS

Patients with UC have a significantly influenced QoL as compared to the general population of the same age and sex. A younger age debut, associated morbidity, and medication affect the patients not only physically but they also limit their social life and their educational, professional and emotional activity.

Although HRQoL is influenced in all UC patients, there are few patients with HRQoL similarly to the general population. Identifying the proper factors (social insertion, medication, etc.) might help improve HRQoL in UC patients.

Therefore, IBDQ-32 has proved to be an efficient test in detecting categories with low HRQoL. HRQoL evaluation helps identify patients in need of professional support.

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CORELAȚII ÎNTRE ASPECTELE CLINICO – BIOLOGICE ŞI VALOAREA LIMFOCITELOR CD4 LA PACIENȚII HIV/SIDA CU INFECȚII BRONHOPULMONARE NON – TUBERCULOASE

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RELATION BETWEEN CLINICAL-BIOLOGICAL ASPECTS AND CD4 LYMPHOCYTE COUNT IN HIV/AIDS PATIENTS WITH NON-TUBERCULOUS PULMONARY INFECTION (Abstract). Pulmonary complications in HIV infection are still an important cause of morbidity and mortality among AIDS patients. An accurate assessment and diagnosis of these patients should include demografic, clinical, and laboratory data associated to the recognition of imaging pattern. When the radiological appearance is atypical and does not suggest pneumonia, the need of interpreting the pulmonary image in relation with HIV viremia level and CD4 lymphocyte count. **Aim**: Analysis of statistical data, diagnosis, course, and particularities of HIV/AIDS cases with non-tuberculous bronchopulmonary infections, and the possible relations between the pulmonary radiological appearance, viremia, and CD4 lymphocyte count. **Material and methods**: Retrospective clinical study of 121 patients with HIV infection and infectious pulmonary disease from Moldova (Romania) diagnosed and treated at HIV-AIDS Regional Centre of the Iasi Clinical Hospital of Infectious Diseases. These patients were followed up in the interval January-December 2011. The studied demographic parameters were: age, gender, area of residence, years of disease. The clinical, laboratory, imaging (pulmonary Xray) data and CD4 lymphocyte count were analysed in dynamics. Data were processed statistically, a direct relation between clinical course and prognosis being found. Patients history, epidemiological history, respiratory symptoms (cough, thoracic pain, dyspnea) associated to the polimorphous clinical picture of HIV infection and the presence of associated comorbidities were recorded. The disease forms found were: lobar pneumonia, acute tracheobronchitis, interstitial pneumonia (non-tuberculous), and bronchopneumonia **Conclusions:** Non-tuberculous bronchopulmonary infectious diseases accounted for a small percentage (13.5%) of the respiratory diseases in HIV/AIDS patients; most infections were found in patients with a CD4 lymphocyte count exceeding 300/mmc; of the clinical forms, acute tracheobronchitis and interstitial pneumonia were the most common; etiological treatment and favorable clinical-biological course were similar to immunocompetent hosts; pneumococci involved in the etiology of bacterial pneumonia in HIV/AIDS patients preserve the same sensitivity as in the seronegative patients. **Key words:** NON-TUBERCULOUS PNEUMONIA, CD4 LYMPHOCYTE COUNT, RESPIRATORY DISEASES IN HIV/AIDS

Cu toate progresele în terapia antiretrovirală, complicațiile pulmonare în infecția HIV rămân o cauză importantă de morbiditate şi mortalitate în rândul pacien-

ților cu boala SIDA. Evaluarea corectă şi diagnosticarea acestora ar trebui să ia în calcul informații demografice, clinice, de laborator, în asociere cu recunoaşterea „pattern-ului" radiologic/tomografic. Aceasta deoarece modificările demografice şi de terapie sunt acompaniate de schimbări ale frecvenței şi etiologiei manifestărilor pulmonare şi implicit ale expresiei radiologice pulmonare.

Sindromul de reconstrucție imună, apărut sub presiunea terapiei antiretrovirale înalt active, ridică probleme de diagnostic diferențial şi dificultăți în decizia de menținere/întrerupere a terapiei antiretrovirale, în lipsa unei bune corelații între clinică, aspectele radiologice şi numărul limfocitelor CD4 (1).

Incidența pneumoniei bacteriene la persoanele infectate cu HIV este de aproximativ 6 ori mai mare ca în populația generală. Pneumoniile şi bronşitele acute sunt cele mai frecvente afecțiuni respiratorii la acești bolnavi în țările dezvoltate, fiind, de obicei, şi prima manifestare clinică a infecției HIV. Mai mult chiar, creşterea recurențelor în cazul pneumoniilor recurente este predictiv pentru scăderea timpului de supraviețuire (2,3). Spectrul etiologic al acestor pneumonii este similar populației generale, doar cei cu imunosupresie avansată putându-se întâlni şi etiologii neobişnuite (*Nocardia asteroides, Bartonella henselae, Bartonella quintana*).

Tuberculoza poate apărea în orice stadiu al infecției HIV, fiind adesea prima manifestare a bolii (reactivarea); în caz de imunosupresie severă (CD4 <200/mm^3), aspectul radiologic poate simula afectul primar TBC.

Majoritatea pneumoniilor sunt determinate de *S. pneumoniae* şi *H. influenzae*, aceiaşi agenți etiologici implicați în patologia infecțiilor pulmonare comunitare. Pneumoniile nosocomiale, frecvent întâlnite la gazdele imunocompetente, sunt cauzate de *S. aureus* şi agenți Gram negativ, în special *P. aeruginosa*, iar rareori poate fi implicată *Legionella pneumophila* şi *Mycoplasma pneumoniae*.

MATERIAL ŞI METODE

S-a realizat un studiu clinic de tip retrospectiv prin urmărirea a 121 de cazuri din Moldova cu infecție HIV şi afectare infecțioasă pulmonară, asistate la Centrul Regional HIV-SIDA din cadrul Spitalului Clinic de Boli Infecțioase Iaşi, în perioada ianuarie - decembrie 2011, diagnosticate şi tratate în cadrul aceluiaşi centru. S-au evaluat vârsta, sexul, mediul de rezidență, anul îmbolnăvirii, datele clinice, de laborator, imagistice (radiografia pulmonară), nivelul viremiei RNA-HIV şi numărul limfocitelor CD4 în dinamică. Datele au fost prelucrate statistic, realizându-se o corelație directă cu evoluția clinică şi prognosticul pacienților.

Au fost definite următoarele criterii clinico – biologice şi radiologice pentru monitorizare:

- debut brutal cu simptome respiratorii traheo-bronşice, cu/fără reactanți de fază acută, cu absența unei imagini radiologice sugestive;
- debut brutal sau insidios, de maxim 14 zile în ambulator, cu simptome respiratorii traheo-bronşice şi imagini radiologice atipice, cu un număr de limfocite CD4 peste 200/mm^3 şi spută negativă pentru BK;
- debut brutal sau insidios, de maxim 14 zile în ambulator, cu simptome respiratorii traheo-bronşice şi imagini radiologice caracteristice de pneumonie lobară;
- fenomene de insuficiență respiratorie acută obiectivate clinic şi prin gazometrie sangvină, cu modificări radiologice pulmo-

nare (bronhopneumonie, infiltrat interstițial).

Între entitățile clinice s-au consemnat: pneumonia lobară, traheobronşita acută, pneumonia interstițială (non tuberculoasă) şi bronhopneumonia.

Alături de acestea s-au luat în considerație includerea în evidență ca seropozitiv HIV şi până la apariția simptomatologiei pulmonare sugestive, tratamentul urmat în momentul internării şi eventualele modificări în terapia antiretrovirală impuse de inițierea tratamentului antiinfecțios.

REZULTATE ŞI DISCUȚII

La finele anului 2011, în evidență activă a Centrului Regional Iaşi se aflau1363 pacienți provenind din 6 județe ale Moldovei, dintre care 1289 (94,6%) aveau peste 20 ani, 41 (3%) avea vârsta între 15 – 19 ani şi 33 (2,4%) aveau vârsta mai mică de 15 ani (tab I).

TABELUL I

Situația pacienților aflați în evidență activă la Centrul Regional Iaşi decembrie 2011

Județul	Grupa de vârstă						Nr. pacienți în evidență activă
	0 – 14 ani		15 -19 ani		> 20 ani		
	Nr.	%	Nr.	%	Nr.	% *	
Bacău	7	2	7	2	331	96	**345**
Botoşani	2	1	4	2	186	97	**192**
Neamț	8	3,13	4	1,6	243	95,3	**255**
Iaşi	4	1,5	15	5,8	239	92,7	**258**
Suceava	8	4,3	16	8,6	172	92,5	**186**
Vaslui	4	3,14	5	3,9	118	92,9	**127**
Total CR Iaşi	**33**	**2,4**	**41**	**3**	**1289**	**94,6**	**1363**

*Pentru valorile mai mici de 10, proporțiile sunt orientative

În anul 2011, la Centrul Regional Iaşi au beneficiat de spitalizare un număr de 895 de pacienți cu infecție HIV/SIDA, reprezentând cazurile nou confirmate şi pacienți cu SIDA stadiul C3, încadrați astfel prin prezența afecțiunilor definitorii şi a numărului limfocitelor CD4, aflați la prima internare pentru afectare infecțioasă respiratorie sau cu pneumonii recurente (tab. II).

Numărul pacienților luați în studiu include cazurile noi declarate şi confirmate respectiv pacienții aflați deja în evidență şi reinternați (unii în repetate rânduri).

TABELUL II

Distribuția pacienților cu SIDA pe stadii evolutive

Stadiu boală	A1	A2	A3	B1	B2	B3	C1	C2	C3
Număr cazuri	27	39	12	11	32	61	18	39	656

Din cei 895 pacienți spitalizați au fost identificate 121 (13,25%) cazuri de infecții respiratorii acute: pneumonie lobară – 34 (28%), traheobronşită acută – 45 (37,2%), pneumonie interstițială (non tuberculoasă) – 40 (33%) şi bronhopneumonie – 2 (1,6%) (fig. 1).

Se observă o proporție relativ redusă (13,5%) a afectării bronho-pulmonare infecțioase netuberculoase în cadrul patologiei generale întâlnite la cele 895 de internări.

Repartiția pe grupuri de vârstă a evidențiat predominanța pacienților de 21 – 30 de ani, urmată de 11 – 20 ani (fig. 2).

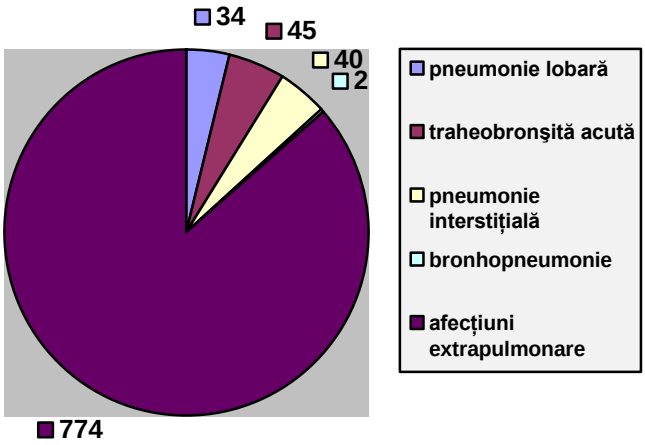

Fig. 1. Proporția afectării infecțioase bronho-pulmonare în cadrul patologiei generale HIV/SIDA la pacienții internați

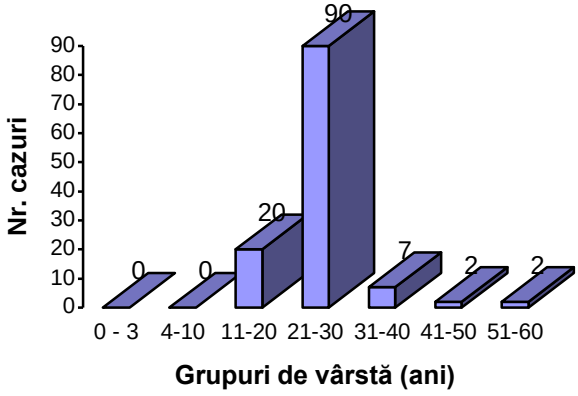

Fig. 2. Distribuția pe grupuri de vârstă a pacienților cu afecțiuni ale aparatului respirator

Am observat o distribuție extrem de polarizată a cazurilor de infecții respiratorii acute la pacienții cu vârste cuprinse între 11 şi 30 de ani, cu un număr foarte redus sau chiar inexistent la celelalte grupuri de vârstă.

Din totalul celor 121 de pacienți, 71 (58,67%) au fost de sex masculin şi 50 (41,32%) feminin.

Un număr de 65 (53,71%) au provenit din mediul rural, faţă de 51 (39,34%) din municipiul Iaşi şi oraşele Paşcani, Târgu – Frumos şi Hîrlău. Un număr de 3 copii erau instituţionalizaţi la Casa de copii Gulliver, iar 2 adulţi proveneau din mediu social defavorizat.

Examenul clinic a evidenţiat simptomatologia respiratorie (tuse, dispnee, dureri toracice) şi manifestările determinate de afecţiunile asociate (tab. III).

Sindromul febril a fost întâlnit în toate cele 121 de cazuri; este o febră persistentă, cu debut cu circa 10 zile anterior spitalizării. Un număr de 40 de pacienţi (33%) au prezentat subfebrilitate (<38^{0}C), iar 81 (66,94%) aveau temperatură între 38 – 40 ^{0}C.

Tusea seacă prelungită a fost prezentă la 66% din cazuri, fără tendinţă la ameliorare. Scăderea ponderală (peste 10%) a fost întâlnită la mai mult de jumătate din lotul studiat. Dispneea de repaus a apărut cu o frecvenţă de 33%, nefiind asociată cu severitatea leziunior pulmonare.

TABELUL III

Simptomele predominante în infecţiile respiratorii non tuberculoase la pacienţii asistaţi pentru SIDA

Simptome	Febră	Tuse seacă	Tuse productivă	Scăderea apetitului	Scăderea ponderală	Dispnee
nr. cazuri	121	80	41	110	90	40
%	100%	66,11%	33,88%	90,90%	74,38%	33%

Ca simptomatologie extrapulmonară în cadrul infecţiei HIV au mai apărut: sindromul diareic (8,3%), adenopatie generalizată (24,8%), greaţă şi vărsături (9,9%), icter sclero-tegumentar moderat (11,6%), tulburări neurologice (encefalopatie intrainfecţioasă şi/sau hipoxică) (16,5%), candidoză oro-faringiană (75%).

Sindromul inflamator a fost prezent la 80 din cei 121 de pacienţi, în proporţie variabilă.

Astfel, VSH-ul s-a situat în intervalul de normalitate la 60 de pacienţi (49,5%), în timp ce creşterea leucocitelor a fost întâlnită în 70 de cazuri (57%). Proteina C reactivă a avut valori de peste 10mg% la 70 pacienţi.

În majoritatea pneumoniilor interstiţiale şi a traheobronşitelor, sindromul inflamator a fost discret sau uşor crescut, în timp ce în cazul pneumoniilor lobare au fost întâlnite valori crescute.

Investigaţii de laborator. Pneumonia bacteriană cu microorganisme încapsulate - *S. pneumoniae* şi *H. influenzae* a reprezentat afecţiunea pulmonară cea mai frecventă la pacienţii HIV/SIDA, ca o posibilă consecinţă a alterării funcţionale a limfocitelor B şi/sau neutrofilelor secundară infecţiei HIV (5). *S. aureus* şi *P. aeruginosa* au fost, de asemenea, implicaţi în patologia respiratorie cu o incidenţă crescută la gazdele imunocompetente, dar pneumonia cu *Pneumocystis jirovecii* a înregistrat valori scăzute.

Din cele 41 prelevate de spută 20 au fost eliminate ca fiind necorespunzătoare, pentru restul s-a calculat scorul Q.

La 5 pacienţi (23,8%) s-a identificat *Streptococcus pneumoniae* care a fost analizat din punct de vedere al rezistenţei la: betalactamine, macrolide, lincosamide, cotrimoxazol, cicline, fenicoli.

Toate cele 5 tulpini au fost sensibile la fluoroquinolone, linezolid, rifampicină şi glicopeptide; un număr de 3 tulpini a prezentat rezistenţă la penicilină şi cicline (tab. IV).

TABELUL IV

Sensibilitatea la antibiotice a tulpinilor de pneumococ identificate

Antibiotic	Sensibilitate	Rezistență
Penicilină		3
Rifampicină	5	
Macrolide	2	3
Vancomicină	5	
Carbapeneme (ertapenem)	5	
Fluoroqiunolone	5	
Clindamicină	5	
Amoxicilină-acid clavulanic	5	

Numărul limfocitelor CD4 şi corelaţia cu aspectul radiologic şi forma clinică: Numărătoarea limfocitelor CD4 a fost efectuată la toţi cei 121 de bolnavi incluşi în lot, rezultând un număr de 90 (74,38%) > 300/mmc, 20 (16,5%) între 200 – 300/mmc şi 11 (9%) < 200/mmc. Toate cazurile de traheobronşite, pneumonii lobare precum şi 29 de pneumonii interstiţiale s-au situat din punct de vedere imunologic în prima şi a doua categorie, în timp ce 11 (9%) cu pneumonie interstiţială au avut CD4 sub 200/mmc (tab. V).

Pneumoniile interstiţiale în cazurile cu un număr al limfocitelor CD4 > 300/mmc au fost interpretate in contextul prezenţei sindromului biologic inflamator ca etiologie mixtă viro – bacteriană, beneficiind de tratament antibiotic (aminopenicilină, cefalosporine, macrolide, fluoroquinolonă), iar cele cu CD4 ≤ 200/mmc au beneficiat suplimentar de asocierea cotrimoxazolului.

TABELUL V

Distribuţia pentru un lot de 121 pacienţi a formelor clinice de boală în relaţie cu numărul limfocitelor CD4

Forma clinică	CD4 > 300/mmc		CD4 200 – 300/mmc		CD4 ≤ 200/mmc	
	Număr	%	Număr	%	Număr	%
Traheobronşită acută	45	37,2 %				
Pneumonie lobară	30	24,8 %	4	3,3 %		
Bronhopneumonie	2	1,65 %				
Pneumonie interstiţială	20	16,5 %	9	7,4 %	11	9 %

CONCLUZII

În anul 2011, la Centrul Regional Iaşi au fost asistaţi 895 pacienţi, din care 121 (13,5%) au prezentat infecţii respiratorii, în majoritate la cei cu un număr al limfocitelor CD4 mai mare de 300/mmc; au predominat traheobronşitele acute şi pneumoniile interstiţiale;

Rezultatele tratamentului etiologic şi evoluţia clinico – biologică au fost favorabile, similar gazdelor imunocompetente, iar numărul limfocitelor CD4 a contribuit la orientarea diagnosticului etiologic şi stabilirea unui tratament de primointenţie;

Tulpinile de pneumococ implicate în etiologia pneumoniilor bacteriene la pacienţii HIV/SIDA au păstrat aceeaşi sensibilitate ca la pacienţii seronegativi.

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NOUTĂȚI

GENELE AR PUTEA EXPLICA LEGATURA DINTRE DIABET ŞI BOALA ALZHEIMER

În ultimii ani, a devenit clar faptul că persoanele cu diabet zaharat se confruntă cu o perspectivă de rău augur - un risc mult mai mare de a dezvolta boala Alzheimer. Acum, cercetătorii de la City College din New York (CCNY) au descoperit unul dintre motive. Ei au evidențiat că o singură genă formează o legătură comună între cele două boli şi au descoperit că gena, cunoscută a fi prezentă în multe cazuri de boală Alzheimer, afectează calea de insulină. Perturbarea acestei căi este un semn distinctiv de diabet zaharat. Constatarea ar putea indica o țintă terapeutică pentru ambele boli. Legătura nu este exagerată. Este cunoscut faptul că persoanele cu diabet de tip 2 au un risc crescut de demență. Insulina este implicată în multe procese metabolice, inclusiv în menținerea sănătății sistemului nervos. Deşi cauza bolii Alzheimer este neclară, un criteriu de diagnostic postmortem este prezența de plăci adezive de proteine amiloid în porțiuni ale creierului pacienților distruse de boală. Mutații ale genei umane "precursor de proteine amiloid" (APP) au fost evidențiate în cazurile familiale de Alzheimer. In cadrul studiului, cercetătorii au analizat o proteină numită APL-1, codificată de o genă de la viermele *Caenorhabditis elegans (C. elegans)*, care se întâmplă să fie un perfect stand-in pentru gena boli Alzheimer umane. Ei au descoperit că mutații ale genei de la vierme, echivalent a genei umane APP, au încetinit dezvoltarea acestuia, ceea ce a sugerat că unele căi metabolice au fost întrerupte. Au examinat modul în care echivalentul APP de la vierme modulează diferitele căi metabolice şi au constatat că acesta inhibă calea de insulină. Acest lucru a sugerat faptul că versiunea umană a genei joacă probabil un rol important atât în boala Alzheimer cât şi în diabet. De asemenea, au constatat că mutații suplimentare în calea insulinei au inversat defecte ale mutație APP şi au explicat modul în care aceste gene sunt legate funcțional. Cercetătorii speră că această perspectivă nouă va duce la noi terapii în tratamentul atât al bolii Alzheimer cât şi al diabetului. Rămâne neelucidat modul în care proteinele precursoare ale amiloid şi produsele disocierii sale intersectează calea insulinei (C. Y. Ewald, D. A. Raps, C. Li. APL-1. the Alzheimer's Amyloid Precursor Protein in Caenorhabditis elegans, Modulates Multiple Metabolic Pathways Throughout Development. *Genetics*, 2012; DOI: 10.1534/genetics.112.138768).

Cătălina Luncă

PARTICULARITĂȚILE REMODELĂRII CARDIACE ESTIMATE ECOCARDIOGRAFIC LA COPIII CU INSUFICIENȚĂ CARDIACĂ SECUNDARĂ CARDIOPATIILOR CONGENITALE ŞI HIPERTENSIUNE PULMONARĂ

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PARTICULARITIES OF THE CARDIAC REMODELLING ECHOCARDIOGRAPHIC ESTIMATED AT CHILDREN WITH HEART FAILURE SECONDARY TO CONGENITAL HEART DISEASES AND PULMONARY HYPERTENSION (Abstract): **The aim** of this study was the determination of the particularities of the cardiac remodeling with echo-cardio graphic methods at patients with chronic heart failure (CHF) secondary to congenital heart diseases and pulmonary arterial hypertension (PAH). **Material and methods:** In the study 204 children were included (average age of 37.1 ± 3.7 months) with CHF secondary to congenital systemic-to-pulmonary shunts (simple, combined and complex) associated with PAH. Depending on the rang of PAH the children were divided into 2 subgroups: IA - 59 patients with CHF and moderate PAH and IB - 145 children with CHF and severe PAH. Using the echocardiography 2D the right and left dimensions of the heart, EF, SF of LV were determined; the Doppler exam - the global function of RV (Tei performance index), systolic and mean pressure in PA. **Results and conclusions:** The particularities of the cardiac remodeling estimated echocardiography with diagnostic value at patients with CHF secondary to congenital shunts complicated with PAH are the following: dilatation of the right cavities of the heart (RVDd and RAD) and of the PA with the tendency of reduction of the LV dimensions, hypertrophy of the anterior wall of the RV, the increasing of the diameter of the fibrosis ring at tricuspid valve, the global dysfunction of RV (Tei index), valvular insufficiency (TVI and PVI), the diminishing of the cardiac index. These parameters were more significant at patients with severe PAH ($p<0.001$), denoting the consequences of the pressure overload on the cardiac remodeling, estimating the level of hemodynamic severity and with certainty can have a special meaning in deciding the therapeutic tactics, prognosis and evolution. **Key words:** HEART FAILURE, CONGENITAL SHUNTS, PULMONARY HYPERTENSION, ECHO-CARDIOGRAPHY, CHILDREN.

Ecocardiografia transtoracică este considerată în prezent cea mai utilizată metodă imagistică non invazivă, standardul de aur în stabilirea şi evaluarea diagnosticului, în aprecierea evoluției, prognosticului şi monitorizarea răspunsului terapeutic la pacienții cu şunturi congenitale sistemico-pulmonare complicate cu insuficiență cardiacă cronică (ICC) şi hipertensiune pulmonară arterială (HTPA) (1). Cateterismul cardiac oferă date hemodinamice mai precise referitor la HTPA şi disfuncția/insuficiența ventriculu-

lui drept (VD), însă investigaţia este o explorare invazivă, cu anumite riscuri, limitată la copiii mici (nou-născuţi şi sugari), scumpă, accesibilă doar în centre specializate (1). Ultimele date din literatura contemporană relatează despre reducerea indicaţiilor la cateterism cardiac în cardiologia pediatrică (2). Din aceste considerente se încearcă în prezent, şi noi ne-am alipit altor studii, identificarea metodelor non-invazive, care să permită un diagnostic precoce, cu o mare sensibilitate, să permită determinarea gradului de remodelare cardiacă şi urmărirea evoluţiei, şi să ofere informaţii prognostice, de stratificare a riscului (1).

Evaluarea ecocardiografică a funcţiei VD, fiind un subiect de mare actualitate este dificilă în prezent, deoarece VD are formă de semilună cu suprafaţă endocardică neregulată şi un mecanism complex al contracţiei în contrast cu VS care are formă eliptică şi uşor de evaluat. VD nu se conformează oricărui model geometric clasic şi estimările de volum pot fi imprecise, fapt ce crează o provocare în interpretarea ecocardiografică (3).

În ultimul timp o atenţie deosebită se acordă evaluării funcţiei globale a VD apreciată prin indicele de performanţă miocardică (IPM) sau indicele Tei, determinat cu ajutorul examenului Doppler (4). El se bazează pe măsurările intervalelor de timp Doppler derivate pe perioada ciclului cardiac, tehnica nefiind limitată de către geometria VD la fel ca alte metode. Acest indice a fost validat în cardiopatiile congenitale, HTP idiopatică, maladiile pulmonare cronice şi alte patologii (5,6).

După datele unor autori evaluarea dimensiunilor VD, atriului drept (AD) şi a prezenţei lichidului în pericard este necesară, constituind factori de prognostic importanţi pentru pacienţii cu HTPA şi IC de dreapta (7,8).

Studiile recente ce vizează estimarea consecinţelor supraîncărcării de volum şi de presiune asupra remodelării cardiace, determinarea performanţei miocardului VD la copiii cu cardiopatii congenitale şi HTPA sunt limitate.

Lucrarea noastră a avut drept scop aprecierea particularităţilor remodelării cardiace prin metode ecocardiografice la pacienţii cu ICC secundară cardiopatiilor congenitale şi HTPA.

MATERIAL ŞI METODE

În studiu au fost incluşi 204 copii (vârsta medie 37,1±3,7 luni) cu ICC secundară şunturilor congenitale sistemico-pulmonare (simple, combinate şi complexe) asociate cu HTPA. În funcţie de gradul HTPA, pacienţii studiului au fost divizaţi în două subloturi: sublotul IA - 59 de copii cu ICC şi grad moderat de HTPA (vârsta medie 52,3±7,4 luni) şi sublotul IB - 145 de copii cu ICC şi HTPA grad sever (vârsta medie 30,9±4,2 luni) (PsAP >70% sau 2/3 din cea sistemică).

Examenul a fost efectuat la ecocardiograful Esaote My Lab 50 (2007) în regim M, 2D, Doppler continuu, Doppler pulsatil, Doppler color şi la aparatul SIEMENS-2007. Ecocardiografia 2D a permis determinarea următorilor indici: dimensiunile cavităţilor drepte ale cordului - AD şi diametrul transversal al VD, a arterei pulmonare (AP) şi a ramurilor sale, grosimea peretelui anterior al VD în diastolă (PAVDd), mişcarea septului interventricular (SIV), prezenţa lichidului în pericard. Au fost apreciate şi dimensiunile ventriculului stâng (VS): diametrul telesistolic (DTS)VS, telediastolic (DTD)VS, cel antero-posterior al atriului stâng (DAS), de asemenea, au fost calculate valorile FE, FS

a VS, fiind comparate cu valorile normale de referință după Kampmann C, 2000 (9).

Funcția globală a VD, IPM sau indicele Tei s-a evaluat cu ajutorul examenului Doppler şi calculat prin raportul dintre timpul contracției isovolumice (TCIV) şi a relaxării isovolumice (TRIV) la perioada de ejecție (PEVD) (fig. 1).

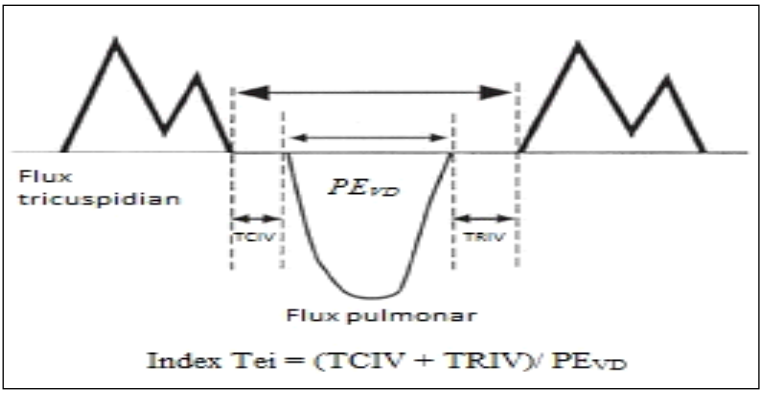

Fig. 1. Metoda de apreciere a indicelui Tei a ventriculului drept (după: 4)

Presiunea sistolică în AP (PsAP) a fost determinată prin măsurarea velocității maxime (V) a jetului de insuficiență tricuspidiană (determinându-se şi gradul de ITr), aplicând ecuația Bernouli: PsVD = $4V_{RTmax}^2$ + PrAD. Presiunea în AD s-a considerat fixă pentru toți pacienții şi egală cu 5 mmHg (conform recomendărilor Ghidului HTP, SEC, 2009). În lipsa unui obstacol la golirea VD, presiuna sistolică din AP este egală cu presiunea sistolică din VD. Presiunea medie în AP (PAPm) am determinat-o prin gradientul protodiastolic maxim dintre AP şi VD pe anvelopa Doppler CW a jetului de insuficiență pulmonară (10). Pentru calculul PAPm am aplicat ecuația Bernoulli simplificată: $4V_{RPmax}^2$ +PrAD. În unele situații am utilizat estimarea PAPm pe baza timpului de ascensiune la AP (TAP), metodă descrisă de Mahan: PAPm = 79 - 0,45 x TAP (11).

Debitul sistemic (Qs) s-a apreciat cu ajutorul integralei vitezei lineare (VTI), suprafața secțiunii transversale a Ao şi FCC, calculând produsul lor, iar raportându-l la suprafața corporală s-a obținut indicele cardiac sistemic (ICs).

Analiza statistică a reziltatelor obținute a fost efectuată cu ajutorul criteriului t-Student de comparare a valorilor medii. Devierea valorii medii a indicelui respectiv față de valoarea martor se consideră statistic semnificativă, dacă probabilitatea erorii nu depăşeşte 5%, deci $p<0,05$.

REZULTATE ŞI DISCUȚII

Ecocardiografia 2D ne-a permis decelarea consecințelor supraîncărcării de volum şi de presiune nu doar a VS studiat de obicei în toate cazurile de IC la adult şi copil (DTDVS, DTSVS, DAS, SIVd) dar mai important a VD, fiind mai rar studiat (tab. I). Dimensiunea diastolică medie a VD (DVDd) a constituit 16,73±0,1 mm în sublotul de pacienți cu HTPA grad sever, față de 15,97±1,0 mm în sublotul cu HTPA grad moderat şi pare a fi fără semnificație statistică ($p>0,05$). Însă dacă ne referim la valori-

le normale corespunzător vârstei şi suprafaţă/corp (după Kampmann, 2000), media normei pentru DVDd în sublotul IB constituie 9,0 mm, iar pentru sublotul IA - 11,0 mm. Astfel, la pacienţii cu HTPA grad sever s-a observat o creştere statistic semnificativă a DVDd - cu 46,2%, faţă de creşterea a DVDd cu 31,1% la pacienţii cu HTPA grad moderat. O altă explicaţie poate fi prevalenţa în sublotul IA a şunturilor combinate cu un defect DSA predominant (n=16) care conduc la dilatarea preponderentă a cavităţilor cardiace drepte. Este bine cunoscut faptul că la supraîncărcare de volum cordul răspunde la stresul hemodinamic prin dilatare de volum, iar la supraîncărcare de presiune - prin hipertrofie; ulterior în evoluţie, odată cu epuizarea mecanismelor compensatorii, survine şi dilatarea cavităţii.

Grosimea peretelui anterior al VD (PAVDd) a constituit 6,51±0,1 mm în sublotul IB *vs* 6,27±0,2 mm în sublotul IA, valorile medii normale pentru aceste subloturi fiind de 2,7 mm şi respectiv 3,0 mm. Deci, se observă o hipertrofie a PAVDd (mecanism compensator local) la pacienţii din lotul cu HTPA severă cu 140,7% faţă de valorile normale şi cu 109% la copiii sublotului cu HTPA moderată, diferenţele fiind semnificative.

TABELUL I

Valorile parametrilor ecocardiografici la copiii cu insuficienţă cardiacă cronică şi hipertensiune pulmonară arterială de diferite grade

Semnele ECO	**Sublot IA ICC cu HTPA grad moderat (n=59)**	**Sublot IB ICC cu HTPA grad sever (n=145)**	**p**
DVDd (mm)	15,97±1,0	16,73±1,0	>0,05
PAVDd (mm)	6,27±0,2	6,51±0,1	>0,05
DITr (mm)	23,16±1,0	24,53±1,0	>0,05
DAD:			
sup-inf (mm)	26,57±1,0	28,37±1,0	>0,05
med-lat (mm)	25,37±1,0	27,22±1,0	>0,05
DAP (mm)	12,95±1,2	16,8±0,4	<0,001
DAP ram. drepte (mm)	10,0±0,9	12,53±0,3	=0,001
Indicele Tei al VD	0,40±0,01	0,47±0,01	<0,001
PsAP (mmHg)	51,31±0,85	75,79±1,28	<0,001
PAPm (mmHg)	37,69±0,48	52,68±0,82	<0,001
DTDVS (mm)	35,28±1,07	34,08±0,91	>0,05
DTSVS (mm)	22,1±0,75	20,42±0,62	>0,05
DAS (mm)	23,08±1,0	23,53±1,0	>0,05
SIVd (mm)	6,48±0,3	6,53±0,1	>0,05
FEVS (%)	69,86±1,0	70,83±1,0	>0,05
FSVS (%)	38,39±1,0	38,82±1,0	>0,05
IC sistemic (l/min/m^2)	2,83±0,15	2,67±0,1	>0,05
IVM (grad)	0,59±0,09	1,3±0,08	<0,001
IVTr (grad)	1,15±0,08	1,87±0,07	<0,001
IVP (grad)	0,85±0,07	1,19±0,05	<0,001

Diametrul inelului fibros la tricuspidă (DITr) a fost de 24,53±1,0 mm la pacienții cu HTPA severă, față de 23,16±1,0 mm la copiii cu HTPA moderată, valorile medii normale în aceste subloturi fiind de 18,0 şi 22,0 mm; înregistrându-se respectiv o majorare a DITr cu 36,3% în sublotul IB, față de 5,3% în sublotul IA (p<0,001).

Dimensiuni crescute ale AD s-au înregistrat în ambele subloturi, dar mai evident au fost la pacienții cu HTPA severă - mărire de 9,2% față de valoarea normală a diametrului superior - inferior al AD şi de 36% al diametrului mediu - lateral, în comparație cu o mărire de 15,4% a dimensiunii medie - laterală a AD la copiii cu HTPA grad moderat. Valorile normale pentru vârstă în loturi sunt: 28 şi 22 mm pentru copiii sublotului IA şi 26 - 20 mm pentru pacienții sublotului IB (după Mayer, Solinger, 2007).

Dimensiunea AP magistrale şi a ramurei drepte au fost statistic semnificativ mai crescute la pacienții cu HTPA grad sever (16,8±0,4 mm şi 12,95±1,2 mm) față de cei cu HTPA moderată (12,95±1,2 mm şi 10,0±0,9 mm) (p<0,001).

PsAP a fost de 75,79±1,28 mmHg la pacienții sublotului IB cu ICC şi HTPA severă, constituind 84,2% din cea sistemică şi 51,31±0,85 mmHg la copiii sublotului IA, reprezentând 54,6% din cea sistemică (p<0,001). De menționat, că TA sistolică la pacienții sublotului IB a fost de 90,1±0,7 iar cea diastolică de 54,4±0,7 mmHg, iar în sublotul IA de 94,3±1,1 şi de respectiv 57,1±1,1 mmHg.

PAPm a fost de 52,68±0,82 mmHg la pacienții sublotului IB, față de 37,69±0,48 mmHg la copiii sublotului IA (p<0,001). Aceste diferențe de presiune în subloturile studiate au şi condus la remodelări variate ale cavităților drepte (dilatare, hipertrofie) şi a AP, dar mai sever exprimate la pacienții cu şunturi congenitale complicate cu HTPA avansată şi disfuncție a VD.

Un aspect particular al valvei pulmonare posterioare datorită valorilor de presiune înaltă, înregistrat prin ecografie mod M, a fost la 109 (76,2%) din pacienții cu HTPA severă față de 21 (35,6%) la copiii cu HTPA moderată (p<0,001).

Indicele de performanță miocardică (parametru cu valoare diagnostică certă) - indicele Tei a VD a fost statistic semnificativ mai crescut la copiii cu HTPA severă - 0,47±0,01 *vs* 0,40±0,01 la pacienții cu HTPA moderată (p<0,001) (fig. 2). Valorile normale citate în literatură pentru vârsta respectivă sunt de la 0,24±0,4 până la 0,28±0,04 (4). Indicele Tei crescut semnifică disfuncția globală a VD, demonstrând consecințele HTPA asupra remodelării cordului drept. Mai multe studii au demonstrat că acest parametru corelează cu FE a VD măsurată prin angiografie cu radionuclizi sau RMN (12,13). Yeo TC şi coaut. încă în anul 1998 au raportat o corelație semnificativă între IPM, simptomele clinice şi supraveţuirea la pacienții cu HTP idiopatică (14).

Noi la fel am observat că acest indice nu depinde de FCC (raportarea TRIV şi TCIV la PE corectează dependența acestora de FCC), de presarcină, de gradul IVTr.

Comparativ au fost estimate şi dimensiunile cavităților cardiace stângi (DTDVS, DTSVS şi DAS), grosimea SIVd, FE a VS şi FS a VS. Datele studiului nostru au relevat dimensiuni mai crescute ale parametrilor VS la pacienții sublotului IA, cu HTPA moderată (DTDVS - 35,28±1,07 şi DTSVS - 22,1±0,75 mm) față de sublotul IB (DTDVS - 34,08±0,91 şi DTSVS - 20,42± 0,62mm), dar fără semnificație statistică (p>0,05). Această particularitate poate fi

explicată prin următoarele: în caz de HTPA severă şi disfuncție VD are loc reducerea volumelor VS cu funcția globală frecvent păstrată; aspectul VS deseori are imagine de literă D. Astfel, o mişcare paradoxală a SIV (de diferită exprimație) cu aplatizarea lui sistolică şi bombarea spre VS a fost observată la 75 (54,7%) din copiii sublotului IB, față de 19 (32,2%) în sublotul IA ($p<0,001$). În ambele subloturi s-a evidențiat hipertrofia SIVd, respectiv 6,53±1,0 şi 6,4±0,3mm ($p>0,05$). DAS a fost crescut față de valorile normale la pacienții cu diferit grad de HTPA, respectiv 23,08±1,0 şi 22,53±1,0 mm, dar fără semnificație statistică ($p>0,05$).

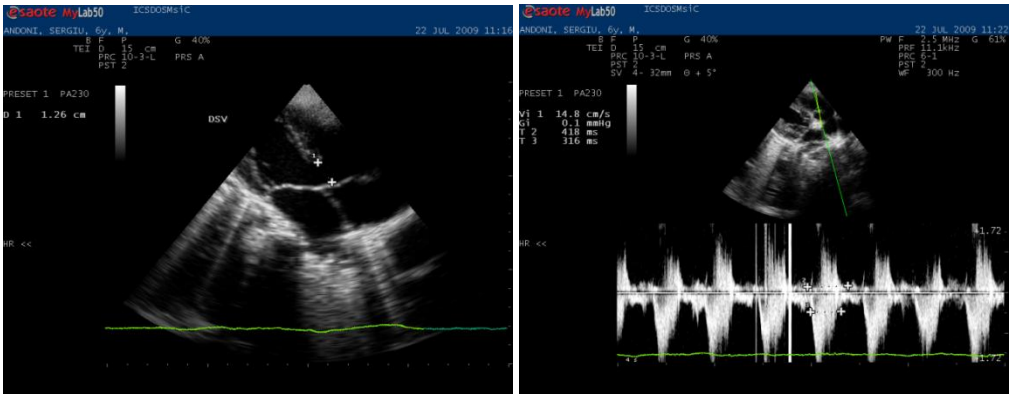

Fig. 2. Determinarea indicelui Tei cu valoare semnificativ crescută (Indice Tei = 0,52) la un băiețel de 6 ani cu DSV membranos de dimensiuni mari, şunt predominant stânga-dreapta şi HTPA severă, disfuncție VD.

FE şi FS a VS în ambele subloturi au înregistrat valori normale: 69,86±1,0% şi 38,39±1,0% la pacienții sublotului IA; 70,83±1,0% şi 38,82±1,0% la cei din sublotul IB ($p>0,05$). Se cunoaşte că determinarea FE pentru estimarea funcției sistolice a VS la pacienții cu cardiopatii congenitale nu este informativă (funcția miocardului este păstrată), valoarea acestui parametru fiind influențat de mulți factori, îndeosebi de mărimea şuntului stânga-dreapta sau de gradul regurgitării atrioventriculare. Astfel, se recomandă la pacienții cu ICC şi şunturi sistemico-pulmonare de a determina DC efectiv, sistemic sau pulmonar. În studiul nostru DC sistemic sau indicele cardiac (IC) a fost diminuat în ambele subloturi, dar mai semnificativ la cei cu ICC şi HTPA severă - 2,67±0,1 față de 2,83±0,15 l/min/m^2. Micşorarea DC este rezultatul necesităților energetice crescute (grație dilatării cavităților) ale miocardului normal la pacienții cu şunturi congenitale.

Majoritatea pacienților cu ICC secundară şunturilor congenitale în asociere cu HTPA au avut insuficiențe valvulare, tricuspide şi pulmonare (IVTr, IVP), dar mai pronunțate au fost în sublotul cu HTPA severă, având semnificație statistică importantă față de sublotul cu HTPA moderată; respectiv gradul IVTr fiind de 1,87±0,07 *vs* 1,15±0,08 ($p<0,001$), iar gradul IVP a fost de 1,19±0,05 *vs* 0,85±0,07 ($p<0,001$). Insuficiențele valvulare estimate denotă remodelarea (dilatarea) cavităților drepte şi prezența cardiomegaliei.

Evidențierea IVTr secundare la aceşti pacienți este posibil legată în primul rând

de dilatarea inelului tricuspidian, modificarea geometriei VD şi deplasarea către apex a cuspelor tricuspidei (15). Gradul de IVM a fost, de asemenea, mai evident la pacienţii cu HTPA severă - 1,3±0,08 faţă de 0,59±0,09 ($p<0,001$).

Prezenţa lichidului pericardic a fost observată în 20 (13,8%) de cazuri doar la copiii cu ICC şi HTPA grad sever. Conform unor studii prezenţa lichidului în pericard constituie un factor de prognostic nefavorabil pentru pacienţii cu HTPA şi IC de dreapta (8).

CONCLUZII

Particularităţile remodelării cardiace estimate la explorările ecocardiografice cu valoare diagnostică la pacienţii cu ICC secundară şunturilor congenitale complicate cu HTPA sunt: dilatarea cavităţilor drepte ale cordului (DVDd şi DAD) şi a AP cu tendinţă de reducere a dimensiunilor VS, hipertrofia PAVDd, majorarea DITr, disfuncţia globală a VD estimată prin indicele de performanţă miocardică Tei, insuficienţele valvulare (IVTr şi IVP), diminuarea DC.

Parametrii sus menţionaţi au fost mai elocvenţi la pacienţii cu ICC şi grad sever al HTPA ce denotă consecinţele supraîncărcării de presiune asupra remodelării cardiace, estimând gradul de severitate hemodinamică.

Indicii remodelării cordului drept din şunturile congenitale complicate cu ICC şi HTPA estimaţi cu siguranţă pot avea o valoare deosebită în deciderea tacticii terapeutice, aprecierea evoluţiei şi a prognosticului acestei suferinţe cardiace la copii.

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NOUTĂȚI

ROLUL PROBIOTICELOR ÎN TRATAMENTUL INFECȚIEI CU *HELICOBACTER PYLORI*

Infecția cu *Helicobacter pylori* este un factor major de risc pentru ulcer gastric, ulcer duodenal, limfom gastric si cancer gastric distal. Tratamentul este reprezentat de asocierea a trei antibiotice şi administrarea de probiotice ca terapie adjuvantă. Există mai multe dovezi care susțin rolul probioticelor în reducerea frecvenței efectelor adverse cauzate de tripla asociere terapeutică la care se adaugă articole care afirmă că utilizarea probioticelor îmbunătățește rata de eradicare a infecției. *Lactobacillus gasseri* OLL2716 s-a dovedit eficace atât în suprimarea colonizării stomacului cu *H. pylori* cât şi în reducerea inflamației mucoasei gastrice. Fujimura et all. au raportat că o tulpină *L. gasseri* OLL2716 conținută în iaurt poate coloniza stratul de mucus gastric la pacienții infectați şi poate suprima multiplicarea *H. pylori*. Ei au investigat efectele probiotice ale *L. gasseri* OLL2716 împotriva *H. pylori* tulpina ATCC 43504 prin cocultivare pe agar cu 5% sânge de berbec, la 37°C, în condiții de microaerofilie. După 24 de ore, microscopia electronică din cultura de *H. pylori* cocultivată cu *L. gasseri* OLL2716 evidențiază transformarea cocoidă. Subcultivarea *H. pylori* din nou pe agar sânge de berbec nu a fost urmată de obținerea culturii. Conversia cocoidă şi suprimarea multiplicării *H. pylori* se datorează producerii de dl-acid lactic de către tulpina *L. gasseri* OLL2716. Producerea de dl-acid lactic este caracteristică şi altor specii de *Lactobacillus* (*L. plantarum, L. salivarius*) care pot fi eficiente împotriva infecției cu *H. pylori*. Aceste rezultate susțin utilizarea asocierii de probiotice *L. gasseri* OLL2716 la terapia de eradicare convențională a infecției cu *H. pylori*, mai ales în contextul creşterii incidenței rezistenței la antibiotice a acestor tulpini. (Fujimura S, Watanabe A, Kimura K, Kaji M. Probiotic Mechanism of *Lactobacillus gasseri* OLL2716 Strain against *Helicobacter pylori*. *JClinMicrobiol* 2012; 50(3): 1134–1136).

Cătălina Luncă

BOWEL OBSTRUCTION MIMICKING HEPATIC HYDATID CYST RUPTURE. CASE REPORT

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BOWEL OBSTRUCTION MIMICKING HEPATIC HYDATID CYST RUPTURE. CASE REPORT (Abstract): The hydatid cyst is generated by a parasite which is endemic in Eastern Europe and the Mediterranean area. Rupture of the cyst can trigger an anaphylactic reaction. Therefore, early recognition of rupture symptoms is critical. We here present the case of a patient who knew she had a hepatic hydatid cyst and had refused surgery. She presented to the emergency department for a rapid deterioration of her health status: nausea, vomiting, intense abdominal pains, and visible chills. The ultrasound image suggested a ruptured and infected hepatic hydatid cyst and slightly dilated, hyperkinetic intestinal loops. CT examination revealed bowel obstruction and hydatid cyst appearance unchanged as compared to the previous examination. Symptoms of rupture of a hepatic hydatid cyst include: intense abdominal pain, biliary tree dilation, free fluid in the abdomen, up to fever and hypotension. There are other medical conditions that may simulate rupture of a hydatid cyst. In this case, ultrasound examination revealed the possible cause of deterioration in patient's state of health (hepatic hydatid cyst). The accelerated peristalsis was wrongly interpreted because of cyst rupture, and not a sign of incipient bowel obstruction. It was the CT examination that helped in making the correct diagnosis, bowel obstruction. However, CT scan did not reveal the cause of bowel obstruction but ruled out some possible causes (wall thickening or tumor). The cause of bowel obstruction (adhesions) was established by the correlation of CT findings with medical history and was confirmed surgically and pathologically. **Keywords:** HEPATIC HYDATID CYST, BOWEL OBSTRUCTION, ULTRASONOGRAPHY, COMPUTED TOMOGRAPHY.

The hydatid cyst is generated by a parasite which is endemic in certain areas of Eastern Europe and Mediterranean area. The most frequent locations are liver and lung. Rupture of the cyst can trigger an anaphylactic reaction. Therefore, early recognition of rupture symptoms is critical.

CASE REPORT

A 31-year-old female patient was brought by ambulance to the Emergency Department four months earlier because of intense abdominal pains. She was an unemployed petite white female. Computed tomography (CT) examination performed at that moment described the following lesions:

1. a 48/48/35mm round structure in liver segment 7, well-delineated to the liver, intensely inhomogeneous, with numerous

peripheral and inner calcifications.

2. a 92/80/100 mm inhomogeneous, predominantly liquid structure, with very few tiny peripheral calcifications in liver segments 5 and 8 (fig. 1).

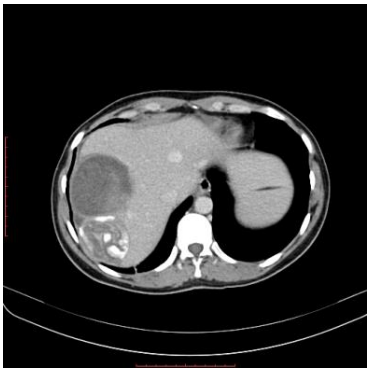

Fig. 1. Hepatic hydatid cysts

Fig. 2. Pus collection under liver

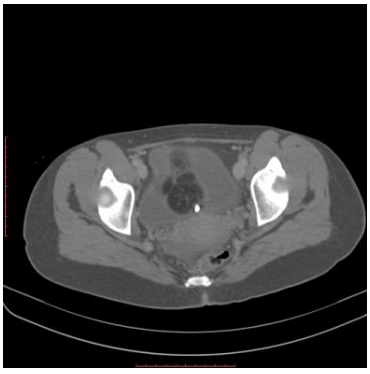

Fig. 3. Ovarian teratoma

3. inhomogeneous lesion of 60/45/80 mm under the liver, with gas bubbles inside, lesion that included the right colic flexure (fig. 2). This structure was considered pus collection from a ruptured hydatid cyst. During surgical intervention it proved to be perforated appendicitis, with pus migrated upwards, towards the liver. Appendicitis was not diagnosed by this CT examination.

4. between the urinary bladder and uterus, an expansive inhomogeneous structure measuring 85/80/85mm, well-delineated, containing fat and two calcifications, diagnosed as teratoma (fig. 3).

In short, CT examination performed at that time revealed: two hepatic hydatid cysts, an abscess (from a perforated appendicitis, with pus migrated under liver), and an ovarian teratoma. The patient underwent appendectomy and right oophorectomy. The patient was discharged 8 days after surgery and was recommended to come back and have the hepatic hydatid cysts removed.

Four months later, the same 31-year-old female patient was brought by ambulance to the emergency department. She had experienced intense abdominal pains for about 14 hours, without any known triggering factor. The patient had a rapid deterioration of her health status: nausea, vomiting, and visible chills. Upon arrival, she also declared she knew she had a hepatic hydatid cyst but had refused surgery. The patient was anxious. She described pain in whole abdomen, but most intense in the right hypochondrium.

Ultrasound examination in the Emergency Department revealed a round structure of about 8 cm x 7 cm, very inhomogeneous in the right lobe of the liver (fig. 4) and slightly hyperkinetic intestinal loops.

No free fluid in the peritoneum and no dilation of the biliary tract were visible. Still, due to the poor state of health of the patient and inhomogeneous appearance of the cyst, the rupture and infection of the hydatid cyst was suspected.

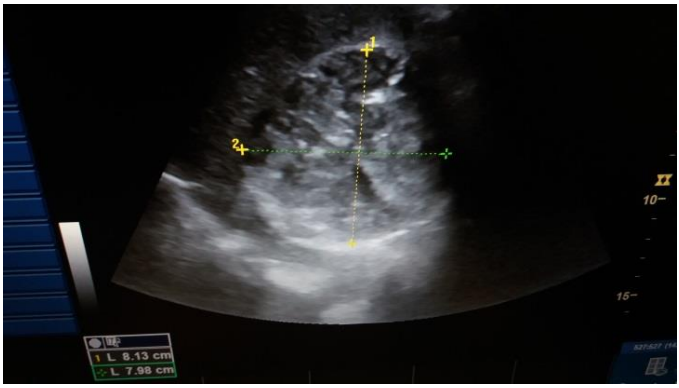

Fig. 4. Hepatic hydatid cyst possibly ruptured

Chest X-ray in the Emergency Department revealed no pulmonary hydatid cyst. Surgical examination revealed a very painful abdomen. The abdomen was moving with breathing and was entirely painful. An abdominal CT was recommended to visualize the hydatid cyst and reveal the possible leakage in the biliary tree or elsewhere.

In the Emergency Department blood was collected. The patient had the following blood values: white blood cells, 10.41 x 10^9 /L; lymphocytes: 14.3%, neutrophils: 81.2%; red blood cells: 4.75 x 10^{12}/L; hemoglobin: 13.8g/dL; hematocrit: 41.3%; Na^+: 138 mmol/L; K^+: 4.1mmol/L; alanine aminotransferase: 18 U/L; aspartate aminotransferase: 19U/L; creatinine: 0.77 mg/dL; serum amylase: 85U/L; glucose: 99 mg/dL.

CT examination revealed that there were two hepatic hydatid cysts, one in segment 7, and a second one in segment 5 and 8, and slightly dilated intrahepatic biliary ducts in segment 8, in contact with the hydatid cyst. Fortunately, images from the previous examination 4 months earlier were found in the CT memory. The radiologist noticed that both hydatid cysts were stationary as compared to the examination four months earlier. The current examination revealed dilated jejunal and ileal loops, up to 35 mm, with air-fluid levels, intense peristalsis, up to the mesogastrium towards the right iliac fossa. No wall thickening was noticed. No tumor was found at that level. Free peritoneal fluid up to 10mm thick could be measured in the pelvic area. No pneumoperitoneum was visible. The CT radiologist concluded that the hepatic hydatid cysts were stationary as compared to the examination four months before, and that there was a bowel obstruction, probably due to adherences, between mesogastrium and right iliac fossa.

The patient was immediately referred to the General Surgery Department, where she was rebalanced with fluids and nonsteroidal anti-inflammatory drugs. That day, the patient did not eat and drink. Gas transit was present. The patient's blood pressure was 100/60 mmHg, with the heart rate 54 beats per minute.

Three days later, the abdominal plain film revealed air-fluid levels in the stomach and small bowel. Blood was collected in the surgery department. The patient had the following blood values: white blood cells: 14.49 x 10^9 /L; lymphocytes: 7.3%, neutrophils: 82.6%. During surgery, numerous dilated small bowel loops were found, with numerous adhesions, and a 10 cm necrotic bowel segment. Adhesiolysis was performed, then segmental enterectomy, followed by enteroenteroanastomosis. Pathological examination of the surgical specimen revealed enter mesenteric infarction.

The next day, gas transit was present. Blood was collected in the Intensive Care Unit, at 9:42 a.m. The patient had the following blood values: white blood cells: 4.97 x 10^9 /L; lymphocytes: 8.9%, neutrophils: 79.6%.

The patient recovered uneventfully and was discharged from hospital with the following recommendations: change the gauze dressing every two days until complete wound healing, avoid physical effort for 3 months, get the metal staples removed two weeks later, wear abdominal belt for a couple of months, follow-up one month later.

DISCUSSION

Though most hydatid cysts can be found in liver and lung, there also are unusual locations, from abdominal wall to heart or hip joint (1). Hydatid cysts are generally single, rarely two or more cysts can be found in the same organ. Our patient was one of the rare cases who had two hepatic hydatid cysts. The hydatid cysts themselves can have unusual aspects (2). Calcification is not synonymous with parasite inactivity (3); it may coexist with still metabolically active cysts.

The severity of hydatid cyst is mainly due to its complications. The most frequent one is rupture into the biliary tract leading to infection, or in the peritoneum (4, 5) and the rarest one is intravascular rupture, considered a lethal emergency. Pulmonary embolism can occur after a fistulization of a hepatic hydatid cyst in the IVC or hepatic veins (6). Symptoms of the rupture of a hepatic hydatid cyst vary from simple discomfort associated with minor signs of allergy to anaphylactic shock (7), and include: intense abdominal pain, dilation of the biliary tree and / or free fluid in the abdomen, up to fever and hypotension. There are other medical conditions which may simulate rupture of a hydatid cyst, like hepatitis (8).

Though the presence of hepatic hydatid cyst can be detected by ultrasonography, detection of dilation of the bile duct or of a small amount of free fluid in the peritoneum is a challenge. On Doppler ultrasound a germinal membrane of the cyst may be found to be extending into the inferior vena cava (9). CT is required to better describe and identify the rupture of a hydatid cyst (10, 11). Magnetic resonance imaging, especially magnetic resonance cholangiopancreatography can be more helpful for the evaluation of dilated intrahepatic bile ducts due to hepatic hydatid cyst rupture (4).

Treatment includes exploratory laparotomy and evacuation of the cyst with deroofing (11). Major hepatectomies (right hepatectomy, left hepatectomy and others) are required for occupation of the entire lobe, severe vascular or biliary involvement, or a combination of the two (12).

In this case, the highly deteriorated health status of the patient was initially believed to be a complication of the known hydatid cyst. The appearance of the hepatic hydatid cysts was not typical on ultrasound

examination, and that is why the right number of the cysts could not be specified, and the numerous calcifications inside the first cyst were wrongly considered as signs of infection. Though neither free fluid in the peritoneum nor dilated biliary ducts were found on ultrasound examination, rupture of the hydatid cyst was suspected, and a CT examination was recommended to identify the point of leakage. It was the CT examination that revealed the cause of the rapid deterioration in health status, bowel obstruction, and the right number of cysts (two). Since neither wall thickening nor tumor was found distally, postoperative adhesions were suspected. Because images at the previous examination were found in the CT memory, the previous and actual appearance of the hepatic hydatid cysts could be compared.

CONCLUSIONS

Bowel occlusion, in the presence of two hydatid cyst, had the same symptoms as a ruptured hydatid cyst: intense abdominal pain, fever, rapid deterioration of the health status and hypotension. Neither free fluid in the abdomen nor dilation of the biliary tree was found upon ultrasound examination. Still, due to the rapid response of radiology and surgery departments, the patient's life could be saved.

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METHODS FOR ESTIMATING "DRY WEIGHT" IN HEMODIALYSIS PATIENTS

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METHODS FOR ESTIMATING "DRY WEIGHT" IN HEMODIALYSIS PATIENTS (Abstract): The correct estimation on dry weight is very important in providing effective and safe hemodialysis to end stage renal disease patients. Optimal fluid removal with minimal adverse reactions from the treatment is one goal of hemodialysis treatment. The aim of this article is to discuss different methods of assessing dry weight and to determine their role in the complex fluid management of end-stage renal disease patient. Recent literature describes various methods of determining optimal hydration state, including clinical or paraclinical methods. Each of these methods has their benefits and limitations. Despite the large number of studies conducted on new technologies for determining dry weight, there is a lack of larger, randomized controlled trials to support these methods. Although the successful use of different methods of dry weight assessment is not supported yet by large clinical trials, some evidence is starting to link the use of some methods to improved outcomes (morbidity, mortality) in this population. **Key words**: HEMODIALYSIS, DRY WEIGHT, BLOOD VOLUME, WEIGHT

One advantage of hemodialysis (HD), as a form of renal replacement therapy, is that it can remove both retained fluid and waste products (1). One of the goals is to ensure that fluid and solute removal is optimal and as comfortable to the patient, with minimal or no adverse reaction from the treatment such as hypotension, cramps and dizziness (2).

Dry weight (DW) assessment is crucial in order to achieve an optimal and comfortable fluid removal in the HD patient. This is often difficult to achieve in the hemodialysis setting because of the lack of reliable criteria to determine whether DW has been reached in a particular patient (3), or the lack of evidence to support a particular method of assessment. This is often complicated by the potential for hemodynamic instability during ultrafiltration. Both doctors and nurses play a pivotal role in dry weight assessment (DW), as they are directly involved with patient treatment and could contribute enormously to better patient outcome. The purpose of this article is to discuss the clinical relevance of dry weight assessment and to identify and discuss various methods of assessing dry weight in adult hemodialysis.

The concept of dry weight has always been opened for debate, as clinicians could not agree on how to clinically define dry weight (4). Some clinicians define DW as the weight at the end of the dialysis session at which the patient is more likely to develop symptoms of hypotension (5). For

some (6, 7) the "ideal" dry weight or the "target weight" (8) is the weight the clinicians hope a patient will be at the end of each HD session, without suffering any of the intradialytic complications associated with intravascular volume depletion. DW and solute removal equates to dialysis adequacy (6). Others define DW based on blood pressure (BP) as the parameter, that is, the absence of hypertension and hypotension (9). For some DW is defined as the patient's current body weight in a physiological extracellular volume (ECV) state (10), that is, the ECV is not expanded. To all these definitions, a further statement of "how the patient feels" can be added (4).

The basic concept of fluid transport between the two major fluid compartments in the body, intracellular and extracellular compartments is essential to the understanding of fluid removal and DW assessment (6, 10). The intracellular space holds approximately 23 liters of fluid (for a 70 kg adult), in comparison to 17 liters in the extracellular space, while 5 liters of blood are in the intravascular space (6). In between dialysis, when fluid is not being removed, the intravascular space fills-up to a certain capacity. As the patient continues to consume fluid, excess fluid volume moves into the interstitial space where it can hold as much as 20-40 liters, sometimes known as the third space (6). At this point, gross edema can be observed on the patient.

During hemodialysis, as fluid is removed from the intravascular space, fluid from the interstitial space moves into the intravascular space, known as the plasma refilling rate (PRR) (6, 10). If the PRR is less than the ultrafiltration rate (UFR), that is, fluid in the intravascular space is not being refilled as quickly as it is being taken-off, the patient could suffer from intravascular volume depletion and associated symptoms (11, 12). The most important factors that affect the PRR include: low albumin level, congestive heart failure, reduced plasma osmolality, hypoxemia, ischemia, septicemia, fever, autonomic dysfunction, and ingestion of anti- hypertensive medications (6, 10). In this situation, fluid removal and dry weight assessment is a challenge, as the patient would exhibit signs of fluid overload and yet dialysis staff are unable to further reduce the weight. Further decreasing the weight would result in the patient experiencing intradialytic symptoms, such as hypotension, cramps and dizziness (12).

Optimal and effective hemodialysis is not possible without a correct dry weight assessment. Several studies have been conducted on the effects of inaccurate assessment or estimation of DW. Hypervolemia (fluid overload) is the major risk factor in the development of hypertension, left ventricular hypertrophy, and cardiovascular disease thus affecting the mortality risk of patients on hemodialysis (6). On the other side, hypotension, cramps, and dizziness - signs of hypovolemia - can appear when the DW is set too low (7). Hypovolemia can also result in reduced blood supply to vital organs causing ischemia, consequently contributing to the loss of residual renal function (6).

Death of cardiovascular causes is extremely high in dialysis population, reaching up to 36%. A prospective study of 97 patients on chronic hemodialysis found that a higher interdialytic weight gain (IDWG), of more than 3% body weight, was associated with cardiovascular events: myocardial infarction, congestive heart failure, aortic aneurysm or intracerebral bleeding

(13). Similarly, another study found that high IDWG (>3%) and non-compliance with the treatment, are independent risk factors for higher blood pressure amongst HD patients (14).

The DRIP study, a randomized controlled trial of 150 chronic hemodialysis patients found that DW reduction has a positive effect on blood pressure (9). However, the incidence of intravascular volume depletion, necessitating fluid repletion and ultrafiltration (UF) volume reduction, was commonly seen in the patients receiving the intervention to low DW. In addition, patients in the intervention group had more episodes of clotted access as compared to the control group. This poses the question does reducing DW contributes to clotting of vascular access, despite better BP control? Although this is possible, other factors could have contributed to the higher incidence of clotted access in the intervention group beside DW reduction. For instance, this study has tried to probe DW in the brink of hypovolemia, which could have contributed in the clotting of access. The clotting of access may have resulted due to poor intravascular refilling and not necessarily due to DW reduction. It is possible that the DW could have been reduced efficiently by increasing the dialysis time to provide adequate intravascular refilling time (6). In this instance, blood volume monitoring could be beneficial in evaluating the refilling status on dialysis (11, 15) whilst assessing the DW and BP.

Intradialytic hypotension (IDH) accounts from approximately 20%, according to some authors (6, 16), to 40% of HD complications (12). Inaccurate estimation of DW is one of the causes of IDH. A dry weight set to low will lead to poor PRR and consequently to signs of volume depletion (6, 12, 17). IDH requires immediate medical intervention to prevent further intradialytic complications, such as ischemia and hypoxemia (6). Interventions include administration of normal saline, stopping ultrafiltration (UF) and on occasions, early treatment termination. These lead to an inefficient fluid removal and decrease dialysis dose (18). A survey on the clinical management of hypotension among nurse managers found that the majority of dialysis staff (81%) agreed that frequent occurrence of IDH leads to decreased efficiency of dialysis (17). In addition, although this study found that each IDH episode only took an average of 10,5 minutes to manage, it can be a time-consuming process, and can add additional pressure on dialysis unit staff.

The usual DW assessment is based on trial and error, as patients weight can change periodically and therefore should be assessed on a regular basis (7). Methods of DW assessments include both clinical methods (19) and non-clinical methods, such as inferior vena cava diameter measurement, blood volume monitoring, bioelectrical impedance, and ultrasound lung water assessment (5, 10 14).

Dry weight cannot and should not be assessed in any patient by a single parameter (1). In order to accurately determine patient's DW, a combination of clinical signs and other parameters are often considered. Clinical assessment, that is relatively easy and quick to perform (20), has always been the most widely used by dialysis staff in assessing patient's DW (19). Clinical methods include the measurement of jugular venous pressure (JVP), inspecting for signs of edema (dependent and peripheral), measurement of BP pre and post-dialysis, chest auscultation, assessing skin

turgor and mucous membranes inspection (6, 21). Blood pressure (BP) must be measured sitting and standing, as orthostatic change in BP is a useful diagnostic tool (19). Elevation of the BP, especially if persistent, usually indicates a need to reduce the DW. On the other side, signs of volume depletion, such as persistent low BP, dizziness, cramping, and vomiting may indicate a need to raise the DW (19). Chronic heart failure can be a cause of low BP, and any increase in the DW must be performed with caution in such cases (9). Jugular venous pressure (JVP) is an useful sign, but unfortunately has been neglected by dialysis nurses and physician (10). Elevation in the jugular venous pressure is a useful sign of fluid overload and should be performed before the commencement of hemodialysis. Elevation of the jugular venous pressure during or at the end of hemodialysis may indicate a need for target weight adjustment (10). On the other hand, one must be aware that the mere absence of an elevated jugular venous pressure during hemodialysis or at the end may still be consistent with fluid overload and should not be considered as a sign of reaching the ideal weight. For example, patients with heart failure may not have elevated JVP, but can still be fluid overload (22). The presence of is a reliable sign of excess body water (6) and usually means that DW should be decreased. Serum albumin must also be high enough to facilitate the movement of interstitial fluid back into the circulation (19). Staff must be aware that some patient may still be overloaded in the absence of edema, known as "silent overhydration" (6). Breathlessness is commonly caused by pulmonary edema in this patient population, and is often caused by fluid overload or left heart failure or both (23). Urgent reassessment of DW and of diagnoses other than fluid overload is required (19) so that appropriate treatment could be instituted.

Treatment history should include parameters such as any recent change in target weight and how well the change was tolerated and average IDWG. A record must be also kept of the average pre/post dialysis BP, including postural changes in BP. Other parameters to include in reviewing patient's history include his antihypertensive treatment and whether these drugs are omitted before each dialysis (1). Total proteins, especially albumin, create the oncotic pressure in the intravascular space, and play an important role in the movement of fluid from interstitial tissue to intravascular space (6). In some patients, fluid removal is not well tolerated if the serum albumin is too low (19). The patient may show specific signs/ symptoms as hypotension and cramps, despite still being fluid overloaded (1). It is therefore very important that clinicians review serum albumin level in combination with other clinical signs when assessing DW.

Clinical methods are the most commonly used for DW assessment, although there are some limitations to their use, most specifically physical assessment (5). Heart function or nutritional status can influence fluid overload, while BP can be affected by more than fluid volume. Non-clinical methods that can help in objectively assessing the patients' ideal weight include inferior vena cava diameter measurement, ultrasound lung water assessment, blood volume monitoring and bioeletrical impedance analysis.

Inferior vena cava diameter (IVCD) and collapsibility measurement can evaluate the amount of intravascular volume in HD

patients, that is, it can detect intravascular volume overload or depletion, and correlates well with central venous pressure (19). This procedure is done through echography of the inferior vena cava diameter, measured in supine position during inspiration and expiration (5, 20, 24). It can be useful in dry weight assessment in hemodialysis patients in combination with clinical assessment and has been shown to predict haemodynamic changes during dialysis (20). This method can be unreliable in patients with right sided heart failure, because the IVCD is strongly related to the right atrial pressure (20). In addition, the usefulness of this method is limited by the high cost involved in performing the procedure and the need of exact timing in performing the measurement (10, 20).

In another study on dry weight assessment, Mallamaci et al. reports the use of chest ultrasound to detect pulmonary congestion and potentially guide ultrafiltration and volume removal (25). “Ultrasound lung comets” are ultrasound artifacts generated from the boundary of thickened pulmonary septa and overlying pleura, the ultrasound equivalent of 'Kerley B lines' on a chest roentgenogram, reflecting lung water and elevated left-heart filling pressures. These linear artifacts have been likened to comets by their appearance, and I will personally attest to the relative technical ease of image acquisition for detecting a “lung comet”. Of the 75 hemodialysis patients studied by Mallamaci et al. nearly two-thirds (47 patients) had moderate to severe lung congestion pre-dialysis, and nearly one-third (21 patients) had persistent congestion of the same magnitude post-dialysis. Among the 56 patients who had no symptoms of congestive heart failure pre-dialysis, 57% had ultrasound findings of significant pulmonary congestion. By regression analysis, only left-ventricular ejection fraction was independently associated with excess predialysis lung water. This type of examination can be done quickly, with portable equipment in a dialysis unit, and the same instrumentation can be used for echocardiography, including IVC evaluation.

Blood volume monitoring (BVM) measures the blood density during hemodialysis through an optoelectronic device or ultrasound transmitter (20, 26). As fluid is removed from the vascular space during hemodialysis, excess body water from the interstitial space would transfer into the vascular space (vascular refilling). Certain blood constituents, such as red cells and plasma proteins are unable to leave the intravascular space thus making the intravascular solute mass relatively constant even when there are changes in blood volume due to ultrafiltration or refilling (15). Changes in relative blood volume or blood density, through haematocrit percentage, are then progressively measured during hemodialysis. Clinicians then interpret the relative blood volume and haematocrit in comparison to the UFR throughout the entire dialysis time (27).

Some authors believed that although the BVM is a non-invasive, easy to perform procedure, its main applicability in the clinical setting is to detect blood volume changes during UF and thereby predict end dialytic hypotension (28). But the usefulness of BVM in assessing DW is limited due to the fact that PRR can be influenced by other patient pathologies (i.e. autonomic dysfunction) (10). In addition, other factors such as dialysate sodium can influence the changes in relative blood volume, making BVM limited in the assessment of DW (20).

In another study of 13 chronic HD pa-

tients over 17 weeks, it was found that the relative blood volume and relative blood volume ultrafiltration ratio has considerable differences between the first and third HD sessions, limiting the use of these parameters in preventing hypotension and as a tool in DW assessment (15). However, the results of this study indicate that BVM should be utilized to monitor changes in the patients' relative blood volume every dialysis over a 2-week period. This will then enable clinicians to adequately interpret the results by the end of the 2-week period. In most patients, IDWG is expected to be higher after a 2-day break (of dialysis session) compared to one-day break, hence are unable to reach their DW on their first dialysis day due to limited time (14). If the patient's relative blood volume remains unchanged over the 3 consecutive hemodialysis sessions, that is, the relative blood volume graph is greater than 95% at the end of each dialysis, then the patient still has a lot of interstitial fluid on board, hence DW should be slowly reduced. If patient's relative blood volume continues to decrease towards the end of the week, then the patient is reaching their DW (15).

Lopot et al. argued that BVM can be useful in determining DW by analyzing how patient responds with different UF profile and varying degrees of hydration for each dialysis session (29). It was further stated that for BVM to be used for DW assessment the following methods must be used in the analyses of relative blood volume: (1) a relative blood volume that does not decrease during hemodialysis despite constant UFR, signifies over-hydration hence DW should be reduced; (2) a relative blood volume that decreases during treatment but refilling is seen after UF cessation and patient is symptomatic of intravascular depletion is mainly due to insufficient refilling time. In this instance, DW can still be decrease but dialysis time is increase, to allow sufficient time for refilling of intravascular space; (3) a decreasing relative blood volume during hemodialysis without refilling after UF cessation, and symptom-free dialysis indicates that the patient is on the correct DW and should be maintained (29).

Last but not least, the most promising method seems to be the bioelectrical impedance analysis (BIA). This method assesses the total body water component, that is, extra cellular and intracellular fluid volume. It is very useful in determining fluid status in all body compartments making it an ideal tool in assessing DW (30). This method has been used for the last 30 years for fluid assessment and various methods of bioelectrical impedance analysis have evolved, such as whole body segmental, single frequency and multi-frequency bioelectrical impedance (10).

In a study on 500 hemodialysis patients from 5 centers, Wabel P. et al. used BIA to place the patients in a "hydration plot", according to the overhydration (OH) value and systolic blood pressure (BP). The study illustrated a wide variability of systolic BP regardless of the degree of overhydration, leading to the need for multiple therapeutic approaches in this population. In another study on 370 dialysis patients a cross-sectional analysis using BIA revealed that 26% of patients would benefit from a correction of body weight (31). Another trial used BIA to guide 52 HD patients to normohydration in one year (32). In the hyperhydrated sub-group the fluid overload was reduced by 2 liters and systolic BP by 25 mmHg. In the underhydrated group there was a 73% decrease in intradialytic adverse events without an increase in BP.

In conclusion, both short and long term effects of poor volume control can be prevented by determining the optimal dry weight. New technologies in DW assessment are now available as an added tool for the clinicians to use. According to recent literature these technologies have assisted clinicians in managing effective fluid removal and DW assessment, but the validity and clinical application of these methods is still limited. The reason could be the cost involved or the lack of larger randomized-controlled trials to examine the use in a greater clinical setting. Although the clinical methods are the most commonly used methods in assessing DW, it is important to note that this does not only include physical assessment, but rather a comprehensive review of other parameters such as blood results, medications and treatment history. The increasing use of new technologies, such as BVM, BIA or ultrasound lung water evaluation must be used in combination with clinical methods of DW assessment in preventing the harmful effects of poor fluid management in HD patients.

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METAPLAZIA MIELOIDĂ CU MIELOFIBROZĂ COMPLICATĂ CU SINDROM CORONARIAN ACUT

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MYELOID METAPLASIA WITH MYELOFIBROSIS ASSOCIATED WITH ACUTE CORONARY SYNDROME. CASE REPORT (Abstract): A 66-year-old man with myelofibrosis and myeloid metaplasia was admitted to hospital where he was diagnosed with acute coronary syndrome. Percutaneous transluminal coronary recanalization followed by drug-eluting stenting resulted in successful revascularization. Because besides thrombus formation the myeloproliferative syndrome has a potential for bleeding, it is very important to carefully follow-up any clotting abnormality. **Key words**: MYELOFIBROSIS WITH MYELOID METAPLASIA, ACUTE CORONARY SYNDROME, DIAGNOSIS

Sindroamele mieloproliferative includ: trombocitemia esențială, policitemia vera şi leucemia granulocitară cronică care sunt caracterizate prin proliferare excesivă şi diferențiere de-a lungul liniilor megacariocitară, eritroidă şi respectiv mieloidă, precum şi metaplazia mieloidă cu mielofibroză caracterizată prin fibroză excesivă a măduvei hematogene. Cele mai comune cauze de deces asociate sunt: transformarea leucemică, mielofibroza secundară, complicațiile hemoragice şi tromboembolice.

Mecanismul patogenic primar în cazul metaplaziei mieloide cu mielofibroză este reprezentat de un defect la nivelul liniilor de celule stem, ducând la eritropoieză ineficientă, hiperplazia megacariocitelor displazice şi creşterea procentului de granulocite imature. Această mieloproliferare clonală este însoțită de mielofibroză reactivă (fibroza măduvei osoase) şi de hematopoieză extramedulară ineficientă. Suspiciunea diagnostică este ridicată de prezența hematiilor „în lacrimă" şi a precursorilor mieloizi pe frotiul de sânge periferic. Trăsăturile clinice tipice includ: splenomegalie masivă, anemie progresivă şi simptome constituționale (fatigabilitate, subfebrilități, scădere ponderală). Mediana supraviețuirii este situată între trei şi şase ani, cauza principală a decesului fiind transformarea leucemică. În majoritatea studiilor privind factorii prognostici, anemia şi vârsta avansată au fost independent corelate cu supraviețuirea redusă. Alți factori de prognostic negativ sunt: prezența simptomelor catabolice, leucocitoza sau leucopenia, numărul mare de precursori granulocitari, trombocitopenia (<100000/mm³) şi anomaliile cariotipale (1,2).

Incidența reală a evenimentelor tromboembolice la pacienții cu sindroame mieloproliferative este greu de apreciat,

majoritatea studiilor clinice fiind retrospective, pe loturi reduse de pacienți, fără o definire clară a criteriilor clinice şi diagnostice (3). În prezent, terapia mielofibrozei este în principal paliativă.

PREZENTAREA CAZULUI

Pacientul P.G. în vârstă de 66 de ani, cunoscut de aproximativ doi ani cu metaplazie mieloidă cu mielofibroză, supus unei cure cu citarabină în urmă cu 5 luni, fumător, normoponderal, fără antecedente personale sau heredocolaterale de tip cardiovascular, se internează în Unitatea de Terapie Intensivă Coronarieni a Institutului de Boli Cardiovasculare Iaşi pentru dureri retrosternale constrictive, de aproximativ o săptămână, cu caracter progresiv agravat, până la apariția acestora la eforturi minime şi în repaus.

Obiectiv, la internare: tegumente palide; TA=140/80mmHg, AV 73/ min, ritmic fără fenomene stetacustice supraadăugate, fără semne de decompensare cardiovasculară; hepatomegalie nedureroasă la 5 cm sub rebordul costal; splenomegalie gradul III, de consistență dură, neomogenă, ce deformează flancul stâng abdominal.

Dintre rezultatele de laborator reținem: anemie hipocromă, microcitară (Hb 9g%, Ht 28%, GR 5,28 milioane/mm³), regenerativă(R 5,2%), cu fier seric 43µg%, feritină 100ng/ml; număr normal al celorlalte elemente figurate (GA 9370/mm³, trombocite 311000/mm³); IP 76%, INR 1,33 spontan; markeri de citoliză miocardică normali(TnT<0,1ng/ml, CK-MB 10 U/l); funcție renală normală (creatinină serică 0,85mg%, Na 139mmol/l, K 4,3 mmol/l); acid uric 8,9 mg%; fosfataza alcalină 265U/l; LDH 830U/l; BT 1,73 mg% (BD 0.76mg%, BI 0,97 mg%); glicemie 80mg%; proteine serice 57,8g/l.

Frotiul de sânge periferic a evidențiat marcată anizopoikilocitoză, cu microcite, schizocite, hematii ''în lacrimă'', hematii „în țintă", hematii policromatofile, hematii cu inele Cabot, eritroblaşti oxifili 5%; deviere la stânga a seriei leucocitare, până la metamielocit; macroplachete (fig. 1).

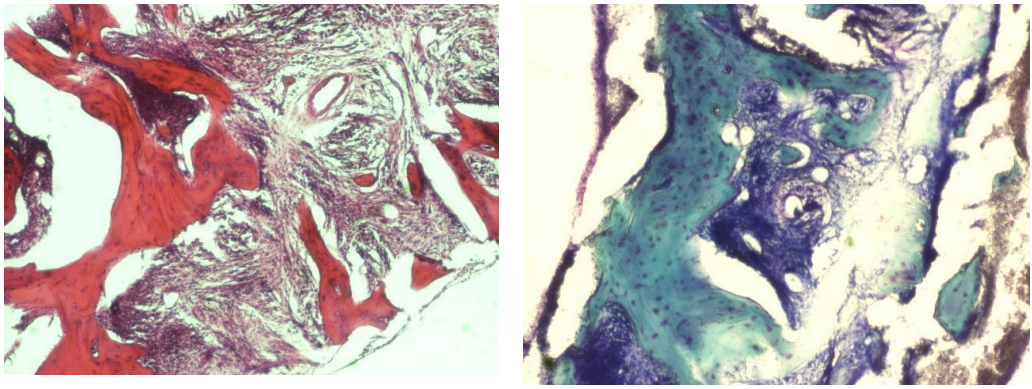

Fig. 1. Coloraţia cu hematoxilin-eozină şi coloraţia May-Grunwald-Giemsa

Electrocardiografic: ritm sinusal 70/min, subdenivelare de segment ST 2 mm, oblic descendentă, urmată de undă T negativă în teritoriul inferior.

Radiologic: cord cu dimensiuni normale (ICT 0,50); plămân fără leziuni evolutive.

Ecocardiografic s-a evidențiat o uşoară hipertrofie concentrică de ventricul stâng,

cu funcţie globală normală (FE 68%), fără tulburări de kinetică segmentară.

Ecografia abdominală confirmă hepatomegalia (lob drept hepatic 173 mm), cu căi biliare principale nedilatate şi splenomegalie masivă (283/130 mm).

S-a iniţiat tratament cu betablocant, heparină, antiagregant plachetar şi nitrat, cu obţinerea controlului simptomatologiei. Având în vedere că anemia severă din cadrul sindromului mieloproliferativ poate fi un factor agravant al anginei, s-a asociat administrarea de eritropoietină şi fier, cu ameliorarea parametrilor hematologici. Ulterior s-a efectuat coronarografia, care a relevat leziuni bicoronariene (artera descendentă anterioară – stenoză ostială 80% şi stenoză 75% în segmentul al doilea, stenoză 80% a arterei coronare drepte în segmentul al doilea). S-a optat pentru angioplastia cu stent acoperit cu everolimus a leziunii ostiale de pe descendenta anterioară, kissing-stenting al originii comune dintre artera descendentă anterioară şi artera circumflexă şi stentare directă a leziunii din segmentul 2 al descendentei anterioare, cu evoluţie ulterioară favorabilă, fără recurenţa anginei la controlul de la o lună postangioplastie.

DISCUŢII

Pacienţii cu sindroame mieloproliferative cronice sunt predispuşi la evenimente trombotice vasculare. Diatezele trombohemoragice caracteristice sindroamelor proliferative prezintă similarităţi calitative: acelaşi tip de diateză hemoragică, o varietate de complicaţii de tip trombotic şi un risc hemostatic care fac evoluţia clinică imprevizibilă. Iniţial acest lucru a fost atribuit numărului crescut de plachete din sângele periferic. Totuşi, numărul evenimentelor trombotice nu este semnificativ mai mare la pacienţii cu trombocitoză; de pildă, în cadrul sindroamelor proliferative, infarctul de miocard complică mai frecvent policitemia vera comparativ cu trombocitemia esenţială, numărul de hematii şi hipervâscozitatea fiind mai degrabă implicate în dezvoltarea trombozei (4,5,6).

Complicaţiile trombo-embolice constituie cea mai importantă cauză de morbiditate şi mortalitate la pacienţii cu osteomielofibroză, cauzând aproximativ o treime dintre decese. Osteomielofibroza a fost considerată o entitate cu risc preponderent hemoragic; recent s-a observat o incidenţă crescută a trombozelor venoase, mai ales în teritoriul portal. Cele mai frecvente complicaţii venoase trombotice sunt tromboza venoasă profundă periferică şi tromboembolismul pulmonar, tromboza de vene hepatice, de venă portă sau de sinusuri venoase cerebrale, producând sindroame clinice caracteristice. Riscul de tromboză arterială este de asemenea înalt, cel mai frecvent fiind afectate arterele membrelor, arterele cerebrale şi mult mai rar arterele coronare sau mezenterice. Sindroamele mieloproliferative sunt caracterizate printr-o lungă perioadă asimptomatică, prediagnostică, când accidentele trombohemoragice pot fi prima manifestare a bolii. Reducerea riscului trombohemoragic pe măsură ce boala evoluează este greu de justificat, emiţându-se ipoteza declinului funcţiei plachetare, cu activare şi degranulare la nivelul spaţiilor din măduva osoasă (2,7,8,9).

Complicaţiile de tip hemoragic sunt mult mai rare, non-fatale, în general implicând vasele mici ale tegumentului şi mucoaselor, putând fi întâlnite şi în prezenţa unui număr normal sau crescut de plachete, fiind cauzate de anomalii calitative plachetare. Tratamentul antiagregant plachetar poate favoriza apariţia hemoragiilor (3).

Literatura citează complicaţii cardio-

vasculare la pacienţii cu sindroame mieloproliferative cronice, de tipul sindroamelor coronariene acute, hipertensiune pulmonară, efuziuni pericardice asimptomatice, tromboză intracardiacă şi chiar leziuni valvulare, acestea din urmă crescând suplimentar riscul ocluziilor vasculare asociate. Relaţia directă dintre sindroamele coronariene acute (pe artere coronare normale sau cu leziuni prezente) şi sindroamele mieloproliferative reprezintă şi astăzi un obiectiv comun al cercetării pentru cardiologi şi hematologi (7,8).

CONCLUZII

Deşi sindroamele coronariene acute sunt complicaţii rare ale metaplaziei mieloide cu mielofibroză, ele trebuie totuşi luate în considerare şi studiate cu atenţie datorită riscului vital crescut pe care îl induc.

O particularitate a cazului o constituie riscul înalt de trombozare precoce a stenturilor. Asocierea tratamentului corect anticoagulant (heparină) şi antiagregant plachetar (aspirină şi clopidogrel) a facilitat evoluţia clinică favorabilă la un pacient predispus trombozelor vasculare. Atragem atenţia însă asupra necesităţii monitorizării atente a anticoagulării şi antiagregării plachetare datorită riscului hemoragic înalt la pacienţii cu sindroame mieloproliferative. Tratamentul cu aspirină în doză mică (100 mg/zi) reduce riscul de tromboză coronariană fără să crească riscul de sângerare (7).

În concluzie, pacientul cu sindrom coronarian acut care asociază metaplazie mieloidă cu mielofibroză necesită o monitorizare atentă a tratamentului anticoagulat şi antiagregant plachetar, alături de corectarea pe cât posibil a parametrilor hematologici şi nu în ultimul rând, controlul factorilor de risc cardiovascular suplimentari.

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EVOLUTION OF CLINICAL FEATURES OF SKIN CANCER IN THE ELDERLY. CASE REPORT

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EVOLUTION OF CLINICAL FEATURES OF SKIN CANCER IN THE ELDERLY. CASE REPORT (Abstract): We present the case of an elderly patient with multiple cardiovascular comorbidities diagnosed with Kaposi sarcoma approximately 10 years ago (the patient having postponed the initiation of the therapeutic protocol) which progressed to dilated cardiomyopathy and procoagulant state because of skin cancer. The onco-geriatric patients represent a distinct category, and as they vary widely in their responses to drugs the individualization of therapy is mandatory and requires the adaptation to the clinical status of each patient.
Keywords: KAPOSI SARCOMA, PRO-COAGULANT STATUS, ONCO-GERIATRICS, CIRS-G SCALE, RISK SCORES.

Kaposi sarcoma (KS) is a multifocal tumor that most commonly manifests in mucocutaneous sites mainly the skin of the lower extremities, face, trunk, genitals and oropharyngeal mucosa (1). KS's relationship with human immunodeficiency virus (HIV) infection is well known and is rare in HIV-negative patients being associated with human herpes virus-8 infection (HHV-8). Based on the clinical ground in which it develops KS is classified into four types: classic or European KS typically appears in middle- or old-aged patients as slow-growing nodules or plaques affecting the oral cavity and skin; endemic or African subtype mainly affects young adults or children and is mostly locally-invasive and/or associated with visceral disease; iatrogenic is mostly observed in organ transplant recipients with immunosuppressive treatment and disappears with the withdrawal of immunosuppression; and AIDS-associated (epidemic KS) which appears in young patients, predominantly homosexual or bisexual men, with multifocal skin lesions (2, 3).

The disease was discovered by the dermatologist Kaposi Moritz in 1872, who

described it as "an idiopathic multiple sarcoma of the skin" (4).

Six cases per million people are reported annually, being the most common neoplasia in HIV-infected patients, and the most aggressive form of the disease (5). Initially, in 1980, the occurrence of KS in AIDS patients suggested that there might be a causal relationship between HIV infection and KS. However, two findings contradicted this theory: HIV proviral DNA was not present in the tumor and not all HIV-positive subjects were equally at risk of developing KS (6).

From a histopathological point of view, there is a double proliferation of fusiform cells and vascular spaces. Being a multifocal affection, it exhibits lesions especially on the lower limbs, with symmetrical disposition in the form of deep, hemispheric, dark red, purple, blue or dark brown dermal nodules (7).

Vascularization in KS takes place prior to the formation of a tumor mass, unlike classical cancers in which angiogenesis begins only after proliferation (8).

CASE REPORT

We present the case of a 79-year-old male, chronic alcohol consumer, former smoker (20 packs-years), known with professional exposure to toxic fumes and contrast temperatures for 20 years, hypertensive for approximately the last 6 years, with maximum blood pressure (BP) of 190/100 mmHg, dyslipidemia, diagnosed 10 years ago with KS (the patient postponing at that time the therapeutic protocol), associating a coronary artery disease in the stage of mechanical (interatrial septal aneurysm) and electrocardiographic (permanent atrial fibrillation) complications. The patient presented to the Emergency Care Unit complaining of dyspnea on low physical exertion, with progressive worsening in the last 3 days, generalized edema and progressive increase in abdominal cavity volume in the last 2 weeks. In the past, chemical conversion was attempted with amiodarone while maintaining sinus rhythm for 2 years after relapse by relative adherence to treatment.

Physical examination revealed that the patient presented anasarca, was in relatively poor general status, afebrile, conscious, normal weight (body mass index BMI=22.5 kg/m^2) and presented cutaneous-mucosal pallor, perioral cyanosis, telangiectasia of the nasal pyramid (fig. 1), purple-colored tumors 0.5-1 cm in diameter at the level of the left lower eyelids (fig. 1), auricular lobes (fig. 2) and abdomen (fig. 3). Moreover, the patient also presented 2 tumors on the tongue (fig. 4) causing swallowing difficulty. Limb examination revealed: keratotic teguments, vascular tumors isolated or in placards on the legs, inferior 1/3 of the forearms and dorsal aspect of the arms, violaceus in color, nonpruritic, superinfected at the level of the right medial malleolus, indurated edema on both lower extremities (fig. 5 and fig. 6). Other findings were: emphysematous thorax, BP=130/90 mmHg, ventricular allure ≈ 120 beats/minute, arrhythmic cardiac sounds, grade 5/6 mitral systolic murmur with radiation to the axilla, grade 4/6 tricuspid systolic murmur, aortic atherosclerosis murmur, turgescent jugular veins. He had increased abdominal volume due to ascitic fluid, sensitive to palpation in the right hypochondrium, liver with sharp inferior border, 3 cm from the costal margin, weakly pulsating leg arteries.

Resting electrocardiogram on admission revealed atrial fibrillation, ventricular rate ≈ 120/minute (asymptomatic patient), micro voltage in the standard and unipolar limb leads, electrical alternation; rS V_2-V_4, mixed terminal phase abnormalities, ventricular extrasystoles some bigeminy (fig.7).

Blood tests revealed: inflammatory syndrome, hypochromic macrocytic anemia syndrome, hepatoprive syndrome, biliary stasis syndrome, hepatic cytolysis syndrome, hyposideremia, hyperuricemia, increased lactate dehydrogenase level, nitrogen retention syndrome. Urinalysis revealed proteinuria and sterile urine culture. The bacteriological analysis of the wound at the right-hand lesions revealed the association of 2 gram-negative bacilli, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*, both susceptible to Levofloxacin. The patient was tested for HIV, the result being negative. Testing for HHV8 was not possible for economic reasons.

Chest X-ray revealed cardiomegaly, right horizontal fissures, aortic knob with calcified contour, accentuated interstitial design (fig. 8). Abdominal and pelvic ultrasound examination documented hepatomegaly with increased reflectivity, inferior vena cava – 26 mm, hepatojugular reflux, presence of the ascitic fluid, gallbladder with edematous walls and without calculi; small size kidneys (right kidney=90 mm, parenchymal index=12 mm, slight cortical echogenicity; left kidney=85 mm, parenchymal index=10 mm, 8 mm lower caliceal stone, echogenic, inhomogeneous renal cortical; prostate=39/40/30 mm with a volume=18 cm^3, inhomogeneous vascular structure. Otherwise, abdominal and pelvic ultrasound did not describe other changes. Echocardiography revealed severely dilated right heart cavities, hypokinetic, severe tricuspid and mitral regurgitation, left ventricular hypertrophy with moderate systolic dysfunction, ejection fraction=40%, associating indirect signs of pulmonary hypertension (fig. 9 and fig. 10).

The initiated complex treatment with anticoagulant, diuretics, coronarodilatator agents, beta blocker, ACE, hepatoprotections, resulted in clinical and biological improvement, with the remission of the phenomena of global cardiac decompensation and improvement of dyspnea. Under antibiotic therapy with new generation fluoroquinolones (according to the antibiogram and recommendations of the infectious physician) and local daily lavatory of skin lesions, the evolution was favorable with the remission of superinfection phenomena. The use of CIRS-G (Cumulative Illness Rating Scale in Geriatric population) lead to the association of oral anticoagulant treatment [score HAS-BLED (Hypertension, Abnormal renal function, Stroke, Bleeding, Labile INR, Elderly, Drugs or alcohol)=6, score $CHAD_2VASC$ (Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke, Vascular disease, Age 65-74 years, Sex category)=4], but, upon evaluating the therapeutic risks and benefits, it was decided in favor of platelet antiaggregating therapy alone (9). The patient timed antiretroviral therapy and oncology assessment.

The prognosis was poor given patient's non-adherence to treatment, with the possibility of developing other cardiovascular or infectious complications. The association of the cutaneous lesions of Kaposi sarcoma can lead to the occurrence of ulcerations and gangrene and ultimately to amputation.

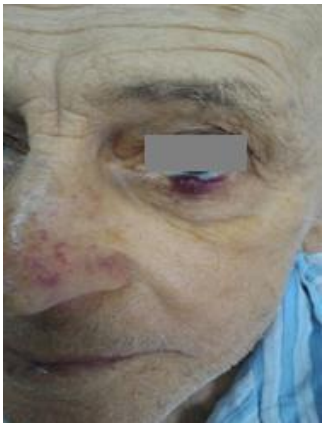

Fig. 1. Violaceous tumors on the left lower eyelid

Fig. 2. Violaceous tumors on the auricular lobes

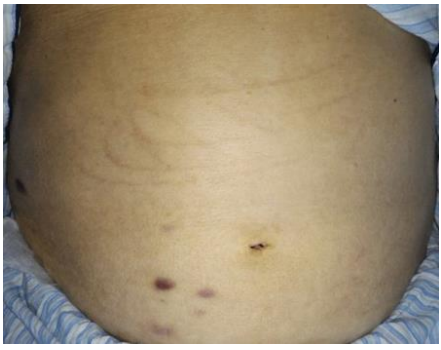

Fig. 3. Violaceous tumors on the abdomen

Fig. 4. Violaceous tumors on the tongue

Fig. 5. Skin lesions at the level of lower limbs

Fig. 6. Skin lesions on the hands

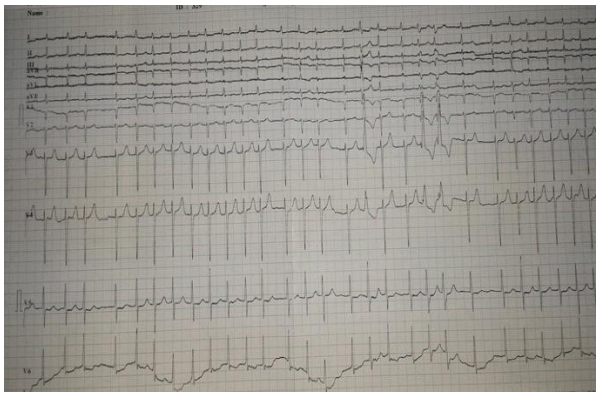

Fig. 7. Admission electrocardiogram

Fig. 8. Chest X-ray

Fig. 9. Biatrial dilatation

Fig. 10. Severe tricuspid regurgitation; $G_{VDAD=}$83 mmHg

DISCUSSION

Atrial fibrillation is the most commonly encountered supraventricular arrhythmia in daily practice. The management of atrial fibrillation includes rate-control or rhythm-control associated with antithrombotic medication aimed at reducing morbidity/mortality, the risk of thromboembolic complications and the degradation of ventricular function (10).

Data in the literature support the assumption that the durable effect of HIV infection is due to the consistent correlation between the decrease in the number of $CD4^+$ T cells (under treatment) and the high risk of cardiovascular diseases. The proatherogenic inflammatory status during the therapeutic protocol may be responsible for the suboptimal increase in $CD4^+$ T cells and higher cardiovascular risk.

HAART (highly active antiretroviral therapy) represents an integral part of the treatment, being recommended as the first step. The response to HAART ranges from 20% to 80%, depending on disease severity and immunological status (11).

The impact and consequences upon the quality of life of KS are various; although the most common form is the cutaneous form, and can occur anywhere in the body, it most commonly involves the lymph nodes (the classic form of KS most often

involves the lymph nodes), the respiratory and gastrointestinal tracts (12, 13).

Advanced immune deficiency associated with HIV infection greatly increases the risk of Kaposi sarcoma and the patients treated with antiretrovirals are at higher risk for premature morbidity and mortality compared to non-HIV-infected patients.

CONCLUSIONS

With this case we describe the course and complications of a skin cancer diagnosed 10 years ago, associated with relative adherence to treatment, which progressed to ischemic and valvular dilated cardiomyopathy.

Kaposi sarcoma presented the particularity of a multicentric cutaneous onset, slow course and a predilection for elderly people living in low socioeconomic status areas. Aging is associated with vascular, structural and functional changes, independent of the conventional risk factors.

The risk of bleeding dominates the logic of the clinician in recommending anticoagulant therapy Clinical practice guidelines and prediction scores are not a substitute for clinical judgement.

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SPIRAL ENTEROSCOPY – A NOVEL METHOD FOR THE MANAGEMENT OF SMALL BOWEL DISEASES – CASE REPORT AND DESCRIPTION OF TECHNIQUE

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SPIRAL ENTEROSCOPY – A NOVEL METHOD FOR THE MANAGEMENT OF SMALL BOWEL DISEASES – CASE REPORT AND DESCRIPTION OF TECHNIQUE (Abstract): Small bowel diseases are associated with difficult management. Recently, new methods such as single or double balloon enteroscopy and capsule endoscopy have been implemented for the investigation of small bowel, but none is perfect. Spiral enteroscopy seems to be an alternative primising technique for the diagnosis and therapy of small bowel diseases. We report two cases in which spiral enetroscopy was useful both for diagnosis and therapy. One was the case of a man with repeated digestive bleeding of unknown cause and spiral enteroscopy helped for diagnosis and treatment with argon plasma coagulation of the vascular lesions. The later was a patient with Gartner Syndrome with multiple duodenal and intestinal polyps and spiral enteroscopy allowed small bowel evaluation. In conclusion, this novel method seems to allow a rapid and efficient diagnosis of small bowel diseases. **Key words:** SMALL BOWEL, SPIRAL ENTEROSCOPY, ANGIOECTAZIA, ARGON PLASMA COAGULATION

Small bowel diseases are generally associated with difficult management due to challenging local access both for diagnosis and therapy. Obscure gastrointestinal bleeding, (overt or occult), along with malabsoption, unexplained diarrhea, small bowel radiological abnormalities, small bowel tumors, surveillance of polyposis syndrome, placement of feeding jejunostomy and thermocoagulation of bleeding lesions are all, indications for the investigation of small bowel (1). Recently, new methods such as single or double balloon enteroscopy and capsule endoscopy have been implemented for these diseases (1). However, none of these investigations are perfect and it is still room for improvement. Spiral enteroscopy is an alternative promising technique which seems to have a positive impact in the management of small bowel disease (2).

DESCRIPTION OF THE METHOD

A special design Endo-Ease Discovery SB (DSB) single-use overtube had been developed by Spirus Medical Inc, Stoughton, Mass, USA to assist advancing a normal enteroscope or pediatric colonoscope in the upper gastrointestinal tract. The DSB be has a working length of 130 cm, internal diameter 12.7 mm and external diameter 17.5 mm with superimposed helical spirals 5 mm high at the distal 21 cm. The overtube lightly connects to the enteroscope by a "gentle lock" and may rotate independently from the enteroscope. The enteroscope may advance independently or may perform spiral enteroscopy depending to the unlock or lock position of the spiral overtube (3).

PROCEDURE

The enteroscopy is done with the pa-

Fig. 1. The Endo-Ease Discovery SD (DSB) single-use overtube special designed for spiral enteroscopy

tient under conscious sedation or general anesthesia. The DSB device positioned 25 cm from the tip together with the enteroscope on which it is mounted is advanced to the ligament of Treitz. The spiral device is than unlocked and the enteroscope is advance through DSB over the ligament of Treitz. The DSB is than advanced by clock-wise rotation about 12 cm from the tip and then relocked. From that moment the DSB is turn clock-wise and the small bowel is retracted over the device, allowing progression in the lumen. When this maneuver is not anymore effective, the DSB is unlocked and the enteroscope is advanced until the maximum length of insertion. Further, the DSB is withdrawn by anticlockwise rotation simultaneously with the small bowel examination.

During 2009, a team of gastroenterologist from USA (Dr. P. Akkerman, Dr. M. Ciocirlan) and Paraguay (Dr. D. Cantero) had been performed lectures and practical demonstration in Spiral Endoscopy in Iaşsi. We present two interesting cases performed during the presentations.

Case 1

A 70 yrs old man, from Iasi, was admitted in the Institute of Gastroenterology and Hepatology Iasi for repeated digestive bleeding (melena) of unknown cause. The patient is known with aortic metal prosthesis, receiving oral anticoagulation therapy (acenocumarol) since 1983 and with chronic viral hepatitis C since 2001.

The patient had several episodes of digestive bleeding (melena) since 1984. Some of them were secondary to hemorrhagic gastritis due to anticoagulation and for some episodes, no cause was revealed even after repeated gastrointestinal endoscopy and colonoscopy. However, most of the time the patient had occult blood lost, iron deficiency anaemia and was under iron repletion therapy.

At admission, the physical exam revealed pallor, heart rate 105b/min, blood pressure 100/65, hepatomegaly. Laboratory findings showed: iron deficiency anemia, thrombocytopenia, and hepatocytolysis.

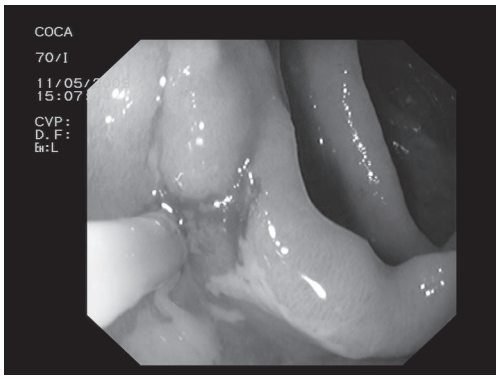
Upper gastrointestinal endoscopy and colonoscopy did not revealed any cause, also at this admission. However, video-capsule endoscopy examination showed multiple angiodisplazia, located probably in the ileum.

Patient underwent exploration with spiral enteroscopy that revealed multiple AVM's in the distal jejunum and proximal ileum and plasma argon coagulation was applied (fig1, 2, 3). Three month follow-up did not reveal any recurrence of bleeding and no anemia.

Case 2

A 25 years old patient known with Gartner Syndrome (Familial Polyposis Coli associated with mandibular osteoma) had recently a total colectomy procedure for removing the colonic polyps. The patient was also known with gastroduodenal polyps. He was admitted in the Institute of Gastroenterology and Hepatology Iasi for the evaluation of the small bowel. A capsule endoscopy procedure for small bowel revealed several small polyps in the duodenum and in the jejunal area.

A spiral enteroscopy procedure was performed in order to evaluate the histology and the possibility of polypectomy. The enteroscopy revealed more than 30 duodenal and jejunal polyps, some $>$ 10 mm in diameter. Polypectomy or biopsies were performed from some of the polyps and the histology showed tubular adenoma low grade dysplasia (fig 4). A spiral enteroscopy through ileostomy



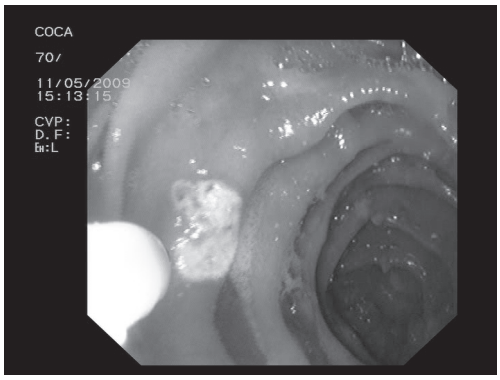


Fig. 2 and **Fig. 3.** Case 1 - Angioectazia of small bowel and thermo-coagulation with plasma argon.

was not done due to the small calibre of the stoma.

DISCUSSION

A patient with small bowel bleeding may be present with melena or hematochezia as overt bleeding or iron deficiency anemia in patients with occult bleeding.

Investigations that allow diagnosis in these cases includes enteroscopy using a colonoscope, sonde enteroscopy (procedures less used nowadays), push enteroscopy or more recent techniques such as single or double balloon enteroscopy, capsule endoscopy. Imaging techniques such as computer tomography angiography/angiography and nuclear scintigraphy are also usefull in detection the site of bleeding however, the later cannot determine the underlying cause.

In our patient, after the exclusion of gastro-duodenal and colonic causes of bleeding, we used the methods available in our unit for the diagnosis and therapy of intestinal bleeding, Capsule endoscopy is now an established technique for detecting obscure intestinal bleeding. The reported diagnostic yield is 63%, superior to push enteroscopy (1,4) and very similar (59.4-80%) to double balloon enteroscopy (42.9-60%) (1,5,6,7). However, capsule endoscopy allows more complete visualization of the small bowel then double balloon enteroscopy (90.6% vs. 62.5%) (6,7). In addition to the diagnostic performance, the non invasive character of capsule endoscopy, allows to be the first investigation to be performed in patients with obscure bleeding (1). There are some concerns on possible severe lesions (tumors) reported to be missed by video-capsule endoscopy and revealed by enteroscopy.

Angioectazia are the single most common cause of bleeding in patients above the age of 50 years (1,8). Thermocoagulation should be used in these patients according to guidelines to treat bleeding and prevent recurrent bleeding. According to location of angioectazia and availability of different enteroscopy techniques (the use of colonoscopy, push enteroscopy, single or double balloon) can be used for the therapy (1).

Patients with polyposis syndromes are at risk of developing small bowel polyps and also complications such as bleeding, small bowel obstruction, intussusceptions or small bowel cancer. Surveillance is recommended for these patients, however the best screening method (capsule endoscopy, enteroscopy, etc.) is yet to be established (1). According to the Spigelman classification for the severity of duodenal polyps, our patient is considered to be stage III (9). The risk for duodenal cancer increases to 36% within 10 years for stage IV patient and the lifetime risk for duodenal cancer is 3-5% (10). Malignancy in the small bowel may also occur but they are unusual. However, severe dif-

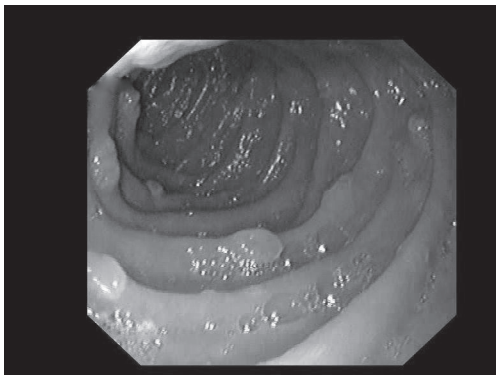


Fig. 4. Case 2 - small bowel polyps

fuse duodenal adenomatosis may require duodenectomy if severe dysplasia is present (10). In our patient, annual surveillance was the option due to low grade dysplasia of the polyps.

Spiral enteroscopy is a novel technique FDA and EC approved which allows along with above mentioned techniques the diagnosis and therapy of small bowel diseases (1, 2). Our case represents the first reported used of spiral enteroscopy in Romania. There are not yet studies comparing spiral enteroscopy with the previous enteroscopic possibilities.

However, published papers suggest that spiral enteroscopy is faster (about half of expected time for double balloon or single balloon techniques). In addition, this method is reasonably safe and the depth of insertion seems to be similar with the double balloon technique. In a recent paper published by Akerman, however on small number of patients (n=27), no major complications were reported. Superficial esophageal mucosal trauma (22% of patients) and sore throat (28%) were the only transient complications reported. In contrast, double balloon enteroscopy, a method on which there are more data, severe complications such as pancreatitis, bleeding and small bowel perforation was reported (3).

In conclusion, spiral enteroscopy is a novel promising method for the investigations of small bowel. However, additional studies are needed to compare spiral enteroscopy with the other diagnostic techniques for small bowel, especially double-balloon enteroscopy.

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ANATOMICAL LOCALIZATION OF ATYPICAL MENINGIOMAS: OUR EXPERIENCE ON 81 PATIENTS

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ANATOMICAL LOCALIZATION OF ATYPICAL MENINGIOMAS: OUR EXPERIENCE ON 81 PATIENTS (Abstract): **Objective:** this study aims to assess a possible relationship between the tumor location and the incidence of atypical meningiomas (AMs). **Material and methods:** from 2010 to 2017, 81 patients with AMs underwent surgical resection. The collected data included: patient age, gender, tumor location, dural venous sinus invasion and tumor laterality (right-left). **Results:** out of the total of 81 patients with AMs, 45 (55.5%) were female patients and 36 (44.5%) were male patients. The male to female ratio was 1:1.3. The mean age of patients who underwent surgery was 62.1 years. There were identified 61 (75.3%) non-skull base meningiomas and 20 (24.6%) skull base meningiomas. Out of the non-skull base meningiomas, the most frequent ones were located at convexity (41.9%) and parasagittal/falcine (32%). Dural venous sinus invasion was reported as present in 18 (22.2%) patients and as absent in 63 (77.7%) patients. **Conclusions:** AMs are more frequently reported in the cerebral convexity than in the skull base. **Keywords:** ATYPICAL MENINGIOMA, SKULL BASE, MENINGIOMA LOCATION, CRANIAL BASE, WHO GRADES.

Meningiomas represent approximately a third out of all primary central nervous system tumors, which makes it the most common brain tumor in adults over 35 years old (1). Out of all these, AMs represent 5-7% of all the meningiomas, according to the 2007 WHO classification (1), with an increasing incidence in recent years (2, 3). At present, AMs account 20% of all meningiomas (4, 5, 6, 7).

Among the multiple prognostic factors of AMs (8) that can predict meningioma grade prior to tissue diagnosis, was also the anatomical localization (9). Several authors noticed the predilection of AMs for cerebral convexity (9, 10, 11).

MATERIAL AND METHODS

We have evaluated 81 patients from the "Professor Dr. N. Oblu" Clinical Emer-

gency Hospital from Iasi, between 2010 and 2016, with histologically proven atypical meningioma (AM) and subsequently underwent craniotomy for resection. We have excluded all patients with neurofibromatosis type 2, multiple meningiomas, and those who had any other intracranial tumor history. The collected data included: gender, age, anatomical localization, dural venous sinus invasion and tumor laterality (right-left) (tab. I). Patient age was defined by the age at the time of surgery. In order to confirm the anatomical localization of AMs, post-contrast T1 magnetic resonance imaging and surgeon's operative note were taken into consideration. Meningiomas were divided into five main categories: (1) convexity, (2) parasagittal/falcine, (3) skull base, (4) posterior fossa and (4) intraventricular. Skull base locations were further stratified as medial and lateral skull base, according to Sade *et al* (10).

RESULTS

Out of the total of 81 patients suffering from AMs, 45 (55.5%) were female patients and 36 (44.5%) were male patients. The male to female ratio was 1:1.3. As for the group distribution, there were: 3 (3.7%) patients <39 years old, 28 (34.5%) patients aged between 40 and 59 years old, 23 (28.3%) patients aged between 60-69 years old and 27 (33.3%) patients older than 69 years. The mean age at surgery was 62.1 years. There were identified 61 (75.3%) non-skull base meningiomas and 20 (24.6%) skull base meningiomas. A more accurate distribution of meningiomas according to localization was performed, as it follows: 34 (41.9%) convexity, 26 (32%) parasagittal/falcine, 13 (16%) skull base (anterior and middle cranial fossa), 7 (8.6%) posterior cranial fossa and 1 (1.2%) intraventricular meningioma. As for the dural venous sinus invasion, this was reported as present in 18 (22.2%) patients and absent in 63 (77.7%) patients. All the patient characteristics are represented in table I and table II.

TABLE I.
Summary of patient characteristics

Characteristic	
No. of patients	81
Age (years)	
Mean	62.1
Median	64
Range	20-85
Age groups	
<39	3 (3.7%)
40-59	28 (34.5%)
60-69	23 (28.3%)
>69	27 (33.3%)
Sex	
Female	45 (55.5%)
Male	36 (44.5%)
Tumor location	
Convexity	34 (41.9%)
Parasagittal/falcine	26 (32%)
Skull base	13 (16%)
Posterior fossa	7 (8.6%)
Intraventricular	1 (1.2%)
Laterality	
Right	32 (39.5%)
Left	28 (34.5%)
Midline	10 (12.3%)
Midline-right	4 (4.9%)
Midline-left	7 (8.6%)
Venous sinus involvement	
yes	18 (22.2%)
no	63 (77.7%)

TABLE II.

Incidence of atypical meningiomas by site of origin

Midline		Lateral			
Tuberculum sellae	1.2% (1)	Orbital roof	1.2% (1)	Convexity	41.9% (34)
Cavernous sinus	4.9 (4)	Orbito-sphenoidal	1.2% (1)	Parasagittal/ falcine	32.0% (26)
Petro clival	1.2% (1)	Lateral / middle sphenoid wing	6.1% (5)	Intraventricular	1.2% (1)
		Lateral tentorial	8.6% (7)		

DISCUSSION

Gender, age. As for the gender, most of the patients were female patients 55.5% (45 cases), while the rest were male patients 44.5% (36 cases). The male to female ratio was 1:1.2. A predominance of males with AM was not found in our study and this gender distribution is different from the ones reported in literature, where grade II meningiomas occur more often in male patients (5). At the same time, other studies have reported a frequency rate of AMs on female patients similar to ours (12, 13). On the other hand, other authors have noticed a gender disparity (14, 15, 16, 17).

The analysis of patient age showed that AMs were diagnosed in the age group with a range between 20 and 85 years (mean age, 62 years). This finding coincides with the data reported so far, which argue that these tumors occur primarily in middle-aged or elderly patients (13, 18). Interestingly enough, in the age group with a range between 40 and 59 years old, there is predominance in women (71.4%) compared to men (28.6%), and a female to male ratio of 2.5:1. In the following age group with a range between 60 and 69 years old, the number of cases in both women and men is similar, with a female to male ratio of 1:1. After the age of 69, AMs are more common in men (59.2%) (fig. 1). This finding can be explained through the sex hormones status in the adult female until menopause and progesterone receptor status, since studies show that grade II or grade III meningiomas have progesterone receptors (19). Also, progesterone receptors play an important role in other neoplasia (20, 21, 22).

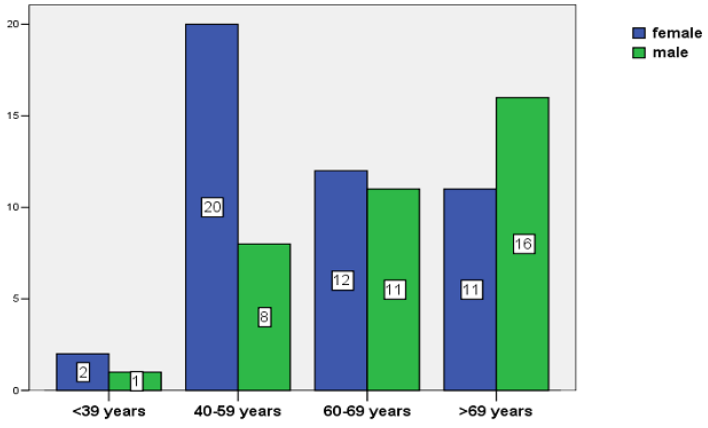

Fig. 1. Distribution by age groups

Tumor location. In our study, we have identified a predominance of AMs for non-skull base locations, in a percentage of 75.3%, compared to skull base meningiomas, which account for 24.6% of the AMs. According to our classification (tab. I), among non-skull base locations, most of the meningiomas were located at brain convexity (41.9%), while the second position was occupied by the parasagittal/falcine location (32%), consistent with studies that show similar percentages (13, 23, 24, 25) (fig. 2). The distribution of anatomical locations of tumors on age groups is similar both in women and men (fig. 3).

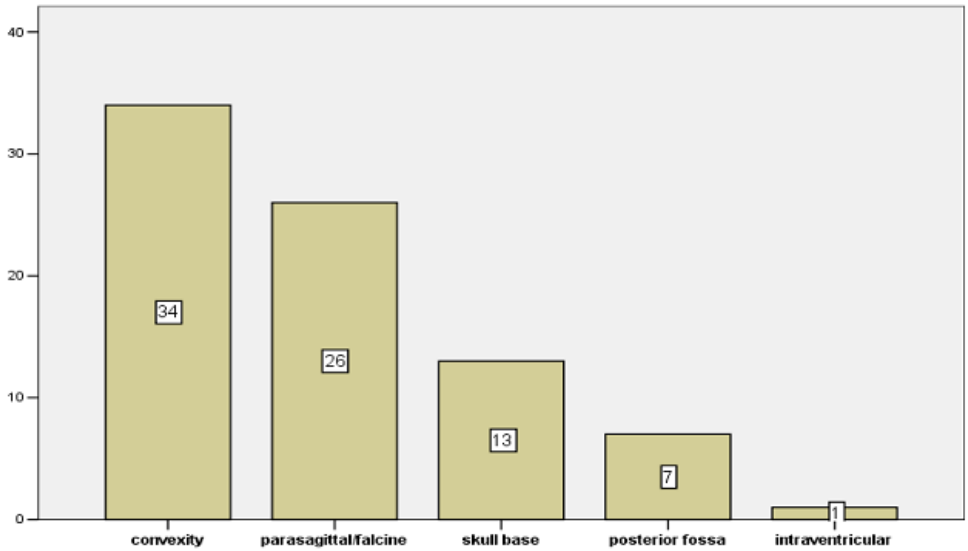

Fig. 2. Incidence of AMs according to anatomical location

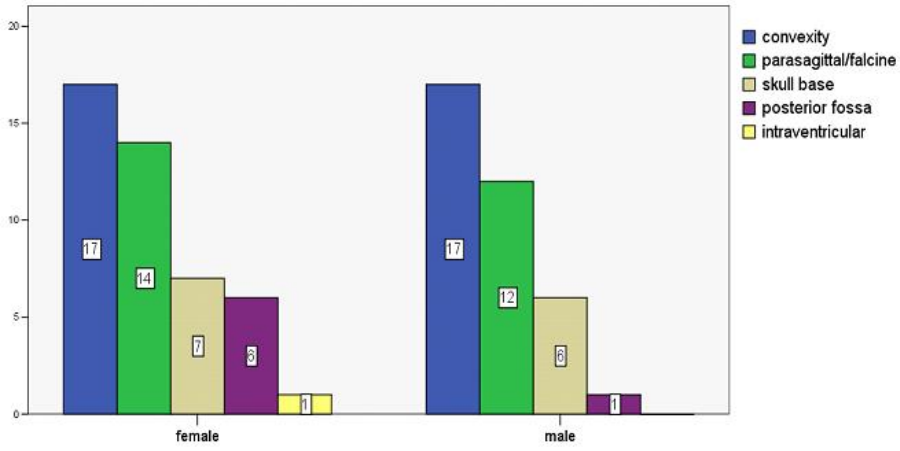

Fig. 3. Incidence of tumor location according to gender

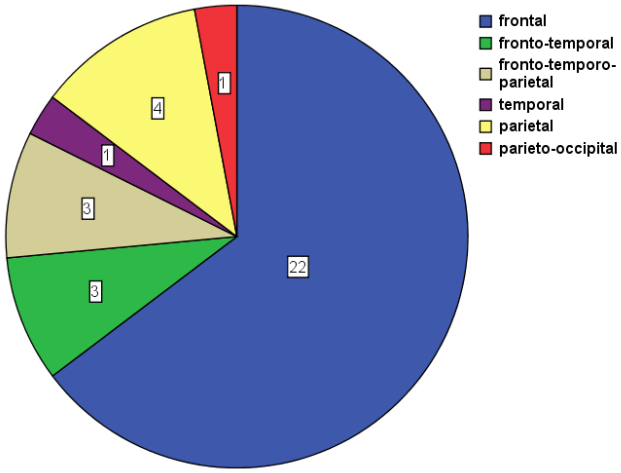

Fig. 4. Incidence of AMs according to anatomical location

Among the first studies on atypical and anaplastic meningiomas with a propensity for cerebral convexity, were those of Mahmood *et al*. and Maier *et al*. (26, 27). In a clinicopathological review of 319 patients, Mahmood *et al.* noticed that most of the atypical and anaplastic meningiomas were located in the cerebral convexities (26). Similarly, Maier *et al.* observed a higher incidence of atypical or malignant meningiomas in falcine or convexity locations in 252 of the patients (27). This preliminary evidence was about to change or at least become a challenge for the traditional notion according to which the histopathological behavior of meningiomas is not at all connected to their localization in the intracranial area (28). Most of the AMs (64.7%, 22 patients) in the brain convexity, were located in the frontal area, with a prevailing occurrence compared to other convexity locations consistent with the studies (29) (fig. 4).

Non-skull base tumors were shown to have an increased risk for grade II or III meningiomas, compared to skull base tumors (9). This disparity can be explained by the distinct embryologic origin of skull-base and non-skull dura. This tissue origin may lead to a propensity for developing different histologic neoplastic subtypes (10, 30, 31). These different subtypes have been associated with different genetic mutations, which could lead to the occurrence of different degrees in the aggressive behavior (32, 33). At the same time, gaining an understanding of these things could lead to the occurrence of specific adjuvant therapies and to the discovery of antiproliferative activity agents against meningioma as well as flavones and flavonols from wine (34, 35). In this regard, medicine faces various legal and ethical issues when it comes to finding the best treatment for the benefit of patients and to respecting their personal values (36, 37) and in which primary concern is the human being (38). Also, adaptation of the patient and his family to the oncological disease is very important (39).

Compared to adult gliomas, there is less available information on the cell origin of

meningiomas or the critical genetic changes that drive tumorigenesis (40). Indeed, gaining an insight into genetic etiology of meningiomas has its roots in inherited cancer predisposition in patients suffering from neurofibromatosis type 2, out of which 50% develop meningiomas (41).

Embryological studies have shown that meninges covering the brainstem may have different origins from those of the lateral skull base and convexities (10). On one hand, the meninges around the brainstem are thought to arise from the cephalic mesoderm, while the telencephalic meninges arise from the neural crest cells (28, 42, 43, 44). Hence, this differential meningeal embryogenesis can result in the predominance of one arachnoid cell type over other certain location, explaining by the aggressive biological behavior of certain meningiomas compared to others, in specific anatomical locations (10).

On the other hand, McGovern *et al.* proved in a study on 216 patients with WHO grade I, II or III, that benign cranial meningiomas arising outside the skull base, are more likely to recur at a higher WHO grade than skull base meningiomas and, moreover, grade I non-skull base meningiomas have a higher rate of cell proliferation than grade I skull base meningiomas. Interestingly enough, in this study, the MIB-1 labelling index of WHO grade I non-skull base meningiomas (2.60%) was significantly higher than that of WHO grade I meningiomas located at the skull base (1.35%). Through these records, the authors suggest that there might be underlying genetic differences in the behavior of non-skull base meningiomas and skull base meningiomas (11).

As for ***skull base meningiomas***, some studies have shown that there is a significantly higher incidence of benign meningiomas in skull base, even if it implies a more benign behavior, compared to non-skull base locations (10, 30). Furthermore, Lee *et al.* proved, in his study on 794 meningiomas of all histologic grades, that meningiomas of the midline/paramedian skull base were predominantly of grade I, namely, of meningothelial histological subtype, with percentages of 83.3-97.3% out of all the meningiomas located in the: cavernous sinus, clinoid process, optic sheath (30), with distorting the anterior visual pathways (45, 46).

Sade *et al.* suggests another explanation for the decreased incidence of malignant meningiomas in the skull base (10). He believes that skull base meningiomas have a higher propensity in showing early signs and symptoms, while the tumors are small-shaped, due to their proximity to cranial nerves and brainstem. At the same time, non-skull base meningiomas remain clinically silent and present large-shape tumors when diagnosed. This aspect gives tumor enough time to undergo additional molecular alterations, resulting in a malignant transformation (10), and may be a reasonable explanation for the higher incidence of grade II and III meningiomas in the elderly (47). In addition, studies have shown that meningiomas originating at the convexity carry more chromosomal anomalies than meningiomas originating at the skull base (48).

In terms of laterality, most of meningiomas (39.5%) were located at right convexity, and the rest (34.5%) at left convexity, with a right to left ratio of 1:1.1, with no significant differences. Other studies showed similar results but could not prove that meningiomas differ in right- or left-sided laterality (12, 49, 50).

CONCLUSIONS

Our study has proved the predominance of AMs in the brain convexity, while rarely occurring in the skull base. By this, we join the preexisting literature studies which reached the same conclusion, and we believe that further studies are necessary to help shed light on the role of genetic factors in the reports between anatomic location and tumor grade. This thing would be of great help in developing specific therapies for patients with aggressive meningiomas, especially AMs.

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UTILITATEA TESTULUI QUANTIFERON TB GOLD ÎN DIAGNOSTICUL MENINGITEI TUBERCULOASE LA COPIL

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THE UTILITY OF QUANTILFERON.TB GOLD IN CHILDREN TUBERCULOUS MENINGITIS DIAGNOSIS (Abstract) : **Material and methods** : We performed QuantiFERON-TB Gold in Tube (QFT-G) in blood and CSF at 40 children who was diagnosed with TB meningitis and at 39 children with non TB meningitis, admitted between October 2006 and December 2009. **Results** : The CSF analyses were suggestive for TB at 27 patients and only 14 had positive culture. The sensitivity of QFT-G in CSF was 72.72% and 69.44% in blood ; specificity 96.96% in CSF and 89.18% in blood ; the positive predictive value was 96% in CSF and 86.2% in blood ; negative predictive value was 78.04% in CSF and 75% in blood. The sensitivity of TST was 61.76% and specificity 82.05%. The sensitivity of the culture from CSF was only 35%. The sensitivity and specificity of QFT-G was higher than TST and culture and better in CSF than in blood. **Conclusions** : The determination of ă -interferon in serum and CSF is useful diagnostic marker of tuberculosis who could improve the management of TB meningitis. **Key words** : TUBERCULOSIS, MENINGITIS, INTERFERON GAMMA, BLOOD TEST, INTERFERON GAMMA, CHILD

Meningita tuberculoasă (TB) este o boală cu gravitate deosebită, impunând un diagnostic precoce şi o terapie etiologică şi patogenică promptă. În ciuda progreselor realizate în acumularea unor noi mijloace de diagnostic şi terapie patogenică, prognosticul rămâne, în multe cazuri. rezervat, decesul survenind la aproximativ 20-30% din bolnavi. Meningita TB este dificil de diagnosticat la copil datorită debutului adeseori atipic şi lipsei unor metode rapide şi sigure de diagnostic. Cultura pentru BK, considerată "standardul de aur", este pozitivă în LCR într-o proporţie de 12-30% din cazuri, iar rezultatele se obţin în 3-8 săptămâni (1, 2).

Apariţia testelor sangvine *in vitro* care măsoară cantitatea de interferon gamma eliberată de către limfocitele T sensibilizate, QuantiFERON TB Gold, pare a fi promiţătoare pentru diagnosticul precoce al meningitei TB. Performanţele testului la copii şi la pacienţii imunocompromişi nu au fost încă suficient clarificate. De asemenea, nu există încă studii extensive privind eficienţa executării testului din LCR. Scopul studiului a fost de a evalua contribuţia testului QuantiFERON TB Gold (QFT-G) în diagnosticul meningitei TB la copii (3,4,5).

MATERIAL ŞI METODE

Studiul se referă la 79 de copii internaţi, în perioada ianuarie 2006- decembrie 2009, în Secţia Boli Infecţioase Suceava şi în Clinica de Boli Infecţioase Iaşi. Lotul a fost împărţit în doua grupuri : primul a inclus

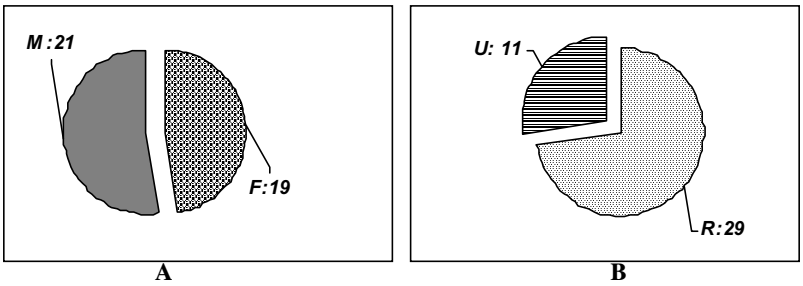

Fig. 1. Repartitia pe sex (A) şi mediul de proveniență (B)

40 de pacienţi diagnosticaţi cu meningita TB, iar al doilea grup martor alcătuit din 39 subiecţi (21- cu meningita virală; 12 – bacteriană; 1 cu toxoplasmoză cerebrală şi 5 fără inflamaţie a meningelui).

Investigaţiile au inclus: hemoleucograma, VSH, ureea, creatinina, glicemia, ionograma, ALAT; examenul LCR (aspect macroscopic, citologie, biochimie, microscopie directă, culturi pe mediul Lowenstein-Jensen); IDR cu 2 unităţi PPD; radiografia toracică; examenul CT cranio-cerebral şi al fundului de ochi; testul QuantiFERON TB Gold în sânge şi LCR, (test elaborat de compania Cellestis), pentru a doza cantitatea de interferon gamma în sângele integral proaspăt, heparinizat, după ce a fost incubat cu o mixtură de proteine sintetice, ESAT-6 şi CFP-10 (6).

Ultima generaţie a testului, QuantiFERON TB Gold in Tube, comportă trei vacutainere (un antigen TB, un control negativ şi unul pozitiv - mitogen). Dozarea interferonului gamma în LCR s-a efectuat folosind kitul QuantiFERON TB Gold cu pachete separate de antigene. După recoltare LCR-ul a fost transvazat într-un microtub uscat steril cu capac. Cu o pipetă sterilă s-a pus câte un ml LCR în minimum 2 godeuri ale unei plăci de culturi celulare pentru a se efectua cel puţin tandemul control negativ - ESAT; dacă a fost posibil s-a adăugat 1 ml şi pentru reacţia cu CFP-10. Deoarece imunocompetenţa pacienţilor a fost stabilită prin folosirea mitogenului în ser, acesta nu a mai fost folosit în cazul LCR-ului. Apoi se adaugă antigenele de stimulare (3 picături/ml), se agită 60 de secunde şi se incubează placa la 37°C. Citirea se face automat cu aparatul de tip ELISA pe baza unui software oferit de Cellestis, obţinând un răspuns calitativ de tip DA/NU. Rezultatele au fost formulate în termenii de negativ, pozitiv sau nedeterminat, considerând valoarea cutt-of de 0,35 UI/ml peste concentraţia controlului negativ.

REZULTATE ŞI DISCUŢII

1. Structura lotului studiat. Lotul de studiu a inclus 40 de pacienţi diagnosticaţi cu meningită TB în perioada 2006-2009, iar cel martor, a cuprins 39 de subiecţi cu meningită non-TB sau fără inflamaţie în LCR, la care s-a efectuat testul QuantiFERON TBGold în sânge şi LCR. Vârsta medie a pacienţilor a fost de 8.85 ani, cu limite între 3 luni - 17 ani, iar raportul dintre sexe a fost, aproximativ, egal (M: F =21: 19), cu diferenţe nesemnificative (p=0,05) pe medii cu creşteri în favoarea mediului urban (fig. 1); cei mai mulţi pacienţi s-au situat în grupul 5-14 ani (fig. 2).

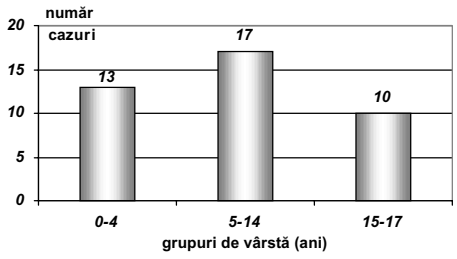

Fig. 2. Repartiţia pe grupuri de vârstă

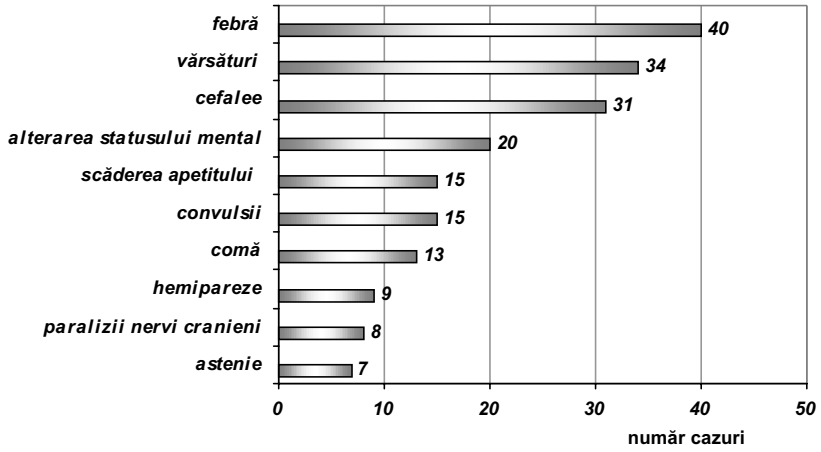

Fig. 3. Semne şi simptome semnificative pentru meningita TB

2. Datele clinice. S-a evidenţiat prezenţa unor semne şi simptome sugestive pentru meningita TB: sindrom febril şi subfebril prelungit (100%); sindrom meningean (77,5%); diminuarea apetitului (37,5%); sindrom encefalitic reprezentat de convulsii (37,5%), alterarea statusului mental, somnolenţă, agitaţie psihomotorie, confuzie (50%), comă (32,5%); paralizii de nervi cranieni (20%); hemipareze (22,5%) (fig. 3).

În unele cazuri simptomele de debut au dus la confuzii cu alte afecţiuni: 9 (22,5%) copii au prezentat tuse şi febră fiind trataţi pentru o afecţiune respiratorie, iar 7 (17,5%) au prezentat dureri abdominale, vărsături, febra, impunând diagnosticul diferenţial cu un abdomen acut chirurgical sau o boală diareica acută; 5 (12,5%) nu prezentau la internare semne de contractură meningeană, acestea fiind semnalate ulterior.

3. Datele epidemiologice. Din anamneza pacienţilor, a rezultat că 24,4% au avut un contact familial de TB şi întregul lot era vaccinat BCG. În ceea ce priveşte factorii favorizanţi s-a remarcat faptul că majoritatea pacienţilor (75,5%) proveneau din mediul rural, având condiţii socio-economice precare.

Din cei 40 de pacienţi cu meningită TB, 6 (15%) erau imunodeprimaţi: 5 cazuri infecţie HIV în stadiul de imunodepresie severă (limfocite CD_4 sub 200 celule/mm^3) şi unul cu leucemie acută limfatică, aflat sub chimioterapie citostatică.

3. Investigaţii paraclinice. Leucocitoza a fost prezentă la internare la 47,5% din cazuri 57,5% au avut un procent crescut de PMN şi 67,5%, VSH-ul a avut valori ridicate; radiografia toracică a fost sugestivă pentru TB pulmonară la 51,1% copii (5 cu formă miliară), 17 opacităţi lobare; o pleurezie TB); examenul CT cranio-cerebral a evidenţiat prezenta hidrocefaliei încă de la internare în 3 cazuri. IDR cu 2 unităţi PPD a fost efectuat la 85% din cei 40 de copii cu meningita TB, fiind excluşi cei şase pacienţi imunodeprimaţi. Citirea IDR-ului s-a efectuat la 72 de ore şi au fost consideraţi pozitivi pacienţii cu indurаţie peste 10 mm. IDR a fost pozitiv în 21 (61,7%) de cazuri. În lotul martor non-TB, IDR-ul a fost efectuat la toţi subiecţii: 32 (82%) au fost negativi şi 7 pozitivi.

Examenul LCR: la 27 (60%) din pacienţi au predominat limfocitele în LCR; glicorahia a fost scăzută la 28 (62,2%), albuminorahia crescută la 35 (77,7%) şi clororurorahia scăzută la 19 (42,2%) subiecţi, aceste date indicând suspiciune de meningita TB (2,3). Examenul microscopic direct nu a evidenţiat prezenţa BAAR la nici unul din pacienţi, iar cultura LCR a evidenţiat

TABELUL I

Rezultatele testului Quantil FERON TB Gold în ser

Rezultat	Meningita TB *		Martori non - TB	
	Nr. cazuri	%	Nr. cazuri	%
Pozitiv	25	62,5	4	10,25
Negativ	11	27,5	33	84,62
Nedeterminat	4	10	2	5,13
Total	**40**	**100**	**39**	**100**

* Pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL II

Rezultatul testului QuantiFERON TB Gold în LCR

Rezultat	Meningita TB *		Martori non - TB	
	Nr. cazuri	%	Nr. cazuri	%
Pozitiv	24	60	1	2,56
Negativ	9	22,5	32	82,05
Nedeterminat	7	17,5	6	15,39
Total	**40**	**100**	**39**	**100**

* Pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL III

Media variabilelor biochimice din LCR în funcţie de rezultatele testului QuantiFERON TB Gold

	QuantiFERON		Independent t test
	Pozitiv	negativ	
Albuminorahie(g/L)	2,13	1,83	t=-0,45, p=0,69
Glicorahie(g/L)	0,37	0,36	t=0,11, p=0,91
Clorurorahie(g/L)	6,90	6,79	t=-0,87, p=0,39

TABELUL IV

Aspecte comparative ale performanţelor testului QuantiFERON TB Gold, în ser şi LCR

	Comparaţie (%)	
	Ser	LCR
Sensibilitatea	69,44	72,72
Specificitatea	89,18	96,96
Predicţie pozitivă	86,20	96,00
Predicţie negativă	75,00	78,04

TABELUL V

Evaluări comparative privind sensibilitatea şi specificitatea metodelor utilizate în investigaţiile de laborator

Metode	Sensibilitatea	Specificitatea
Cultivare Lowenstein-Jensen	35,00	100,00
IDR 2 unităţi PPD	61,76	82,05
QFT – G ser	69,44	89,18
QFT – G LCR	72,72	96,96

prezenţa *Mycobacterium tuberculosis* în 14 (35%) cazuri. La un copil s-a izolat o tulpină cu rezistenţă la HIN. Intervalul mediu necesar cultivării *M. tuberculosis* a fost de 28,14 zile.

Rezultatele testului *QuantiFeronTB Gold* în ser şi LCR (tab. I, II, III) au evidenţiat diferenţe semnificative statistic ($p=0,05$) între constantele biochimice din LCR în funcţie de rezultatul testului QFT-G in LCR (tab. III). Performanţele testului Quanti-FERON în sânge şi LCR sunt comparabile

cu cele semnalate şi în alte studii. S-a realizat şi o evaluare comparativă privind sensibilitatea si specificitatea diferitelor metode utilizate pentru diagnosticul meningitei TB (6, 7, 8) (Tab. IV, V)

CONCLUZII

Testul QuantiFERON TB.GOLD a permis, coroborat cu datele clinice, epidemiologice şi examenul LCR, un diagnostic rapid (în 48 de ore) a unui număr de 25 cazuri de meningită TB, 12 (48%) dintre ele fiind confirmate şi bacteriologic.

Sensibilitatea şi specificitatea testului QuantiFERON TBGOLD în LCR a fost superioară IDR la tuberculină fiind mai mari în LCR decât în sânge. Performanţele testului QuantiFERON TBGOLD au fost influenţate negativ de terapia antiTB anterioară testării.

Rezultatele negative sau nedeterminate ale QuantiFERON TBGOLD sunt mai frecvente la copiii mici şi în cazul unei reacţii inflamatorii reduse în LCR. Din cei 6 pacienţi imunodeprimaţi diagnosticaţi cu meningita TB, 4 au avut rezultate negative la QFT-G.

Deşi cultura pentru micobacterii rămâne „standardul de aur" în diagnosticul meningitei TB, noile teste imunologice bazate pe eliberarea interferonului gamma par a fi promiţătoare pentru managementul acestei boli, testul QuantiFERON TBGOLD fiind avantajos prin tehnica sa simplă, timpul scurt de realizare şi costurile medii. De aceea, se consideră că testul QuantiFERONTB Gold este o metodă rapidă de diagnostic al meningitei TB la copil, care ar putea îmbunătăţii prognosticul printr-o iniţiere precoce şi adecvată a terapiei TB.

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MATERNAL-FETAL ATTACHMENT - UPDATE ON THE ROLE OF THREE-DIMENSIONAL, FOUR-DIMENSIONAL AND HD LIVE ULTRASOUND

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MATERNAL-FETAL ATTACHMENT - UPDATE ON THE ROLE OF THREE DIMENSIONAL, FOUR-DIMENSIONAL AND HD LIVE ULTRASOUND (Abstract): The continuous improvement in obstetrics imagery during the last few decades has contributed in the refining of the prenatal diagnosis but also to enhance the maternal fetal bonding. Two-dimensional (2D) ultrasound images have a positive influence on the attitude of expectant parents toward the pregnancy by enhancing the parental-fetal bond and reducing anxiety in parents. The introduction of three-dimensional (3D) ultrasonography (US) created the opportunity of having a non-invasive technique with easily understood images of the fetus. HD Live technology is a novel US technique that improves the quality of the 3D/4D images. HD Live uses an adjustable light source and software that calculates the propagation of light through surface structures in relation to the direction of light. The HD Live photos are more impressive than the classic 3D pictures, and parents usually request photos. Three-dimensional HD Live enables detailed observation of the fetal external anatomy beginning with the first trimester and doesn't increase significantly the examination time. HD Live may play a role in strengthening this relationship between parents and their future child. **Keywords**: MATERNAL-FETAL ATTACHMENT, HD LIVE ULTRASONOGRAPHY, THREE-DIMENSIONAL, FOUR-DIMENSIONAL ULTRASONOGRAPHY.

The spectacular development in obstetrics imagery during the last few decades has contributed to improving and refining prenatal diagnosis. Previously, what could only be suspected by traditional methods (clinical examination and sometimes medical intuition) became certainty by using ultrasonography.

Maternal-fetal attachment (MFA)

Pregnancy is characterized as a physiologically and biologically interdependent experience, wherein mother and child develop a bond that is qualitatively distinct, unlike any other. The maternal-fetal bond is the strongest bond in humans, and this bond begins often shortly after conception (1). The terms bonding and attachment are used interchangeably in the literature to indicate the feelings that parents have toward their fetus (2). Bonding is the parent's tie to the neonate during the first hours after birth (3); attachment is the relationship between the infant and caregiver that develops during the

first year of life (4). Cranley defined maternal-fetal attachment as: "the extent to which women engage in behaviors that represent affiliation and interaction with the unborn child" (5, 6).

Research on the impact of 2D ultrasound on fetal-parental bonding suggests that these images have a positive influence on the attitude of expectant parents toward the pregnancy by enhancing the parental-fetal bond, reducing anxiety in parents, and increasing awareness on the importance of maintaining maternal health (7, 8).

Three-dimensional and four dimensional (4D) US were first demonstrated nearly 15 years ago but are becoming part of a clinical routine in recent years. The use of 3D and 4D US in obstetrics has many advantages, including providing of an enhanced diagnosis of fetal anomalies, allowing accurate volume measurement of organs with irregular shape, and offering the possibility of storing the volumes (9, 10, 11).

The introduction of 3D US impacted not only the medical community, but also attracted attention from the patients and media because for the first time, technology created the opportunity to have non-invasive, realistic, easily understood images of the fetus. Moreover, the introduction of 4D US into clinical practice allows a better viewing of facial expressions, such as mouth opening, tongue protrusion, yawning, smiling, eye opening and blinking. Visualization of the fetus by the mother may arouse emotions capable of triggering or improving maternal-fetal bonding and may lead to changes in behavior and lifestyle that promote maternal and fetal health (9).

Research on the impact of 3D/4D US on maternal bonding has reported contradictory results. Pretorius et al. analyzed 124 mothers and 65 fathers after 3D US examination using a questionnaire relating to how they felt about their fetus before and after 3D/4D US. They concluded that parents have a change of attitude regarding their fetus after seeing 3D/4D US. (2) Ji et al. compared maternal-fetal bonding between 50 mothers exposed to 2D US only and 50 mothers exposed to both 2D US and 3D US. For all categories of maternal-fetal bonding, patients who had 3D US examinations consistently scored higher than those who had a 2D US examination only (10, 11).

In his study with over 68 pregnant women, Segmen stated that both 2D and 3D US have a positive impact, but 3D US offers no enhanced benefits in comparison to 2D US. (13) Rustico *et al.* conducted a randomized clinical trial with 100 pregnant women during the second trimester to evaluate whether adding 4D US to the conventional 2D US could influence maternal emotional status. No difference in the proportion of women with a positive response to 2D US or 2D US plus 4D US was observed. In addition, when the investigators applied a validated instrument to evaluate maternal-fetal bonding (Maternal Antenatal Attachment Scale) to a subset of 46 patients enrolled in the study, no difference between the two groups in quality and intensity of attachment or global attachment score was identified. (14)

To be relevant and useful for improving fetal-parent attachment, both 2D and 3D images should be as clear and real as possible. With 3D US, technical developments, such as the electronic fetal scalpel, which allows the removal of unwanted information from the volume data set, have been reported to improve the image quality for visualization of the fetal face in approximately 70% of the cases. (15) The latest technological development in US is high-definition HD Live technology and surely

deserves attention regarding MFA.

HD Live technology

HD Live technology is a novel US technique that improves the 3D/4D images. HD Live uses an adjustable light source and software that calculates the propagation of light through surface structures in relation to the direction of light (16). The virtual light source produces selective illumination with the respective shadows created by the structures where the light is reflected (17). This possibility of using HD Live technology to create lighting and shadowing effect increases depth perception. The combination between lights and shadows produces remarkable images that are much more natural than those obtained with classic 3D US. The virtual light can be placed in the front, back or lateral sides, actually where viewing is desired.

HD Live could be used throughout the whole pregnancy. In the first trimester of pregnancy, images that resemble those usually seen in atlases or in media could be very impressive for parents (18, 19). The introduction of 3D/4D US with HD Live in the first trimester of pregnancy has resulted in remarkable progress in ultrasonographic visualization of early embryos and fetuses, and development of new fields of 3D sonoembryology (fig. 1). The HD Live photos are more impressive than the classic 3D pictures, and parents usually request photos or record them. According to Ji et al, mothers exposed to 3D US had a higher tendency to show their US images to other people and to form a mental picture of the baby after the examination than those having only 2D US (82% *vs.* 39%; $p < .001$) (12). Due to natural and artistic image of the fetus, surely the HD Live pictures would be shared with relatives or friends at least as often as classic 3D US.

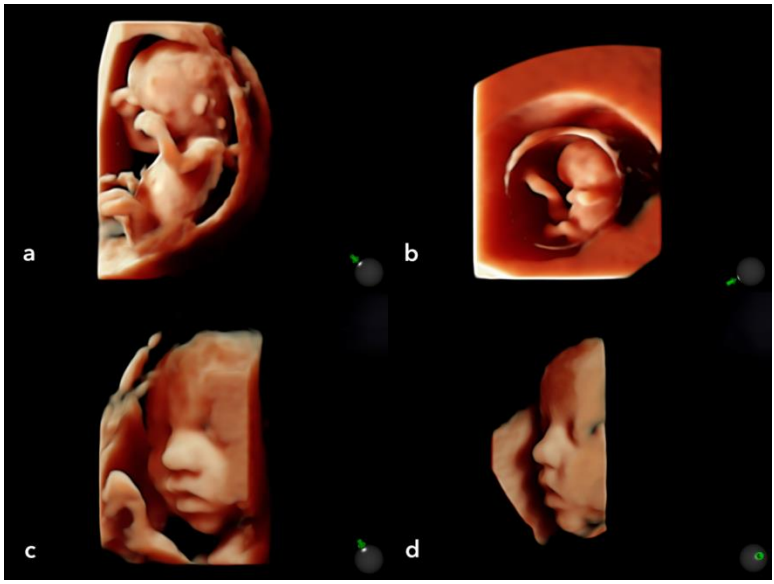

Fig. 1. a. Pregnancy in the first trimester of pregnancy- 12 weeks of amenorrhea.
b. Pregnancy in the first trimester of pregnancy-9 weeks of amenorrhea.
c. Pregnancy in the third trimester of pregnancy- Fetal face.
d. Pregnancy in the third trimester of pregnancy- Fetal profile.

Although the messages from patients are clearly positive, the exact role of HD Live technology in the maternal fetal attachment remains to be established in the future. Because of the more natural and realistic images, it is expected that HD Live will favor and enhance this bonding. Being a new technology, no studies are available yet in the literature to analyze the relationship between the bonding and HD Live. We believe that studies regarding the role of HD Live in improving the fetal-maternal bonding are necessary and will be available in the near future.

Current and future development

Embryonic development is a fascinating area, which might be followed using the ultrasound scanning technique. There has been an immense acceleration in understanding the early human development in the last years due to the newest ultrasound techniques: 3D/4D ultrasound, Doppler ultrasound, tomographic ultrasound images, HD Live (16, 20).

HD Live enables a detailed evaluation of the fetal face from the first trimester and reveals that a small fetus is not more a fetus but a "person" even in the first trimester of pregnancy.

Three-dimensional HD live enables a detailed observation of the embryonic anatomy beginning with the first trimester and in this way, it makes possible to develop new relationships inside the family (19). HD Live represents one of the most promising forms of non-invasive diagnostics in order to study and follow embryo development.

CONCLUSIONS

In actual practice, 3D and 4D US are becoming an important part of state-of-the-art ultrasonographic imaging in obstetrics. HD Live is a new, emerging technology that shows remarkably high-quality images of the embryo and fetus.

Motherhood is an extraordinary opportunity, for both the mother and child, to participate in a relationship that is irreplaceable in value and meaning.

HD Live may play a role in further strengthening this relationship between parents and their future child, but the exact role remains to be established.

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UNELE EVALUĂRI EPIDEMIOLOGICE ŞI MEDICO-LEGALE ASUPRA INCIDENŢEI SINUCIDERILOR ŞI A ALTOR CAUZE ALE MORŢII VIOLENTE, COMPARATIV, LA NIVEL NAŢIONAL ŞI ÎN JUDEŢUL IAŞI, ÎN PERIOADA 1997-2006

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SOME EPIDEMIOLOGIC AND FORENSIC MEDICAL ASSESSMENTS ON SUICIDE INCIDENCE AND OTHER CAUSES OF VIOLENT DEATH IN ROMANIA AND IAŞI COUNTY BETWEEN 1997 AND 2006 (Abstract): The study included information collected in the reports of Forensic Medicine Institutes, at the national level, processed and interpreted using epidemiological and biostatistical methods. **Results**: In Romania, at the national level, the annual values of suicide incidence increased from 18 to 30% and in 70-80% of cases, other causes led to violent death (intoxication, precipitation, submersion, burning, cut wounds, shooting a.s.o.). In Iaşi County, the annual values of suicide incidence varied between 1.1 and 2‰ inhabitants, and the main cause was hanging registered in 60.2% of cases in 31-60 age groups, with male predominance. **Conclusion**: Results are useful for the orientation of educational prevention programs. **Key words**: SUICIDE, VIOLENT DEATH, EPIDEMIOLOGY, FORENSIC MEDICINE

În mod obişnuit, se consideră că suicidul este un act de autoprivaţiune, conştientă, de viaţă a individului care, deseori, interferează cu accidentele şi omorurile. Aceste trei tipuri de privaţiune de viaţă a omului au simptome similare şi se raportează ca moarte violentă (1,2,3). De aceea, nu poate fi considerat suicid autoprivaţiunea de viaţă a unui individ aflat în stare de inconştienţă sau moarte care a survenit din neglijenţa victimei (4,5).

În unele situaţii, individul comite anumite acţiuni autoagresive, care demonstrează suicidul, prevăzând posibilitatea morţii, dar sperând că va rămâne în viaţă („suicid demonstrativ"). Dacă în asemenea cazuri se înregistrează sfârşitul letal, fapta va fi calificată nu ca suicid, ci ca deces din lipsă de precauţie (6,7).

Caracterul complex al unor evaluări epidemiologice şi medico-legale asupra morţii prin suicid este determinat de faptul că, deseori, familia victimei încearcă, din anumite considerente, să ascundă cauza adevărată a decesului, prezentându-l ca fiind urmarea unui accident, modificându-se, astfel, imaginea generală asupra cazului aflat în cercetare (8,9,10).

În cele ce urmează, s-a urmărit evaluarea descriptivă a factorilor incriminaţi în inducerea suicidului, la nivel naţional, comparativ cu judeţul Iaşi, cu implicaţii educa-

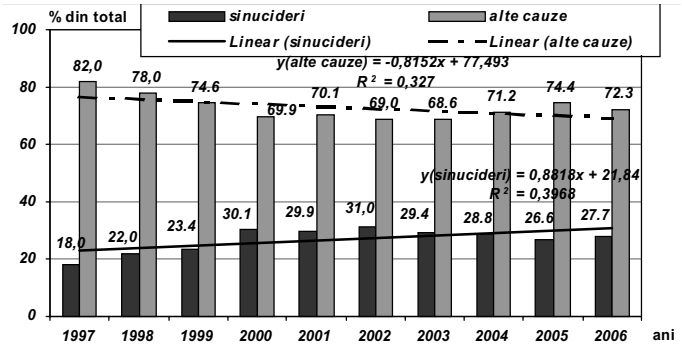

Fig. 1. Incidenţa sinuciderilor şi altor cauze de moarte violentă, înregistrate în România, în perioada 1997-2006

ţional-prevenţionale.

MATERIAL ŞI METODE

În studiu au fost incluse informaţiile acumulate la nivel naţional, prin evaluarea rapoartelor privind sinuciderile şi alte cauze ale morţii violente, realizate de Institutele de Medicină Legală, prelucrate şi interpretate prin metode epidemiologice şi biostatistice, în perioada 1997-2006.

REZULTATE ŞI DISCUŢII

1. Sinuciderile şi alte cauze ale morţii violente. Evaluarea dinamicii fenomenului suicidar, la nivel naţional şi, comparativ, în judeţul Iaşi, a evidenţiat, pentru perioada 1997-2006, valori ale incidenţei anuale ale sinuciderilor cu creşteri semnificative ($p < 0,05$) de la 18-23,4% în anii 1997-1999, la valori apropiate de 30% în ceilalţi ani (fig. 1). În afară de sinucideri, moartea violentă a fost provocată şi de alte cauze, cu diferenţe anuale nesemnificative ($p > 0,05$), cuprinse într-o medie de 70-80% din total (tab. II).

2. Evoluţia anuală a incidenţei sinuciderilor în judeţul Iaşi. În perioada 1997--2006, valorile de incidenţă a sinuciderilor de toate cauzele au înregistrat diferenţe anuale nesemnificative ($p > 0,05$). În 4 ani, acestea au fost de 1,1‰ locuitori, în alţi 4 ani, 1,2-1,3, cu valori de 1,6 în 2006 şi 2‰ în 2003 (tab. II).

În anul 2003, cu rata cea mai ridicată a sinuciderilor (2‰ loc.), analiza mecanismelor de producere a leziunilor tanato-generatoare a evidenţiat situarea, pe primul loc, a spânzurării (70%), urmată, la mare dis-

TABELUL I

Principalele cauze ale sinuciderilor evidenţiate prin autopsii medico-legale, înregistrate la nivel naţional, în perioada 1998-2006

Anul	Cauze - % din total *						
	Precipitare	Spânzurare	Intoxicații	Plăgi tăiate	Submersie	Împuşcare	Altele
1998	14	6	4	1	1	0	74
1999	10	65	14	0	8	1	2
2000	31	50	12	0	0	1	6
2001	23	52	14	0	0	2	9
2002	9	40	10	1	3	1	6
2003	9	72	8	0	4	0	7
2004	8	70	10	3	5	1	3
2005	3	77	11	2	3	1	4
2006	6	78	8	2	3	1	2

* pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL II

Evoluţia numărului de sinucideri înregistrate în judeţul Iaşi, în perioada 1997-2006

Anul	Populaţia	Cazuri	
		Nr.	‰ooo
1997	813713	90	1,1
1998	818103	94	1,2
1999	822005	93	1,1
2000	825855	92	1,1
2001	829437	94	1,1
2002	807777	105	1,3
2003	808887	147	2,0
2004	808595	107	1,3
2005	808886	107	1,3
2006	824083	130	1,6

tanţă, de precipitare (9%), intoxicaţii (8%), submersie (4%), arsuri (1%), electrocuţii (1%). În categoria "alte metode", s-au inclus 5% din cazuri (fig. 2).

3. Repartiţia pe grupuri de vârstă a sinuciderilor, în judeţul Iaşi. În perioada 1997-2006, cumulativ, la % din total, grupurile de vârstă implicate în sinucidere au prezentat diferenţe semnificative ($p < 0,05$): 41-50 ani (22,2%); 31-40 ani (19,1%); 51-60 ani (18,9%). S-a observat că intervalul demografic 31-60 ani a înregistrat 60,2% din total cazuri de sinucidere. Aceste grupuri de vârstă se caracterizează printr-o intensă inserţie socială, care le expune unor stări de stres, adesea conflictuale, cu frecvente riscuri suicidare (fig. 3).

4. Evoluţia sezonieră şi pe sex a sinuciderilor, înregistrate în judeţul Iaşi. În cei 10 ani luaţi în studiu, incidenţa, evaluată cumulativ, a sinuciderilor, a avut o dispersie multilunară, cu creşteri semnificative ($p < 0,05$) în intervalul aprilie – iulie, situaţie care poate fi explicată, cel puţin în parte, prin accentuarea sezonieră a stărilor conflictuale, sociale şi familiale, inclusiv cu implicarea consumului, în exces, a alcoolului.

Repartiţia pe sex a cazurilor de suicid, a evidenţiat o diferenţă puternic semnificativă ($p < 0,001$), cu predominanţa bărbaţilor, mai frecvent implicaţi în stări conflictuale, deseori asociate consumului excesiv de alcool.

CONCLUZII

Caracterul complex al unor evaluări epidemiologice şi medico-legale asupra morţii prin suicid este determinat şi de faptul că, deseori, familia victimei încearcă să ascundă cauza adevărată a decesului,

În perioada 1997-2006, la nivel naţional, valorile anuale ale incidenţei sinuciderilor au înregistrat creşteri semnificative, de la 18-23,4% în 1997-1999, la valori apropiate de 30% în ceilalţi ani. În 70-80% din total cazuri, moartea violentă a fost provocată de alte cauze.

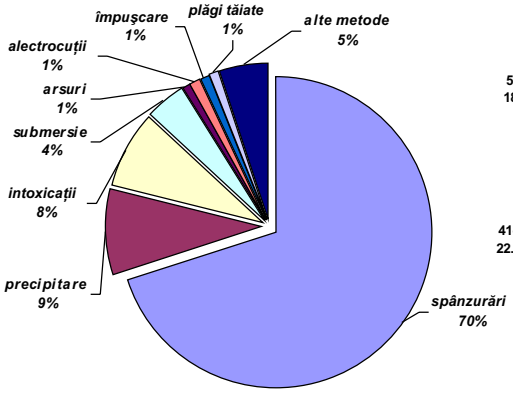

Fig. 2. Modalităţi de sinucidere, înregistrate în judeţul Iaşi, în anul 2003 (sinucideri – 2‰ooo loc.)

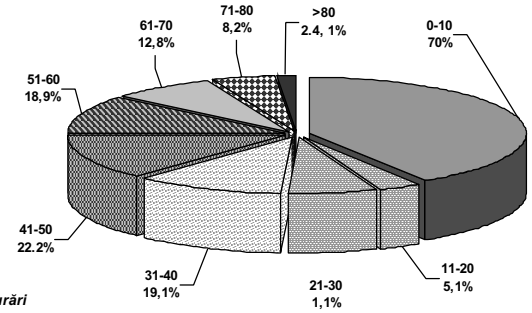

Fig. 3. Repartiţia fenomenului suicidar pe grupuri de vârstă, în judeţul Iaşi, cumulativ pentru perioada 1997-2006

În judeţul Iaşi, în aceeaşi perioadă, valorile anuale ale incidenţei sinuciderilor au variat între 1,1‰ loc, cu excepţia anului 2006 (1,6‰) şi 2003 (2‰) când, între cauzele suicidului, pe primul loc s-a situat spânzurarea.

Cele mai multe cazuri de sinucideri s-au înregistrat, în fiecare an, în lunile aprilie-iulie, cu o diferenţă puternic semnificativă, cu predominanţa bărbaţilor, mai frecvent implicaţi în stări conflictuale, deseori asociate consumului excesiv de alcool.

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NOUTĂŢI

VIRULENŢA QUORUM SENSING-MEDIATĂ A TULPINILOR DE *Pseudomonas aeruginosa* CE COLONIZEAZĂ PACIENŢII CU VENTILAŢIE MECANICĂ

Pseudomonas aeruginosa colonizează frecvent pacienţii intubaţi şi este cauza pneumoniei asociată ventilaţiei (ventilator-associated pneumonia -VAP). *Köhler* şi colaboratorii au încercat să determine rolul quorum sensing (QS) ce reglează expresia factorilor de virulenţă în cursul colonizării cu tulpini de *P aeruginosa* şi dezvoltării VAP. În acest scop au fost studiate izolatele de *P aeruginosa* din aspirate traheale recoltate de la 29 de pacienţi intubaţi, dintre care 6 au dezvoltat VAP. Genotipurile şi factorii de virulenţă QS-independenţi (exoU, exoS, PAPI-1 şi PAPI-2) ale izolatelor de *P aeruginosa* ce colonizează aceşti pacienţi au fost identificate *in vitro* utilizând testele CLONDIAG. Pentru a evalua virulenţa QS-dependentă a fost măsurată producţia de elastaza şi ramnolipide. Pentru a monitoriza activitatea QS *in vivo*, ARN-ul total a fost extras direct din aspiratele traheale şi a fost măsurată expresia genelor QS. Dintre toţi factorii de virulenţă testati, numai ramnolipidele au fost asociate cu dezvoltarea VAP ($p = 0,003$), iar VAP a apărut mai frecvent la pacienţii colonizaţi cu tulpini producătoare de niveluri ridicate de ramnolipide ($p = 0,001$). Monitorizarea genelor QS *in vivo* a arătat expresia genelor ţintă la pacienţii cu VAP. În concluzie, producţia de ramnolipide QS-dependentă la izolatele de *P aeruginosa* este în legătură cu dezvoltarea pneumoniei asociată ventilaţiei mecanice (Köhler T, Guanella R, Carlet J, van Delden C. Quorum sensing-dependent virulence during *Pseudomonas aeruginosa* colonisation and pneumonia in mechanically ventilated patients. *Thorax* 2010 ; 65 (8) : 703-710).

Teodora Vremera

THE IMPORTANCE OF THE MICROBIOME IN THE ALZHEIMER DISEASE PATHOLOGY - THE POSSIBLE RELEVANCE OF SOME METABOLIC DEFICIENCIES

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THE IMPORTANCE OF THE MICROBIOME IN THE ALZHEIMER DISEASE PATHOLOGY-THE POSSIBLE RELEVANCE OF SOME METABOLIC DEFICIENCIES (Abstract): With the latest increased awareness on the connections that might exist between the modifications of microflora and most of the neuropsychiatric disorders, we focused our efforts from the present mini-review on discussing the possible interactions between the pathophysiology of Alzheimer's disease (the most important neuropsychiatric disorder, as judged by the number of patients affected worldwide) and microflora functioning. In addition, we speculate here about the importance of some related metabolic modifications on the context of the aforementioned importance of the microbiome in the Alzheimer's disease pathology. Keywords: MICROBIOME, ALZHEIMER'S DISEASE, METABOLISM.

Alzheimer disease (AD) is the most frequent, irremediable and gradual cause of dementia and neurodegenerative disorder among people over the age of 60, with the symptoms of the disease being described for the first time (early 19th century) by the German psychiatrist Aloysius "Alois" Alzheimer.

It can be characterized by either a late-onset (more than 95% are sporadic - sAD), but, at the same time, it can be considered a "genetic heritage" with a predisposition of appearance before the age of 60, defined by an early-onset (about 5% of them are familial - fAD).

This disease leads to a generalized damage to the brain, especially at those levels involved in cognition and behavior, by 2018 accounting for around 70% of the cases (almost 50 million people), generating gigantic costs, with a tripling of numbers expected by 2050.

Living in a microbial world, evolving in tandem with the microbiome - the total of microorganisms that populate different regions of the body, and in particular our intestines (1), lately, a growing interest has emerged in this "symbiosis", that highlights a close relationship between gut microflora and the brain.

It has been found that each individual hosts numerous communities of microbes (bacteria, viruses, fungi and archaea), this

collectivity fulfilling key functions for our health, such as biosynthesis of vitamins, provides protection against pathogen overgrowth, energy modulation and immunological, producing small bioactive molecules that mediate interactions with the host and contribute to the neurohormonal axes. Although the tendency to believe that each individual possesses the same microflora, various genetic studies indicate an inter-individual variation between the human gut microflora of each individual. For example, twins present an almost identical microbiome compared to that of siblings. However, a lot of variations are not completely elucidated, these, possibly having to do with the co-incidental colonization and stabilization of an ecosystem in a niche formed by genetics, age, biotope and nutrition (2, 3, 4).

Deregulation and dysbacteriosis of the gut microflora is correlated with a multitude of diseases in human including endocrine disorders (type 2 diabetes, obesity), dysmetabolic syndrome X, allergies, inflammatory bowel disease, colorectal cancer and Alzheimer disease (5), interactions between host and its intestinal microbiota being quite complicated, influence exerted on the adjacent organs as well as on those at a greater distance taking place through multiple pathways (6).

Current state of knowledge regarding microbiome and pathology of AD

With the completion of the human genome project in 2003, the total number of genes constituting our own DNA was estimated to about 25,000 gens. Higher pluricellular organisms represent the ideal amphitrions of a microscopic world, better known as microbiota or microbiome, forming an "super-organism", populating it from the early hours of life, total number of microbial genes being approximately 150 times higher. Compared to an individual adult whose body is estimated to be formed from about 10^{13} cells, it is currently speculated that the human microbiome is composed from about ten times more cells, ~ 10^{14} (a hundred thousand billion bacteria). Subsequently, they were grouped into three enterotypes: *Bacteroides*, *Prevotella*, and *Ruminococcus*.

Because most of them are found in the intestine, initially was thought that the gut microflora comprised between 500-1,000 species of microbes, nearly 3,500 were identified so far (7, 8). Researchers claim that they produce about 1.5 kilograms of biomass, the main genres being divided into three categories (9), containing strictly anaerobic entities, apparently, the most important role being attributed to those facultatively anaerobic such as enterobacteria and enterococci found in a rather large number in the gastrointestinal tract (GI) of neonates, their whole existence depending on the supply of dietary carbohydrates that have escaped digestion. It can be concluded that the carbohydrate mixture that enters the colon resulting from food intake, nutritional digestion, and absorption, exerts significant action upon them, creating favorable conditions for the subsequent development of those anaerobic (10).

1. *Firmicutes*, *Bacteroides*, *Escherichia coli*, *Enterobacteriaceae* and *Veillonella* - neutral;

2. *Bifidobacterium*, *Lactobacillus*, *Eubacterium*, *Fusobacterium*, *Campylobacter jejuni* - potentially beneficial;

3. *Clostridia*, *Staphylococcus*, *Proteus*, *Pseudomonas aeruginosa* -potentially harmful.

In his review, Szablewki summed up the composition and concentrations of human gut microflora in a healthy digestive tract system according to the location of the main classes of microorganisms (11).

As mentioned above, gastrointestinal tract (role in maintaining the homeostasis

and symbiosis between host and his "tenants") houses trillions of symbiotic microbes due to its interface (250-400 m^2) between the host, antigens and exogenous factors that influence normal physiology and alter the host's susceptibility to disease, essential and beneficial as well for an appropriate development of central nervous system and brain responses (12, 13, 14). Cells from the central, enteric and peripheral nervous systems form a neural network capable of sensing and responding to endo- and exogenous, as well as to the environmental ones (6), in association with hypothalamic-pituitary-adrenal axis giving birth to the so-called gut-brain axis (15).

Results of studies published over the past 10 years support the concept of bidirectional gut-brain interactions and *vice versa*, which are insufficiently understood, including metabolic, immune, endocrine and neural pathways. Central nervous system, gut and immune functions are modulated through this axis, germ free models (GF) used reflecting a role in host cognition or AD-related pathogenesis, moreover, in maintaining the integrity of the blood-brain barrier, also dependent on the microbiota and especially microbial products, short-chain fatty acids (16, 17, 18, 19).

Recent studies in which murine lines and human subjects (alive and post-mortem) have been used in the relevance of AD pathology are summarized in tab. I and II.

TABLE I

Recent studies that highlights the close relationship between AD and microbiota deregulation

Model	Procedure/Aim	Observations	Reference
Rat	intrahippocampal injection of β-amyloid	Alzheimer probiotics *Lactobacillus acidophilus-fermentum* and *Bifidobacterium lactis - longum* improves learning process, memory and ameliorates oxidative stress	(20)
Rat	injection with D-Galactose	*Lactobacillus plantarum* MTCC1325 ameliorates cognitive deficits, restored acetylcholine level and reduced amyloid plaques formation	(21)
5x fAD male mice	analysis of fecal proteins	A reduced quantity of trypsin, as well as a change of the microflora, closely related to aging.	(22)
3x transgenic AD mice	mixture of lactic acid bacteria and bifidobacteria	SLAB51 diminishes the oxidative stress in the AD mice brain by triggering the activity of a class III deacetylase histone (SIRT1)	(23)
ApoE$^{-/-}$ mice	infection with *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia* and *Fusobacterium nucleatum*	Results indicate that *Porphyromonas gingivalis* has the ability to access the ApoE$^{-/-}$ mice brain	(24)
APP/PSEN1 transgenic mice	comparative study	*Odoribacter* and *Helicobacter* genus significantly increased in APP/PSEN1 mice, and *Prevotella* genus in wild type mice	(25)
APP/PSEN1 transgenic mice	long-term broad-spectrum combinational antibiotic treatment	In APP/PSEN1 mice treated ABX, take place changes in gut microbiome as well as at circulant inflammatory mediators. Likewise, they display reduced Aβ deposition, but increased soluble Aβ, reactive gliosis surrounding the plaques being reduced	(26)

Model	Procedure/Aim	Observations	Reference
APP/PSEN1 transgenic mice	comparative study	All specimens used exhibit the same bacterial profile in the first 3 months. After 6 months, *Turicibacteriaceae* and *Rikenellaceae* increased in all groups, *Bacteroidetes* total remaining invariable. Transgenic mice were defined by an increase of *Proteobacteria* (*Sutterella*), also encountered in autism spectrum disorders cases. Furthermore, *Erysiopelotrichaceae* family was more abundant in transgenic mice, whose activity is related to inflammatory reactions.	(27)

TABLE II

Recent studies realized on human subjects (alive and post-mortem) that highlights the close relationship between gut microflora and the brain

Number of subjects	Observations	Reference
53 AD patients	AD patients infected with *Helicobacter pylori* presented a pronounced deficiency of cognitive processes	(28)
25 AD patients and 25 as control	A change in the gut microflora has been observed; a decrease in abundance of *Firmicutes* and *Bifidobacterium*, and an increase in abundance of *Bacteroidetes*	(2)
35 AD, 46 MCI and 77 as control	Of the seven oral bacteria associated with periodontitis, following testing of immunoglobulin G antibody levels, only in the case of *Fusobacterium nucleatum* and *Prevotella intermedia* levels were significantly increased in AD compared to the controls	(29)
60 participants; 30 treated with milk or a mixture and 30 as control	At the end of the twelve weeks, Mini-Mental State Examination score was considerably higher	(30)
38 elderly	An association between periodontal diseases and Aβ load was observed by using C-Pittsburgh compound B and positron-emission tomography imaging	(31)
28 individuals	Amyloid precursor protein, interleukin 1β and complement C1q subcomponent subunit A mRNA levels were considerable upregulated in periodontitis affected gingival tissues	(32)
10 AD and 10 as control	Lipopolysaccharides from periodontal bacteria can access the AD brain during life as much or even longer after the patient's death (relatively equivalent range).	(33)
25 AD, 19 MCI and 21 as control	Results revealed an increased levels of tumor necrosis factor alpha and interleukin 6 in the patients with AD/MCI and periodontitis after performing the following tests: MMSE, FAQ, periodontal and oral evaluation, blood concentrations of interleukin 1, 6 and the tumor necrosis factor alpha.	(34)
24 AD and 18 as control	The levels of *Escherichia coli* K99 and lipopolysaccharides were higher in the case of patients with AD compared with controls, lipoglycans colocalized with Aβ1-40/42 in amyloid plaques and around vessels in AD brain	(35)

Number of subjects	Observations	Reference
10 AD and 4 as control	Spirochete-specific DNA of *Borrelia burgdorferi* found in senile plaques	(36)
5 AD and 5 as control	Results indicate a characteristic presence intracellular and one non-conformal extracellular of *Chlamydia pneumoniae* antigens present in the frontal and temporal cortices of the AD brain. Furthermore, *C. pneumoniae*, amyloid plaques and neurofibrillary tangles are found in the same region of the brain there being a incongruity one to another	(37)

The relevance of metabolic and oxidative stress related deficiencies in AD-microbiome interactions

Complex pluricellular organisms, such as mammals, are provided with adequate mechanisms, ensuring the transport of oxygen to all the cells that need it (38). However, being strictly aerobic organisms can have serious repercussions on us, the reduction of molecular O_2 to H_2O during the process of cellular respiration drives both the ATP synthesis necessary to sustain life, as well as the production of free radicals (39). These reactive species, generated by nicotinamide adenine dinucleotide phosphate oxidase and nitric oxide synthase nevertheless perform important functions, such as the defense of organism against infections and activation of various signaling pathways (40).

For example, it was showed that in the patients with Alzheimer and mild cognitive impairment there is a similar decrease of the superoxide dismutase and glutathione peroxidase, concomitant with an increase of malondialdehyde level compared to the control group (41). It seems that the human brain is extremely susceptible to oxidative stress, in literature existing consistent evidence that attests that free radicals play a crucial role in triggering by reflecting the etiopathogenesis of AD (42).

Perhaps the most well-known hypothesis is the amyloid cascade who dominated the AD field over the past decades. A new one has been issued recently, more precisely the mitochondrial cascade, where dysfunctions of this organelles, combined with an increased production of reactive oxygen species, could lead to AD development (43). Du *et al.*, detailed in his review all the current hypothesis about AD known to date (44).

Although it is well known that colonization of the infant gut at birth influences normal development (45), caesarean section delivery rates have increased rapidly over the last decade (46), becoming clear the fact that caesarean section can influence in negative ways the infant gut colonization. It seems that vaginally delivered infants, compared with those by caesarean section are colonized with less beneficial microbes (*Bifidobacterium* and *Bacteroides*) and higher numbers of potentially harmful such as *Clostridium difficile*. A major impact in the early development of the new-born is assigned to breastfeeding and dietary interventions using pre- and probiotics (47, 48, 49).

The main precursors involved in the modeling and induction of intestinal microflora variations during an individual's life, which may then generate a dysbiosis, are mass industrialization and aging process, affecting the production of immune mediators and induces both chronic inflammation and metabolic dysfunction. The main contributing factors in this context are: uncontrolled drug intake, excessive hygiene, changes in diet, addictions, chronic stress, even drinking water (50, 15, 8). Nelson *et al.*, provide essential data about the genotoxic and hepatocarcinogenic effect exerted by halo acetic acids (HAA) from the drinking water upon cecal microbiota from mice.

Noteworthy is that a mutagenesis in a *Salmonella* micro suspension was observed after an incubation at different time intervals, with and without cecal microbiota (51). For example, in 2014, another group reported a possible association between alcohol consumption, cognitive functions and type 2 diabetes, after the examination of 219 patients. Their results indicate that moderate consumption can reduce some of the neuropathological aspects of type 2 diabetes, as well as a decrease of glycemic levels in patients that consume higher levels of alcohol (52). In the same year, other group confirmed a significant correlation between the serum levels of uric acid and the specific parameters of the metabolic syndrome (53).

Also, our group provided additional evidences that cardiovascular risk factors are involved in cognitive regression, results obtained after the examination of 15 patients with mild cognitive impairment and 15 with AD by mainly focusing on aspects such as body mass index importance, cholesterol levels, hypertension and their related effects (54).

It's well known that obesity and diabetes in humans are associated with increased rates of anxiety and depression, which is why Soto *et al.*, evaluated behaviors and insulin action in brain of mice with diet-induced obesity (DIO), usually characterized by a decreased insulin signaling and increased inflammation in the nucleus accumbens and amygdala. After the treatment of DIO mice with oral metronidazole or vancomycin, they observed a decreased inflammation as well as an improvement of insulin signaling in the brain (55).

Those, the aforementioned aspects we presented here could exert a fundamental importance in studying the neurobiological mechanisms of these complex neurological processes and their pathophysiological manifestations, complications and associations (56, 57, 58). By using GF rats and rats associated with human fecal bacteria, Bowey *et al.*, demonstrated that isoflavones and ligans, in particular, equal, is not produced by some subjects mainly due to a lack of specific components of the gut microflora (59).

In addition, multidisciplinary studies have revealed that various non-psychiatric systemic diseases like obesity (60, 61, 62) diabetes (63, 64, 65), cardiovascular diseases (66, 67, 68) irritable bowel syndrome (69, 70, 71) and CRC (72, 73, 74) are the result of an unstable microbial profiles.

Also, all these speculations we described in the present "metabolic" subchapter can be related to the fact that there is scarce information on this matter/no previous study on the metabolic influence on the microflora-AD interactions, according to our best of knowledge.

CONCLUSIONS

The current literature, as well as our mini report are clearly demonstrating that there are clear interactions between the pathophysiology of Alzheimer's disease and microflora functioning. In addition, we speculate here about the importance of some related metabolic modifications on the context of the aforementioned importance of the microbiome in the Alzheimer's disease pathology.

Conflict of interests

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NEWS

HPV and colorectal cancer

In the last decade, several studies have suggested that HPV might have a role in colorectal carcinogenesis. Detection of HPV in colonic tissue has led to a wide range of work proposing a causal association between HPV and colorectal cancer. The presence of HPV in colon tissue remains a highly controversial topic of discussion because of inconsistencies in result reproducibility. Inactivation of the E2 gene through genomic integration promotes the expression of the E6 and E7 oncoproteins, which antagonize the function of p53 and pRB, respectively. The resulting degradation of p53 and pRB mediated by these oncoproteins facilitates viral DNA proliferation within the host and leads to neoplasia by mechanisms well described throughout the literature. In this study based on a well-characterized Hispanic population reported to have a high incidence of HPV-associated malignancies, HPV-16 was detected in 12 of the 19 HPV-positive colorectal samples (63.2%); the remaining 7 (36.8%) corresponded to other HPV genotypes (non-typed). The distributions of HPV-16 positive tumors regarding tumor location were as follows: 33.3% (4 of 12) of the samples were found in the proximal colon (cecum, ascending colon, and transverse colon), 50.0% (6 of 12) were found in the distal colon (splenic flexure, descending colon, and sigmoid colon), and 16.7% (2 of 12) were detected in the rectum. For non-HPV-16 tumors, 2 out of 7 (28.6%) were located in the distal colon and out 5 of 7 (71.43%) were found in the rectum. A high prevalence of HPV infection (42.2%) was detected in the tumor tissues analyzed. Only 1 of the 36 (2.8%) control samples was positive for a non-HPV-16 type. In conclusion, this study reported a high prevalence of HPV infection, a high prevalence of HPV-16 and integration of the HPV-16 genome in colorectal tumor tissue from Caribbean Hispanics. (Bernabe-Dones RD, Gonzalez-Pons M, Villar-Prados A, *et al*. High prevalence of human papillomavirus in colorectal cancer in Hispanics: A Case-Control Study. *Gastroenterol Res Pract* 2016; 2016:7896716).

LATE FAT EMBOLISM COMPLICATION AFTER EARLY STABILIZATION OF FEMORAL FRACTURE. CASE REPORT

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LATE FAT EMBOLISM COMPLICATION AFTER EARLY STABILIZATION OF FEMORAL FRACTURE. CASE REPORT (Abstract): Fat embolism syndrome is a multiorgan disease and a fearsome complication of traumas associated with fractures or orthopedic surgeries. Most commonly it occurs after femoral fractures and its major clinical features include pulmonary dysfunction, hypoxia, mental status changes, petechial rash, fever, tachycardia, anemia and thrombocytopenia. The here presented case is a 20-year-old male patient diagnosed with a right femoral fracture following a trauma with direct impact to the thigh. The medical team recommended immediate surgical treatment, but surgery was delayed for technical reasons for 12 hours and consisted of centromedular osteosynthesis with the interlocking nail system. The peculiarity of this case is the late occurrence (5 days after trauma) of this potentially fatal complication which fortunately was diagnosed when the patient was still hospitalized thus making possible the rapid initiation of specific therapeutic measures that ensured patient survival and complete remission of symptoms, without sequels. **Keywords:** FAT EMBOLISM, REAMING, OSTEOSYNTHESIS, PULMONARY DYSFUNCTION, FRACTURE.

Fat embolism syndrome (FES) was first described by Zenker in 1861 as fat droplets in the lung capillaries of a railroad worker who sustained fatal thoracoabdominal injuries (1). Later, the femoral fracture was identified as the main cause of this potentially fatal complication with pulmonary, neurological, hematological and dermatological manifestations. Studies have established that the incidence of this syndrome ranges from 0.5% to 11% in the patients who sustained long bone fractures (2) and mortality rate is high, of about 20% (3). Differential diagnosis should be made with other acute respiratory diseases such as pulmonary embolism, ARDS, pneumonia, thoracic trauma (4). Neurological manifestations are rare but may be dramatic and loss of consciousness has been described for more than two months (5). The main therapeutic resources are represented by the rapid recognition of this complication, initiation of supportive therapy and high-pressure PEEP (6). Initially the disease was explained by the mechanical theory, namely the passage of particles resulting from injury of the adipose tissue into the venous circulation of the traumatized region. The identification of an aseptic inflammatory process in the brain and lung tissue has led to the development of the second theory, namely the biochemical

theory (7). Fat embolism is represented by the occurrence of fat in the vascular system and is different from the fat embolism syndrome (8). Gurd and Wilson have proposed three major and eight minor diagnostic criteria. Major criteria are respiratory changes (radiological included), petechial rash and neurological symptoms. Minor criteria include tachycardia, pyrexia, retinal fat or petechiae, oligoanuria, fat globules in urine, sudden drop in platelet and hemoglobin levels, elevated ESR and the presence of fat particles in the sputum. Diagnosis is made by the presence of at least one major criterion and four minor criteria (9). The here reported case is that of a male patient who had a late onset of FES, 5 days after trauma, and the surgical treatment of the femoral fracture consisted in centromedular osteosynthesis with interlocking nail, performed 12 hours after trauma.

CASE REPORT

A 20-year-old pedestrian sustained a road accident in which the direct contact with the car was at the level of the right thigh. He was immediately picked up by the ambulance service and arrived at the emergency department 30 minutes after the accident. At the time of presentation, he was conscious, hemodynamically and respiratory stable, the only complaints being related to the right thigh, without any other thoracoabdominal or neurologic symptoms. Personal and family medical history did not contain significant details, the patient being a smoker for 6 years. Biological samples collected at the time of presentation showed levels close to normal for the complete blood count (Hgb 14.8 g/L, PLT-245*10^3/μL, WBC 12.5*10^3/μL), while biochemical determinations showed moderately higher levels of muscle enzymes, explained by the significant muscle contusion. Pulmonary and pelvic X-rays showed no pathological changes, heart rate was 110, respiratory rate 24, no craniocerebral trauma was detected, and the neurological examination revealed no changes. The initial diagnosis was isolated right femoral fracture and the medical team recommended the immediate surgical stabilization with a centromedular nail. Surgery could not be performed urgently for technical reasons (lack of complete nail insertion tools) and was delayed for 12 hours, while the patient received antalgic medication.

Surgery was performed under spinal anesthesia and consisted in close reduction and fixation with a 10-mm austenitic stainless steel antegrade nail. The medullary canal was prepared with 9-12 mm reamers, which allowed the easy insertion of the implant (fig. 1).

Fig. 1. Preoperative and postoperative aspect of the right femur. Centromedular osteosynthesis with a reamed antegrade nail

Operative time was approximately 60 minutes, the patient remained stable throughout it, and the blood loss from surgical incisions was insignificant. The biological constants were checked immediately after surgery revealing a 3.4 g/dL decrease in hemoglobin level (postoperative Hgb 11.4 g/dL), explained both by the

preparation of the medullary canal and by the hemodilution induced by the intravascular infusion of intraoperatively perfused solutions. During the first three postoperative days the course was favorable both locally and in terms of general state, the patient being neurologically normal and mobilizing himself the same day of surgery, moving actively and completely the joints in the proximity and resuming walking with partial weight-bearing on the operated lower limb. Biological samples collected three days after trauma showed a Hgb level of 10.1 g/dL and still elevated creatine-kinase level (1434 U/L), while the remaining muscle enzymes tended to return to normal. The clinical picture of the patient changed suddenly on the fourth postoperative day when he first presented a temperature rise (38°C) and later the mental status deteriorated and he became confused, complaining of bilateral chest pain followed by respiratory dysfunction requiring oxygen administration. Emergency collection of biological samples revealed a significant drop in hemoglobin level compared with the previous day (from 10.1 to 6 g/dL, HCT-18.8%), which could not be explained by a prolonged bleeding at the fracture focus. For the first time after trauma, coagulation tests showed modified levels and the muscle enzymes which previously showed a tendency to return to normal were rising again. Higher levels of leukocytes, ESR, C-reactive protein and fibrinogen indicated an inflammatory syndrome of suspected septic origin with a starting point in the lungs or operated fracture. An emergency chest X-ray revealed changes in lung appearance compared to the initial one, which was normal. At the level of both pulmonary areas, the patient presented an alveolar filling syndrome in small and confluent foci, picture known in the literature as ground-glass airspace disease (fig. 2).

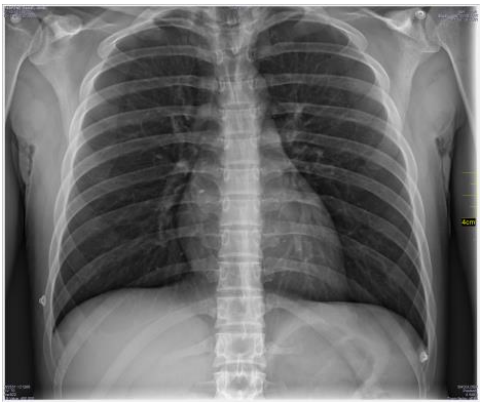

Fig. 2. Normal chest X-ray on admission

The occurrence of severe respiratory failure required patient's transfer to the intensive care unit in order to benefit from the best therapeutic measures and BiPaP respiratory support. Intravenous broad-spectrum antibiotics (Levofloxacin), corticotherapy, volemic repletion, blood transfusion, and antalgic medication were initiated. During the first 24 hours, no changes were recorded both in respiratory and neurological terms, and the biological constants were at the same level, with the exception of hemoglobin which continued to drop to a minimum of 5.8 g/dL although two units of red cell concentrate were transfused. Inflammatory markers (ESR, CRP, FBG) were still elevated while presepsin levels were within normal range throughout hospital stay. Blood cultures during the inflammatory syndrome remained negative for both aerobic and anaerobic germs, and thus the suspicion of respiratory failure due to lung infection was refuted.

The second diagnostic hypothesis was pulmonary thromboembolism, the patient not receiving on admission the usual dose of anticoagulant because it was intended that surgery to be performed as soon as possible. Due to technical problems surgery was delayed for 12 hours, the patient not being on anticoagulant protection for 24

hours from the time of the trauma. Cardiology consultations after the transfer to the ICU, supported by the ultrasound appearance of the heart and large vessels did not find clinical and laboratory elements able to explain respiratory failure as a result of pulmonary thromboembolism. The sudden drop of hemoglobin level has raised the possibility of autoimmune hemolysis due to hypersensitivity to a product administered in the hospital or the occurrence of FES. Following the hematological consultation, the administration of corticotherapy, methylprednisolone 250 mg/day, was recommended and the therapeutic effect was spectacular, with the normalization of the neurological status, the infiltrative appearance of the lungs progressively remitted, and hemoglobin level reached 9.5g/dL after 7 days of administration, when corticotherapy was stopped. The patient was discharged 14 days after trauma without neurological or pulmonary sequels and came for clinical and radiological follow-up three months postoperatively when the healing of the femoral fracture was confirmed, which made possible the resumption of pretraumatic activities.

DISCUSSION

Fat embolism is usually the complication of high energy trauma, especially in the case of pelvic and femoral fractures. Among the factors predisposing to this syndrome are younger age, the polyfractured patient, bilateral femoral fracture, closed fracture, the conservative treatment of the long bone fractures (10). There are exceptions when low energy trauma by falling from the same level and simple fracture of the femoral neck caused fat embolism syndrome (11). The main manifestation is pulmonary, which is the expression of pulmonary vascular bed obstruction by fat globules and the aggression exerted by free fatty acids on the pulmonary and brain capillary endothelium. The late occurrence of respiratory dysfunction is an argument in favor of lesions induced by biochemical mechanism. Mortality is estimated by Wildsmith to range from 5% to 15% and increases up to 36% in patients requiring mechanical ventilation (12). Prompt diagnosis is the one that offers the chance of survival and healing without sequels. Consultation and specific cardiology investigations are absolutely necessary to rule out the possibility of a cardiovascular cause of respiratory failure, chest X-ray remains the most accessible investigation that captures the characteristic infiltrate scattered throughout both lung fields, CT scan provides additional data on lung and large vessel changes and the cerebral MRI identifies the anatomical substrate of neurological manifestations. Corticotherapy is an important treatment resource and has proven its effectiveness in the case treated by us. Its prophylactic administration is not a practice that is routinely recommended and applied to patients at risk. The purpose of treatment is to support pulmonary function and to fight other complications by maintaining a level as close to normal as possible of blood gases and oxygenation, volemic repletion, parenteral nutrition, prophylaxis of deep vein thrombosis, gastrointestinal protection. McDermott recommends the administration of albumin both for its protective effect on the vascular bed as well as by its binding with free fatty acids, thus limiting their harmful effect on lung endothelium (13). Recent and older studies recommend the early stabilization of long bone fractures and especially of femoral fracture for the prophylaxis of FES (14, 15). Our case, of a patient operated relatively quickly, 12 hours after the accident, and who developed late FES, 5 days after the trauma, signals the high risk in a

young patient who sustained a high-energy femoral fracture and in which centromedular osteosynthesis with reamed femoral canal was performed.

CONCLUSIONS

Of all medical specialties, traumatology and orthopedic surgery are at the highest risk of complications by FES, which once present has a high rate of sequels and mortality. The young patients with femoral fracture, especially those with bilateral fracture, should be considered as a risk group and require careful clinical and laboratory supervision to surprise the manifestations type tachycardia, tachypnea, hypoxia, temperature rise, thrombocytopenia. Immediate fixation of long bone fractures and especially of femur fractures has been shown to reduce the incidence of this complication. High-energy trauma, 12-hour delay of surgery, young age, femoral canal reaming are causes that favored the occurrence of FES in the here reported case. In conclusion, our patient developed a less common form, particular by the late occurrence of FES, but presented its main specific manifestations. Early diagnosis, supported by the chest X-ray appearance and the rapid initiation of corticotherapy and supportive therapy made possible the complete remission of neurological and pulmonary lesions.

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PREVALENCE AND ANNUAL RISK OF TUBERCULOUS INFECTION TO CHILDREN AND ADOLESCENTS IN TWO HIGH ENDEMIC TB COUNTIES FROM ROMANIA

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PREVALENCE AND ANNUAL RISK OF TUBERCULOUS INFECTION TO CHILDREN AND ADOLESCENTS IN TWO HIGH ENDEMIC TB COUNTIES FROM ROMANIA (Abstract): The prevalence of Latent Tuberculosis Infection (LTBI) and Annual Risk of Tuberculosis Infection (ARTI), key epidemiologic indicators used to estimate the transmission of tuberculosis in the community, have not been evaluated in Romania. The aim of the study is to estimate the prevalence of LTBI and ARTI in children and adolescents from two areas with increased TB endemics: Constanta and Timis. **Material and methods:** We performed an epidemiological observational study to estimate the prevalence of LTBI, using tuberculin skin test (TST), in a representative sample size of the population aged 0-16 years in both areas, regardless of the presence or absence of BCG post vaccination scarring. **Results:** 1,928 children and adolescents were evaluated, 1,088 from Constanta (CT) and 840 from Timis (TM). The LTBI prevalence for the studied group was 12.81% (CT-11.86% and TM - 14.05%), for boys 12.46% (CT-11.34% and TM-14.11%) and girls 13.21% (CT-12.53% and TM-13.98%). The prevalence of LTBI was 8.2% in the urban area (CT-9.19%, TM-7.02%) and 4.5% in the rural area (CT-2.67% TM -7.02%). ARTI for the study cohort was 1.64% (1.58% CT and 2.19% TM). In 2016, the TB global incidence (GI) in the two analyzed areas (CT = 73.7% per 100,000, and TM = 70 per 100,000) was higher than the national one (GI = 64.8 per 100,000). **Conclusions** The values of LTBI and ARTI prevalence in the two analyzed territories were comparable, but significantly higher than in other developed countries, and similar to those with high TB global incidence. **Keywords:** LATENT TUBERCULOSIS INFECTION**,** THE ANNUAL RISK OF TUBERCULOSIS INFECTION, COUNTY EPIDEMIOLOGICAL SURVEY, CHILDREN.

Tuberculosis (TB) continues to be an important public health problem in Romania (RO), with a global incidence of 64.8 per 100,000 in 2016 (1). The TB incidence in children 0-14 years was 19.4 per 100,000 (1). Although there has been a steady decrease in the TB endemic since 2002, it is necessary to remain vigilant in the control of this disease in the territory, as long as Romania has the highest amount of cases in the EU region, with an important mortality and fatality rate (2). The prevalence of latent TB infection (LTBI) is a useful tool to assess the trend of the TB endemic in one territory (3,4). Annual Risk of Tuberculosis Infection (ARTI), although

controversial in the model proposed by Styblo to predict the incidence of contagious TB cases, remains a key epidemiological indicator of appreciation acquiring TB infection during a year (5,6). Both indicators, the prevalence of LTBI and ARTI, are calculated using the tuberculin skin test (TST). Despite its simplicity, efficiency, and reliability, Romania does not routinely apply the LTBI prevalence and ARTI to assess the trend of infection and to make TB control measures more effective at a national level.

THE OBJECTIVE of our survey was to estimate the prevalence of LTBI and to estimate ARTI in the population of children and adolescents, in two areas with increased endemics in Romania (Constanta – CT, and Timis - TM).

MATERIALS AND METHODS

Study cohorts

We conducted a cross sectional survey to estimate the prevalence of LTBI in two counties with high TB incidence from Romania: CT and TM. The sample was randomly selected from preschool and school college children living in the two counties. We excluded from the study those who showed signs of fever, cutaneous atopy, childhood eruptive diseases, immunosuppressive illness, malnutrition, active TB, or other vaccination done a month before TST, and those whose parents or tutor refused to sign the informed consent. The size of BCG scar was not an exclusion criterion. 1,088 children from CT and 840 children from TM, aged 0-16 years, who are attending school, have been tested using TST. For the two cohorts of participants, the basic demographic characteristics were collected. The clinical exam for all participants was performed by the pulmonologist. The Mantoux test was performed using 5 UT tuberculin units (TU) of PPD RT23 provided by the Statens Serum Institut, Copenhagen, Denmark, according to the standard protocol (7,8). The correctness of the technique was followed by the occurrence of a pale skin papule of 5-6 mm. The induration was measured 72 hours later (8). The TST was considered positive if the transverse diameter of the induction was equal to or greater than 10 mm. We calculated the prevalence of LTBI using cut-off points of 10 mm (positive TST), as it is widely accepted for the diagnosis of LTBI in the conditions of vaccination of over 95% of the Romanian population. To avoid errors on the validity and reproducibility of the test, the same team of doctors and nurses participated in the whole study.

Statistical analysis

The experimental data was processed using the statistical processing program IBM *SPSS Statistics v.20.0* software *(SPSS Inc., Chicago, IL, USA)*. We have been used descriptive statistics (to characterize discrete and continuous variables defined at the database level), and nonparametric statistical tests (chi-squared test for the comparison of two proportions). The continuous variables were presented as means and standard deviations, and the categorical variables were presented as frequencies and percentages. Chi-square test was used to compare the prevalence of LTBI in the two geographical areas in relation with different variables: age, sex, and home environment (8, 9). P-value <0.05 was considered significant (9).

Measurements

Prevalence of LTBI - was calculated by reference to the number of positive TST in 100 subjects (children and adolescents)

tested, according to the formula below (10, 11):

$$P=\frac{\text{Number of children with positive TST x 100}}{\text{Total number of children analyzed}}$$

Estimation of ARTI - The standard formula used to estimate the probability of being infected in one year (ARTI) was: $R = 1-(1-p)^{1/age+0,5}$ **(**where R is the annual risk of infection, P represents the prevalence of LTBI, and age indicates the average age of children and adolescents studied) (5,9). For a percentage estimation of ARTI, the result of the standard formula was multiplied by 100 (8).

Population

According to the ARTI protocol, the estimation of the sample size survey was made by using the formula: N=d (1.96)2 (1-P)/ε2P (8), where **N** is sample size, **d** represents the design effect (the value after protocol is 3), **P** is the expected prevalence of LTBI, ε is the relative precision which reflects the error degree between the estimated prevalence chosen at the beginning of the study, and the real prevalence of LTBI after the study (15%) (10, 11). Due to the lack of other epidemiologic studies in Romania regarding the prevalence of LTBI in children and the fact that the incidence of contagious forms of TB is high, we chose a hypothetical prevalence of 20%. According to these calculations, the estimated population for our study was 2,148, and finally, the group studied included 1,928 children and adolescents.

Ethics statement. Written informed consent for tuberculin testing was obtained from the parent or tutor of every child. The Ethics Committees of the Clinical Pneumology Hospital Constanta and Clinical Hospital of Infectious Diseases and Pneumology "Dr. Victor Babes" Timisoara approved the study protocol.

RESULTS:

Study setting and population characteristics

The study was conducted in two high endemic TB counties, geographically located in opposite areas of Romania: CT and TM. TB global incidence (GI) in these areas was always above the national values. Since 2002, the evolution of the TB endemic in both counties has followed the same decreasing tendency as at the national level. The global incidence values over the last four years are shown in Table I.

This study was conducted during 2014-2015. 1,928 children and adolescents aged between 0–16 years participated in this survey: 1,088 from CT and 840 from TM, irrespective of their BCG scar status. In our country, the BCG vaccination is given at birth or as early as possible within the first month of life, and national BCG vaccine coverage has been >95%.

The baseline participants' characteristics and LTBI calculation by population categories are presented in Table II.

The average age was higher in TM county, 8.84 years compared to CT, 7.9 years ($p<0.001$). The TM subjects included a greater proportion of adolescents aged 15-16 years (14.6% TM vs. 5.5% ($p<0.001$). The LTBI prevalence for the study group was 12.81%. The proportion of subjects with a positive TST test was 11.86% in CT and 14.05% in TM ($p=0.153$). There was a significantly higher proportion of absence in CT of 10.85% compared to TM-3.93% ($p<0.001< \alpha = 0.05$). In the studied group, the LTBI prevalence was 6.69% in boys (CT-6.43% and TM-7.02%), and 6.12 in girls (CT-5.42% and TM -7.02 %) showed in table II. LTBI prevalence values by age

group compared in the counties are shown in Figure 1 and Table III.

The highest value of LTBI prevalence was recorded at the age of 2 years in CT (2.85%) and the age of 14 in TM (1.67%) (table III). There are significant differences between the TST positive score recorded in CT (2.85%) and TM (0.71%) for the age of 2 years ($p<0.001$).

TABLE I

Global incidence (GI) in Romania (RO) compared to CT and TM counties and TB global incidence in children (GI) between 2013-2016 (1)

	2013			2014			2015			2016		
	RO	CT	TM	**RO**	CT	TM	**RO**	CT	TM	**RO**	CT	TM
GI %000	**73.5**	89.9	91.4	**70.2**	87.6	85.2	**71.7**	88.8	81.5	**64.8**	73.7	70
No. of TB cases	**15505**	651	623	**14938**	634	581	**14269**	607	566	**12836**	503	487
GI in children %000	**22.4**	27.9	15.7	**20.3**	34.8	28.4	**21.3**	33.9	17.9	**19.4**	27.4	20.7
No. of TB cases in children	**708**	31	15	**642**	38	27	**657**	37	20	**597**	30	17

TABLE II

Baseline characteristics of subjects and LTBI prevalence by sex and home environment

Variable	CT	TM	p value
(n)	1088	840	
Mean Age, years	7.9 (±4.12)	8.84 (±4.7)	**<0.001**
male	617 (56.71%)	418 (49.76%)	**0.002**
female	471 (43.29%)	422 (50.24%)	**0.002**
0-4	255 (23.4%)	203 (24.15%)	0.709
5-9	410 (37.8%)	264 (31.43%)	0.004
10-14	363 (33.3%)	250 (29.76%)	0.092
15-16	60 (5.5%)	123 (14.64%)	**<0.001**
TST (positive≥10mm)	129 (11.86%)	118 (14.05%)	0.153
TST (+) urban	100 (9.19%)	59 (7.02%)	0.086
TST (+) rural	29 (2.67%)	59 (7.02%)	**0.000**
TST (+) male	70 (6.43%)	59 (7.02%)	0.607
TST (+) female	59 (5.42%)	59 (7.02%)	0.146
TST (+)≥15	49 (4.5%)	30 (3.57%)	0.306
TST (negative)	841 (77.30%)	689 (82.02%)	**0.011**
Absent for read TST	118 (10.85%)	33 (3.93%)	**<0.001**

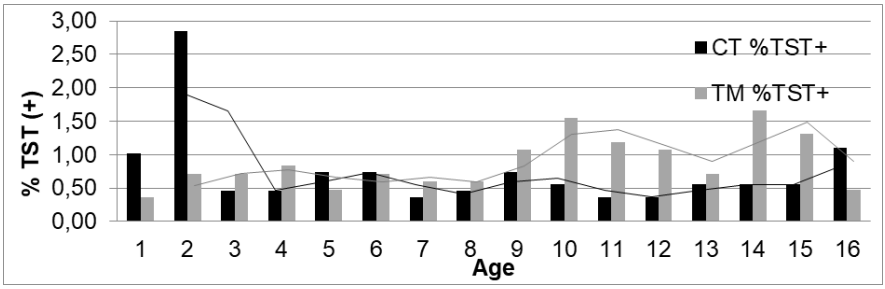

Fig. 1. Trendline of the LTBI Comparative prevalence of CT and TM by age category.

TABLE III

Comparative prevalence of LTBI and ARTI in CT and TM counties

	CT	TM		CT	TM	Total CT+TM
Age	% TST(+)	% TST(+)	p	%ARTI	% ARTI	% ARTI
1	1.01	0.36	0.094	1.01	0.36	0.73
2	2.85	0.71	**0.001**	1.43	0.36	0.96
3	0.46	0.71	0.462	0.15	0.24	0.19
4	0.46	0.83	0.301	0.12	0.21	0.16
5	0.74	0.48	0.473	0.15	0.10	0.12
6	0.74	0.71	0.957	0.12	0.12	0.12
7	0.37	0.60	0.467	0.05	0.09	0.07
8	0.46	0.60	0.681	0.06	0.07	0.06
9	0.74	1.07	0.434	0.08	0.12	0.10
10	0.55	1.55	0.028	0.06	0.16	0.10
11	0.37	1.19	0.035	0.03	0.11	0.07
12	0.37	1.07	0.061	0.03	0.09	0.06
13	0.55	0.71	0.652	0.04	0.06	0.05
14	0.55	1.67	**0.017**	0.04	0.12	0.07
15	0.55	1.31	0.077	0.04	0.09	0.06
16	1.10	0.48	0.133	0.07	0.03	0.05

BCG vaccination could influence a high prevalence of LTBI in CT at 2 years of age, in an urban area; the differential diagnosis of LTBI and post-vaccinal positive TST reaction could be made by the T-SPOT-TB assay which is more specific for the positive diagnosis of LTBI than the TST (12). In CT the prevalence of LTBI was higher in the urban area (9.19%) *versus* rural area (2,67%). Compared to CT, in TM there were no differences between environmental field and sex. The evaluation of the induration diameter revealed a percentage of 8.07% of subjects with a TST diameter of > 15 mm, suggesting either a recent TB infection requiring further investigations to

exclude an active disease, or a person at risk for the transformation of a latent infection into the disease, requiring careful monitoring. ARTI on the cohort studied was 1.64%. ARTI was 1.58% in CT and respectively, 2.19% in TM, given that in 2016 the global incidence in the two analyzed areas was 73.7% in CT and 70% in TM respectively. As $\chi^2_{calc} = 2.13 < \chi^2_{cr} = 7.81$, respectively $p = 0.547 > \alpha = 0.05$, it is considered that there are no significant differences in percentage of ARTI values registered by age groups in the two counties. The ARTI was 0,42% in CT and 0.50% in TM for 5-9 age group. For both counties ARTI decreases with age.

DISCUSSION

Until now, no LTBI or ARTI prevalence studies have been conducted in Romania. In our country, TST is currently used in epidemiological investigations for LTBI detection in TB outbreaks or for primary TB symptoms in children. According to the National Guidelines, a TST ≥ 10mm response is considered positive, meaning a natural infection with *Mycobacterium tuberculosis;* whereas, a TST over 15 mm is considered an intense reaction, with the probability of an increased risk of lesion evolution. Our study was the first large survey to estimate the prevalence of LTBI and ARTI in Romania. LTBI prevalence was 12.8% in the study cohort. The ARTI value was 1.58% in CT and 2.19% in TM and was highest in the 1-4 years age group. The study was conducted in two regions with a high tuberculosis endemic, but with some socio-economic and cultural particularities.

CT is located in the SE of Romania, influenced by the sea climate, and the harbor on the Black Sea. In 2014 CT had an incidence of TB of 87.6 per 100,000. As a harbor, which by itself brings heterogeneous and migrant population, we considered that besides commonly known TB risk factor such as malnutrition, smoking (including electronic cigarette vaping in adolescents), TB-HIV coinfection, and low socio-economic status (13-15), there are other specific local conditions that can influence the level of LTBI in Constanta, such as the emigration phenomena, or institutionalization in care homes and prisons (16,17). Environmental and occupational exposure due to a petroleum refinery in the area could also compromise the systemic immune response and increase the risk for LTBI as well as lung cancer (18,19).

TM, situated in the western region of Romania, with Western influences, is known for a higher living standard, where TB incidence was 85.2% per 100,000 in 2014 (Table 1) (1).

ARTI results in our study on incidence confirms the Styblo coefficient - each 1% ARTI corresponds to an incidence of 50 per 100,000 inhabitants (6). The estimated values of TB incidence after Styblo rule (79 %000 inhabitants in CT and 109,5 %000 in TM) are close to the real ones.

Although the ARTI is the simplest and the most effective and reliable indicator to estimate the epidemiological situation of TB, many developing countries do not use ARTI. For instance in Saudi Arabia, two studies were published: one by Al Kassimi in 1993 where the prevalence of LTBI was 5.6%, and ARTI was 0.5 % for the 5-8 year group, and another by Balkhy, published in 2017, where the LTBI in the population under 15 years old was 2.9% (3.6% in girls and 1.5% in boys) (20,21). In Saudi Arabia the global incidence of

TB was 50% per 100,000 in 1990, and 12% per 100,000 in 2014 (22). A nationwide cross-sectional study of primary school children aged 6–11 years conducted in 2011 in Gambia revealed a prevalence of LTBI using a 10 mm cut-off point of 10.8% in girls and 12.3% in male, and an ARTI of 1.27% (23). Another survey from 2004 performed in the south of India on children aged between 1-9 years, without a BCG scar, found a prevalence of LTBI of 5.9%, and a corresponding ARTI of 1.0% (24). In Europe, there are very few studies on the prevalence of LTBI and ARTI, and these are targeted at at-risk populations such as health workers, HIV-infected individuals, patients treated with anti-tumor necrosis factor agents, and foreign-born people from countries where TB is endemic. The study of Gallegos Morales found an LTBI rate in foreign-born students in Lübeck Germany of around 10% (25). However, a comparison and interpretation is difficult to produce, due to the notable differences in the methodology of the TST survey: different population groups studied, different immunosuppressed condition (26), severe disseminated forms of TB (27), associated comorbidities (28), different tuberculin concentrations (1 TU, 2 TU, 5 TU PPD), different cut-offs for TST test, the presence of BCG vaccination or the use of IGRAs in TST place for LTBI confirmation (11,23).

A limitation of this study could be the small size of the cohort analyzed on different age groups. Another limitation of the survey is that the studied population included age groups 10-16 years old in a BCG vaccinated population. The ARTI protocol suggests that it is preferable to conduct a survey on children under 10 years old (8). It is also possible that the small number of non-vaccinated BCG children introduced in the study could influence the results (29). For a better evaluation of LTBI, Interferon Releasing Assays (IGRAs) are recommended because TST has several limitations (12), but both of them are inconsistent with active TB diagnosis (30,31). However, the both tests do not confirm the diagnosis of active TB, but in same circumstances could be a suggestive tool to doubt TB etiology of disease (32,33).

The age group that appears to be the most affected by BCG vaccination and interferes with the results of the study is age group 0-4 years, as it seems to have happened in CT. Between 5 - 9 years old, LTBI prevalence is relatively stationary and comparable in the two counties: 0.42% in CT and 0.50% in TM. Considering the gender difference regarding transmission of LTBI, we should to be aware of the young women with childbirth potential who need to be included in the TB annual screening when the above-mentioned risk factors are present (34). Education has proven to be instrumental in promoting a healthy and smoke free behavior in the younger population (35). In the same time, it has become critical to control smoking as a public and personal responsibility, not only for the smoking cessation but also for the prevention of COPD (36). Therefore, education in the prevention of the contagious infectious disease, such as tuberculosis, remains the most important method to decrease the prevalence of one of the most frequent disease in Romania.

We believe that the strength of the study consisted of the fact that it was carried out in two counties with similar endemics and the results on LTBI prevalence

and ARTI were not significantly different.

CONCLUSIONS

The high risk of TB infection estimated in our study (1.58% CT and 2.19% TM) indicates that it is necessary to provide sustained and committed quality services for tuberculosis control at national level with a periodic repetition of prevalence surveys. Case detection should be improved by maintaining DOT and quality of care.

This study provided all the necessary data to assess the impact of the disease and to evaluate future national epidemiological trends.

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AGGRESSIVE ANGIOMYXOMA OF THE VULVA IN POST-MENOPAUSAL PERIOD: LITERATURE REVIEW AND CASE REPORT

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AGGRESSIVE ANGIOMYXOMA OF THE VULVA IN POSTMENOPAUSAL PERIOD: LITERATURE REVIEW AND CASE REPORT (Abstract): Aggressive angiomyxoma (AA) is a rare, benign and slowly growing mesenchymal tumor which affect women especially on the reproductive age. Because over 80% of cases are misdiagnosed and most of them are often confused with lipomas, the diagnosis is made by histological examination. We describe here a case of an 89-year-old female, at menopause, that presented with a large pedunculated angiomyxoma on the right upper of major labia. The vulvar AA diagnosed was made due to clinical-pathologic findings and histologic result after the local excision of the tumor. The patient was discharge 7 days after surgery and along of 7 months of follow-up no recurrence or metastasis were documented. In this case we showed the diagnosis, management and prediction for vulvar angiomyxoma in postmenopausal period, but further follow-up period is necessary for surveilling the recurrence, knowing that the tumor it may reappear in between 2 months and 12 years. **Keywords**: VULVAR AGGRESIVE ANGIOMYXOMA, VULVAR BENIGN TUMOR, VULVAR LIPOMA.

Steeper and Rosai, firstly described an aggressive angiomyxoma (AA) in 1983 and, at the moment, there have been reported in the literature around 300 cases of this tumor type (1, 2). Aggressive angiomyxoma of the vulva is a benign, invasive myxoid tumor that primarily occurs in the pelvic, perineal and genital region. This tumor affects especially adult women in their reproductive age, having a women-men ratio of 6.6 : 1. When occurs in men, usually is being spread in different places like scrotum, perineum, spermatic cord, testicular/spermatic cord neoplasm, spermatocele and hydrocele (3, 4).

Its development is mainly from the connective tissue, having a fibroblastic or myofibroblast origin. Mostly, AA appears in premenopausal women, and resembles with another similar lesions from this areas like Gartner's duct cyst, Bartholin's gland cyst, inguinal hernia, lipoma and leiomyoma (2). Even if it is a benign tumor, in the literature has been reported an increased rate of local recurrences and invasion, around 30% to 72%; metastases cases and even death

(3). The pathophysiology of the tumor remains unclear and the diagnosis has to be certified by histological examination, due to its large number of misdiagnose, more than 80% (5).

After analyzing the tumor cell biology, the researchers had reached the conclusion that suppressive hormonal therapy with estrogen and progesterone plays a crucial role in the pathogenesis of AA, due to its favorable clinical response after treatment with GnRH analogues, estrogen/progesterone receptor blockers and aromatase inhibitors (6). In this pathology, another treatment possibilities with successful results are represented by radiotherapy and angiographic embolization (7).

CASE REPORT

We report the case of an 89-year-old female with vulvar aggressive angiomyxoma who entered on the menopause since she was 45 years old and had no significant pathological conditions at the moment of the examination. She presented in our department accusing the arising of a slow growing mass that started two years ago. The patient did not accuse any pain and had no other complains about it, except the unpleasant appearance. The clinical examination revealed a tumor mass that measured approximately 18 cm and developed from the 1/3 upper level of the right labia with a pedicle that has a length of 4 cm (fig. 1). The tumor was soft, had a gelatinous consistency and its pedicle root did not infiltrate the vaginal wall; on gynecological examination, the vaginal and cervical mucosae were normal. No associated lymphadenopathy were found. The patient' laboratory investigations were found within normal limits including complete blood count (CBC), liver and renal function tests, serum ionogram test and Ca-125 marker (10.8U/mL).

During the vaginal ultrasound examination in the left parameter, on ovarian topography, a circle nonhomogeneous isoechogenic image was detected, with a hyperechogenic aria of 76/47 mm diameter; color Doppler imaging showed random and irregular dispersed blood vessels toward the center of ovarian tumor.

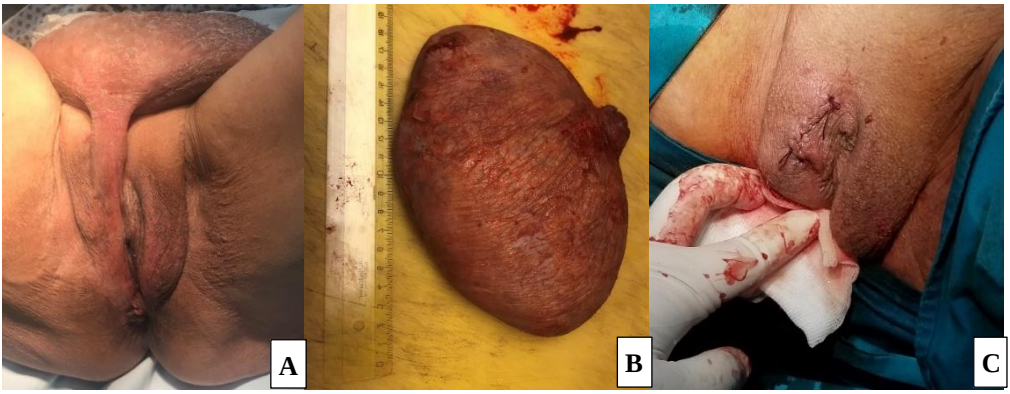

Fig. 1. A- Gross vulvar mass which developed from the right labia.
B - Image with the tumor after surgical resection.
C - Image after surgical excision of the lesion in immediate postoperative period.

The patient refused the surgical treatment for the ovarian tumor. She only consented the surgical treatment for labial tumor. This it was performed with section of the tumor pedicle (4 cm length) that was localized on the right major labia; the excision of the lesion was made with a clear resection margin. The tumor resected was sent for histological extemporaneous exploration (fig. 2). A fine local hemostasis was obtain and the lesion was closed performing an intradermal suture with Vicryl® suture (polyglactin 910). The results of extemporaneous examination of the tumor objectified a labial lipoma with the reserved result of paraffin processing of the tissue.

The patient postsurgical evolution was favorable under Ciprinol 200 mg, two tablets per day, and analgesia treatment. She left after 7 days of hospitalization. The histological examination of the tumor mass certified the vulvar angiomyxoma diagnose.

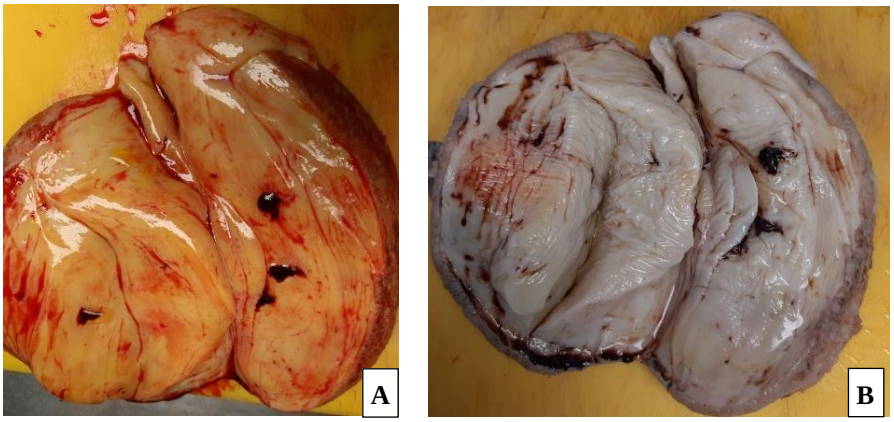

Fig. 2. Images of post operatory dissection of the labial tumor

Macroscopic histopathological examination revealed the presence of an 18/13 cm diameter tumor mass, covered by skin with ulcerated areas; the growth mass included a 3 cm thick pedicle without any visible tumor infiltration. The surface sections were gray-white with a gelatinous and glistening appearance and congestion areas.

Microscopy presented paucicellular proliferation of cells with oval or fusiform nuclei, a difficult visible cytoplasm and lack of nuclear pleomorphism or atypical mitoses. The tumor mass had relatively well delimited margins and contained abundant myxoid stroma, collagen bands and numerous blood vessels of different diameters with thin walls that were observed in hematoxylin and eosin (HE) staining (fig. 3). No hemorrhagic or necrotic areas were found. The pedicle and the excision margins found no signs of tumor infiltration.

The histopathological diagnose revealed an aggressive angiomyxoma of the major right labia. The patient had been followed during 7 months after the surgery and no recurrence or metastasis signs were remarked.

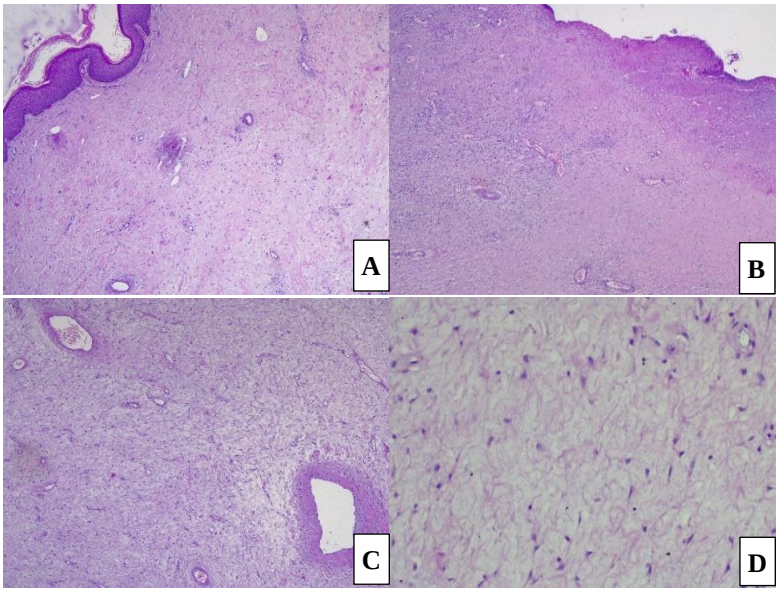

Fig. 3. The histologic aspects of aggressive angiomyxoma. **A** – Surface squamous epithelium (HE x4); **B** – Ulcerated surface epithelium (HEx4); **C** – Dilated blood vessels with different diameters and thick walls, myxoid stroma (HE x4); **D** - Myxoid stroma and fusiform cells without atypia (HEx10).

DISCUSSION

The vulvar aggressive angiomyxoma is threatening due to its characteristic of regional infiltration and recurrence (8). Nine cases were firstly reported in the literature by Stepeer and Rosai, who described different mesenchymal tumors located in the pelvis or perineum of youth females (9). Even if the number of cases increased over the years, and at this moment are counted approximately 300 cases, there are few cases reported in postmenopausal period (10, 11).

The pathogenesis of AAM is still unclear, it is believed that AAM develops from dysfunctional mesenchymal cells with genetic defects. There had been commonly found some chromosomal abnormalities that determine a translocation in the region of 12q 14-15 (12).

There are two clinico-histopathological entities: superficial angiomyxoma (SAM) and aggressive angiomyxoma (AAM). Both of them are diagnosed by the pathologist. The first category has a decreased rate of local recurrence compared with AAM, which can rise from 30% to 72%, causing the necessity of long-term monitoring (13, 14). Even if AAM has me*et al*so a favorable prognostic, two reported cases in the literature encountered a poor evolution which converted into cancer (15). The average of recurrence is one time for AAM (14).

In cases where AAM is suspected is necessary to perform preoperative imaging in order to establish the extension of the surgical excision. Computed tomography (CT) scan and Magnetic resonance imaging (MRI) offer a good predictability to what regard the tumor`s extent, both being superior to physical examination (12).

Superficial angiomyxomas are normally negative for IHC markers, except for vimentin and CD34, whilst AAMs are estrogen receptor (ER) negative and/or progesterone receptor (PR)-positive. This situation explains why clinical response is that good after treatment with GnRH analogues, estrogen/progesterone receptor blockers and aromatase inhibitors.

The "gold" standard cure is represented by radical excision of the tumor with negative margins, offering the optimum outcomes and evolution of the treatment. Aggressive angiomyxoma had shown variable responses to several adjuvant therapies such as hormonal blockade, radiotherapy and angioembolization. Hormonal therapy with GnRH agonists (leuprolide acetate or goserelin) are valuable in decreasing the tumor size in situations where surgical excision is not manageable. Radiotherapy and chemotherapy do not provide any benefits due to the tumor`s decreased mitotic activity (16). Adjuvant therapy, using arterial embolization it may be needed in cases with partial excision of the tumor mass (17).

CONCLUSIONS

The outcomes of the vulvar aggressive angiomyxoma strategies have not been properly evaluated because of the limited amount of cases for study, and evidence relies on small series and case reports. In this situation, AAM is a topically benign but aggressive mesenchymal tumor in which the surgical excision is the optimal treatment with no recurrence sign during 7 months of follow-up.

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NEWS

THE INTERPLAY BETWEEN VAGINAL MICROBIOTA AND INTRAUTERINE ADHESION USING NEXT GENERATION SEQUENCING METHODS

Intrauterine adhesion represent a disease that is a very common cause for female infertility. Since it has an increased recurrence rate after surgical interventions, its pathogenesis and recurrence causes are important to understand better this condition. Liu *et al*. (2019) analysed, using high-throughput sequencing method, the vaginal microbiota of females diagnosed with intrauterine adhesion and healthy patients` vaginal secretion. The results shown that intrauterine adhesion does not bring any important changes for vaginal microbiota, having a slighty effect on the number of vaginal bacterial species. The patients with intrauterine adhesion presented at the phylum level a reduced percentage of Firmicutes and increased percentage of Actinobacteria whereas in the healthy females group the rate was more lower ($P<0.05$). Moreover, in about half of the intrauterine adhesion vaginal samples was identified at the genus level a remarkable decreased number of Lactobacillus and enhanced growth of Prevotella and Gardnerella ($P<0.05$).The interplay between synechiae and vaginal microflora can reveal remarkable changes in the vaginal microbiota diversity in patients with intrauterine adhesion. The increase of Lactobacillus population may bring interesting benefits in the endometrial regeneration process but also may help to decrease de recurrence rate of intrauterine adhesion (Liu Z, Kong Y, Gao Y, *et al*. Revealing the interaction between intrauterine adhesion and vaginal microbiota using high-throughput sequencing. *Mol Med Rep* 2019; 19(5): 4167-4174).

EXTENDED NECROTIZING FASCIITIS LOCALIZED AT THE LEVEL OF THE PERINEUM, BUTTOCK AND THE LOWER LIMB. CASE REPORT

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EXTENDED NECROTIZING FASCIITIS LOCALIZED AT THE LEVEL OF THE PERINEUM, BUTTOCK AND THE LOWER LIMB. CASE REPORT (Abstract): Necrotizing fasciitis, as the name suggests, is a rare, but potentially lethal infection, with a rapidly progressive character, of the deep or superficial fasciae of skeletal muscles. In the current paper, we aim at highlighting the importance of diabetes mellitus, particularly of undiagnosed diabetes, as well as the delayed presentation to the doctor, as important risk factors for the occurrence of necrotizing fasciitis and the rapid evolution of the infection. To this respect we exemplified through the case of a diabetic patient (unknown by the time of admission) who developed an extremely severe form of necrotizing fasciitis (actually, a necrotizing fasciomyositis), extended to several areas of the body (perineum, buttock, lower limb). **Keywords:** DIABETES MELLITUS, FASCIITIS, NECROSIS, INFECTION, MORTALITY, SURGERY.

Necrotizing fasciitis, as the name suggests, represents a rare, but possibly lethal infection, with a rapid progression, of the deep or superficial fasciae of skeletal muscles, more frequently at the level of the lower limbs, perineum and trunk walls, with interest in the subcutaneous adipose tissue as well and possible (secondary) integumentary damage in the course of evolution (1, 2).

The infection and the edema produced in the muscular fascia and then in the subcutaneous tissue lead to ischemia and the necrosis of the tissues affected due to compression at the level of the vessels in that region (3). The lack of firm and rapid surgical interventions, despite the administration of the antibiotic treatment makes mortality even higher and it reaches 90% because of the occurrence of the systemic inflammatory response syndrome (SIRS) which can lead to death through its evolution to sepsis and other septic complications (4).

CASE REPORT

We present the case of a 53-year-old male urgently hospitalized with fever, chills and the presence of a bulky tumor mass (10/7 cm), fluctuance in the right ischiorectal fossa that associates local inflammatory signs, suffering which had begun 7 days before. Biologically on admission: significant leukocytosis (22,000 / mm^3), blood glucose level 510 mg/dL (unknown as a diabetic patient), serum sodium 133 mmol, urea 142 mg/dL, total lipids 478 mg/dL, C-reactive protein 20 mg/dL, fibrinogen >1,000 mg/dL. The diagnosis of right ischiorectal phlegmon was made and emergency surgery was performed by making an incision, by pus evacuation, debridement and the excision of dead tissue. At approximately 15 hours postoperatively, the patient begins to experience intense pain in the right lower limb, posterior perineum and right buttock, and the clinical examinations of those areas reveals erythema, swelling of the skin, crackling sensation on palpation and local heat. The scrotal skin and the external genitalia were not affected by the suppurative process. The tentative diagnosis of extended necrotizing fasciitis was made, localized in the posterior perineum (hence a Fournier gangrene), of the buttock and the right lower limb, with a starting point of the suppuration in the ischiorectal fossa.

Emergency surgery was performed by making wide and multiple incisions in the posterior perineal region, in the buttock and right lower limb, finding significant necrosis in the large gluteal muscle as well as in the muscle mass of the thigh and calf, liquefaction necrosis in the fasciae and subcutaneous adipose tissue (necrotizing fasciomyositis) (figs. 1, 2).

In the following days, there were also multiple areas of skin necrosis in the affected areas. The devitalized skin together with the compromised subcutaneous tissue and the muscular tissue and the necrotic tissue were excised to healthy tissue in serial surgical sessions by a team of plastic surgeons. The daily cleaning of the wounds was performed, the completion of the excisions of necrotic tissue was performed under intravenous general anesthesia, with dressings with serum and betadine. The germs identified in the wounds were *E. coli* (pus from ischiorectal phlegmon), but 2 other multidrug-resistant germs were identified, *Kluyvera ascorbata* and *Acinetobacter baumannii* (identified in the pus collected from the level of the areas with necrotizing fasciomyositis).

Fig.1. Intraoperative appearances

Fig. 2. Intraoperative appearances

In parallel, sustained treatment of hydro electrolytic rebalancing was administered, parenteral nutrition (patient on food fasting for the sterilization of the perineal region), antalgic treatment, antidiabetic anti-inflammatory (insulin with dose adjustment depending on the blood glucose level) and initial empirical broad spectrum antibiotic therapy, and then, according to the antibiogram, the patient being monitored in the intensive care unit. After obtaining granular wounds, the covering of multiple excisional skin defects was performed through several plastic surgery interventions with free split skin (fig. 3). The evolution was favorable, with small residual granular wounds, treated conservatively (fig. 4).

Fig. 3. Skin plasty - early postoperative appearance

Fig. 4. Skin plasty - postoperative appearance

Whenever possible, the active and passive monitoring of the patient was performed, and an adequate recovery treatment adapted to the patient's clinical state was performed. The patient was discharged after he had cured (fig. 5), with laboratory tests within normal limits (blood glucose level 110 mg/dL under insulin treatment), being referred to a nutrition and metabolic disease facility in order to determine the therapeutic scheme for diabetes mellitus. The control performed after 6 months shows a patient with a good general condition and healed wounds that has a normal mobility. (fig. 6)

Fig. 5. The appearance of postoperative wounds at the discharge of the patient

Fig. 6. Scarred wounds at 6 months after surgery

DISCUSSION

Diabetes mellitus predisposes to increased mortality compared to the general population (the low immune reactivity of the body of diabetic patients being known) and the necrosis of the affected necrotic tissues being more common and severe in these patients than in the general population (5), (6), (7),(8). The diagnosis of necrotizing fasciitis is made after the clinical examination, but the diagnosis of certainty is made intraoperatively through the identification of necrosis in the muscle fascia in the affected area.

In 2004, Wong and his team imagined a scoring system for the assessment of the risk of developing necrotizing fasciitis. It is called The Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) (tab. I). A score higher or equal to 6 is equivalent to a significant risk that the patient may develop necrotizing fasciitis (9), (10).

TABLE I

The Laboratory Risk Indicator for Necrotizing Fasciitis (10)

PARAMETER	RANGE	SCORE	PARAMETER	RANGE	SCORE
Hb (g/dL)	>13.5	0	Sodium (mmol/L)	<135	2
	11–13.5	1	Creatinine (μmol/L)	>141	2
	<11	2	Glucose (mmol/L)	>10	1
White cells (10^9/L)	<15	0	C-reactive protein (mg/dL)	>15	4
	15-25	1			
	>25	2			

In our patient's case, the LRINEC score on admission was 7, so there is a significant risk for him to develop necrotizing fasciitis, even though at the local examination he presents signs of inflammation only at the level of the right ischiorectal fossa.

All the studies performed in the last 30 years have concluded that the rapid and correct establishment of the diagnosis and the firm emergency surgical intervention with debridement and excision of the affected structures to healthy tissue and abundant lavage with antiseptic solutions combined with broad spectrum antibiotic therapy (empirical at first, and then adapted to the antibiogram), hydroelectrolitic and vasopressor support whenever needed (the patients being often unstable from a hemodynamic point of view) and adequate nutrition (preferably the patient be treated in an intensive care unit) are absolutely necessary measures for the attempt of saving these patients (11, 12).

CONCLUSIONS

Necrotizing fasciitis is an extremely severe affliction with a high mortality and only the prompt establishment of an adequate surgical treatment, in parallel with other measures of supported rebalancing of the patient and specific antibiotic treatment for a long time, treatment established by a multidisciplinary team (general surgeon, plastic surgeon, anesthesia and intensive care physician, recovery physician) can significantly reduce the risk of death.

Diabetes mellitus is a significant risk factor for the occurrence of necrotizing fasciitis and if we talk about an undiagnosed and implicitly untreated case of diabetes, it can significantly influence the prognosis of the patients due to the extent and severity of the lesions.

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PARTICULARITĂŢILE INFECŢIEI URINARE LA MALNUTRIŢI

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CHARACTERISTIC FEATURES OF URINARY TRACT INFECTION IN MALNOURISHED CHILDREN (Abstract): The aim of this study is to prove a relationship between urinary tract infection and malnutrition in children 0-3 years old, hospitalized in the IVth Pediatric Clinic, Hospital *St. Mary* Iaşi, between January 2000 and December 2004. **Material and methods**: We have made a retrospective study for 298 infant and young children with urinary tract infection, 237 eutrophic and 61 malnourished. We studied comparatively the both groups with urinary tract infection (UTI), and we applied statistic methods for results. **Results**: The statistic methods prove that relative risk for UTI increases in malnutrition, predictive positive value is 72.5%. The clinical manifestation is similar in malnourished and eutrophic, but many co morbidities were associated with dystrophic status. Malformation of urinary tract was associate two times more in malnourished child. The etiology of infection was dominated by *Escherichia coli, Proteus* and *Klebsiella pneumoniae*. In malnourished children 5% of UTI was determinate by opportunist etiological agents like: *Enterobacter, Enterococcus, Acinetobacter*. More frequently, bacterium develops resistance of antibiotics like aminopenicilina, Trimethoprim and Cephalosporin. **Conclusion**: Accurate and fast diagnosis and treatment of UTI in infant and child with malnutrition is the best way for nutritional rehabilitation and prevention of serious consequence. **Key words**: URINARY TRACT INFECTION, MALNUTRITION, PRETERM INFANT, EUTROPHIC, DYSTROPHIC

COMPLICATIONS IN ANTICOAGULATION THERAPY FOR PREGNANT WOMEN WITH MECHANICAL HEART VALVE. CASE REPORT AND REVIEW OF THE LITERATURE

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COMPLICATIONS IN ANTICOAGULATION THERAPY FOR PREGNANT WOMEN WITH MECHANICAL HEART VALVE. CASE REPORT AND REVIEW OF THE LITERATURE (Abstract): **Aim:** Bleeding complications happen more frequently in pregnant females who carry mechanical heart valves (MHVs) compared with biological prosthesis valves (BPVs). The main objective for these patients represents a rigorous and individual monitoring of the anticoagulant treatment in order to minimalize the rate of complications that may appear. We present from "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology from Iasi, the case of a 30-year-old pregnant woman with dichorionic-diamniotic twin pregnancy, barrier of MHV, who suffered postoperative bleeding complication under guidelines recommended doses of anticoagulant. The hematoma that developed five days after the caesarian section need it surgical intervention for hemostasis correction. **Conclusions:** The incidence of bleeding complications is higher during pregnancy and postpartum in MHV carriers. Thrombosis or hemorrhage usually occur when the monitoring of anticoagulation is improper or when the treatment must be interrupted for different reasons. **Keywords:** ANTICOAGULANT DRUGS, HEART VALVE PROSTHESIS, PREGNANCY.

Valvular heart disease represents the most widespread entity of cardiovascular pathologies among pregnant women, having the most ordinary etiologies, such as congenital heart conditions and rheumatic heart disease (1).

A brittle issue represents the management of women with mechanical heart valves (MHV) during pregnancy and per partum period. Over the years, the development of pharmaceutical industry and technological fields improved the usage of anticoagulants and biocompatibility of the MHV. By increasing the adhesion of a slender biomaterial layer to the biocompatible surface of the mechanical valves, the feasibility of MHVs augments, decreasing the rate of thrombosis complications (2-4).

The management of valvular heart diseases has developed tremendously, improving the quality of life for the population with congenital or acquired cardiac diseases. Therefore, the birth rate between fertile women with MHV is continuously increasing and requires more improvements regarding the control of anticoagulation (CA) among pregnancy (5).

Pregnant women with MHV present a significant risk of mortality and morbidity, with raised costs for their management as the oncology patients. Obstetrical complications are very common in the per partum period and often include postpartum hemorrhage (PPH), wound hematoma or intra-abdominal hemorrhage. The management must be multidisciplinary, made by a maternal-fetal medicine specialist, a cardiologist, a hematologist, an anesthesiologist, and a nursing personnel experienced in obstetrics and cardiology, in order to consider and handle the risks and benefits for every patient (5).

CASE REPORT

A 30-year-old Caucasian women, 72 kg, 172 cm, pregnant III, para III with dichorionic–diamniotic twin pregnancy of 36 weeks + 2 days (first fetus – complete breech presentation; second fetus – transverse presentation) known with intrahepatic cholestasis of pregnancy was admitted to the Obstetrics Department of "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology from Iasi, complaining for non-systemic uterine contractions. Her medical history revealed two anterior caesarean sections, MHV, heart failure NYHA class II, chronic venous insufficiency, and atrial septal defect closure which required surgery at young age. Clinical examination at that moment was found normal. The pathologic results of patient`s blood test revealed: ALT= 56 U/L, AST= 47 U/L, Serum Bile Acids= 27.0 μmol/L, Fibrinogen= 522 mg/dL and INR= 0.97.

One month before, the patient performed a cardiologic examination and echocardiogram. The results shown a preserved systolic function of the left ventricle, without segmental kinetic disturbance, normal ejection fraction, mild tricuspid regurgitation, no signs of pulmonary hypertension and normal quantity of pericardial fluid. The patient was under treatment with Acenocoumarol 4 mg per day during the pregnancy and switched it to Enoxaparin 8000 UI/0.8mL x 2 / day, as the cardiologist recommended.

Two days after, she was admitted to Intensive Care Unit, having systematic uterine contractions, and the caesarean section was perform without any bleeding complications or others. The patient gave birth to twin who weighed 3,100 g, respectively 3,300 g, both of them having 7 Apgar score at 5 minutes. The bacterial endocarditis prophylaxis was made and in postpartum period the patient was treated with Cefuroxime 1.5 g/12 hrs, Enoxaparin 7,000 UI/0.7 mL and 6,000 UI/0.6 mL/24 hrs., and anti-inflammatory drugs. The next day after surgery, the coagulation parameters were found within normal values.

In the 5th day, the patient accused high intensity pain in the hypogastrium. Her clinical examination revealed a tendered and distended lower quadrant; a round, rough consistency mass (15 cm diameter) was noticed between the two abdominal flanks. Abdominal ultrasound revealed a normally decreased uterine size without any retention of placenta or membranes fragments and a hypoechoic area measuring 150 x 100 mm, being suggestive for preper-

itoneal hematoma (fig. 1). Blood tests were abnormal within WBC=13.88 10^3/uL, NEU=11.5 10^3/uL, HGB= 7.5 g/dL, HCT= 22.4%, CRP= 96 mg/L, Fibrinogen= 647 mg/dL.

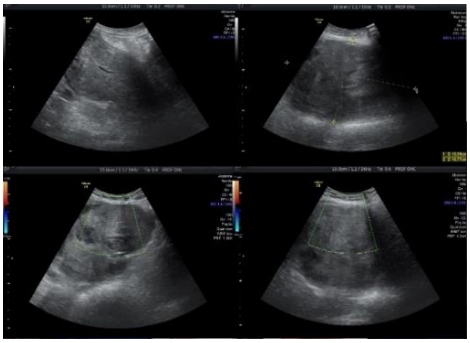

Fig. 1. Abdominal ultrasound examination of the uterus and hypoechoic mass measuring 15/10 cm suggestive for preperitoneal hematoma after cesarean section

Surgical treatment was performed in order to evacuate the preperitoneal hematoma and to establish the hemostasis. Intraoperative, it was found preperitoneal organized blood clots and approximately 1,000 mL of dark blood (fig. 2). A good hemostasis was obtained with no intraoperative complications. Postoperative, the patient went under treatment with Enoxaparin 6,000U/ 0.6 mL/12 hrs., Metronidazole 500 mg/100 mL, and Cefuroxime 1.5g/12 hrs. Three days after the intervention, the patient change Enoxaparin anticoagulant therapy with Acenocoumarol 4 mg 3 tb/day. Blood test results shown: HGB= 8.5 g/dL, HCT= 16.2%, aPTT= 31.1, D-Dimer tests= 200-400 ng/mL, Fibrinogen= 681 mg/dL, Quick time= 14.6/sec, INR= 1.09, IQ= 93, ISI= 1.24.

The postoperative patient's evolution was favorable, without any other bleeding complications. In day she went out the hospital her vital signs were stable and blood test in physiologic parameters.

Fig. 2. Massive (subaponeurotic) preperitoneal hematoma

DISCUSSION

In the presented case, the patient was under VKA (Vitamin K Antagonists) since the beginning of pregnancy and switched to 8,000 UI/0.8 mL Enoxaparin, twice per day, one month before the admission in the Obstetric Department, as the cardiologist recommended. Among the pregnancy, no bleeding or thrombosis episodes and no signs of fetal toxicity were reported. During the surgery, the intraoperative bleeding was normal with no hemorrhagic episodes and a rigorous hemostasis was performed. In postpartum, the anesthesiologist reduced the anticoagulant doses to 7,000 UI/0.7 mL and 6,000 UI/0.6 mL, respectively, per 24

hours. Even though the coagulogram parameters were within normal before and post-surgery, in the 5th day after the C-section the patient developed a large hematoma that need it reintervention.

The optimal dose of anticoagulant should be establish in a manner to create a balance between the risk of hemorrhage and thrombosis (fig. 3). After the second intervention, the Enoxaparin was reduced again to 6,000 UI/0.6 mL every 12 hours and three days after was switch to 4 mg of Acenocoumarol/day, with no further bleeding or thrombosis episodes.

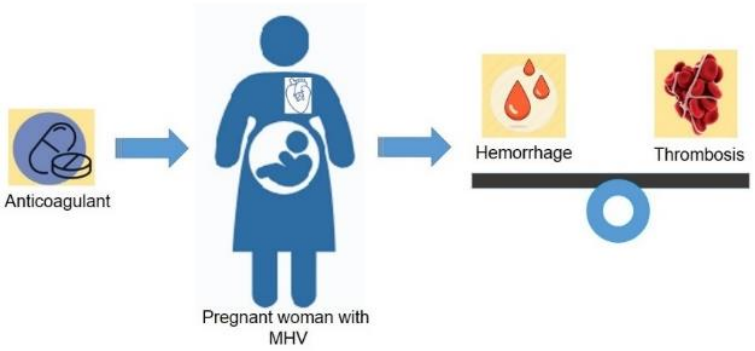

Fig. 3. The expectant response of optimal dose of anticoagulant treatment in pregnant women with MHV

The flawless anticoagulant indicated in pregnancy should be safe for the fetus, to not cross through hemato-placental barrier, in order to avoid fetal hemorrhage and teratogenicity. Vitamin K antagonists, unfractionated heparin and low-molecular-weight heparin represent the allowed anticoagulant drugs for pregnant patients who carry MHVs, even though all these treatment classes are associated with feto-maternal adverse events (6).

VKA are teratogenic drugs that cross the hemato-placental barrier, causing osseous and cartilaginous maldevelopment as well as anomalies of the central nervous system. It must not be administered between 6 and 12 gestational weeks due to its higher risk of embryopathies (7). Its fetal risk outcomes depend on the dose that is administered, usual ≤ 5 mg of warfarin per day has a reasonably safer profile (8).

European Society of Cardiology (ESC) guidelines and American Heart Association (AHA)/American College of Cardiology (ACC) advise VKAs sequel during pregnancy at the lowest doses with the administration under strict control of UFH (Unfractionated Heparin) or LMWH (Low Molecular-Weight Heparin) between 6-12 gestational weeks period. To what regards the birth plan, natural delivery is common advisable. Cesarean section is recommended when is need it to deliver rapidly due to the obstetrical indications or in a full VKA anticoagulated patient with premature labor in order to decrease the risk of fetal intracranial hemorrhage during vaginal delivery. Interruption of VKA and induction of intravenous UFH (requires an activated partial thromboplastin time– aPTT –more than two

times followed) or LMWH (requires a target of anti-Xa level between 0.8-1.2 U/mL, every 4-6 hours after de administration) should be initiated with 2 weeks anterior the planned vaginal birth due to the risk for premature labor. Low-molecular weight heparin must be changed with UFH before delivery, whilst UFH should be administrated during the labor and restarted after 4-6 hours the delivery happened (9, 10).

A meta-analysis, conducted by Steinberg *et al.* (2017), found that VKA therapy experienced the most decreased rate of adverse maternal effects compared with LMWH which presented the lowest risk for adverse fetal effects. Also, they observed an equivalent fetal risk in women treated with ≤ 5 mg of warfarin per day and those who received LMWH (11). Another study reported that VKAs encounter the smallest rate of maternal complications and live births, whereas LMWH associate the highest rate of live births (12).

Bleeding complications are quite frequent when anticoagulation therapy is used. Cardiology Registry of Pregnancy and Cardiac Registry (ROPAC) showed 23% rate of bleeding complications whilst United Kingdom Obstetric Surveillance System (UKOSS) study presented a higher rate, up to 29% (13, 14). In some situations, the warfarin therapy can be delayed to prevent PPH but requires a careful obstetric monitoring. Patients with MHV present low pregnancy outcomes, especially when high doses of warfarin are administered in the first trimester of pregnancy. It was reported an increased rate miscarriages, stillbirths, neonatal death, preterm labors, and certain fetal abnormalities. Bleeding complications can be hard to control in gynecology pathologies (15) and it becomes more complicated when the patient associates an MHV due to a cardiovascular disease.

Thus, the results we are presenting here on this gynecological-cardiovascular multidisciplinary context could have a further relevance in these complex pathophysiological approaches, as this could be an important management strategy for cases like this, as our team demonstrated in previous related works (16-18).

In our case, the patient had a favorable prognosis after second time reduction of the anticoagulant therapy. The increased dose of Enoxaparin and a rigorous monitoring prevented another episode of hemorrhage. Although the patient received the correct doses of anticoagulant, according to her weight and associate pathology, as the guidelines recommend, she still manifested hemorrhagic complication, reaching the conclusion that a rigorous monitoring is necessary even under a correct therapy.

CONCLUSIONS

Pregnant women who carry mechanical heart valve acquire an increased risk to develop thrombotic or hemorrhagic complications, and chronic anticoagulation is need it, but the long-term anticoagulation needs to be safe for both mother and fetus.

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LAPAROSCOPY IN ACUTE ABDOMINAL DISORDERS

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LAPAROSCOPY IN ACUTE ABDOMINAL DISORDERS (Abstract): In emergency preoperative diagnosis is most accurate in acute cholecystitis and least accurate in pelvic conditions and mesenteric ischemia. Laparoscopy has a double goal: establish or confirm diagnosis and treating the disorders. Laparoscopy identifies those patients who do not need surgery. Our experience includes 2,778 patients with surgical acute abdomen: 1,857 cases with acute cholecystitis, 719 cases with acute appendicitis, 57 cases with perforated peptic ulcer, 10 cases with intestinal obstruction on postoperative adhesions, 19 cases with abdominal trauma, 87 cases with acute adnexal pathologies, 4 cases with acute pancreatitis, 12 cases with mesenteric ischemia, 12 cases with abdominal abscesses and one case with false acute abdomen. Our experience clearly shows the feasibility and safety of the laparoscopic appendectomy. The minimal access approach brings back suture in the treatment of perforated peptic ulcer in the conditions of an efficient postoperative medical treatment. Keywords: LAPAROSCOPY, ACUTE ABDOMEN, UNNECESSARY LAPAROTOMIES.

The recent expansion of both diagnostic and therapeutic laparoscopy has added a new tool to the surgical armamentarium. Laparoscopy is an efficient method for diagnostic in surgical acute abdomen because usually allows a good view of the uterus and adnexa, the appendix, the gallbladder, the stomach, the pylorus, and the first portion of the duodenum.

The laparoscope was introduced to determine whether the laparotomy would be required. With the development of new techniques, it has become possible to perform definitive therapeutic procedures under videoendoscopic control in the setting of the acute abdomen.

Laparoscopic appendectomy and closure of perforated duodenal ulcer have become common procedures, and laparoscopic cholecystectomy is now the standard of care in acute cholecystitis.

Laparoscopy can be used to assess intestinal viability if bowel ischemia is suspected.

MATERIAL AND METHODS

Between January 2013 and March 2020, in the First Surgical Clinic, from "Sf. Spir-

idon" County Clinical Emergency Hospital, Iasi were performed 8,367 laparoscopic interventions; 2,778 interventions of these (33,2%) were performed in emergency.

Laparoscopies establish the following diagnostic: Perforated peptic ulcer – 57 cases; Abdominal trauma – 19 cases; Intestinal infarction – 12 cases; Intestinal obstruction – 10 cases; Acute appendicitis – 719 cases; Acute adnexal pathologies – 87 cases; Acute cholecystitis – 1,857 cases; Acute pancreatitis – 4 cases; abdominal abscesses – 12 cases; False acute abdomen – 1 case.

RESULTS

8031 laparoscopic cholecystectomy was performed in our clinic for various anatomoclinical form of cholecystitis. Laparoscopic cholecystectomy for acute cholecystitis was performed in a series of 1857 patients (71.9% women and 28.1% men) with mean age 50.4 years (between 18 and 82 years). Obesity was encountered in 78% of the cases. Clinical aspect was typical for acute cholecystitis in 93.7% of the cases. In all cases diagnosis was confirmed or suggested at ultrasound examination. In most cases acute cholecystitis was related to gallstones presence; only 5.7% of the cases were not determined by lithiasis.

At pathology, the lesions were: edematous acute cholecystitis (72.3%); phlegmonous acute cholecystitis (8.1%); gangrenous acute cholecystitis (9.5%); plastron (11,1%).

Intraoperative in 80% of the cases we found pericholecystic adhesions. In 31.2% Hartmann pouch was placed behind porta hepatis. This was not surprising because with an inflamed gallbladder the anatomy in the area may be severe distorted. If the dissection was difficult or the ductal anatomy was not clear we converted to open cholecystectomy, because in this conditions the risk of ductal lesions is rather high. Conversion rate in this series was 9.1% (169 cases) as compared to a 2.8% rate in the entire series of laparoscopic cholecystectomies. The rate of biliary lesions was 0.1%.

Decompression of the enlarged gallbladder was needed in 80% of the cases.

We encountered the following intraoperative complications: gallbladder perforation (31.8%); hemorrhage (16.8%); gallstone loss (11.2%).

Mean operating time was 78 minutes (25-130 minutes), much longer than the mean value for laparoscopic cholecystectomy in general. Bottom line is that the intervention was easy in only 7.8% of the cases. In most cases we gave antibioprophylaxis. Postoperative morbidity was low, 5.6%. Mortality was 0.18% (2 cases – the cause was pulmonary thromboembolism). Mean postoperative hospitalization was 3.8 days. Laparoscopic cholecystectomy can be performed routinely for acute cholecystitis. Laparoscopic appendicectomy was performed on a series of 719 patients (81% women and 19% men). Their age was between 12 and 68 (mean 23) years.

Laparoscopic appendicectomy was indicated in the following situations: women at the reproductive age, because of the difficulties of establishing an accurate preoperative diagnosis in their cases; in our series the diagnosis error rate in this group was 20%; obesity, present in 32% of the cases; young patients; sportsmen.

At pathology, the lesions were: edematous appendicitis (61.5%); phlegmonous appendicitis (25.3%); gangrenous appendi-

citis (7.6%); plastron (5.6%).

The technique used was: "in" in 120 cases (16.7%); "out" in 252 cases (35%) and mixed in 298 cases (41.49%). Conversion rate was 6.81% (49 cases).

Morbidity was: 4 cases with parietal hematomas (0.55%); 5 cases with wound infections (0.69%).

Complications encountered were: 2 cases with peritonitis (6th day postoperative) 0.27%. Mean hospital stay was 3.2 days.

In our experience laparoscopic appendicectomy has certain advantages allows diagnosis of other conditions; decreases wound infection rate; deceases hospital stay; decreases recovery time for the patient; best cosmetic results.

Laparoscopic closure of duodenal peptic ulcer was performed in a series of 57 patients (all men) with mean age 28 years (between 18 and 58 years). The presentation was at 1-10 hours after symptoms onset. Preoperative diagnosis was perforated ulcer in most cases, pneumoperitoneum being present in 55 cases. At presentation, the patients had acute abdomen symptoms; perforations were small (< 5 mm) in all cases. Suture closure with an omental patch was performed in 57 cases. It is very important to wash and drainage very well the peritoneal cavity. Laparoscopic intervention offers this possibility. In only one case an inadequate closure leaded to a leak and after two days we performed a classic intervention. There were no other complications, and the morbidity was zero. Mean postoperative hospitalization was 4.6 days. Postoperative all patients received medical treatment for ulcer and sterilization of *Helicobacter pylori*. At follow-up after 3 months and a year there was no recurrent peptic ulcer in all cases.

Open laparoscopy was performed in 10 cases of intestinal subacute obstruction by postoperative adhesions. In only 5 cases was possible to performed adhesiolysis. In all other cases we had to convert to open procedure.

Laparoscopic surgery for abdominal trauma was performed in 19 cases, 5 cases for wound trauma and 14 cases for abdominal blunt trauma (hemoperitoneum). In abdominal blunt trauma intraoperative diagnosis was 3 hepatic lesions (II-III Moore degree) for who was performed exploration and hemostasis with Argon beam and TachoComb® (superficial injury of the liver) and 11 splenic lesions (II-III Moore degree) for who we performed laparoscopic splenectomies in 3 cases (rupture hematoma spleen), laparoscopic diagnosis with conservative treatment and spleen preservation in 2 cases and 6 conversions with spleen preservation in one case (packing with resorbable prosthesis).

Laparoscopic procedures were performed for acute adnexal pathologies in 87 patients. In cases with torsion of the ovary was required oophorectomy. In 6 cases with tubal pregnancies, we performed 3 salpingostomy and 3 salpingectomy, respectively. Otherwise, there were cases of ruptured ovarian cysts hemorrhagic. We did not include cases of pelvic inflammatory disease.

Other causes of acute abdomen in which we intervened by means of laparoscopy were: 4 cases of acute pancreatitis and 10 cases with bowel ischemia. In all this 4 cases an open laparotomy was avoided.

DISCUSSION

Many of the causes of the acute abdomen can be managed by laparoscopic ap-

proach (1). The therapeutic approach in these patients is based on clinical suspicion and on the results of diagnostic laparoscopy. Each procedure started by performing diagnostic laparoscopy to determine the specific abnormality present. Laparoscopy has become a routine procedure in the management of acute abdominal disease and can be considered both an excellent therapeutic and additional diagnostic tool in selected cases (2).

The diagnostic of acute cholecystitis is usually made preoperatively by clinical examination and ultrasound exploration. Sometimes acute cholecystitis may present as an acute abdomen if the gallbladder is gangrenous or perforated. Grasping of the gallbladder can be difficult when an acute state is present. We do not hesitate to decompress the gallbladder to improve the ability to grasp an acute inflamed gallbladder.

Laparoscopic cholecystectomy can be routinely performed in acute cholecystitis, but the surgeon must be prepared to convert to open cholecystectomy. If there is necrosis or the gallbladder cannot otherwise be retracted, then the operation should be converted to open approach.

It is also important to not spend to long with the laparoscopic technique if the dissection is difficult or the ductal anatomy is not clear.

The complications are more frequent in laparoscopic cholecystectomy than in open method. Bleeding from the liver can be a significant problem. Bile leaks can occur the cystic duct where a clip has fallen off.

Endoscopic retrograde cholangiopancreatography is used to identify the site of the ductal leak and to rule out injury to the common bile duct.

Common bile ducts injuries typically occur when the common duct or the right hepatic duct have been misidentified as the cystic duct. This injury is preventable by performing meticulous dissection beginning at the junction of the neck of the gallbladder and the cystic duct.

Indications for laparoscopic appendectomy include patients with an uncertain diagnosis (elderly, reproductive age females), obese patients, and those with suspected perforated appendicitis.

Laparoscopic management of perforated appendicitis is superior to open technique.

Dissection of adhesions and the irrigation of the entire abdominal cavity can be realized without extension of the incision. Simultaneously, another cause of acute abdomen can be identified and operatively managed. Obese patients have an increased wound infection rate and require larger incisions for open procedure. Further advantages of the laparoscopic technique include decreased exposure of subcutaneous tissue to the infected appendix and therefore a lower infection rate (3). It is better to performing appendectomy even if the organ appears normal (sometimes the appendiceal inflammation can be detectable only at the microscopic examination).

The preoperative diagnosis of perforated peptic ulcer is usually simple established by clinical examination and presence of air in peritoneal cavity on the abdominal radiographic films. The laparoscopic approach brings back the suture closure with an omental patch in the treatment of perforated duodenal ulcer. The modern medical treatment of peptic ulcer avoids the recurrent ulcer.

If the perforation is small and surrounding tissue is healthy it is possible to per-

formed direct closure using 3-0 polyglactin suture closure on atraumatic needles. It is better to close the defect in a transverse direction to prevent narrowing of the lumen.

Large perforations or those with large amount surrounding tissue friability and edema can be closed with an omental plug. The peritoneal cavity must be thoroughly irrigated with warm saline solution. Tilting the table in different positions helps with irrigating the entire peritoneal surface (4).

"Kissing ulcers" can occurs in which there is an anterior perforation and posterior penetration with bleeding. In these conditions it may be necessary to convert to laparotomy.

Acute abdominal pain from gynecologic sources is a common problem in surgery. The etiology of this pain in reproductive age females is often obscure (5). Many of these gynecologic sources of acute abdominal pain can be managed laparoscopically. Laparoscopy is indicated when the diagnosis is uncertain, the patient is obese, and the definitive operative management can be handled with the laparoscopic technique. The only contraindication is the patient with hemorrhagic shock.

Another cause of acute abdomen can be the acute pancreatitis. The laparoscopic diagnosis is made by opening the lesser sac with scissors dissection.

Ischemic bowel can be found at laparoscopy for an acute abdomen. It depends on the judgment of the surgeon to determine if it is hopeless situation (6). If the bowel is ischemic and not necrotic, the patient should have emergency mesenteric angiography.

The use of laparoscopy in blunt abdominal trauma has been expanded from merely a diagnostic tool to a therapeutic modality in selected cases (7). This has resulted in a quicker and more specific diagnosis and avoidance of unnecessary laparotomies.

Regarding the outcome measures for cases of the acute abdomen as a whole, Agresta *et al.* (8) included 1,272 patients admitted with acute abdomen who were approached laparoscopically. In comparison with open surgery, there was a significant reduction of total complication in laparoscopic treatment than open surgery (1.9 *vs.*13%).

Hemodynamic instability, huge abdominal distension, fecal peritonitis, and perforated cancer are relative contraindications for the laparoscopic approach. In recent years, abdominal emergencies have increasingly been managed successfully by laparoscopy. In acute appendicitis, acute cholecystitis and perforated peptic ulcer, randomized controlled trials have proven that the laparoscopic approach is as safe and as effective as open surgery, with fewer complications and a quicker postoperative recovery. Other indications such as blunt and penetrating trauma to the abdomen, small bowel occlusion and perforated diverticular disease are under debate, indicating that more randomized controlled trials comparing laparoscopic and open surgery are still necessary (6).

CONCLUSIONS

Laparoscopy is very important in the diagnosis and treatment of acute abdomen conditions. This is due to certain advantages as the ability to examine the entire peritoneal cavity and perform a definitive procedure, and of course when needed there is always the possibility to convert to an open procedure. Laparoscopy also reduces unnecessary laparotomies.

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NEWS

LONG TERM CONTROL IN ACROMEGALY - IS IT ACHIEVABLE?

Acromegaly is a rare endocrine disorder characterized by an excessive chronic secretion of GH and IGF-I, that, in time, results in numerous comorbidities. Biochemical control is not easily achievable but is of paramount importance for a reduction in morbidity and mortality. The presented results come from an extension to a prospective, multicenter, randomized, parallel-group, 6-month core study (Phase III PAOLA study). In the core study, patients were randomized to double-blind long-acting pasireotide 40 mg/28 days or 60 mg/28 days, or continued treatment with open-label long-acting octreotide 30 mg or lanreotide 120 mg every 28 days. As much as half of the enrolled patients had at least three comorbidities (mostly cardiovascular and glucose- or lipid-related disorders) at study entry. The conclusion of this study is that long-acting pasireotide was an effective and consistent therapeutic option for up to 5.8 years of treatment in patients with previously uncontrolled acromegaly and a high prevalence of comorbidities (cardiovascular, metabolic, including glucose-related disorders). (Colao A, Bronstein MD, Brue T, *et al*. Pasireotide for acromegaly: long-term outcomes from an extension to the Phase III PAOLA study. *Europ J Endocrinol* 2020; 182(6): 583-594).

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PONSETI METHOD AS A TREATMENT OPTION FOR NEGLECTED CLUBFOOT. CASE REPORT AND LITERATURE REVIEW

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PONSETI METHOD AS A TREATMENT OPTION FOR NEGLECTED CLUBFOOT. CASE REPORT AND LITERATURE REVIEW (Abstract): Congenital clubfoot is a common congenital malformation, whose conservative treatment using the Ponseti method achieves excellent initial correction rates and good long-term results. On the other hand, neglected clubfeet, which are typically more frequent in underdeveloped and developing countries, pose treatment difficulties and render corrective surgery necessary. The main problems with correction are raised by the increasing stiffness present in the soft tissues and the dysplastic development of bones. Furthermore, the hyperlaxity of young children decreases with growth, contributing to the reduced overall elasticity of the affected soft tissues and clubfeet. Deciding upon which type of treatment would be appropriate is not always facile. In our clinic, we tend to start the treatment of neglected clubfeet with the modified Ponseti method, which can achieve partial correction or full correction in most cases, diminishing the need for extensive surgical procedures. The case report of a long time neglected bilateral clubfoot successfully corrected using the modified Ponseti method is presented below, followed by a relevant review of the literature. **Keywords**: NEGLECTED CLUBFOOT, STIFF CLUBFOOT, LATE PRESENTING CLUBFOOT, POST IMMOBILIZATION FRACTURE, POST-IMMOBILIZATION OSTEOPOROSIS.

Congenital talipes equinovarus is a frequent congenital malformation with an incidence of 1-2 cases per 1,000 births in Europe (1-3). Most clubfeet are idiopathic as the etiology is still unknown and they should be distinguished from syndromic clubfeet occurring in association with neuromuscular diseases, genetic syndromes, teratogen-induced malformations. Regardless of etiology, the diagnosis of clubfoot remains clinical, and it is based on the presence of four deformities which are almost always present: cavus, adductus, varus and equinus. The treatment of clubfoot is usually done before walking age and the Ponseti method (4), which is the gold standard, achieves correction in over 90% of the cases (5, 6). In these cases, surgery is reserved for resistant clubfeet and treatment of recurrences and residual deformities. Neglected clubfeet are defined as those clubfeet diagnosed and treated after walking age, which are more difficult to treat due to increased stiffness. Probably manipulative treatments have been

tried as initial treatment, but surgery remains the main method for their correction so far, especially in those severe cases which were neglected for a long

Fig. 1. Clinical aspect of neglected clubfeet before, during (swelling around the ankle and medial side of the foot were present during treatment and disappeared completely until the end of treatment) and after treatment (clinically moderate persistent cavus in the right foot).

CASE REPORT

We present the case of an 8-year and 11-month-old boy referred to our clinic for severe bilateral deformation of the feet associated with difficulty in maintaining balance during walking. Informed consent was obtained from his legal guardians. According to his parents, the deviations started developing progressively in both feet at the age of 2.5-3 years. The reason for the late presentation was his medical history, which included: growth delay of undetermined cause, extragonadal stage IV germ cell tumor of the left internal obturator muscle with pulmonary metastases treated by resection and chemotherapy (in complete remission at presentation), pulmonary emphysema, and post-thrombotic syndrome of the left common and internal iliac veins, both of which apparently developed during oncological treatment. Electromyography revealed a peripheral axonal demyelination in both lower limbs suggestive of severe sensory and motor polyneuropathy, further diagnostic studies being required. Clinical examination revealed severe stiff clubfeet, both graded Pirani 4 and Dimeglio grade IV, bilateral calf hypotrophy; calluses on the dorsolateral aspect of both feet; bilateral *genu flexum*, and no active toe extension. As

treatment, we used the modified Ponseti technique (7) which practically follows the same steps as the original Ponseti method, except the cast change interval (every two weeks). Due to patient's medical history, we prescribed Enoxaparin 2,000 IU per day for the deep vein thrombosis (DVT) prophylaxis during the casting period. In-between cast change interval, the patient routinely underwent Doppler ultrasound examination of both limbs and pelvis in order to exclude occult DVT. The duration of the correction phase was 14 weeks, and we applied a number of 6 casts according to the modified Ponseti Protocol (fig. 1). The cavus deformity was corrected during the first 4 weeks (2 casts), while the adductus and varus deformity were corrected in the following 6 weeks (3 casts). Lastly, the equinus deformity was corrected by performing a percutaneous Achilles tenotomy under general sedation and local anesthesia due to patient's history of pulmonary emphysema, achieving approximately 10 degrees of dorsiflexion bilaterally. The patient was immobilized in a long-leg plaster cast for 4 weeks maintaining the foot in dorsiflexion and abducted to approximately 60 degrees. After last cast removal, we prescribed a full-time foot ankle abduction orthosis for the following 3 months, but the patient had a hard time tolerating it.

Full-weight bearing was allowed only after 4 weeks due to the osteoporotic appearance of bones on the X-rays taken at the end of the correction phase (fig. 2).

The improvements of the clubfeet during the correction phase were slow but encouraging. Nevertheless, starting with week 4 of the correction phase the patient complained of occasional pain at the level of the talar head and anteromedial aspect of the ankle joint during immobilization (counterpressure point). The pain typically disappeared the same day. Several days after weight bearing during physical therapy, the patient described pain in the middle third of the right calf, which disappeared completely after one month. The X-rays taken 8 weeks later showed the presence of a healed stress fracture of the tibia and fibula, with no misalignment (fig. 3).

Fig. 2. Anteroposterior X-ray of the feet before tenotomy showing osteoporotic appearance in both feet, normal talocalcaneal relationship, well-reduced navicular covering the talar head.

Fig. 3. Weightbearing lateral X-ray of the right lower leg.

Healed diaphyseal stress fractures of the right tibia and fibula two months after weight bearing was permitted. Plantigrade aspect of the foot.

DISCUSSION

The purpose of clubfoot treatment is to obtain a plantigrade, functional, painless foot, which we achieved in the end (fig. 1). To our knowledge, this is the first published case report of a secondary neglected clubfoot treated with the Ponseti method. In this particular patient, the associated comorbidities were the main reason for the late presentation. Furthermore, had a corrective surgical release been chosen as a treatment option, anesthesia would have carried more risks due to our patient's associated conditions. Surgery is still the first option of treatment in many countries despite the fact that it is technically more difficult, carries more risks of complications, is more painful and more expensive (8-10).

Starting with the worldwide adoption of the Ponseti method as first option in clubfoot treatment, the age interval for using this method has increased. Usually, the mean age at treatment initiation is between 2 and 5 years, but children of older age can be found dispersed in the literature throughout different published studies (9, 11, 12). Correction rates ranging from 67% to 100% have been reported using the Ponseti method, without associated extensive surgery, which is remarkable taking into consideration that almost all neglected clubfeet are very stiff and severe (7, 13, 14). The mean age at presentation and mean number of biweekly applied casts found in the literature is similar to ours: 3 to 4 months and 7 to 12.8 casts (7, 13). The dorsiflexion range obtained after tenotomy tends to diminish in older children (13), but it is still compatible with a plantigrade foot and ability to wear normal shoes, so the family is generally satisfied with the result. Although in his initial study Lourenco (7) maintained the post-tenotomy plaster for 5 weeks to allow healing of the Achilles tendon, other authors did not encounter any complications regarding Achilles tendon healing after a 4-week post-tenotomy immobilization (13, 15). Regarding the bracing protocol used to prevent recurrences, most authors prescribe it for one year in case of children older than 4 years. However, as in the case of our patient, tolerance to bracing might be reduced in older children, hence compliance rates may diminish. Different dermatologic reactions like swelling and skin erythema (fig. 1) might be seen during the correction phase and are thought to be caused by excessive pressure, but they are usually mild and do not need treatment. On the other hand, osteopenia induced by long periods of immobilization is a concerning aspect as it increases the risk of iatrogenic fractures. They are considered to have a risk incidence of 1.3% in children younger than 3 years whose feet are forcefully manipulated (16). Besides this aspect, as seen in our case report (fig. 2), osteoporosis may facilitate the development of stress fractures during weight bearing even when precautions are taken.

CONCLUSIONS

Although surgical treatment is probably the first choice of treatment in patients with neglected clubfeet in many pediatric orthopedics centers, the modified Ponseti technique shows encouraging results and is worth to be taken into consideration as a treatment option. We believe this modified manipulative method can at least partially correct the deformities in most cases, reducing the extent of further surgical procedures required to complete the correction.

CONFLICT OF INTERESTS AND FUNDING

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EPIDEMIOLOGY OF PURULENT-SEPTIC INFECTIONS CAUSED BY METHICILLIN-RESISTANT *STAPHYLOCOCCUS* IN THE REPUBLIC OF MOLDOVA

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EPIDEMIOLOGY OF PURULENT-SEPTIC INFECTIONS CAUSED BY METHICILLIN-RESISTANT *STAPHYLOCOCCUS* IN THE REPUBLIC OF MOLDOVA **(Abstract):** *Staphylococcus* infections are a major global concern, caused by the annual growth of methicillin-resistant *Staphylococcus* strains and the emergence of strains resistant to reserve anti-staphylococcal antibiotics. **Aim:** The aim of the study was to determine the risk factors, the degree of spread of methicillin-resistant *Staphylococcus* (MRS) strains in health care facilities in the Republic of Moldova, as well as their resistance to antibacterial drugs. **Material and methods:** The research of the real incidence, epidemiological peculiarities and the degree of sensitivity/resistance of MRS infections to antibiotics was performed based on the descriptive cross-sectional observational epidemiological study, overall being analyzed 10,055 staphylococcal strains. The risk factors and socio-economic impact were determined based on a retrospective case-control study. The research group included 269 patients with bacteriological investigations positive for methicillin-resistant *Staphylococcus* (MRS), the control group - 159 patients with bacteriological investigations positive for methicillin-sensitive *Staphylococcus* (MSS). **Results:** The share of MRS strains among *Staphylococcus* strains in the Republic of Moldova is on average 35.97%. A higher level of spread of MRS strains is recorded in maternity hospitals - 61.81%, followed by multi-profile health care facilities - 36.32%, slightly lower in rural health care facilities - 22.36%. The prevalence of MRS strains varied depending on the profile of departments and pathological products. Most often, MRS strains were isolated from patients in the departments of surgery, traumatology, orthopedics, resuscitation and intensive care. A high level of MRS was found in blood cultures - 76.11%, and 96.3% of MRS strains isolated from blood proved to be polyresistant. **Keywords:** HEALTHCARE-ASSOCIATED INFECTIONS, METHICILLIN-RESISTANT *STAPHYLOCOCCUS*, METHICILLIN-SENSITIVE *STAPHYLOCOCCUS*, POLYRESISTANCE, ANTIBIOTICS.

Healthcare-associated infections caused by antimicrobial-resistant microorganisms are today one of the most important challenges for modern medicine. There are 426,277 healthcare-associated infections in the EU each year caused by antimicrobial-resistant microorganisms, the number of deaths being estimated at 33,110 per year (1, 2).

Currently, *Staphylococcus* infections are mostly produced by multidrug resistant strains to a very wide range of antibiotics used in the inpatient setting (2, 3).

As the last decades have been marked

by a progressive and rapid increase in the number of resistant bacterial strains involved in human diseases and a low rate of introduction of new antibiotics into therapy, in 2017, the WHO established the most important categories of multidrug resistant germs which require the introduction of new therapeutic strategies, with 3 priority levels: critical, high and medium, the methicillin-resistant *Staphylococcus aureus* (MRSA) strains belonging to the high level (4, 5).

Staphylococci which rank first in the etiology of bacterial infections, the annual increase in the number of methicillin-resistant staphylococcal strains and the emergence of strains resistant to reserve anti-staphylococcal antibiotics, place this infection among emerging infectious diseases (2, 6, 7, 8).

From the first cases of MRSA to date, the scale of the problem has increased dramatically, the incidence of MRSA infections reaching endemic proportions in some hospitals. In Europe, a north-south gradient is observed, MRSA strains being rare in Scandinavian hospitals (<2%) and much more widespread in hospitals in Mediterranean countries (> 40%) (9, 10, 11, 12).

Although in recent years there has been a decrease in the percentage of methicillin-resistant *Staphylococcus* strains in the EU/EEA, from an average of 19.6% in 2014 to 16.9% in 2017, MRSA remains a major pathogen in the EU/EEA, MRSA levels being high in several countries, and resistance to many antimicrobial groups is steadily increasing (12).

Knowledge of risk factors, transmission mechanisms and local epidemiology in methicillin-resistant *Staphylococcus* (MRS) infections will contribute to the implementation of surveillance and control measures as well as to the improvement of the given recommendations.

MATERIAL AND METHODS

The research of the real incidence, epidemiological peculiarities, nosological forms and the degree of sensitivity/resistance of MRS infections to antibiotics was performed based on the descriptive cross-sectional observational epidemiological study. The study included the results of bacteriological investigations of patients with purulent-septic infections (PSIs) from two multi-profile health care facilities over a four-year period, 2014-2017, as well as from a maternity hospital and the results of bacteriological investigations of patients in rural areas (on the model of three districts).

A total of 10,055 *Staphylococcus* strains were analyzed, 8,639 being isolated from multi-profile health care facilities, 1,090 from rural areas and 326 from a maternity hospital. Isolation of *Staphylococcus* strains and determination of methicillin resistance and antibiotic resistance / sensitivity were performed both by the classical method and the automated VITEK 2 Compact system (bioMérieux).

To determine the risk factors and the socio-economic impact, a retrospective case-control study was performed, based on the survey data collected from patient observation forms with PSIs caused by staphylococci. Two research groups were created: the basic group included patients with bacteriological investigations positive for MRS and the control group comprised patients with bacteriological investigations positive for MSS.

RESULTS

Resistance of *Staphylococcus* strains to antibacterial drugs varied depending on the

group of antibiotics, the type of antibiotic and the profile of the inpatient department where it was isolated. The highest prevalence of methicillin-resistant *Staphylococcus* was found in maternity hospitals, in which, of the total number of isolated *Staphylococcus* strains, 61.81% were found to be MRS, followed by multi-profile health care facilities, with a share of MRS strains of 36.32%, and slightly lower in rural areas - 22.36% (fig. 1).

The situation regarding PSIs caused by MRS in the health care facilities of the Republic of Moldova in the multiannual dynamics is worsening. The share of MRS strains isolated from patients with PSIs during 4 years of studies is increasing, from 29.62% in 2014, to 42.70% in 2017, the increase being statistically significant ($t = 5.17$, $p<0.001$) (fig. 2).

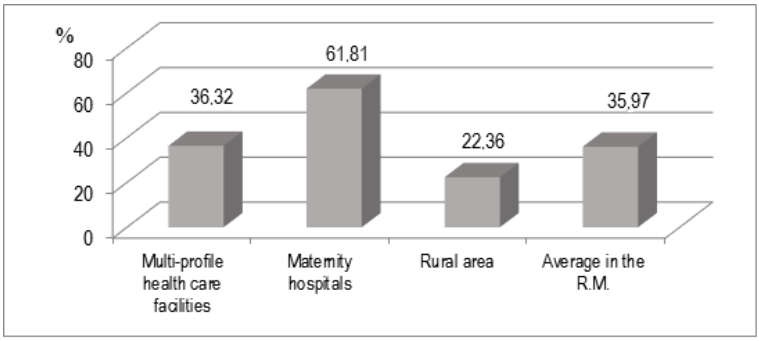

Fig. 1. Share of MRS strains in the total number of *Staphylococcus* strains isolated from patients with PSIs in the Republic of Moldova

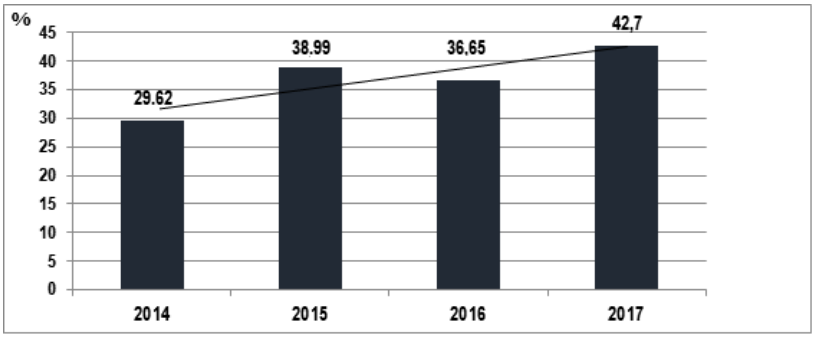

Fig. 2. Share of MRS strains in the total number of *Staphylococcus* strains in multiannual dynamics

Purulent-septic MRS infections are caused by both coagulase-positive staphylococci and coagulase-negative staphylococci. The analysis of the share of methicillin-resistant *Staphylococcus* strains depending on the staphylococcal species revealed different levels (fig. 3).

Of the total number of *S. aureus* strains tested for sensitivity / resistance to oxacillin, 19.43% showed to be resistant, while *S. hominis* strains were found to be resistant to oxacillin in 94, 44% of cases, *S. epider-*

midis - in 61.34%, *S. saprophyticus* - in 51.54%, and *S. hemolyticus* - in 48.71% (fig. 3). Although coagulase-positive *Staphylococcus* is more common in human purulent-septic infections, the degree of resistance to methicillin is lower compared to that of coagulase-negative *Staphylococcus* species (fig. 3).

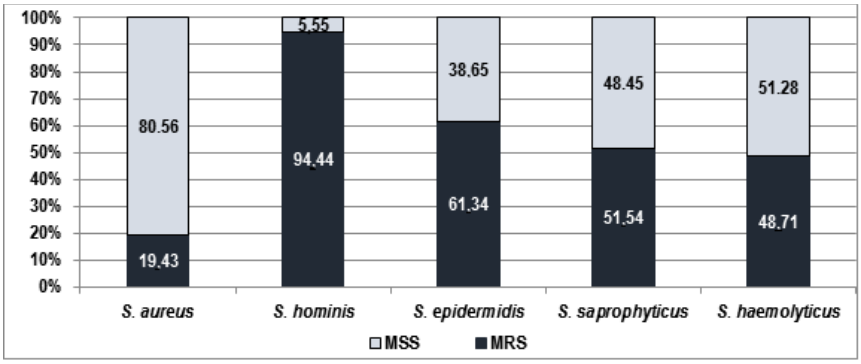

Fig. 3. Share of methicillin-resistant *Staphylococcus* strains by staphylococcal species

Staphylococcus strains showed varying resistance to methicillin, depending on the biological substrate investigated (tab. I). An obviously high share of MRS was found in blood cultures. Of the total number of *Staphylococcus* strains isolated from blood (n = 201), 76.11% (n = 153) of the strains were found to be methicillin resistant. The high share of MRS strains isolated from the blood allows to consider the given problem as a serious one, because it is a question of generalized infections (pneumonia, septicemia).

The isolation of MRS strains also varied depending on the profile of the inpatient department. A higher rate of MRS strains were isolated from patients with PSIs in the departments of surgery - 26.08%, traumatology and orthopedics - 19.84%, resuscitation and intensive care units - 15.20%. However, 14.84% of MRS strains were isolated from patients undergoing outpatient treatment.

A significant share of MRS infections was also found in the therapeutic departments: general therapy and allergology - 5.81%, hepatology - 3.86%, rheumatology/arthrology - 3.64%.

TABLE I

Share of methicillin-resistant *Staphylococcus* strains in various pathological products

Pathologic material	Total	Including MRS	
		N	%
Taken from the respiratory airways	1,230	205	16.66
Taken from the urogenital tract	510	117	22.94
Bile	326	158	48.46
Blood	201	153	76.11
Other bio substrates	837	257	30.70
Total	3,104	890	8.67

TABLE II

MRS/MSS strains resistance/sensitivity to non-β-lactam antibiotics, isolated from patients with septicemia

Antibiotic group	Type of antibiotic	Causative agent						Statistical difference
		MRS			MSS			
		Total	R		Total	R		
		n	n	%	n	n	%	
Aminoglycosides	Gentamicin	265	163	61.50	74	2	2.70	t=3.53, p<0.001
	Tobramycin	316	193	61.07	121	3	2.47	t=5.08, p<0.001
	Netilmicin	58	4	6.89	31	0	0	-
	Amikacin	314	78	24.84	120	2	1.66	t=1.69, p<0.001
	Total	**953**	**438**	**45.96**	**346**	**7**	**2.02**	t=7.06, p<0.001
Glycopeptides	Vancomycin	212	0	0.00	77	0	0.00	-
	Total	**212**	**0**	**0.00**	**77**	**0**	**0.00**	-
Macrolides	Clarithromycin	264	195	73.86	87	25	28.73	t=4.62, p<0.001
	Erythromycin	76	54	71.05	28	5	17.85	t=2.64, p<0.05
	Azithromycin	2	2	100	0	0	0	-
	Total	**342**	**251**	**73.39**	**115**	**30**	**26.08**	t=5.57, p<0.001
Tetracyclines	Doxycycline	276	86	31.15	110	2	1.69	t=2.13, p<0.05
	Total	**276**	**89**	**31.15**	**110**	2	1.69	t=2.13, p<0.05
Phenicols	Chloramphenicol	237	93	39.24	69	11	15.94	t=1.84, p>0.05
	Total	**237**	93	39.24	**69**	**11**	**15.94**	t=1.84, p>0.05
Fluoroquinolone	Ciprofloxacin	313	135	43.13	123	4	3.25	t=3.59, p<0.001
	Moxifloxacin	320	97	30.31	128	22	17.18	t=1.38 p>0.05
	Gatifloxacin	262	177	67.55	93	5	5.37	t=5.57, p<0.001
	Levofloxacin	317	100	31.54	126	3	2.38	t=2.48, p<0.05
	Total	**1212**	**509**	**41.99**	**470**	**34**	**7.23**	t=7.02 p<0.001

MRS strains were also found to be resistant to non-β-lactam antibiotics. A fairly high resistance to antibiotics was found in MRS strains isolated from the blood (tab. II).

The resistance of MRS strains to macrolides accounted for 73.39%, including to erythromycin - 71.05% and clarithromycin - 73.86%. MSS strains showed much lower resistance values to this group of antibiotics - 26.08%, with a statistical difference t = 5.57, p<0.001 (clarithromycin –28.73% (t = 4.62, p <0.001), and erythromycin - 17.85% (t = 2.64, p<0.001)).

Differences in MRS and MSS strains resistance / sensitivity were also determined in the case of aminoglycosides, being 45.96% in the case of MRS strains, and only 2.02% in the case of MSS, (t = 7.06, p <0.001), including gentamicin - 61.50% (MRS) and 2.70% (MSS), (t = 3.53, p <0.001), tobramycin - 61.07 (MRS) and 2.47% (MSS), (t = 5.08, p <0.001), amikacin - 24.84 (MRS) and 2.02 (MSS), (t = 1.69 p <0.001).

MRS strains showed resistance to tetracyclines - 31.15%, compared to MSS strains - 1.69% (t = 2.13, p <0.05). MRS strains also showed high resistance to fluoroquinolones - 41.99%, compared to a much lower resistance of MSS strains - 7.23% (t = 7.02, p <0.001). MRS strains also showed resistance to ciprofloxacin - 43.13%, and MSS - 3.25% (t = 3.59, p <0.001), moxifloxacin - 30.31% and 17.18%, respectively (t = 1.38, p > 0.05), gatifloxacin - 67.55% and 5.37% (t = 5.57, p <0.001), levofloxacin - 31.54% and 2.38% (t = 2.48, p <0.05).

MRS strains isolated from the blood of patients with septicemia proved to be polyresistant in 96.3% of cases and sensitive to antibiotics in only 3.69%, while most strains of MSS - 91.66% proved to be sensitive to antibiotics, and only in 8.33% - polyresistant, the difference being statistically significant (t = 9.99, p <0.001).

TABLE III

Risk factors for PSIs caused by MRS strains

Risk factors	Research group (n=269)	Control group (n=159)	t-Student Test
Age ≥ 50 years	189 (70.26%)	95 (59.74%)	t = 1.74, p>0.050
Repeated hospitalization	176 (65.42%)	68 (42.76%)	t = 3.24, p<0.010
Microbial associations	158 (58.73%)	41 (25.78%)	t = 4.18, p<0.001
Exposure to intensive care	174 (64.68%)	56 (35.22%)	t = 4.01, p<0.001

Analysis of morbidity according to age groups (tab. III) shows that PSIs caused by MRS strains are more common in elderly patients (≥ 50 years) - 70.26%, compared to those with MSS - 59.74% (t = 1.74, p > 0.05). The character of repeated hospitalizations can be highlighted among the risk factors fPSIs caused by methicillin-resistant *Staphylococcus* strains. A higher incidence of patients exposed to repeated hospitalizations is observed in patients with MRS. Of the total number of patients, from whom MRS strains were isolated, 65.42% underwent repeated hospitalizations, and only 34.58% of them underwent the first hospitalization. In the control group, patients with MSS infections underwent the first hospitalization - 57.24% and only

42.76% - repeated hospitalization (t = 3.24, p <0.01).

Infections caused by MRS were found to have a higher proportion - 58.73%, being in combination with other species of microorganisms, compared to those caused by MSS strains - 25.78%, producing mostly (41.10%) generalized forms (pneumonia, septicemia, etc.). Another risk factor is exposure of patients to intensive care, 64.68% of patients with MRS infections being subjected to treatment in intensive care units, and only 35.22% of patients with MSS infections underwent treatment in the intensive care units (t = 4.01, p <0.001).

TABLE IV

Number of hospitalization days of patients with PSIs caused by MRS and MSS strains

Number of days	Indices	MRS		MSS	
		Number of patients	Total days	Number of patients	Total days
1-20	n	135	1,686	130	1,442
	%	63.38		86.09	
21-40	n	64	1,773	19	488
	%	30.04		12.58	
≥ 41	n	14	827	2	123
	%	6.57		1.32	
Total	n	213	4,286	151	2,053
Average hospitalization days		**20.12**		**13.59**	

Patients with PSIs caused by MRS strains had a much longer hospital stay, which averaged 20.12 days, compared with those with MSS - 13.59 days. Of the total number of patients with MRS, 6.57% followed treatment in the inpatient department for more than 41 days, compared to those with MSS - 1.32%. The lethality of patients with purulent-septic infections caused by MRS accounted for 21% (tab. IV).

DISCUSSION

Excessive and irrational use of antibiotics remains one of the main risk factors for increasing bacterial resistance to antibiotics. In the first report about antimicrobial resistance, WHO notes that it has become a major global threat to public health (4, 5).

Infections caused by antibiotic-resistant microorganisms, including MRS infections, significantly increase economic costs by extension of hospitalization, increasing the period of incapacity for work, increased use of antibiotics and other means and methods of diagnosis and treatment. Methicillin resistance was shown to have a negative impact on clinical and economic outcomes, especially in terms of increased morbidity and mortality, long-term hospitalization, as well as the need for additional interventions to reduce the clinical impact (13, 14).

Patients with MRS proved to have more comorbidities, more complications during hospitalization, requiring additional investigations, long-term hospitalizations, including intensive care, more complex and expensive treatment. Much higher costs for the treatment of MRS infections than for

MSS infections are due to both the higher number of treatment days and additional costs, which include costs for screening, use of personal protective equipment, and costs of antibiotics and drugs used in the treatment and decolonization of patients (15, 16). Patients with SPI caused by the MRS strains included in the current study had a much longer duration of hospitalization compared to those with MSS, the difference in average being 6.5 days.

Shortly after the appearance of MRSA, it has been realized that the problem of methicillin resistance involves not only coagulase-positive staphylococcal strains, but also coagulase-negative staphylococcal strains. Although in the beginning, these species of microorganisms were given little importance, being considered part of the normal flora of the skin and nasal mucosa, today because of resistance to antibacterial preparations have become a source of concern for the health system (17, 18). Our results demonstrated that in Republic of Moldova, although coagulase-positive *Staphylococcus* is frequently involved in septic-purulent human pathology, the degree of resistance to methicillin is lower (21.2%) compared to the same in staphylococcal species coagulase-negative (59.6%).

Given the continuous evolution of the phenomenon of antibiotic resistance, including methicillin-resistant *Staphylococcus*, it is important to establish the level of distribution and the degree of antibiotic sensitivity/resistance at both levels, namely, health care facilities and national level. Promoting global surveillance of antibiotic resistance through programs such as *ICARE*, *SENTRY*, *MYSTIC*, *EARSS*, also emphasizes the importance of implementing local studies or national surveillance programs to highlight circulating phenotypes in order to guide selective or empirical antibiotic therapy in clinical situations, thus requiring the initiation of an early antibacterial therapy (6, 19).

CONCLUSIONS

Methicillin-resistant *Staphylococcus* infections present a major public health problem both worldwide and in the Republic of Moldova. The degree of spread of methicillin-resistant *Staphylococcus* strains in the Republic of Moldova is on average 35.97% and varies depending on the type of health care setting: maternity hospitals - 61.81%, multi-profile health care facilities - 36.32%, health care facilities in rural areas - 22.36%. A very high resistance to methicillin is found in coagulase-negative *Staphylococcus* strains (48.71-94.44%). More frequently, MRS strains were isolated from patients in the inpatient departments of surgery, traumatology, orthopedics, and intensive care. MRS strains have a much higher resistance to non-beta-lactam antibiotics compared to MSS strains, and 96.3% of blood-isolated MRS strains have been shown to be polyresistant. People over 50 years, repeated hospitalizations, microbial associations, and exposure to intensive care have been shown to be risk factors for infections caused by MRS. In MRS infections, the medical, economic and moral impact is significant, the lethality being 21%. Rational and selective use of antibiotics based on antibiotic susceptibility test is the only chance to improve the situation.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received specific funding regarding this scientific research in the Project, code 20.80009.8007.05.

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TOTAL HIP ARTHROPLASTY FOLLOWING OPEN REDUCTION AND INTERNAL FIXATION FOR DISPLACED ACETABULAR FRACTURES

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TOTAL HIP ARTHROPLASTY FOLLOWING OPEN REDUCTION AND INTERNAL FIXATION FOR DISPLACED ACETABULAR FRACTURES (Abstract): Fractures of the acetabulum are difficult to treat and even after fixation they can lead to posttraumatic arthritis and avascular necrosis of the femoral head. Total hip arthroplasty has been the most common surgery performed for the complications of posttraumatic arthritis and avascular necrosis of the femoral head. **Aim**: To evaluate the links between acetabular fracture type, radiological and clinical outcomes of open reduction and internal fixation, and clinical outcomes of total hip arthroplasty. **Material and methods:** One hundred thirty-two patients with acetabular fractures were treated surgically at the Trauma Department of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi. Fifteen patients underwent THA for posttraumatic osteoarthritis or femoral head avascular necrosis following open reduction and internal fixation for acetabular fractures. Clinical and radiographic examinations were performed at 6 weeks, 3 months, 6 months, 1 year, and annually thereafter. Patients were assessed clinically using the Harris Hip Score and radiologically with Matta outcome grading. Heterotopic ossification was classified according to Brooker grading. **Results:** Mean Harris Hip Score has improved from 55 before conversion to 90 at 6 months after THA. No dislocation, deep vein thrombosis or pulmonary thromboembolism, new nerve injury, and heterotopic ossification occurred. **Conclusions:** The conversion to total hip arthroplasty after posttraumatic arthritis and avascular necrosis of the femoral head in acetabular fracture can lead to reasonable pain relief and functional improvement. **Keywords:** ACETABULAR FRACTURE, INTERNAL FIXATION, TOTAL HIP ARTHROPLASTY, POSTTRAUMATIC ARTHRITIS, AVASCULAR NECROSIS OF THE FEMORAL HEAD.

Acetabular fractures are serious intra-articular injuries which can lead in time to impairment of the hip joint function (1). Most frequently, these fractures emerge in young patients following high energy trauma, especially road accidents, in which the acetabular fracture is often associated with hip dislocation. The increase in life expectancy is associated with a higher number of elderly patients suffering fragility fractures. These are low energy fractures that affect the pelvic ring, but they can also be located at the acetabulum. The number of patients not receiving therapy for osteoporosis is

still high and the damaged bone structure causes delayed healing and recovery after pelvic fractures (2).

Most studies have shown that only the surgical treatment of these fractures, with anatomic reduction and rigid internal fixation, can restore the hip function and prevent long-term complications. Technological developments have made possible the routine surgical approach to acetabular fractures. The use of computed tomography (CT) in most traumatology services has allowed surgeons to better organize the stages of surgery (3). The three-dimensional printing technique allowed to obtain a model on which the reduction techniques can be planned and the plates to be used can be contoured preoperatively (4). Surgery duration is thus reduced. Unfortunately, despite modern fracture reduction techniques, 27% to 37% of the patients develop posttraumatic arthritis or avascular necrosis of the femoral head (AVN) (5, 6). Favoring factors for the development of these complication are early cartilage damage, residual articular incongruity, improperly placed implants, disruption of femoral head blood supply and associated comorbidities (7, 8).

The procedure indicated in case of any hip arthritis and/or femoral head necrosis is total hip arthroplasty (THA). Following acetabular fractures, this procedure is more complex due to the scar tissue, acetabular bone defects, and retained internal fixation implants (9, 10). The presence of osteosynthesis materials in the immediate vicinity of the acetabulum makes it sometimes necessary to partially remove it. These surgeries have a high degree of complexity and increased risks. Particular situations have also been described in the literature in which the acetabular fracture appeared in the context of a hip dislocation associated with ipsilateral trochanteric fracture, osteosynthesis implants being now present in the proximal femur (11).

Geriatric patients with acetabular fractures are currently being operated on. In the literature there are studies on the outcomes of surgeries including patients over 80 years of age. The complication rate is higher due to insufficient bone stock, and many patients require conversion to THA.

Additionally, another factor which needs to be considered when deciding for THA is that most patients with acetabular fractures are young, with high activity level, and subsequent high failure rates of THA (12, 13, 14).

The aim of the study was to evaluate the links between acetabular fracture type, radiological and clinical outcomes of open reduction and internal fixation (ORIF), and clinical outcomes of THA.

MATERIAL AND METHODS

Patient data. From July 1, 2011, to December 31, 2019, 132 patients with acetabular fractures were treated surgically, ORIF, at the Trauma Department of the University Hospital, Iasi, Romania, by the same senior surgeon. Out of these, fifteen patients underwent THA. Informed consent was obtained from all the individual participants included in the study. The study was conducted according to the Helsinki Declaration and was approved by the Ethics Committee of the 'Sf. Spiridon' Emergency Hospital Iasi, Romania.

Inclusion criteria were patients > 18 years of age, diagnosis of posttraumatic osteoarthritis or femoral head avascular necrosis following ORIF for acetabular fractures, patients suitable for surgical treatment and a minimum follow-up of 1 year after THR.

After identifying the patients, we retro-

spectively reviewed the demographic data, type of fracture according to Letournel classification (15, 16), mechanism of trauma, initial treatment of the fracture, time between fracture treatment and THR, type of prostheses, complications, follow-up time.

Surgical Technique. All THAs were performed through a modified Hardinge approach. The acetabulum was prepared for routine primary THA. Hardware from ORIF was not routinely removed. Heterotopic ossifications (HO) were removed only if they interfered with the reaming and placement of the cup/acetabular reinforcement ring. Acetabular bone defects were assessed based on the American Academy of Orthopedic Surgeons (AAOS) classification (17). In large bone deficiency, for acetabular reconstruction we used a cemented acetabular fixation combined with an acetabular reinforcement ring and morselized cancellous bone grafts from the resected femoral head. Cementless acetabular fixation was used in cases without large bone defects using press-fit technique, and/or additional screw fixation. All femoral stems were cementless. Joint articulation was either ceramic-on-cross-linked polyethylene or ceramic-on-ceramic.

Perioperative protocol. Antibiotic prophylaxis was started 30 minutes before the incision in the operating room and within the first 24 hrs. postoperatively. To prevent thromboembolism, low molecular weight heparin was routinely administered for one month. Prophylaxis against HO consisted in the administration of indomethacin 75 mg daily for 21 days. All patients were allowed to do passive movements following surgery and were restricted to touch-down weight-bearing walking for 6 weeks and gradually increase this to full weight-bearing thereafter.

Clinical and radiological assessment. Postoperative follow-up was performed at 6 weeks, 3 months, 6 months, 1 year and annually thereafter. Patients were assessed clinically using the Harris Hip Score (HHS) (18) and radiologically with Matta outcome grading (19). HO was classified according to the system developed by Brooker *et al.* (20)

Statistical analysis. The database was created in EXCEL and was statistically processed with the *SPSS version 18.0* software (IBM, Armonk, New York, USA).

RESULTS

All acetabular fractures were caused by high-energy trauma (motor vehicle accidents, falls from height) and were treated primarily with open reduction and internal fixation after fracture reduction. Most patients had complex fractures and were all first treated with plates and screws.

The mean patients age of 42±11.25 years (range from 22 to 78 years). Ten patients (66.6%) were men and 5 were women (male/female=2/1). Among the fifteen patients who underwent THR, left side was affected in 9 cases (tab. I).

Three patients had isolated posterior wall fractures and 12 patients had complex fractures (when >2 simple fractures were combined): transverse and posterior wall fractures were found in 9 patients, posterior wall and posterior column fractures in one, T-shaped fracture, and anterior column and posterior hemi transverse fracture in one patient each. Posterosuperior dislocation was found in 7 patients and central dislocation in 3 patients. For the posterior wall fracture component Kocher - Langenbeck approach and for all other fractures the modified Stoppa approach or combined anterior and posterior approaches were used (3, 21, 22).

TABLE I
Demographic data of the study group

Variables	Values
Mean age (years)	42±11.25
Gender	
Male/Female	10/5
Mean body mass index (kg/m^2)	26.2±9.31
Preoperative diagnosis	
Posttraumatic osteoarthritis	9 (60)
Femoral head necrosis	6 (40)
Fracture patterns	
Posterior wall	3 (20)
Posterior wall + posterior column	1 (6.6)
Transverse + posterior wall	9 (60)
T-shaped	1 (6.6)
Anterior column + posterior hemi transverse	1 (6.6)
Dislocation	
Posterosuperior	7 (46.6)
Central	3 (20)
Interval time between fracture ORIF and THA (months)	18±16.33
Median follow-up (months)	12±8.41

Values are expressed as mean ± SD, frequency and percentage (%)
ORIF=open reduction and internal fixation, THA=total hip arthroplasty

After ORIF for acetabular fractures, patients were assessed radiologically using Matta outcome grading. Here the patients are divided into three groups: anatomical, congruent and incongruent group. Based on this division 9 outcomes were in anatomical group and 6 outcomes in congruent group. Functional outcomes were divided into four groups according to HHS: poor, fair, good and excellent. Thus, 9 outcomes were excellent, 3 good and 2 fair. HO was observed in 4 patients. Posttraumatic sciatic nerve palsy was recorded in 3 cases.

The most common cause for THA was posttraumatic osteoarthritis (9 patients) followed by femoral head necrosis (6 patients). THA was performed after an average of 18±16.33 months after acetabular fracture ORIF (range from 7 to 30 months).

During THR we found protrusion of the hardware into the acetabulum (a screw in one case, bone necrosis of the posterior wall fragment with subsequent posterior plate and/or spring plates in other 7 cases). In these cases, protruded hardware needed to be removed prior to THA (fig. 1).

Cementless cup was used in 7 cases, in others (due to large bone defects) cemented cup combined with an acetabular reinforcement ring and morselized cancellous bone grafts from the resected femoral head were use. All the femoral stems were uncemented (fig. 2).

As for postoperative complications, we recorded one case of superficial infection treated with debridement and vacuum-assisted closure (23) and in another case increased of HO from Brooker 2 to Brooker 3 at 6 months follow-up without clinical impairment.

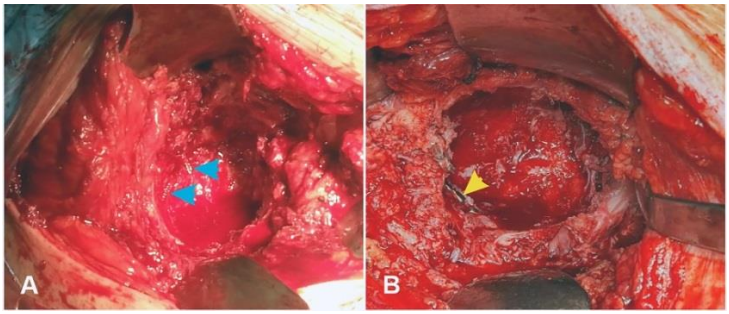

Fig. 1. Intraoperative imagines during THA, intraacetabular view: **A** - protrusion of screws into the acetabulum (blue arrows), **B** - posterior wall fragment necrosis with subsequent protrusion of the posterior reconstruction plate into the acetabulum (yellow arrow).

Fig. 2. Example of transverse + posterior wall fracture (29-year-old female): preoperative 3D CT scan (**A, B**); ORIF for acetabular fracture: postoperative X-rays (**C** - antero-posterior, **D** - iliac oblique and **E** - obturator oblique views) - internal fixation with omega plate throughout anterior (modified Stoppa) approach of the transverse component and additional fixation with a reconstruction plate and two spring-plates throughout posterior (Kocher-Langenbeck) approach of the posterior wall fragments, postoperative CT scan (**F** - axial, **G** - coronal, **H** - sagittal) - articular congruence with anatomical reduction of the acetabular surface (Matta 1), 15-monhts follow-up (**I** - anteroposterior X-ray view and **J** - coronal CT scan) - avascular necrosis of the femoral head (Ficat and Arlet stage IV); THR: postoperative X-ray (K - anteroposterior view) - uncemented THR, fracture fixation hardware was kept in place except the two spring plates because the plate hooks protruded into the acetabulum, 6-months follow-up (**L** - anteroposterior X-ray view).

The HHS was used to evaluate the outcomes of the conversion procedure (THR) with regard to pain alleviation and functional improvement at 12 months, even though the HHS at the final follow up was documented for all patients. Based on the outcomes, HHS improved from an average of 55 before THA to an average of 90 at 12 months after THA.

DISCUSSION

Posttraumatic osteoarthritis and femoral head necrosis may occur in patients with acetabular fracture treated with open reduction and internal fixation. Although the complications are quite numerous and require complex solutions, the surgical treatment of displaced fractures is clearly superior to the conservative one where the complication rate is much higher (21, 22). It is predictable that surgical treatment will become the first option even in people at extreme old age, the advantages being shown by studies on the postoperative survival rate of patients who have suffered hip fractures (24).

Out of the factors that affect the outcome of acetabular fractures surgery, in our study the surgeon-dependent variables could be excluded because all the fractures were treated by the same surgeon. The surgeon-independent variables are: the mechanism of the injury, damage to the femoral head, sciatic nerve injury, dislocation, fracture pattern, associated injuries, the patient's age and comorbidities. The external examination of the patient is thus important and identifying associated injuries can significantly impact treatment outcome (25). The implication of the age and gender could not be substantiated because the patients in our group are young or in the middle age (the mean patient age was 42±11.25 years). The hip dislocation is another variable that is known to negatively affect the outcome (26). Related to fracture pattern, most published studies reported poorer outcome in posterior wall, posterior column, posterior wall + posterior column and T fractures due to cartilage damage, impaction and comminution (26, 27, 28, 29). It was suggested that primary THR of these fractures is a better option, but large randomized clinical trials are needed to validate available data (30). From our patients that underwent THR, in 10 cases (66.6%) the acetabular fracture was associated with dislocation. Posterior wall/column fracture component was encountered in 13 patients (86.6%) and T-shape fracture in one patient. All of our patients but one (78-years old female) were treated primarily with ORIF of the acetabular fracture.

THA, although considered a reliable procedure, has a higher incidence of complications and poorer outcomes than non-traumatic primary THA (31, 32). Cemented THA following acetabular fractures was preferred in earlier studies, however, later studies showed increased revision and loosening rates in the acetabular side. Authors suggested that a sclerotic bone bed in the acetabulum is inappropriate for cement fixation (33, 34). Improved outcomes of cementless THA after acetabular fractures have been reported. These studies suggested that the improved outcomes were due to the development of cementless acetabular fixation in THA and fracture management (31, 32).

In our study we recorded good clinical, radiological and patient-related outcomes, without the presence of serious complications after the surgical treatment of acetabular fracture or THA and no differences

between cemented and uncemented THA.

CONCLUSIONS

The findings of the present study suggest that the THA of posttraumatic arthritis or femoral head necrosis after acetabular internal fixation is clinically successful without increased risk of complications and provides good functional results.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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VERBASCUM NIGRUM L. AND *AJUGA CHIA* SCHREB.: ANTIOXIDANT ACTIVITY AND CORRELATION WITH PHENOLIC CONTENT

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VERBASCUM NIGRUM L. AND *AJUGA CHIA* SCHREB.: ANTIOXIDANT ACTIVITY AND CORRELATION WITH PHENOLIC CONTENT (Abstract): **Aim**: The aim of the present study was to investigate the antioxidant potential of *Verbascum nigrum* L. and *Ajuga chia* Schreb. aerial parts in correlation with their phenolic contents. **Material and methods**: Each plant material was subjected to a five-step sequential extraction procedure using diethyl ether, dichloromethane, ethyl acetate, methanol and water. The total phenolic content was determined using the Folin-Ciocalteu method. Antioxidant activity was assessed using six *in vitro* assays: DPPH radical, ABTS radical cation, superoxide anion radical and hydroxyl radical scavenging assays, reducing power and ferrous ions chelating assay. **Results and discussion**: The highest phenolic contents were found in *Verbascum nigrum* ethyl acetate and methanol extracts (199.35 ± 3.63 and 181.94 ± 2.38 mg/g, respectively). *Verbascum nigrum* methanol extract developed the most potent scavenging effects against DPPH, ABTS and superoxide radicals and showed the strongest reducing power (EC_{50} = 28.03 ± 0.40, 17.77 ± 0.00, 990.00 ± 0.01 and 17.30 ± 0.12 µg/mL, respectively). *Verbascum nigrum* aqueous extract strongly chelated the prooxidant ferrous ions (EC_{50} = 0.05 ± 0.01 mg/mL). There were found strong positive correlations between the antioxidant activity (DPPH and ABTS scavenging activity, reducing capacity) and phenolic content of *Verbascum nigrum* extracts. **Conclusions**: *Verbascum nigrum* is a rich source of antioxidant compounds and needs further investigations on the metabolite profiling and bioactivity. **Keywords**: *VERBASCUM NIGRUM*, *AJUGA CHIA*, ANTIOXIDANT ACTIVITY.

Oxidative stress, a state in which a biological system becomes unable to neutralize the highly reactive oxygen species, is involved in the initiation and progression of various diseases (malignant, inflammatory, metabolic and autoimmune diseases) (1). *Verbascum* genus belongs to the Scrophulariaceae family, the latter including more than 2,500 species (mulleins) distributed around the world. Mulleins have various uses in the traditional medicine, with reported expectorant, emollient, antiseptic, antimalarial, antiulcerogenic, hepatoprotective and cytotoxic properties (2). Chemical studies have revealed the presence of flavonoids, iridoids, saponins and phenylethanoids as

main classes of bioactive compounds in *Verbascum* species (3). *Ajuga* (Lamiaceae family) is a genus including around 300 species (4). Sterols, iridoids, withanolides, polyphenols, polyketides and alkaloids, as well as essential oils (4-7) confer various activities (antimicrobial, antimalarial, anti-inflammatory, hypoglycemic, antioxidant, wound healing, neuroprotective) to *Ajuga* extracts (4, 8-12).

Although *Verbascum* and *Ajuga* species have been thoroughly investigated, there is scarce information about the chemical composition and bioactivity of *Verbascum nigrum* L. (black mullein, dark mullein) and *Ajuga chia* Schreb. (syn. *Ajuga chamaepitys* subsp. *chia* (Schreb.) Arcang., yellow bugle, ground-pine). In this respect, the present study evaluated the antioxidant potential of the aforementioned species in correlation with their phenolic contents.

MATERIAL AND METHODS

Plant material: The aerial parts of *V. nigrum* and *A. chia* were collected in the Republic of Moldova (Merenii Noi and Hlinaia, respectively) in May 2014. The aerial parts were dried, powdered and deposited in the Department of Pharmacognosy, Faculty of Pharmacy, *Grigore T. Popa* University of Medicine and Pharmacy Iasi, Romania.

Extraction: The dried and powdered aerial parts of *V. nigrum* and *A. chia* (20 g each) were extracted by a five-step sequential extraction procedure, using solvents with increasing polarities (diethyl ether, dichloromethane, ethyl acetate, methanol and water, 3 × 200 mL, 1 h) at room temperature. The resulting extracts were concentrated in a rotary vacuum evaporator at 40-45°C, freeze-dried and kept at -20°C until further use.

Total phenolic content: Total phenolic content was assessed using the Folin-Ciocalteu method, the results being expressed as gallic acid equivalents (mg gallic acid/g extract) (13, 14).

Antioxidant assays: The scavenging capacity against synthetic free radicals (1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation) and reactive oxygen species (superoxide anion radical, hydroxyl radical), reducing power and capacity to chelate the prooxidant ferrous ions were evaluated using previously described methods with minor changes (15-22).

Statistical analysis: The results are expressed as means ± standard error mean (SEM) of three replicates. *Microsoft Home and Business 2019* was used for the statistical analysis.

RESULTS

Total phenolic content: The phenolic content of *V. nigrum* extracts ranged from 0.25 ± 0.01 mg/g (VN5) to 199.35 ± 3.65 mg/g (VN3), VN4 and VN3 extracts showing the highest phenolic amounts. In case of *A. chia* extracts, the phenolic content varied between 35.07 ± 0.70 mg/g in AC1 to 90.55 ± 2.29 mg/g in AC5 (tab. I).

DPPH radical scavenging activity: At 84 µg/mL, two of *V. nigrum* extracts, VN3 and VN4, exhibited significant and similar scavenging effects (86.80 ± 0.02% and 85.53 ± 0.01%, respectively). At the same concentration, VN1, VN2 and VN5 extracts showed weak activity (7.35 ± 0.12%, 10.55 ± 0.25% and 6.55 ± 0.23%, respectively). At the highest concentration tested (333 µg/mL), VN3 and VN4 extracts scavenged 98% of the DPPH free radical. With respect to *A. chia* extracts, AC5 was the most active,

with 82.26 ± 3.00% scavenging activity at 333 µg/mL. According to the EC_{50} values (tab. II), VN3 and VN4 extracts proved to be efficient DPPH radical scavengers.

TABLE I

Extracts obtained by five-step sequential extraction of *Verbascum nigrum* and *Ajuga chia* aerial parts and their phenolic contents

Extraction solvent	*V. nigrum* aerial parts			*A. chia* aerial parts		
	Extract	Amount (g)	Total phenolic content (mg/g)	Extract	Amount (g)	Total phenolic content (mg/g)
Diethyl ether	VN1	0.35	26.70 ± 1.59	AC1	0.15	35.07 ± 0.70
Dichloromethane	VN2	0.17	50.77 ± 1.54	AC2	0.22	38.36 ± 1.82
Ethyl acetate	VN3	0.60	199.35 ± 3.63	AC3	0.20	38.71 ± 0.72
Methanol	VN4	5.72	181.94 ± 2.38	AC4	3.42	50.85 ± 1.11
Water	VN5	3.06	0.25 ± 0.01	AC5	3.23	90.55 ± 2.29

ABTS radical cation scavenging activity: At 50 µg/mL, VN3 and VN4 extracts exhibited 100% ABTS scavenging activity. At the same concentration, VN2 and VN5 extracts developed two-fold weaker scavenging effects (52.59 ± 0.02% and 56.45 ± 5.82%, respectively). VN1 extract was almost inactive, showing only 9.52 ± 0.02% scavenging activity at 100 µg/mL. According to the EC_{50} values, none of the *V. nigrum* extracts reached the scavenging potency of the positive controls, catechin and quercetin (tab. II). *A. chia* extracts showed weak scavenging activity. At 300 µg/mL, AC2 and AC3 extracts scavenged the ABTS radical cation by only 23.00 ± 1.83% and 21.97 ± 3.56%, respectively, whereas AC4 and AC5 extracts showed slightly higher scavenging activities (35.10 ± 5.54% and 33.22 ± 8.49%, respectively).

Reducing power: VN3 and VN4 extracts were the most active, with 100% reducing activity at 60.6 µg/mL. VN2 and VN5 extracts showed weaker effects (58.51 ± 0.14% at 182 µg/mL and 66.81 ± 4.42% at 121 µg/mL, respectively). No activity was observed for VN1. With respect to the EC_{50} values, catechin showed the strongest reducing potential (6.50 ± 0.11 µg/mL), followed by VN3 and VN4 extracts, with comparable EC_{50} values (18.22 ± 0.32 and 17.30 ± 0.12 µg/mL, respectively). Among *A. chia* extracts, AC4 and AC5 were the most potent (39.84 ± 1.44 and 92.31 ± 6.29 µg/mL, respectively) (tab. II).

Ferrous ion chelating activity: Except VN3, all other four *V. nigrum* extracts exhibited dose-dependent ferrous ion chelating effects. At 2 mg/mL, VN2 was the most potent (97.63 ± 0.01%), followed by VN4 (94.06 ± 0.00%) and VN1 (76.72 ± 0.00%). However, according to the EC_{50} values, the strongest chelating activity was exhibited by VN5 extract (0.05 ± 0.01 mg/mL) (tab. II). With respect to *A. chia* extracts, AC2, AC4 and AC5 exhibited strong chelating effects at 2 mg/mL: 99.29 ± 0.00%, 97.80 ± 0.01% and 96.54 ± 0.01%, respectively. According to the EC_{50} values, the ferrous ion chelating ability of *A. chia* extracts varied in the following order: AC5 > AC3 > AC2 > AC4 > AC1 (tab. II).

Superoxide anion radical scavenging activity: Within 1-3 mg/mL concentration range, *A. chia* extracts showed mild super-

oxide anion radical scavenging effects, with the highest activity for AC2 (30.70 ± 0.02% at 3 mg/mL). Among *V. nigrum* extracts, VN4 was the most active (EC_{50} = 990 µg/mL), but significantly less active than the positive control, glutathione (EC_{50} = 1.09 ± 0.01 µg/mL) (tab. II).

Hydroxyl radical scavenging activity: VN3 was the most potent *V. nigrum* extract, showing 93.36 ± 1.11% scavenging activity at 1.87 mg/mL and an EC_{50} value of 1.10 ± 0.03 mg/mL (tab. II). At the same concentration, VN4 and VN5 extracts had moderate activity (67.18 ± 3.87% and 54.13 ± 1.36%, respectively). VN1 and VN2 extracts exhibited weak scavenging effects (below 30%). According to the EC_{50} values, quercetin (0.16 ±0.01 mg/mL) was 6.8 times more potent than VN3 (tab. II). *A. chia* aqueous extract (AC5) scavenging activity varied from 5.96 ± 1.90% to 69.66 ± 1.91% within 0.23–0.94 mg/mL concentration range (EC_{50} = 1.24 ± 0.04 mg/mL) (tab. II). All other *A. chia* extracts showed weak effects (maximal inhibition below 30%).

TABLE II

Antioxidant activities of *Verbascum nigrum* and *Ajuga chia* aerial part extracts

	EC_{50}					
Sample	**DPPH radical scavenging activity (µg/mL)**	**ABTS radical cation scavenging activity (µg/mL)**	**Reducing power (µg/mL)**	**Ferrous ion chelating activity (mg/mL)**	**Superoxide anion radical scavenging activity (µg/mL)**	**Hydroxyl radical scavenging activity (mg/mL)**
VN1	ND	ND	ND	1.06 ± 0.00	ND	ND
VN2	ND	267.53 ± 0.44	148.60 ± 0.03	1.02 ± 0.00	ND	ND
VN3	35.84 ± 0.84	21.50 ± 0.01	18.22 ± 0.32	ND	ND	1.10 ± 0.03
VN4	28.03 ± 0.40	17.77 ± 0.00	17.30 ± 0.12	0.66 ± 0.00	990.00 ± 0.01	1.36 ± 0.09
VN5	312.32 ±14.19	85.82 ± 0.11	86.90 ± 4.37	0.05 ± 0.01	ND	1.72 ± 0.05
AC1	ND	ND	ND	1.43 ± 0.01	ND	ND
AC2	ND	ND	ND	0.21 ± 0.00	ND	ND
AC3	ND	ND	ND	0.18 ± 0.00	ND	ND
AC4	332.00 ± 0.08	ND	39.84 ± 1.44	0.51 ± 0.00	ND	ND
AC5	159.41 ± 0.06	ND	92.31 ± 6.29	0.16 ± 0.00	ND	1.24 ± 0.04
Q	3.09 ± 0.10	2.34 ± 0.01	NT	NT	NT	0.16 ± 0.01
Cat	NT	3.86 ± 0.01	6.50 ± 0.11	NT	NT	NT
GSH	NT	NT	NT	NT	1.09 ± 0.01	NT
EDTA-Na_2	NT	NT	NT	6.65 ± 0.25*	NT	NT

ND – not determined (weak activity); NT – not tested; Q – quercetin; Cat – catechin;
GSH – reduced glutathione; EDTA-Na_2 - ethylenediaminetetraacetic acid disodium salt; *µg/mL.

DISCUSSION

The present study reports on the antioxidant potential of different extracts prepared from the aerial parts of *V. nigrum* and *A. chia* in relation to their phenolic contents. The highest amounts of phenolics (199.35 ± 3.65 and 181.94 ± 2.38 mg/g) were determined in *V. nigrum* ethyl acetate (VN3) and

methanol (VN4) extracts, respectively. According to literature data, in other *Verbascum* species extracts, the phenolic contents ranged from 39.47 ± 1.68 mg/g (*V. densiflorum*) to 51.94 ± 2.30 mg/g (*V. sinuatum*) (23), being significantly lower than those determined in VN3 and VN4 extracts. In *V. blattaria* ethyl acetate extract, the phenolic content was 4.5-fold lower (18) compared to *V. nigrum* ethyl acetate extract (VN3). A low phenolic content (28.70 ± 0.9 mg/g) was determined in *V. betonicifolium* mucilage free water extract (24). The phenolic content in *A. chia* methanol extract VN4 (50.85 ± 1.11 mg/g) was comparable to or higher than those reported in other *Ajuga* species methanol extracts (56.76 ± 0.92, 22.63 ± 0.61, 20.86 ± 0.53 and 5.94 ± 1.98 mg/g in *A. laxmanii, A. genevensis*, *A. reptans* and *A. bracteosa*, respectively) (15, 25, 26).

The antioxidant potential of *V. nigrum* and *A. chia* extracts was assessed by six *in vitro* assays. In brief, different mechanisms involved in the antioxidant activity, such as hydrogen donation, electron transfer and transition metal ions chelation were investigated.

Previous studies on the DPPH scavenging activity of other *Verbascum* species extracts reported various results: *V. antiochium* methanol extract and *V. blattaria* ethyl acetate extract had weak effects (EC_{50} values of 4.80 mg/mL and 667 µg/mL, respectively) (18, 27), whereas *V. speciosum, V. songaricum, V. cheirantifolium, V. thapsus, V. densiflorum* and *V. sinuatum* ethyl acetate extracts (EC_{50} values ranging from 9.81 ± 0.71 to 23.56 ± 0.94 µg/mL) (23) were more active as DPPH scavengers than *V. nigrum* ethyl acetate extract VN3 (EC_{50} = 35.84 ± 0.84 µg/mL). Mihailović *et al.* reported EC_{50} values varying from 60.7 ± 1.7 to 121.5 ± 2.9 µg/mL for *V. nigrum* (collected in Serbia), *V. phlomoides* and *V. thapsus* methanol extracts and higher ones (EC_{50} = 105.7 ± 1.9 - 257.0 ± 4.6 µg/mL) for the aqueous extracts obtained from the same species (28). *V. pseudoholotrichum* methanol and aqueous extracts were less active against DPPH radical than *V. nigrum* extracts VN4 and VN5 (EC_{50} = 775 *vs.* 28.03 µg/mL and EC_{50} = 539 *vs.* 312.32 µg/mL, respectively) (29). Similarly, *V. ovalifolium* methanol extract (EC_{50} = 40.97 ± 0.54 µg/mL) (30) was less active than VN4 extract. In contrast to the present study, good DPPH scavenging abilities were found for *A. genevensis, A. reptans, A. laxmanii* and *A. bracteosa* methanol extracts, with EC_{50} values of 72.08 ± 6.02, 83.16 ± 5.21, 24.89 ± 0.83 and 36.90 µg/mL, respectively (15, 25, 26).

With respect to ABTS radical cation scavenging capacity, the methanol extract VN4 (EC_{50} = 17.77 ± 0.00 µg/mL) was more active than the methanol extracts obtained from *V. nigrum* collected in Serbia (EC_{50} = 90.0 ± 1.6 µg/mL) and other *Verbascum* species (EC_{50} values of 121.4 ± 2.1 and 114.4 ± 2.6 µg/mL for *V. phlomoides* and *V. thapsus* methanol extracts, respectively) (28). Other studies on the antioxidant activity of *Ajuga* species expressed the ABTS scavenging activity as trolox equivalents and therefore, a comparison with our results is not feasible. However, the antioxidant activity varied among *Ajuga* species (30.38, 66.13 ± 2.87 and 60.98 ± 1.52 mg trolox equivalents/g extract for *A. orientalis*, *A. genevensis* and *A. reptans* methanol extracts, respectively) (26, 31).

The present study found high reducing power values for the ethyl acetate and methanol extracts of *V. nigrum* aerial parts, namely VN3 and VN4 extracts (EC_{50} = 18.22 ± 0.32 and 17.30 ± 0.12 µg/mL, respectively). Luca *et al.* reported good reducing capacities for *V. ovalifolium* ethyl

acetate and methanol extracts (EC_{50} = 15.19 ± 0.37 and 22.56 ± 1.04 µg/mL, respectively) (30). On the other hand, *V. ovalifolium* aqueous extract had lower reducing power than VN5 extract (EC_{50} = 114.56 ± 2.59 *vs.* 86.90 ± 4.37 µg/mL) (30). *V. atlanticum* ethyl acetate extract was slightly less active than VN3 (EC_{50} = 23.81 ± 0.16 *vs.* 18.22 ± 0.32 µg/mL) (32). Reducing properties were also reported for various *Ajuga* species. *A. bracteosa* methanol extract exhibited a reducing activity of 718.4 ± 36 mg ascorbic acid equivalent/g at 1 mg/mL (15).

Ferrous ions can generate reactive radicals, including the hydroxyl radicals and therefore, ferrous ions chelation reduces the oxidative stress (33). According to Mihailović *et al.*, methanol extracts of *V. sinuatum*, *V. nudicaule* and *V. speciosum* showed ferrous ion chelating abilities of 14.44 ± 4.89%, 19.06 ± 8.80% and 5.42 ± 0.72%, respectively, at a concentration of 1 mg/mL (28). Within 25–200 µg/mL concentration range, *A. iva* extracts had moderate metal chelating effects, with the methanol extract being the most active, followed by the aqueous and chloroform extracts (34). *A. chamaecistus* aqueous extract was reported to have a ferrous ion chelating potential equivalent to 5.25 ± 0.21 mg ethylenediaminetetraacetic acid equivalents/g (10).

Among all investigated extracts, only VN4 extract exhibited a weak scavenging activity against superoxide anion radical (EC_{50} = 990 µg/mL). Luca *et al.* found superoxide scavenging abilities for *V. ovalifolium* ethyl acetate, methanol and aqueous extracts (EC_{50} = 527.26 ± 2.00, 573.31 ± 2.05 and 2417.43 ± 39.93 µg/mL, respectively) (30). According to El Gizawy et *al.*, *V. nubicum* methanol extract had a weak superoxide scavenging potential (only 40% scavenging capacity at 0.8 mg/mL) (35). *A. chia* extracts obtained in the present study showed no superoxide scavenging activity within 1.0 - 3.0 mg/mL concentration range. AC2 extract was the most active (30.70 ± 0.02% scavenging activity at 3 mg/mL). To the best of our knowledge, literature data on superoxide scavenging activity of other *Ajuga* species is lacking.

Hydroxyl radicals, highly reactive species generated by Fenton reaction, are responsible for DNA oxidative damage and other deleterious effects (oxidative damage of lipids and aminoacids, protein unfolding) (36). The hydroxyl radical scavenging effects of *V. nigrum* extracts obtained in the present study are weaker than those reported in literature for extracts obtained from other *Verbascum* species (EC_{50} values of 6.0 ± 0.6 and 166.5 ± 3.6 µg/mL for *V. phlomoides* methanol and aqueous extracts, respectively; EC_{50} values of 9.8 ± 0.8 and 171.6 ± 5.0 µg/mL for *V. thapsus* methanol and aqueous extracts, respectively) (28). Literature data on the hydroxyl radical scavenging capacity of *Ajuga* species is limited. A methanol: chloroform leaf extract of *A. bracteosa* (grown *in vitro*) scavenged the hydroxyl radical with an EC_{50} value of 122.5 ± 0.9 µg/mL (37).

The correlation between the antioxidant activity of *V. nigrum* and *A. chia* extracts and their phenolic contents was evaluated using the Pearson's test (tab. III, fig. 1). There were found strong positive correlations between the antioxidant activity (DPPH and ABTS scavenging activity, reducing capacity) and phenolic content of *V. nigrum* extracts. There was no correlation between the antioxidant activity of *A. chia* extracts and their phenolic contents. The negative correlations between the ferrous ion chelating capacity of *V. nigrum* and *A. chia* extracts and their phenolic contents suggest that other compounds than phenolics are responsible for chelation ability.

TABLE III

Pearson's correlation coefficients (r) between the antioxidant activities of *Verbascum nigrum* and *Ajuga chia* extracts and their phenolic contents

	DPPH radical scavenging activity	ABTS radical cation scavenging activity	Reducing power	Ferrous ion chelating activity	Superoxide anion radical scavenging activity	Hydroxyl radical scavenging activity
***Verbascum nigrum* extracts TPC**	0.9522**	0.9175*	0.9532*	-0.5100	-	0.8872
***Ajuga chia* extracts TPC**	-	-	-	-0.5400	-	-

*significant at $p < 0.05$; ** significant at $p < 0.1$; TPC, total phenolic content.

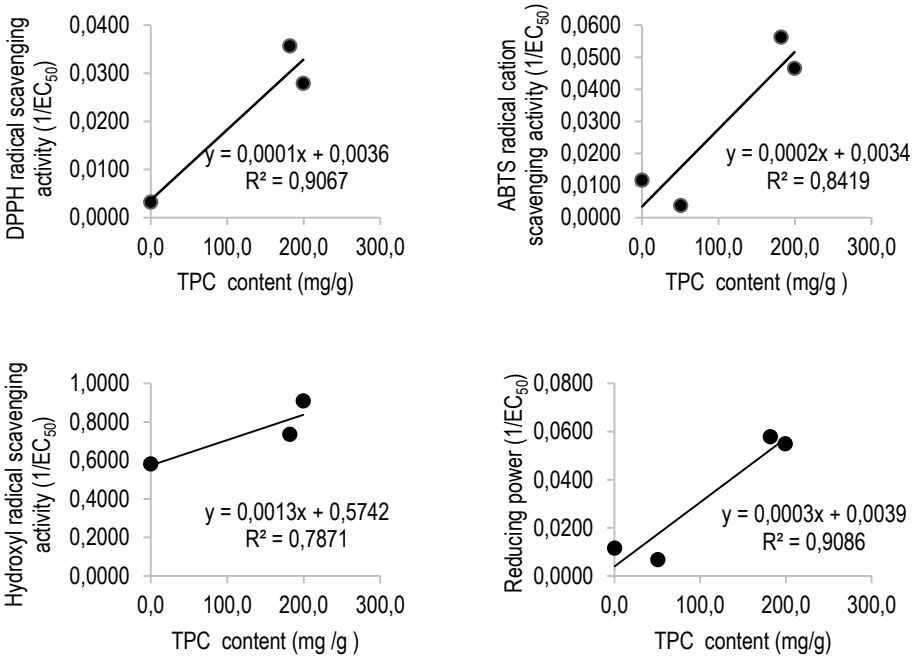

Fig. 1. Correlation between the antioxidant activity of *Verbascum nigrum* extracts and their phenolic contents

CONCLUSIONS

The current research evaluated the antioxidant potential and possible correlation with the phenolic content of ten extracts obtained from the aerial parts of *Verbascum nigrum* and *Ajuga chia* using five different polarity solvents. The assays revealed that *Verbascum nigrum* extracts had stronger antioxidant effects in comparison with *Ajuga chia* extracts. *Verbascum nigrum* ethyl

acetate and methanol extracts were efficient radical scavengers and reducing agents, with methanol extract being the most active in DPPH, ABTS and superoxide radicals scavenging assays, but also in the reducing power assay. On the other hand, *Verbascum nigrum* aqueous extract was the strongest ferrous ion chelator. Strong positive correlations were found between the antioxidant activity (DPPH and ABTS scavenging activity, reducing capacity) and phenolic content of *Verbascum nigrum* extracts. To conclude, *Verbascum nigrum* is a rich source of antioxidant compounds and needs further investigations on the metabolite profiling and bioactivity.

CONFLICT OF INTEREST AND FUNDING

Authors declare that there is no conflict of interest, and they received no funding for this study.

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DESIGN AND OPTIMIZATION METHOD FOR OBTAINING NEW CHITOSAN-BASED NANOFIBERS FOR WOUND HEALING APPLICATIONS

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DESIGN AND OPTIMIZATION METHOD FOR OBTAINING NEW CHITOSAN-BASED NANOFIBERS FOR WOUND HEALING APPLICATIONS (Abstract): **Aim**: The aim of the present study was to establish the optimal parameters for obtaining electrospun nanofibers (NFs), in correlation with their nanometer size range. **Material and methods**: Several types of nanofibers were designed: CS_PEO@P, CS_PEO@M_L_P, CS_PEO@P_C, CS_PEO@I_P, which have incorporated different active components (ACs), such as propolis (P), Manuka honey (M), *Calendula officinalis* infusion (C), L-arginine (L) and insulin (I) into a chitosan-polyethylene oxide (CS_PEO) matrix. The nanofibers were obtained *via* electrospinning method, the optimal electrospinning parameters were established, the average diameter was determined with Fiber metric software and the morphology was assayed using SEM microscopy. **Results and discussion**: Only homogenous, randomly-oriented and continuous NFs were considered proper for the study. Furthermore, after determining the average diameter of the fibers, a value of 600 nm was considered a reference maximum. By incorporating ACs, the morphology and the average diameter of NFs changed, with the most notable difference in the case of CS_PEO@M_L, when an increase from 164 ± 17 nm (CS_PEO matrix) to 201 ± 6 nm (CS_PEO@M_L_P) was noted. **Conclusions**: Although the Manuka honey-based NFs have displayed a larger diameter, they presented a smoother morphology and homogenous distribution, which means that this type of NFs could be proper for biomedical applications. **Keywords**: CHITOSAN, NANOFIBERS, WOUND HEALING, ELECTROSPINNING.

Developing new options for the management of wound care is a major focus in the biomedical field, as there is an ever-growing need to improve wound healing materials while minimizing processing costs (1). This, in turn, gives the patient a better and more efficient possibility to adjust the therapy according to the healing

stage. If a wound fails to heal within three months, the wound becomes chronic, and complications are more likely to occur (2). This may be due to an impairment caused by the oxidative stress (under the form of reactive oxygen species: superoxide, hydrogen peroxide, and even hydrochloric acid) in the inflammatory phase, at the wound site (3). Furthermore, other factors such as diabetes (insulin deficiency) may affect the lipid, protein or carbohydrate metabolism, respectively, thus delaying the wound healing (4). The natural extracellular matrix (ECM) consists of fibrous proteins with diameters between 50 nm and 150 nm. Novel nanomaterials such as electrospun nanofibers (NFs) provide a proper matrix for wound healing (5), demonstrating beneficial effects regarding tissue formation (6). NFs are highly porous systems with a large surface which are obtained using electrospinning method. By acting as artificial ECM they promote cellular adhesion, proliferation, and differentiation. NFs can be designed and their properties could be adjusted, according to the features of polymer matrix and of the active compounds included to the matrix (7). Chitosan (CS) is a biodegradable polysaccharide, derivative of chitin, with a wide range of biomedical applications, based on its properties, such as polycationic structure, nontoxicity, biodegradability, mucoadhesive, antibacterial and antifungal effects (8). It consists of *β*-(1,4)-D-glucosamine and N-acetyl-D-glucosamine units and the glucosamine unit content depends on the degree of deacetylation (9). Polyethylene oxide (PEO) is often used as a copolymer to aid the electrospinning technique and facilitate the NFs obtaining process (10).

Having the polymer matrix consisting of CS and PEO (CS_PEO), the following active components (ACs) were chosen to be incorporated into the NFs: propolis, Manuka honey, L-arginine, *Calendula officinalis* infusion, and insulin.

Propolis is a resin-like product made by bees. It has a complex chemical composition which depends on the local flora or weather. Flavonoids, phenolic acids and their derivatives are relevant for their antioxidant, anti-inflammatory and antimicrobial properties, which are of great importance in wound healing (11).

Manuka honey acts as a natural re-epithelization agent, which also displays antibacterial properties, due of its major constituent, methylglyoxal. In addition, due to its antioxidant components, Manuka honey also reduces the inflammation and through osmotic mechanisms, absorbs wound exudate, regulates moisture at the wound site and therefore promotes angiogenesis and production of granulation tissue. Finally, wound contraction and epithelialization are promoted (12).

L-arginine is an *alpha*-aminoacid with basic character, containing a guanidine group, which may synergistically promote antioxidant activity when it added to other compounds. More notably, it also promotes epithelialization (13).

Based on its content of flavonoids and saponins, *Calendula officinalis* is found in various pharmaceutical forms (tincture, ointments), used for epithelialization, anti-inflammatory and healing effects (14).

Insulin is a hormone which promotes healing of injured skin if applied topically. It promotes granulation tissue formation and therefore accelerates the contraction of the wound in the healing process (15).

In this work, NFs were prepared *via* electrospinning of a polymer solution consisting of a blend of CS and PEO. After established the optimal electrospinning parameters (concentration of the polymers, ratio CS:PEO, flow rate, distance, voltage), several ACs were further incorporated. To balance the biological effects of the NFs, either a trophic and an antimicrobial (Manuka honey, propolis, insulin) was used together with an angiogenic (L-arginine) or an antioxidant (*Calendula officinalis* infusion) component.

MATERIAL AND METHODS

Reagents

Chitosan (CS) with medium molar weight, polyethylene oxide (PEO) with high molecular weight (1 mill g/mol), L-arginine HCl, and acetic acid (≥99.7%) were purchased from Sigma Aldrich. *Calendula officinalis* was provided by Fares, Romania, propolis by Apiland, Romania, Manuka honey 550+ from Manuka Health New Zealand Ltd and insulin (NovoRapid Penfill 100 U/ mL) from NovoNordisk, Denmark. All other reagents were of analytical grade and were used as received without further purification.

Preparation of CS_PEO and CS-PEO@ACs solutions

The CS_PEO solutions were prepared according to a protocol previously reported (16). Briefly, CS 0.5-3% (wt/v) and PEO 0.5-2% (wt/v) were dissolved in 50% (v/v) acetic acid solution. After the complete dissolution, the ACs (propolis, Manuka honey, L-arginine, insulin) were added to obtain CS-PEO@ACs solutions (CS_PEO@P, CS_PEO@M_L_P, CS_PEO@I_P). A similar protocol was followed for preparation of CS_PEO@P_C solution, with the note that instead of the distilled water used to make the acetic acid solution, a *Calendula officinalis* infusion was used.

Electrospinning of CS_PEO and CS-PEO@ACs solutions

The NFs were prepared using a laboratory-scale electrospinning system (Nanospinner Inovens device) set up with a generator, pump, and a grounded collecting plate used as negative electrode. The CS-PEO and CS-PEO@ACs solutions were electrospun from disposable 5 mL syringe with Luer fitting. The 23-gauge needles were the positive electrode.

Characterization of the CS-PEO and CS_PEO@ACs NFs

The NFs' morphology and average diameter were assayed with Scanning Electron Microscopy (SEM, Inspect S electron microscope with acceleration voltages in high vacuum and a secondary electron beam using energies of 30 keV). All samples were covered with a thin gold film before being analyzed to diminish the electrical charging. After micrographs were taken for all samples, the fiber distribution was determined using the Phenom Fibermetric software. This was carried out by measuring at least 150 fibers from every image (three images from each sample). The data obtained is shown as average diameter ± standard deviation (SD).

RESULTS

În order to obtain randomly-oriented CS_PEO NFs, with small diameter, different electrospinning parameters were changed, such as concentration of CS (0.5-

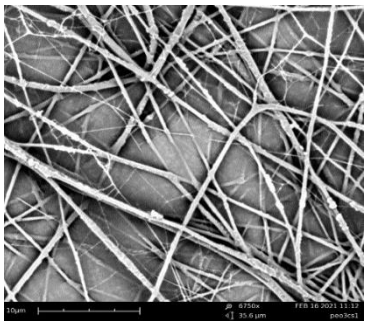
3% wt/v), PEO (0.5-2% wt/v), CS:PEO ratio (3:1, 2.5:1, 2.5:1.5, 2:1, 1:2, 1:1.5, 1:1, 1:0.5, 0.5:2, 0.5:1.5, 0.5:1), flow rate (1.5-0.5 mL/h), voltage (18-25 kV), the distance between syringe needle and collector (20-27 cm). The results about the morphological aspects of the developed fibers in terms of surface, diameter, distribution and orientation are presented in table I and figure 1.

The optimal parameters to form CS-PEO NFs with proper morphological characteristics (small diameter, continuous aspect, randomly-orientated and distribution was set to CS 1% (wt/v), PEO 0.5% (wt/v), CS:PEO = 1:0.5 and flow rate 0.6 mL/h). Except the flow rate, these parameters were used further to electrospinning the CS_PEO@ACs solutions in order to obtain the corresponding NFs. As results, well-aligned, unidirectional CS_PEO@ACs NFs, with small diameters, no beads and smooth surfaces were obtained (tab. II).

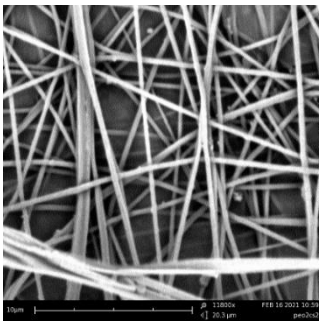
TABLE I.
The electrospinning parameters used for preparation of CS_PEO NFs

CS (g/100 mL)	PEO (g/100 mL)	Flow rate (mL/h)	Distance (cm)	Voltage (kV)	Notes
3	1	0.75	25	18-25	dripping, NFs with large diameter, >600 nm
3	1	1	25	18-25	
3	1	1.25	25	18-25	
3	1	1	27	18-25	absent NFs
3	1	1	27	18-25	absent NFs
3	1	1	27	18-25	dripping, NFs with large diameter, >600 nm
2.5	1	1	25	18-25	dripping, NFs with large diameter, >600 nm
2.5	1.5	1	25	18-25	
2	1.5	0.9	25	18-25	
2	1	0.9	25	18-25	
1	2	0.9	25	18-25	
1	2	1	20	18-25	dripping, NFs with bead, average diameter of ~400 nm
1	2	1	27	18-25	
1	1.5	1	25	18-25	
1	1.5	1	20	18-25	
1	1.5	0.75	25	18-25	
1	1.5	0.75	20	18-25	
1	1.5	0.5	25	18-25	
1	0.5	1	20	18-25	dripping, NFs with bead, average diameter smaller than 400 nm
1	0.5	0.75	22	18-25	
1*	**0.5***	**0.6***	**25***	18-25	
0.5	2	1.5	25	18-25	NFs with discontinuous aspect and large diameter; they were not homogenously deposited on the collecting plate and the distribution was not homogenous
0.5	1.5	1.25	25	18-25	
0.5	1	1	25	18-25	

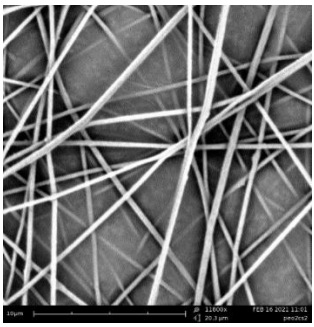
*The parameters considered optimal and were further used for incorporation of ACs.



CS: 3% (wt/v)
PEO: 0.3% (wt/v)
fibers with rugous structure, both microfibers and NFs are formed



CS 2% (wt/v)
PEO 0.4% (wt/v)
fibers with smoother surface, microfibers are still present



CS 1% (wt/v)
PEO 0.5% (wt/v)
NFs with smooth surface with average diameter below 600 nm

Fig. 1. Correlation between CS_PEO concentration and fiber morphology.

TABLE II.

The optimized electrospinning parameters used for preparation of CS_PEO NFs

Sample	ACs	Flow rate (mL/h)	Average diameter ± SD (nm)
CS_PEO	-	0.6	163 ± 16
CS_PEO@P	P: 7.5% (wt/v)	0.2	169 ± 12
CS_PEO@I_P	P: 7.5% (wt/v I: 46 IU/mL	0.2	169 ± 11
CS_PEO@P_C	P: 7.5% C: infusion	0.2	170 ±11
CS_PEO@M_L_P	M: 7.5% (wt/v) L: 7.5% (wt/v) P: 7.5% (wt/v)	0.5	201 ± 6*

*p< 0.05

DISCUSSION

Setting up a yield-effective electrospinning process is a time-consuming operation because it implies the adjustment of every parameter involved in the process. To sum up all obstacles that were encountered in the optimization phase, not only the difficulty is to be mentioned, but also the solution that was found to counter it.

Longer stirring times of polymeric solutions also helped against the formation of droplets, by increasing the homogeneity of the electrospinning solutions. Therefore, a minimum 36 h was the optimal time to ensure proper conditions. When no fibers seemed to be forming, the electric field was increased to values higher than 22 kV (16). In case of discontinuous fiber formation, an increase of both the electric field and flow rate helped with to optimize the process. A particular case also appeared when using CS 2% (wt/v), forming of flat fibers. This was countered by increasing the distance between the syringe needle and the collector.

Often the electrospinning of the polymeric solution leaded to the formation of beaded chains. Since the humidity was kept at low values during the experiments (17-20%) and the room temperature was at a constant value of 21-22℃, other values needed to be adjusted. When changing the molecular weight of CS to a higher one, little to no fibers were formed, therefore the experiment resumed at the initial medium molecular weight. The key to the success of the experiment was adjusting the polymers (CS, PEO) concentrations and the ratio. As can be seen in fig. 1, higher concentration led to wider fiber diameters, and rough surfaces of the fiber. A smooth surface was obtained at a ratio of 1:0.5 (CS : PEO) and a maximum total concentration of 1.5% (wt/v) (CS 1% PEO 0.5%) polymer solution. The higher the weight concentration of the chitosan, the more the solution seemed to be extruded from the syringe rather than simply flow. Although an increase of the electric field seemed to help with this problem, there were still fragments which seemed to be building at the tip of the needle. Also, in this case fibers seemed to be climbing back from the collector to the needle. This was another factor that contributed to choosing the medium molecular weight CS (17).

The SEM analysis determined for optimized types of NFs incorporating ACs that they had smooth surface with no beads, possibly due to the properties induced by the ACs. Indeed, the average diameter has increased after adding the ACs into the CS_PEO matrix, our results agreeing with other literature data (18).

A more comprehensive analysis through SEM (fig. 1) shows a compact 3D nanofiber mesh containing randomly settled fibrils, which generate an irregular surface with an interwoven porous frame. This 3D frame shapes a large contact surface with docking points for cell-NF interaction helping cellular processes as adhesion, migration, and proliferation (19). Also, electrospun NFs have also given a unique means for Manuka honey delivery while contributing as artificial matrix for wound healing thanks to their geometry (20).

The widest diameter was noted for the CS_PEO@M_L_P (201 ± 6 nm), while for the NFs incorporating propolis this value was approximately 170 nm (169 ± 12 nm for CS_PEO@P, 170 ± 11 nm for CS_PEO@P_C, and 169 ± 11 nm for CS_PEO@I_P). The only statistical difference, when compared with CS_PEO (163 ± 16 nm) appeared for CS_PEO@M_L_P (p value <0.05). This may be due to honey's viscous properties and smaller possibility for the electrospinning jet to stretch rapidly and permit the solvent to evaporate in a very short amount of time.

Other studies included NFs that incorporate Manuka honey up to 10-20% (wt/v), the average diameter also displayed a rise from 357 to 400 nm (18). This data also supports our results and demonstrates the successful incorporation of the Manuka honey into the CS_PEO matrix.

CONCLUSIONS

New blended NFs consisting of CS and PEO incorporating ACs were designed, and their obtaining process was optimized, considering the morphology and fiber diameter as key elements to successful preparation. After the optimization of the electrospinning process, in terms of the polymer (CS, PEO) concentration, polymer ratio (CS:PEO), flow rate, voltage and distance between syringe needle and collector, proper CS_PEO NFs were obtained. Based on optimized process of CS_PEO NFs new CS_PEO@ACs NFs were

developed. The results revealed that the Manuka honey-based NFs (CS_PEO@M_L_P) displayed a good diameter, smoother fiber morphology and homogenous distribution that could be useful for further applications. The new blends are a starting point to explore new possibilities for modern wound healing therapy and can be candidates for further tests regarding wound healing potential.

CONFLICT OF INTEREST AND FUNDING

Authors declare that there is no conflict of interest.

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NEWS

VITAMIN D AND OPHTHALMOPATHIES. A REVIEW

The importance of hydroxycholecalciferol as one of the liposoluble vitamins is known in the prevention and treatment of metabolic bone diseases (rickets, osteomalacia, osteoporosis). In recent years, however, information has increased on the importance of vitamin D3 in numerous organ systems and in the pathogenesis of various diseases, e. g. ophthalmopathies. The immunological functions of vitamin D3 are the subject of studies dealing with autoimmune optic nerve disorders and their results appear to have a positive effect on demyelinating diseases. It also plays an important role in maintaining the thickness of the retinal nerve fiber layer, but its additional administration has not been successful. It appears that sufficient serum vitamin D3 levels may protect patients from deterioration in the form of a further attack of demyelination. The course of diabetic retinopathy is probably also influenced by vitamin D3, by correlating the fact that its receptor and the enzymes of its metabolism are expressed on the retina. Low serum levels of vitamin D3 may even trigger age-related macular degeneration. Conversely, higher dietary intake of vitamin D3 may positively affect neovascularization. The optimal level of hydroxycholecalciferol is between 60 and 200 nmol /l, the severe deficit represents a decrease below 25 nmol/l. However, the demonstration of its protective character in connection with the mentioned diseases of the retina and optic nerve will require a sufficient number of studies to confirm the facts found so far about this rediscovered vitamin (Hribová P, Sotak S. Vitamin D and ophthalmopathies. A review. *Ceska a slovenska oftalmologie: casopis Ceske oftalmologicke spolecnosti a Slovenske oftalmologicke spolecnosti* 2022; 2: 1001-1004).

EVALUATION OF GINGIVAL EPITHELIAL CHANGES THROUGH OPTICAL COHERENCE TOMOGRAPHY AT PERIODONTAL PATIENTS WEARING FIXED DENTAL PROSTHESIS

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EVALUATION OF GINGIVAL EPITHELIAL CHANGES THROUGH OPTICAL COHERENCE TOMOGRAPHY AT PERIODONTAL PATIENTS WEARING FIXED DENTAL PROSTHESIS (Abstract): The current *in vitro* investigation **aim** is to identify changes in optical coherence tomography images of gingival tissue samples by analyzing the epithelial thickness and its pixel density, trying to find connections with the gingival inflammatory condition of periodontitis patients wearing fixed dental prosthetics. **Material and methods**: A total of 25 patients were admitted in this study, and were undergone a clinical periodontal examination, where probing depth (PD) and gingival inflammation index (GI) were recorded, and gingival samples also were collected from teeth with and without dental prosthesis, consequently two groups were obtained: control group CG-25 gingival samples from the teeth not wearing a dental prosthesis and prosthetic group PG -25 gingival samples from the teeth wearing a dental prosthesis. The samples were analyzed through optical coherence tomography. **Results**: The results show a statistically unsignificant difference in PD between the two groups, but also a statistically significant difference in GI between the two groups. A statistically significant difference was found between the epithelial thickness (ET) between the two groups. A strong inverse and statistically significant correlation were discovered between ET and GI. **Conclusions**: The OCT analysis has proved useful in showing that there could be a possible association between periodontal changes and fixed dental prosthesis, and it also showed that fixed prosthodontics has a significant negative impact on the periodontium, making possible not only the treatment but the prevention of periodontal clinical changes in patients wearing dental prosthesis, thus improving the quality of life of these patients.
Keywords: PERIODONTAL, OCT, DENTAL PROSTHESIS.

Periodontal disease is an inflammatory condition that affects the teeth's supporting tissues. The illness is caused by bacteria and begins at the gingival level, when perio pathogen microorganisms create inflammation of the soft tissues around the tooth. The inflammation advances progressively into the deeper structures that support the teeth. Periodontal pockets build around the tooth as the disease progresses, containing more bacterial plaque and calculus deposits, thus exacerbating the inflammation (1).

If the onset of the disease is influenced by the microbial composition of the subgingival biofilm and the type and intensity of the host immune response, subsequently, a series of local and systemic predisposing factors are involved. Generally, local risk factors act as bacterial biofilm retentors and prevent the patient from efficiently removing plaque deposits. These local risk factors include morphological abnormalities such as crowded teeth and malocclusions, dental treatments such as orthodontic and prosthodontic appliances and dental calculus. Most local risk factors are modifiable and correctable so as to offer to the patient and practitioner a favorable status for a good plaque control (2).

The technology of optical coherence tomography (OCT) generates optical sectional pictures. Low-coherence interferometry is used to determine the echo time delay and magnitude of backscattered light reflected by a given studied surface. The laser beam directed at the surface is reflected differently by the surface's structural irregularities, resulting in interferences that are detected by a sensor (3).

OCT has been researched in preventive dentistry as a technique to check the integrity of dental sealants used in youngsters (4). It has been employed in endodontics as an adjuvant approach for identifying root canals in teeth with unique endodontic anatomical systems and in restorative dentistry to identify tooth cracks, their morphology, and location, making it easier to diagnose and treat challenging dental disorders such the fractured tooth syndrome (5).

The experimental use of OCT in periodontology found that OCT can visualize periodontal pockets and attachment loss, and that it can even be used to measure pocket depth utilizing axial resolution of the images, with findings that are comparable to manual periodontal probing(6). Using the measurement of gingival tissues thickness or pixel density of images, OCT proved to be useful in assessing inflammation in epithelial or connective tissue in periodontal patients with systemic diseases (7).

The current in vitro investigation intends to identify changes in optical coherence tomography images of gingival tissue samples by analyzing the epithelial thickness and its pixel density, trying to find connections with the gingival inflammatory condition of periodontitis patients wearing fixed dental prosthetics.

MATERIAL AND METHODS

Patients. The subjects for the study addressed the Periodontology Department at Craiova University of Medicine and Pharmacy in Romania from 01.11.2018 to 01.06.2019. The Ethics Committee permission of the University of Medicine and Pharmacy of Craiova, number 104/ 22.10.2018, was acquired for the study, as well as written informed consent from all participating patients. All participants were told about the study's purpose and that it was being carried out in line with the Hel-

sinki Declaration.

To be included in this study, all periodontal patients must have been diagnosed with periodontitis, had at least one periodontal pocket ≥ 5mm on a tooth wearing a dental fixed prosthesis in the lateral premolar-molar region, allowing for standardized gingival tissue collection, regardless the material from which the prosthesis were made or their age and at least one periodontal pocket ≥ 5mm on the correspondent tooth from another premolar-molar region without dental prosthesis. Patients were diagnosed with periodontitis (8) at the Periodontology Department, had initial periodontal therapy, and then underwent surgical periodontal therapy following a reevaluation. To reduce individual variability, all patients underwent periodontal examinations by the same previously trained practitioner. Criteria for exclusion: diagnostics of diabetes, rheumatoid arthritis, liver or cardio-vascular diseases, treatment with non-steroidal anti-inflammatory medication or antibiotics in the three previous months, pregnancy, smoking.

A total of 25 patients were admitted in this study, aged 32-62 years (51.04±9.87), 14 females (F), 11 males (M). The patients were undergone a clinical periodontal examination and gingival samples were collected from teeth with and without dental prosthesis, as mentioned above and two groups were obtained: control group CG-25 gingival samples from the teeth not wearing a dental prosthesis and prosthetic group PG -25 gingival samples from the teeth wearing a dental prosthesis.

Clinical Periodontal parameters. From each patient, the probing depth (PD) expressed in millimeters was collected from the periodontal chart for every tooth included in the study, calculating the average for each group and the gingival inflammation index (GI – Loe and Silness, 1963) expressed in grades 0-3 (0 = normal gingiva; 1 = mild inflammation: slight change in color, slight edema, no bleeding on probing; 2 = moderate inflammation: redness, edema, and glazing, or bleeding on probing; 3 = severe inflammation: marked redness and edema, tendency toward spontaneous bleeding, ulceration) also calculated as the average for each group (9).

Gingival tissue sampling. Gingival samples were taken from the periodontal pocket during periodontal surgery conducted as part of a conventional periodontal treatment for patients with periodontal disease. Samples were standardized to a square dimension of 3/3 mm. They were then put in 10% formalin and refrigerated at +8 degrees Celsius until their processing.

OCT analysis. Thorlabs (Thorlabs Inc., New Jersey, USA) provided the OCT setup, model number OCS1300SS, with a power swept laser source at a central wavelength of 1310 nm, a spectral band-width of 100 nm, and an average power of 12 mW. The gingival samples were withdrawn from their containers, and the residual formalin solution was dried from their surfaces. They were then placed on Petri dishes with the gingival epithelium facing upwards and subjected to the OCT system's laser beam.

The samples were scanned in air with axial resolutions of 12 m and lateral resolutions of 15 m. The 3 mm square samples were scanned across a range of 1024 x 1024 x 512 pixels (length, width, depth, x:y:z), with the refraction index set at 1.41 (10). Each sample scanning yielded 512 sections, which were stored as JPEG images. The photos were analyzed using ImageJ, a free image processing program provided by the National Institutes of

Health in the United States. For the pixel density study, five consecutive photos were chosen from a total of 512 for each sample, including the median image (256), two prior (254, 255) and two following images (257, 258), and the density was assessed in terms of both pixel counts and gray-scale values (7). Consequently, 125 images were investigated in each group (CG and PG).

A measuring segment in the form of a linear shape was inserted perpendicular to the center of each picture, from the higher to the lower edge of the gingival epithelium (fig. 1). Through the ImageJ application, the length of the segment, representing the epithelial thickness (ET), was calculated in µm. The application produced a set of data reflecting the gray-scale intensities of the pixels (7), as well as the number of pixels present in each examined region and the length and area filled by the measurement segment, which were then recorded in *Microsoft Excel* format. The average values (Pix) were computed for each research group.

Statistical analysis. The data were expressed as mean ± standard deviation (SD). The t-Student test was used to compare the acquired data for the two research groups, different characteristics of the research groups were correlated using the Pearson statistical correlation test, with the statistical significance level set at $p<0.05$ (*SPSS version 19.0*, IBM, USA)

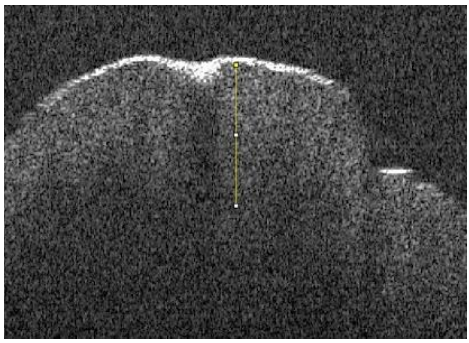

Fig. 1. Measurement of ET and Pix on the OCT image of the periodontal patients gingiva

RESULTS

The results show a difference in PD between the two groups, but statistically unsignificant ($p>0.05$) but also a statistically significant difference in GI between the two groups ($p=0.046$). A statistically significant difference was found between ET ($p=0.003$) and Pix ($p=0.000013$) between the two groups.

A strong and statistically significant correlation was found between Pix and GI in each group and a strong inverse and statistically significant correlation was discovered between ET and GI (tab. I).

TABLE I.

Statistically correlations between Pix, GI and ET

	PD	GI	Pix	r_1	ET	r_2
PG	5.96±1.13	2.68±0.43*	52.68±303**	-0.955#	283.81±31.4***	-0.812§
CG	5.64±0.86	2.04±0.56	47.64±4.14	-0.840##	343.65±43.5	-0.867§§

*p<0.05 significant difference in GI between the two groups; **p<0.05 significant difference in Pix between the two groups; ***p<0.05 significant difference in ET between the two groups; #p<0.05 strong significant correlation between GI and Pix in the PG group; ##p<0.05 strong significant correlation between GI and Pix in the CG group; §p<0.05 strong inverse significant correlation between GI and ET in the PG group; §§ p<0.05 strong inverse significant correlation between GI and ET in the CG group.

DISCUSSION

Maintaining a healthy periodontal status at the level of teeth that benefit from restorations with edges in contact with the free gingival margin is a challenge for practitioners, as the odonto-periodontal unit is constantly affected by the microbial flora, as its load can be stimulated by dental restorations (11). The most common mechanism by which prosthetic restorations may affect the status of the marginal periodontium is by favoring the accumulation of bacterial plaque (12).

The positioning of the edges of the crown in relation to the free gingival margin, the material from which the dental prosthetic restoration is made, its contour and profile, all these elements influence the health of the periodontal structures (13). In our study, the PD of teeth wearing a dental prosthesis are higher than that of those without it, but the differences are not significantly, while the GI in group PG is significantly higher than in group CG, suggesting the influence of dental prosthesis on the gingival inflammatory status, possibly by plaque retention and, consequently, the exacerbated inflammation.

There are histological changes that can be observed within the inflamed gingival epithelium, with the appearance of inflammatory oedema and the decrease of tissue density. The OCT assessment of gingival tissue samples suggests an association between pixel density and the degree of periodontal inflammation, as it was previously shown in periodontal patients suffering from systemic conditions (7). In the present study, it was found that Pix inversely correlated with GI, sustaining the previous finding. The images of the gingival samples, obtained by OCT revealed that the pixel density of the epithelium was lower in PG samples as compared to those of group CG. This fact could be explained by the exacerbated periodontal inflammation in the gingiva of teeth wearing a dental prosthesis.

The major epithelial thickness (MET–distance between the external epithelial surface and the epithelial crista tip) and the epithelial base thickness (EBTdistance between the external epithelial surface and basal membrane located between two cristae) were evaluated on the histological or OCT images of the gingiva of periodontal patients (14-16) or the epithelial thickness (ET) in different areas of marginal gingiva (17). These studies shows that the inflamed epithelium is thinner as our study found also in PG, between the ET and GI being found an inverse correlation. These results sustain the idea that the presence of dental prosthesis exacerbates the inflammation in periodontitis patients.

However, the study was limited in the number of participating patients, the purpose was to draw attention to the possible influence of dental prosthesis, regardless the material or the initial placement of prosthesis margins on the periodontal inflammation in the presence of the preexisting periodontal diagnosis.

CONCLUSIONS

The OCT analysis has showed that there could be a possible association between epithelial inflammatory changes and fixed dental prosthesis, and it also showed that fixed prosthodontics has a negative impact on the periodontium, making important not only the treatment but the prevention of periodontal clinical changes in patients wearing dental prosthesis, thus improving the quality of life of these patients.

CONFLICTS OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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INFLUENCE OF BODY MASS INDEX ON DENTAL DEVELOPMENT AND GINGIVAL INFLAMMATION IN CHILDREN OF 6-12 YEARS OLD

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INFLUENCE OF BODY MASS INDEX ON DENTAL DEVELOPMENT AND GINGIVAL INFLAMMATION IN CHILDREN OF 6-12 YEARS OLD (Abstract): **Aim**: Our study aims to assess the influence of BMI (Body Mass Index) on dental development and gingival inflammation in children aged 6-12 years old. **Material and methods**: The study was conducted on a group of 153 children aged 6 to 12 years, which were divided into three categories: normal weight, overweight and obese patients. Dental development estimation was performed using the Demirjian method, by using orthopantomographies. For each patient, chronological age, BMI, plaque index (PI) and gingival index (GI) were recorded. **Results**: The mean difference between chronological and dental age for the whole study group was 0.66±0.5 years. The mean value of the difference between chronological age and dental age for overweight and obese subjects was 0.81±0.43 years and 1.1±0.45 years, respectively. A significant correlation between BMI and GI was observed for male subjects. No correlations were found between BMI and chronological age, or between BMI and dental age. It was found correlation between BMI and the difference between chronological age and dental age. **Conclusions**: In the group of obese children there was a greater difference between chronological age and dental age compared to overweight and normal weight children, indicating that permanent teeth are likely to erupt at a younger age among obese and overweight pediatric patients. **Keywords**: GINGIVAL INFLAMMATION, DENTAL DEVELOPMENT, OBESITY.

Dental development is a complex biological process, studies showing that obesity can be associated in many cases with an acceleration of odontogenesis, regardless of gender or socioeconomic status (1-4).

Obese pediatric patients have been shown to have low levels of growth hormone and, at the same time, increased lev-

els of insulin-like growth factor1 (IGF-1) (5). Studies suggest that IGF-1 is associated with accelerated growth and development by suppressing the activity of the hypothalamic-pituitary system, which may lead to steadily decreasing growth hormone levels (5, 6).

Body Mass Index (BMI) is the most widely used indicator of obesity (National Institute for Clinical Excellence, 2006) (7).The Body Mass Index could be calculated dividing the weight in kilograms by the square of height in meters.

Recent studies also suggest that obese adolescents exhibit early development of craniofacial massif (8), which may lead to worsening of the existing dento-maxillary anomaly, requiring orthodontic intervention through interceptive therapeutic methods (9).

Numerous methods have been developed to assess dental age in children and adolescents. The method described by Demirjian in 1973 (10) is the most widely used method of dental age assessment due to its accuracy and feasibility. The effectiveness of the Demirjian method has been evaluated among different ethnicities (11-14). The correlation between dental development, height and weight has also been assessed and found to be a significant correlation in many of the studies (2, 15-18).

Our study aims to assess the influence of BMI on dental development and gingival inflammation in children aged 6-12 years.

MATERIAL AND METHODS

The study was conducted on a group of 153 children aged 6 to 12 years, 70 male and 83 female patients who presented to the dental office for routine dental treatments. For the study to be carried out, the approval of the Ethics Committee of University of Medicine and Pharmacy from Craiova, Romania was obtained (No.14/21.01.2019). and the informed consent was signed by the tutors.

Inclusion criteria: pediatric patients with no history of systemic disease, who had not received orthodontic treatment in the past and whose panoramic radiological investigations provided good quality images. Exclusion criteria: patients with an incomplete medical history, congenital malformations such as cleft lip and palate (whether they were surgically corrected or not), developmental dental anomalies or patients with previous extractions of permanent teeth, and radiological investigations of poor quality.

Dental age estimation was performed on the 153 orthopantomographies using the Demirjian method (10) based on the eight stages of development of the tooth, starting with mineralization from the cusp tip to apex closure. As the method involves a degree of subjectivity, the analysis of each orthopantomography was performed by an examiner who transformed the scores that have been obtained using gender-specific conversion tables.

Chronological age was determined according to the date of birth of each patient.

The BMI was calculated as the ratio of weight to height (expressed in kg/m^2). Patients' weight status was assessed using the International Obesity Task Force (IOTF) recommended classification system for childhood obesity (19). The study group was divided into three categories: normal weight, overweight and obese patients.

The clinical examination was performed by a single well-trained dentist.

The bacterial plaque index (PI) (20) was used to assess supragingival plaque deposits, which has a grading scale with values

from 0 to 3.The Gingival Index (GI) scores on a scale of 0 to 3, where 0 - normal appearance to 3 - severe inflammation characterized by oedema, redness and spontaneous bleeding (21).

For statistical analysis, *Graph Pad Prism 9.4.0* was used. The data are described as mean ± standard deviation (SD) for continuous variables and as number (percentages) for discrete variables. The direction and magnitude of correlation were assessed with Pearson or Spearman correlation or 3-way ANOVA, depending on that values from variables are sampled from populations with a Gaussian distribution or not. Data were tested for normality using Shapiro-Wilk test.

RESULTS

The mean age of the study group was 9.22±1.69 (54.2% female and 45.8% male), while the background was predominantly urban. The mean weight with a value of 32.88±7.34 (72.5% of them with normal weight) (tab. I)

The mean difference between chronological and dental age for the whole study group was 0.66±0.5 years. The mean value of the difference between chronological age and dental age for overweight and obese subjects was 0.81±0.43 years and 1.1±0.45 years, respectively (tab. I). The differences between the two ages according to gender are shown in Table II.

A low correlation was identified between BMI and PI, on the limit of statistical significance for male patients (ρ=0.23, p=0.055), but the data could not be correlated for the female patients (ρ = -0.119, p=0.286).

A significant correlation between BMI and GI was observed for male subjects (ρ= 0.398, p=0.001), while for the female group there was none (ρ=0.167, p=0.131). No statistically significant correlation was identified between GI and the difference between chronological age and dental age (ρ=0.059, p=0.468).

TABLE I.

Batch distribution according to variables analyzed

Variables analyzed	
Chronological age	
Mean (±SD)	9.22 (±1.69)
Range	6-12
Age category	
6-9 years old	61 (39.9%)
9-12 years old	92 (60.1%)
Dental age Mean (±SD)	9.87 (±1.8)
Difference between chronological age and dental age Mean (±SD)	
whole study group	0.66 (±0.5)
normal weight children	0.58 (±0.5)
overweight children	0.81 (±0.43)
obese children	1.1 (±0.45)
Gender	
Male	70 (45.8%)
Female	83 (54.2%)
Background	
Urban	124 (81%)
Rural	29 (19%)
Weight	32.88 (±7.34)
Height	135.05 (±10.15)
BMI	
Normal weight	111 (72.5%)
Overweight	35 (22.9%)
Obese	7 (4.6%)
Plaque Index (PI)	
0	24 (15.7%)
1	72 (47.1%)
2	54 (35.3%)
3	3 (2%)
Gingival Index (GI)	
0	55 (35.9%)
1	68 (44.4%)
2	29 (19%)
3	1 (0.7%)

Also, no correlations were found between BMI and chronological age (ρ=-0.101, p=0.213), or between BMI and dental age (ρ=-0.02, p=0.81), but there was between BMI and the difference between chronological age and dental age (ρ=0.281, p<0.001). Thus, increased BMI is associated with accelerated dental development in children aged 6-12 years old.

TABLE II.
Difference between chronological age and dental age by gender

AGE	Male (n=70)	Female (n=83)
Chronological age Mean (±SD)	9.36 (±1.73)	9.1 (±1.66)
Dental age Mean (±SD)	9.97 (±1.82)	9.79 (±1.79)
Difference between the chronological age and the dental age Mean (±SD)	0.62 (±0.57)	0.69 (±0.42)

No statistically significant correlation was identified between age, gender and BMI. The distribution is normal with the mean=0.66 and SD=0.50. No possible outliers were detected for the dependent variable. Dental development was significantly accelerated with increasing BMI, but not after adjusting for age and gender (p>0.05). These three factors did not correlate in terms of acceleration of the dental development.

Accelerated dental development was observed to be correlated with BMI only in male patients (ρ=0.363, p=0.002), but for female patients the correlation was low and to the limit of statistical significance (ρ=0.208, p=0.059).

The magnitude of the correlation between variables differs by gender without being statistically significant. A greater difference in dental development can be observed for female patients in the age group of 9-12 years old compared to the age group of 6-9 years old(figs. 1, 2)

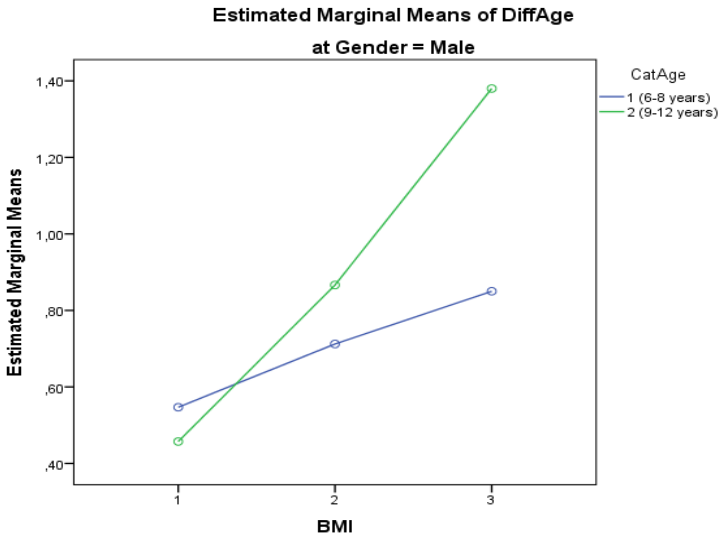

Fig. 1. Correlation between BMI and difference between chronological age and dental age for male patients

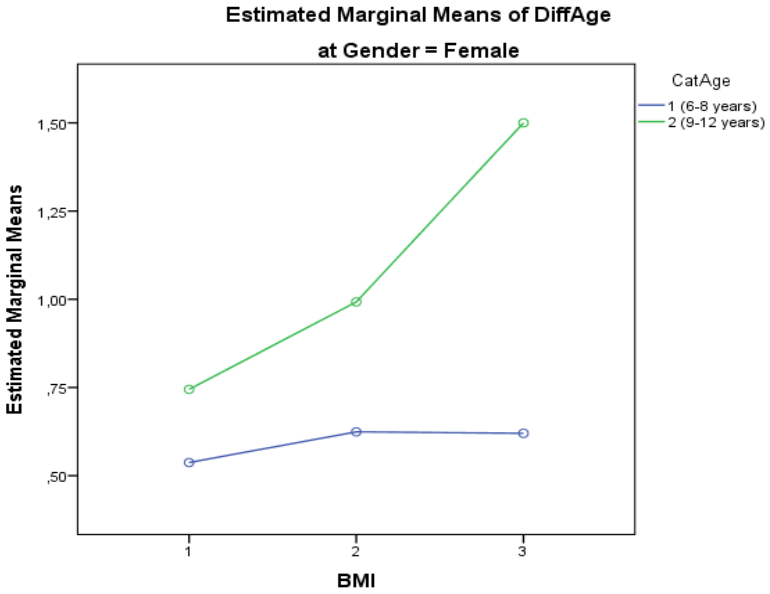

Fig. 2. Correlation between BMI and difference between chronological and dental age for female patients

DISCUSSION

Childhood obesity is frequently associated with accelerated dental development, an increased incidence of carious lesions located mainly on permanent molars, an increased risk of periodontitis in late adolescence and early adulthood (22). In the study by Hedayati Z *et al.*, the dental age of normal weight children was found to be lower than the chronological age, while among obese children the dental age was higher than the chronological age and there was a direct correlation between BMI and the process of dental development (3), data which were not according to the study by Panchbhai A *et al.* in which a negative, non-significant correlation was observed between BMI and mean dental age (23).

Our study found a significant positive correlation between accelerated dental development and BMI values, results also found in other studies (3, 15, 17, 24-28). Also, studies have reported accelerated dental development/age in obese children compared to children with weight within normal values (2, 29, 30).

In contrast to the results of the present study, it was reported a negative correlation indicating delayed tooth eruption for the group of overweight and obese children of 6-7 years old compared to the group of children with normal weight, authors explaining these results through the differences in ethnicity, racially, and dietary habits between Indian and Western population (31). Also, Khan *et al.* observed that the dental eruption time of Pakistani children are different in many aspects with to other nationalities. (32).

The results of our study show that accelerated dental development correlated with the BMI in male patients. In some studies, it was shown that among female patients, the difference between chronolog-

ical and dental age in both obese and normal weight patient groups was greater compared to male patients (24, 25). Du-Plessis *et al.* evaluated the correlation between BMI and dental development by gender and observed a non-significant difference between boys and girls (28).

Early onset of dental development refers to the importance of establishing and performing proper oral hygiene in children from an early age. In a longitudinal study (17), it was observed that overweight pediatric patients have a higher number of erupted teeth compared to other children of the same age ($p<0.001$) and also that there is a correlation between BMI and oral health in children and adolescents. Evan in our study it was found a low correlation to the limit of statistical significance between BMI and PI, there are other results that showed a bad attitude of obese/overweight children and teenagers towards oral hygiene (33).Our study showed significant correlation between gingival inflammation and BMI for male patients, possibly due to a combination of factors: not only to the inappropriate attitude toward dental hygiene but to the metabolic and inflammatory changes (33).

If permanent teeth erupt early, there is concern that the young patient will not have the ability and responsibility to brush them correctly. Therefore, their poor oral hygiene will result in an increased frequency of carious lesions in the mixed and permanent dentition. In addition, the timing of eruption could affect the planning of orthodontic therapy. If interceptive orthodontic treatment is recommended, which may result in altered growth patterns (34), the timing of therapeutic intervention will involve a reassessment of the clinical case to consider gender and BMI (8).

CONCLUSIONS

In the group of obese children there was a greater difference between chronological age and dental age compared to overweight and normal weight children, indicating that permanent teeth are likely to erupt at a younger age among obese and overweight pediatric patients.

CONFLICTS OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest to disclose, and they received no specific funding regarding this scientific research.

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NEWS

IS THE PATIENT WITH PSORIASIFORM SKIN LESIONS SUSCEPTIBLE TO PARADOXICAL REACTION UNDER THE TNF A ADMINISTRATION?

Paradoxical reactions (PR) are immune-mediated disorders that would normally respond to the biologic therapy. The exacerbation of existing psoriasis after administration TNF-α antagonist is a well-described problem. This study analyzes, 125 patients during the therapy with a TNF antagonist. Of these, 14 patients experienced PR under TNF antagonist therapy (adalimumab, infliximab, certolizumab pegol, etanercept). Thirteen patients discontinued TNF antagonist therapy and only one patient continued the biologic therapy. The present study showed no improvement in patients who were switched to another TNF antagonist, except one patient who was switched to infliximab. In addition, this study suggests that focal infections in psoriasis patients may be a risk factor for the development of PR due to TNF antagonist administration. Our study demonstrates that there are some disease (acrodermatitis continua of Hallopeau and palmoplantar pustular psoriasis) which may predispose the patients to PR under TNF antagonist therapy. Finally, this finding has relevance for the physician to recognize these clinical problems and helps to identify the potential therapeutic targets (Miho Mori, Rie Tobita, Chizu Egusa, *et al*. Clinical background of patients with psoriasiform skin lesions due to tumor necrosis factor antagonist administration at a single center. *J Dermatol* 2021; 48(11): 1745-1753).

OPTIMIZATION OF PCR BASED ON DNA QUALITY IN ONCOLOGICAL RISK POPULATION DNA SAMPLES FROM BENIN

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OPTIMIZATION OF PCR BASED ON DNA QUALITY IN ONCOLOGICAL RISK POPULATION DNA SAMPLES FROM BENIN (Abstract): In Benin, like other countries in the sub-Saharan region, breast cancer ranks first among all cancers. **Material and methods**: A highly heterogenic collection of 120 DNA samples from breast and prostate cancer patients and matched controls was available for further study on detection of BRCA1/2 gene mutations pattern. **Results**: Initially, the PCR amplification of BRCA1 exon 5 was performed for all samples, with poor results in terms of PCR efficiency. Only about a third of the samples showed visible bands of the expected size and with no contamination. A re-evaluation of DNA samples showed high variability on DNA concentration and quality, due to different extraction methods, sample origin and preservation condition. **Conclusions**: Our study primarily illustrate that DNA extracted from different tissue by different extraction methods may be damaged but still usable, by increasing the DNA amount in the PCR reactions and adjusting the other PCR parameters. However, appropriate caution should be exercised when analyzing samples derived from different tissue sources. By appropriate optimization, heterogenous samples can be simultaneously used for PCR amplifications with single parameters.
Keywords: BRCA1, BREAST CANCER, PROSTATE CANCER, MULTIPLEX-PCR.

Breast cancer (BC) is now ranked as the most common cancer worldwide, with an incidence of 2.3 million new cases (11.7%) and it's continuously increasing during the last decade. Breast cancer mortality rates have markedly increased across Africa where estimated age-standardized rates in 2020 ranged from 20 deaths per 100,000 women across Northern Africa to 27 deaths per 100,000 women in Western Africa (1). In Benin, like other countries in the sub-Saharan-region, breast cancer ranks first among all cancers of all sexes. Its incidence is constantly increasing with a

high mortality rate (2).

Knowledge of genetic factors contributing to BC risk has important implications for patients and their families both for genetic counseling and for potential access to new therapies, which improve survival and reduce side effects. BRCA1 (3) and BRCA2 (4) are major cancer predisposition genes, associated with a greatly increased risk for breast cancer development, with risk estimates ranging from 59-87% for BRCA1 and from 38-80% for BRCA2 mutations (5). Screening for mutations in these two genes is now standard practice for Hereditary Breast and Ovarian Cancer Syndrome (HBOC) cases in Western Europe and North America and allows early detection and efficient medical follow up and treatment (6, 7, 8, 9). However, in sub-Saharan Africa the exact burden of cancer mutations remains largely unknown and understudied. A few reports on BRCA1 and BRCA2 mutations and sequence variations unique to Africans has been reported: the presence of 3 common BRCA1 mutations: 185delAG in exon 2, 4184del4 in exon 11, and 5382insC in exon 5 was reported in black South African women with breast cancer; 2 novel BRCA1 truncating mutations, Q1090X and 1742insG in Nigerian breast cancer patients (10); four BRCA1 missense variations; one BRCA2 truncating mutation, 3034del4, previously unreported in anyone of African descent; and 20 no truncating variants were detected in BRCA2 (11). However, no study was conducted in Benin, so to start some genetical screening on Beninese breast cancer patients is very important in order to address these knowledge gaps.

Through to technological advances, NGS, Sanger and others molecular screening techniques are now available, there are still expensive and practically hard to use in low economical developed countries. For this reason, the polymerase chain reaction (PCR) technique remains the basis of molecular analysis in these countries.

To overcome the inherent disadvantage of cost and to improve the diagnostic capacity an allele specific multiplex PCR method for detection of BRCA1 5382insC mutation has been developed by Pak Cheung R and *et al.* (12). Early studies highlighted the obstacles that can jeopardize the production of sensitive and specific multiplex assays. Although more recent studies have provided systematic protocols and technical improvements for simple test design the method still need optimization to enhance sensitivity and specificity and to facilitate the application of multiplex PCR for mutation detection in specific conditions.

The objective of this paper was the optimization of the PCR amplification process using a heterogeneous DNA collection from different tissue sources of breast and prostate cancer (PC) patients and controls from Benin. BRCA1 Exon 5 amplification is simple and relatively rapid method for DNA quality testing. This assay was used for a bigger study to investigate the presence of BRCA1 5382insC and other common worldwide mutations in the Beninese population with cancer risk.

MATERIAL AND METHODS

A highly heterogenic collection of 120 DNA samples provided by University of Abomey Calavi, Benin and represent consecutive series of breast cancer (BC) patients (tissue and blood n=38) prostate cancer (PC) patients (blood, n=32) and their respective sex, age, and ethnicity matched controls (39 blood control for BC

patients and 11 blood control for PC) was evaluated. The micro fragments of breast cancerous tissues were collected by the oncologist surgeon by micro biopsies by the patients diagnosed with breast cancer were treated at the National University Hospital Center-HKM (CNHU-HKM) of Cotonou (Benin) in the department of Visceral Surgery and Oncology. The blood of patients with breast and prostate cancer was collected in other hospitals in Benin.

Genomic DNA was extracted from peripheral blood collected on EDTA, by 2 different methods: using the Wizard™ Genomic DNA purification kit (Promega Inc, Madison, WI, USA) and using phenol chloroform method.

Two 3 mL samples were processed in parallel, for the first method and DNA was eluted in 1 mL TE Buffer.

The phenol-chloroform method was used both to extract DNA from microtissue and blood cells. Lysis buffer containing proteinase K (20 mg/mL) and RNAse was added to the sample to disaggregate the cell membrane. Then, an equal volume of chloroform was added to the collected upper aqueous phase, followed by another centrifugation (10,000 rpm at 4°C for 10 min). The supernatant was collected in a new Eppendorf tube and the DNA was extracted as a pellet after addition of ice-cold 96% ethanol and incubation at -20°C for 4h (13).

DNA amount and quality was estimated by spectrophotometry, using Biochrom WPA Biowave II UV/Visible Spectrophotometer.

Exon 5 of BRCA1 gene was amplified in 20 µL final volumes, starting from 50 ng of gDNA. The amplification mixture included 1 × PCR buffer, forward and reverse primers, dNTPs, and 2 U of *Taq* DNA polymerase. Thermal profile used for amplification was a first denaturation step at 94°C for 5 minutes, followed by 35 cycles: denaturation at 94°C for 20 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for one minute. Final extension was accomplished at 72°C for 7 minutes.

Allele-specific multiplex-PCR was performed for detection of 5382insC BRCA1 mutation, using the 3-primers multiplex-PCR as reference method (14, 15). PCR was finally performed in a final volume of 20 µL, using 50 ng gDNA and containing 0.5 units of GoTaq Polymerase (Promega) in appropriate 1X no magnesium containing buffer, 3.25 mM MgCl2 (2.6 µL used from a 25 mM stock solution), 0.2 µM of each primer (0.4 µL of 0.01 mM solution for common reverse, wild-type specific forward and mutant-specific forward), 0.2 mM each dNTP (0.4 µL of a pre-mixed dNTP solution with 10 mM each), and nuclease-free water to final volume of 20 µL.

The amplifying program was optimized alternatively on thermocyclers as Mastercycler™ Gradient (Eppendorf AG, Hamburg, Germany) (12). Each PCR reaction consisted of an initial denaturation of 10 minutes at 95°C, followed by 35 cycles of 15 seconds of denaturation at 94°C, 15 seconds of annealing at 57°C, and 30 seconds of extension (with an increment of 1 second for each subsequent cycle) at 72°C, and a final extension step of 5 minutes at 72°C using GeneAmp® 9700 PCR System, Applied Biosystems.

Amplification aliquots were migrated, at 5 V/cm on 2% or 3% agarose gels containing 0.5 µg ethidium bromide for 1 mL gel solution. Following electrophoresis, gels were visualized under UV and photographed using the DigiGenius gel documentation system, Syngene.

RESULTS

Initially, the PCR amplification of BRCA1 exon 5 was performed for all samples using the same DNA amount, based on the provided concentration information. The required assay DNA input amount was 50ug. Unfortunately, the results of this first attempt were far from satisfactory. We obtained for all the 120 samples analyzed only 64 (53%) visible bands but just 42 (35%) of the expected size and without contaminations. The best results was obtained for BC controls extracted from blood tissue, 69% of which has visible bands, and the worst was for the PC patients DNA extracted from blood tissue, where only 31% of samples shown acceptable amplified bands.

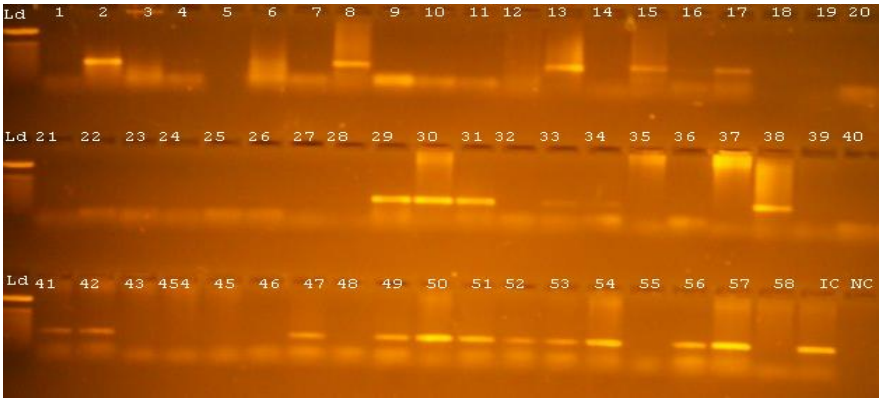

Fig. 1. PCR analysis of BRCA1 exon 5 regions 1.5% agarose gel electrophoresis of the PCR products to verify the correct amplification. Ld: molecular weight marker. IC: internal control DNA- a sample with known 50ng amount in reaction mixture. NC: negative control. Each lane corresponds to the amplicon relative to the DNA extracted from different sample

In attempt to elucidate the cause of such poor PCR amplification results we decide to remeasure the concentration and the quality of DNA using the spectrophotometer and the optical density (OD) ratio at 260 nm / 280 nm. We got a highly variated pattern of value ranged from very low concentration and DNA quality to pretty high value (tab. I). Only about half of them was more than 50 ng/μL concentration and with 260 nm / 280 nm ratio ranged between 1.8 and 2.

As we were unable to have the DNA at the recommended input concentration for a high proportion sample, we increased the input DNA volume. Samples was processed with a series of input DNA amounts from the recommended 1μL of DNA for samples that could be diluted to 50ng/μL to 2 or even 3 μL of DNA for these lower than 50ng. This variable volume was used for more than 50% of our sample set. In accordance to the new DNA concentration value we adjust the DNA amount added to reaction mix to be 50 ng - the same for the all samples. This time we obtained more uniform results, 82% of the samples has exon 5 amplified, but the bands intensity was very low for most amplicons (fig. 2).

TABLE I.
Summary of BRCA1 exon 5 amplification results after initial analysis and on remeasured samples

Samples	Sample number	DNA concentration ng/µL	R260/280 ratio	Before remeasuring		After remeasuring	
				Average efficiency amplification	Good efficiency amplification	Average efficiency amplification	Good efficiency amplification
BC blood	38	18-452	1.142-1.889	21	14	31	26
BC control	39	18-162	1.467-1.938	27	19	34	31
PC blood	32	14-430	1.309-1.998	10	6	27	22
PC control	11	15-501	1.556-1.882	6	3	7	6
Total	120	14-501	1.142-1.998	64	42	99	85

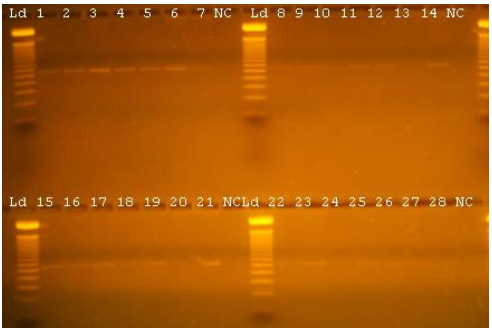

Fig. 2. PCR analysis of BRCA1 exon 5 regions after remeasuring of the DNA samples. 1.5 % agarose gel, Ld: molecular weight marker. IC: internal control DNA- a sample with known 50ng amount in reaction mixture. NC: non-template control. Each lane corresponds to the amplicon relative to the DNA extracted from different sample

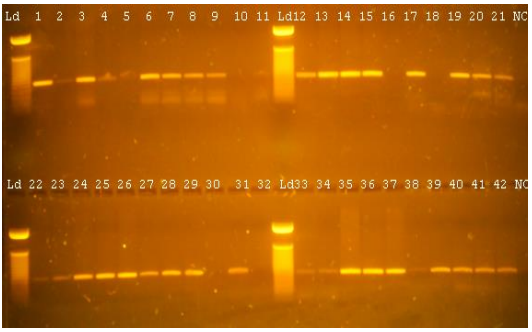

Fig. 3. PCR analysis of BRCA1 exon 5 regions with increased amount of DNA from 50 to 100ng per reaction mixture. 1.5 % agarose gel, Ld: molecular weight marker. IC: internal control DNA- a sample with known 100 ng amount. NC: non-template control. Each lane corresponds to the amplicon relative to the DNA extracted from different sample

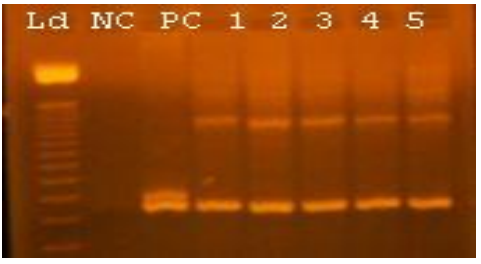

Fig. 4. Electrophoretic profiles of multiplex-PCR products issued from a 20 µL amplification volume, in 3% agarose gel Ld: DNA 50 bp step ladder; NC: No template control; PC: Positive 5382insC DNA control amplification; Lanes 1-5: BC patients DNA amplifications

We decided to increase the DNA impute amount from 50 to 100 ng per reaction mix and the band intensity increased to a satisfactory level (fig. 3). At least for breast cancer samples more than 90% of amplicons had bands with expected size, good intensity and without contaminations. We repeated the PCR amplification for the samples without clear bands to ensure that all working errors were excluded. The percent of samples with satisfactory bands increase from 90 to 95%.

Once all the amplicons covering the exon 5 BRCA1 were obtained, we chose a 3 primers multiplex-PCR method previously described (fig. 4**)** to detect the BRCA1 exon5 5382insC mutation on several BC samples from our collection. Method require a good quality of DNA samples so it would be a good way to confirm the validity of our results and the good start for further experiments on the DNA collection. The results have shown a simultaneous amplification of the control and the wild-type specific products, generating in this way a functional multiplex-PCR for several BC DNA samples and confirmed the good quality of provided samples.

DISCUSSION

Most of the methods for mutation analysis currently available rely on polymerase chain reaction (PCR) amplification of the target DNA. Unfortunately, PCR is more difficult with DNA of less-than-optimal quality and concentration. Low amounts of DNA samples are associated with decreased PCR yields and a progressive inability to amplify longer templates (16). Beside concentration the relative difficulty of producing PCR products is often related to DNA due to damage both from extraction process or preservation. Damage at even a single base can block polymerase elongation and destroy the template. However, DNA is a complex molecule, and it has been difficult to distingue between quantity and quality when it is about the PCR efficiency, especially because damage is likely to be randomly distributed or different between templates (17). Although to a lesser extent, the impact of other PCR components such as salt, dNTPs, oligonucleotide primers, as well as the effect of regionally stable secondary structures of DNA template on the productivity of PCR amplification have also been studied (18).

In theory, a single copy of DNA or a single cell is sufficient for amplification by PCR under ideal conditions. In practice, however, amplification efficiency of a specific template amount is highly dependent upon reaction components and parameters, as well as sensitivity of the DNA polymerase. In the present study, we show that very low concentration of DNA template in PCR mix less than 30ng can lead to low DNA template concentration-dependent production. Benita and co-workers (19) in an extensive study of factors influencing PCR success demonstrated that the template rather than the primer was most often the cause of PCR failure.

CONCLUSIONS

We concluded primarily that DNA extracted from different tissue by different extraction methods may be damaged but still readable by increasing the DNA amount in the PCR mix and adjust the other PCR components. However, appropriate caution should be exercised when analyzing small numbers of templates or DNA

derived from different tissue sources. These conclusions could improve our ability to provide PCR conditions equally favorable for amplification of all templates in complex samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The author Sara Y. Houngues received the *Eugen IONESCU* scholarship from the Romanian State, the Agence Universitaire Francophone (AUF) Notification no. 9006/20.04.2022.

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NEWS

EVALUATION ON THE CORROSION RESISTANCE, ANTIBACTERIAL PROPERTY AND OSTEOGENIC ACTIVITY OF A NEW BIODEGRADABLE MG ALLOYS

the last decade, being seen as a viable alternative to osteofixation systems with biomaterials like Co-Cr, stainless steels and titanium in view of their use in the dental, alveolar and maxillofacial field. The orientation towards the third category of biomaterials, which also includes biodegradable Mg-based materials, is given by the fact that no new surgery is required to remove osteofixation systems, they have good biocompatibility, with mechanical properties and the modulus of elasticity similar to cortical bone, high capacity for osseointegration and could have the corrosion rate close to the healing rate of bone tissue. The rapid degradation of magnesium (Mg)-based implants in physiological environment limits its clinical applications, and alloying treatment is an effective way to regulate the degradation rate of Mg-based materials. In this work we aimed to obtain a new alloy based on Mg-Ca-Zn-Ag for which we performed *in vitro* and *in vivo* tests. The results showed that after alloying Mg with Ca the Mg-based alloy has a corrosion rate compared to the bone recovery period and after addition of Zn and Ag, the antibacterial potential of Mg alloys was also enhanced. After implantation in a rat femoral showed significantly osteogenic activity and bone substitution rate due to its higher corrosion resistance as well as the stimulatory effects of the released metallic ions. The current work not only evaluated the clinical potential of the biodegradable Mg alloys as bone grafts but also provided a feasible approach for predicting the in vivo degradation behavior of biodegradable materials. (Hewei C, Yuan B, Zhao R, *et al*. Evaluation on the corrosion resistance, antibacterial property and osteogenic activity of biodegradable Mg-Ca and Mg-Ca-Zn-Ag alloys *Journal of Magnesium and Alloys* / doi.org/10.1016/j.jma.2021.05.013).

110 YEARS SINCE THE BIRTH OF CONSTANTIN ARSENI – THE FOUNDER OF ROMANIAN NEUROSURGERY

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110 YEARS SINCE THE BIRTH OF CONSTANTIN ARSENI–THE FOUNDER OF ROMANIAN NEUROSURGERY (Abstract): The history of Romanian neurosurgery and the evolution of ideas in this field are inextricably linked to the evolution of the brain chronography process in its complexity. This process, which has shown an uneven but ascending evolution, in terms of morphology, function and their implications in medical practice, in the spiritual and material life of man, continues today. Neurosurgery is the frontier specialty with Neurology, Ophthalmology, Otolaryngology and Maxillofacial surgery, which has managed to snatch from each of these chapters of pathology, exploration methods and surgical approach, creating its own methods of exploration and diagnosis, technique and specific operator instrumentation, which led to the formation of his own personality. The authors will present one of the great personalities of Romanian neurosurgery, Dr. Constantin Arseni, the descendant of Prof. Dr. Dumitru Bagdasar, who founded it in Bucharest, creating the largest university center of neurosurgery in Europe at that time (1975). **Keywords:** HISTORY, NEUROSURGERY, FOUNDER, DUMITRU BAGDASAR, CONSTANTIN ARSENI.

In Romania, Neurosurgery developed as a separate specialty starting in 1932. Until then neurosurgical activity was sporadically, represented by operations on the nervous system performed by the great surgeons of the time in Romania. Some of the operations were, however, outstanding for a period in which anesthesia and resuscitation techniques in the postoperative phase were insufficient, and rehabilitation hardly known. In 1895, Dr. Bardescu was the first in the world to perform a resection of Gasser's ganglion for trigeminal neuralgia, operation he also repeated in 1902.

Subsequently, other Romanian surgeons – Toma Ionescu, Amza Jianu, Iancu Jianu, Iacob Iacobovici – carried out surgery for the removal of brain tumors, and Leonte operated on the spinal cord for tumoral compression or applied antero-lateral cor-

dotomy in cases of refractomy pains. It is certain that these Romanian surgeons, preoccupied by the management of nervous lesions, were stimulated by their cases, imposed the Romanian School of Neurology at International level. The anatomical, physiologic and clinical investigations of Gheorghe Marinescu, Minea, Noica, Radovici, Paulian, Danielopolu, I. T. Niculescu and others represented this stimulus promoting ideas and therapeutical methods diseases of the brain and spinal cord.

In Iasi, Prof. Dr. Alexandru Moruzzi (1900-1957) is a key Romanian Medical Personality who was formed by the French School of Medicine. After completing his studied in Paris under the first Neurosurgeon Thierry de Martel (1875-1940), he worked in Romania as a General Surgeon in Prof. Amza Jianu's Clinic at Charity Hospital in Iasi. In 1933, with help of Prof. Leon Ballif (1892-1967) Prof. Moruzzi creates the first department of neurosurgery in Romania at Socola Psychiatry Hospital in Iasi (the third psychiatry hospital in Europe after Stockholm and St. Petersburg).

If we talk about the Bucharest Universitary Center, Prof. Dr. Dumitru Bagdasar (1893-1946) is considered the founder of the Romanian School of Neurosurgery. He is direct student at Prof. Harvey Cushing School of Neurosurgery (1926-1931). His activity in Boston was greatly appreciated by his mentor, being regarded as Cushing's Honorary Assistant.

In 1929, after returning from the United States of America and considering Romania did not yet recognize neurosurgery as an independent surgical specialty, he was employed as a neurologist by Jimbolia Neuropsychiatric Hospital. In 1931 is promoted to chief of Neurology Department at Cernauti Hospital. From 1929 to 1934 he performed over 200 surgical inventions on neurosurgical pathologies. After numerous pressures, in 1935 he succeeds in establishing the first independent neurosurgical department in Bucharest Central Hospital of Nervous Disease – the second as a national scale, after Socola Psychiatry Hospital in Iasi. After his death, Prof. Constantin Arseni continued the devoted activity of Prof. Bagdasar, being considered as the founder of the Romanian School of Neurosurgery (1, 2).

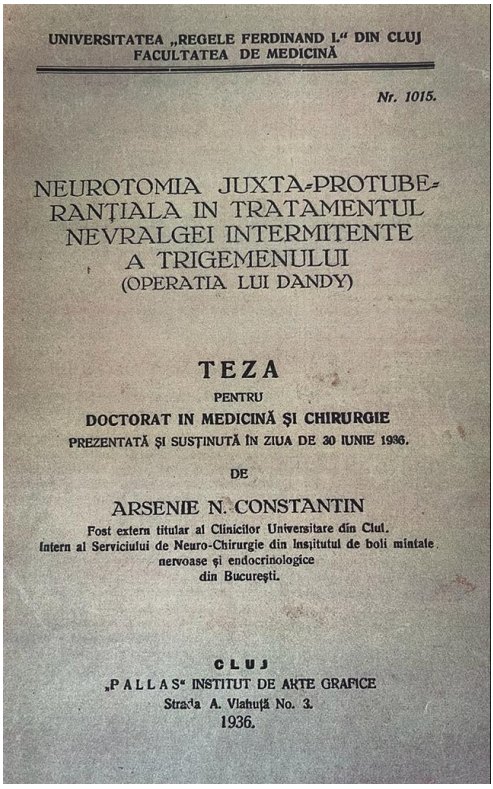
EARLY LIFE AND EDUCATION

Dr. Constantin Arseni was born in Dolhasca Commune, Suceava County, on February 3rd, 1912 (fig. 1). He attended primary school in Dorohoi. After graduating from Bacau High School, he enrolled at the Faculty of Medicine and Pharmacy in Cluj-Napoca, where he distinguished himself as an eminent student and graduated in 1935 as head of promotion. As a student, he had the chance to hear elite professors of Romanian medicine: Prof. Iacob Iacobovici (1879-1959) - General Surgery, Prof. Iuliu Hatieganu (1885-1959) - Internal Medicine, Prof. Victor Papilian (1888-1956) - Anatomy, Prof. Grigoriu Cristea (1883-1951) - Obstetrics-Gynecology.

Fig. 1. Prof. Dr. Constantin Arseni (1912-1994)

On June 30th, 1936, he presented his doctoral thesis entitled *"Juxtaprotuberential Neuroanatomy in the treatment of Trigemen's Neuralgia* being a premiere in Romania, as until then no theses with Neurosurgical titles had been approached, the subject arousing the attention of great professors (fig. 2).

Dedicating his life to medicine, the young Constantin Arseni quickly promoted and entered medical education as a University Assistant at the Department of Pathological Anatomy, led by Professor I. T. Niculescu (1895-1957) (3).


UNIVERSITATEA „REGELE FERDINAND I." DIN CLUJ
FACULTATEA DE MEDICINĂ

Nr. 1015.

NEUROTOMIA JUXTA-PROTUBERANȚIALA IN TRATAMENTUL NEVRALGEI INTERMITENTE A TRIGEMENULUI
(OPERATIA LUI DANDY)

TEZA
PENTRU
DOCTORAT IN MEDICINĂ ȘI CHIRURGIE
PREZENTATĂ ȘI SUSȚINUTĂ ÎN ZIUA DE 30 IUNIE 1936.
DE

ARSENIE N. CONSTANTIN
Fost extern titular al Clinicilor Universitare din Clui.
Intern al Serviciului de Neuro-Chirurgie din Insțitutul de boli mintale nervoase și endocrinologice
din București.

CLUJ
„PALLAS" INSTITUT DE ARTE GRAFICE
Strada A. Vlahuță No. 3.
1936.

Fig. 2. Doctoral Thesis of Dr. Constantin Arseni entitled *"Juxtaprotuberential Neuroanatomy in the treatment of Trigemen's Neuralgia" (Dandy's Method),* Cluj-Napoca, 1936

A NEW LIFE AS A NEUROSURGEON

Pursuing a career in the operating room, he worked for three years at the Emergency Hospital in Bucharest, where he sharpened his talent as a surgeon, after which he will work with Prof. Dr. Dumitru Bagdasar, helping to raise the foundations of Romanian Neurosurgery (fig. 3).

Fig. 3. Prof. Dr. Dumitru Bagdasar (1893-1946)

After the tragic loss of his mentor Prof. Dr. Dumitru Bagdasar in 1946, Dr. Constantin Arseni, a 34-year-old primary care physician, took over the management of the neurosurgery service at the Central Hospital for Mental, Nervous and Endocrinologic Diseases, the only department of this kind in this country. After taking up duty in 1946, in the Department with 80 beds and after strenuous endeavors, Constantin Arseni was able to have the first Clinic of Neurosurgery from Romania (4).

He operated in difficult conditions, together with Dr. Ionescu Ion, Dr. Sofia Ionescu-Ogrezeanu with carpeted gloves,

reduced medical staff and emergency avalanches (fig. 4).

He had at his disposal a single Boovie electrocoagulation device brought by Prof. Dr. Dumitru Bagdasar from the United States of America. The radiological examination was performed in the radiology service located at a distance from the clinic, the patients being transported at that time with a stretcher.

The need to practice new techniques involved conducting numerous studies in the autopsy room. Following a totally unpleasant incident, Dr. Constantin Arseni contracted sepsis and was extremely rescued by the risky penicillin obtained from the American Embassy.

The multitude and difficulty of the cases, the small number of collaborators (Dr. I. Ionescu, Dr. V. Miclaus, Dr. T. Iacob and Dr. S. Ionescu) imposed a spartan work schedule.

Dr. Constantin Arseni was in the hospital at 5 o'clock in the morning, even on Saturdays, to deal with this program. The examination of the patients and the operating program ended in the afternoon, but Dr. Arseni continued his activity until late in the evening (consultations, follow-up of operated patients, scientific activity) (5).

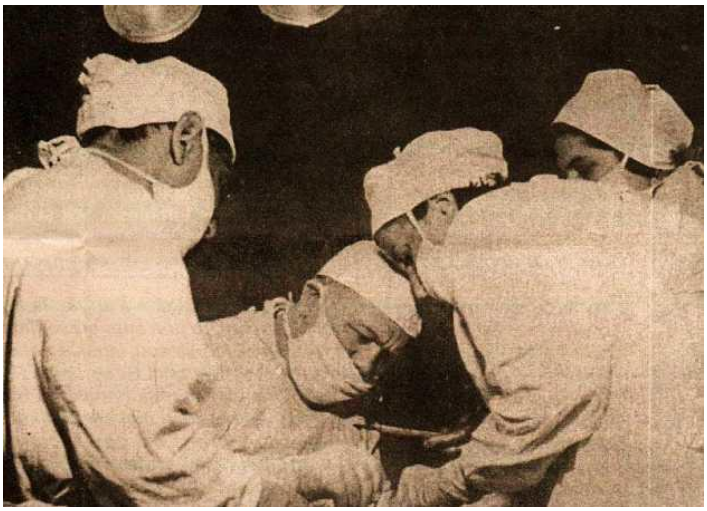


Fig. 4. Prof. Dr. Constantin Arseni and Dr. Sofia Ionescu in the surgery theatre

"FATHER" OF ROMANIAN NEUROSURGERY

For his merits, Dr. Constantin Arseni, became an Associate Professor in 1952 and University Professor of "Carol Davila" University of Medicine and Pharmacy in 1963.

As a result of this activity, the prestige of the clinic increases with the confidence in this specialty, the patients no longer being directed in outdated stages. Under these conditions, through the intervention of Professor Constantin Arseni, the hospitalization capacity reaches 220 beds, often being exceeded.

The Clinic of Neurosurgery of Bucharest developed rapidly, approaching the most varied aspect of neurosurgical pathology. The increased number of subjects with which the Clinic was concerned ne-

cessitated a constant effort of organization, technical development and research work. In 1964 the number of beds had increased to 220, distributed in the various sections strictly according to the specialties: two surgical sections for cerebral expanding processes, one each for vertebro-medullary surgery, infantile neurosurgery, cerebro-vascular surgery, epilepsy and dyskinesia, head and spinal injuries, with a subsection of general traumatology. There are two operative blocks with 7 operation theaters. In 1960 a special anesthesia and intensive care unit was opened and equipped according to the most modern requirements.

Paraclinical activity and scientific research was carried out at that time (1964) by a laboratory of electroencephalography with 4 modern apparatus, a laboratory of electro-myography, of neuromorphopathology and biochemistry.

The neuroradiology department developed both in the direction of standard investigations and complex research work with contrast media: angiography, ventriculography, iodoventriculography, air and iodine ventriculography. The surgery of epilepsy may be applied owing to a stereotaxic surgical equipment.

Under Professor C. Arseni, the Neurosurgical Clinic of Bucharest is transformed in 1975 into the "Prof. Dr. D. Bagdasar" Clinical Hospital of Neurosurgery, the largest hospital of Neurosurgery in the Europe, with 550 beds and, subsequently, 650 beds (fig. 5).

It appears as a crowing and a confirmation of the value of Neurosurgery withing the framework of medical specialties (5).

This passion for the study of the morpho pathology of the central nervous system materialized in the realization of an important collection of Anatomo-Pathological pieces specific to various intracranial expansive processes and especially of brain tumors, whose biology has preoccupied him all his life (fig. 6).

Fig. 5. Neurosurgical Clinic "Prof. D. Bagdasar" opened in 1975 with all the facilities.

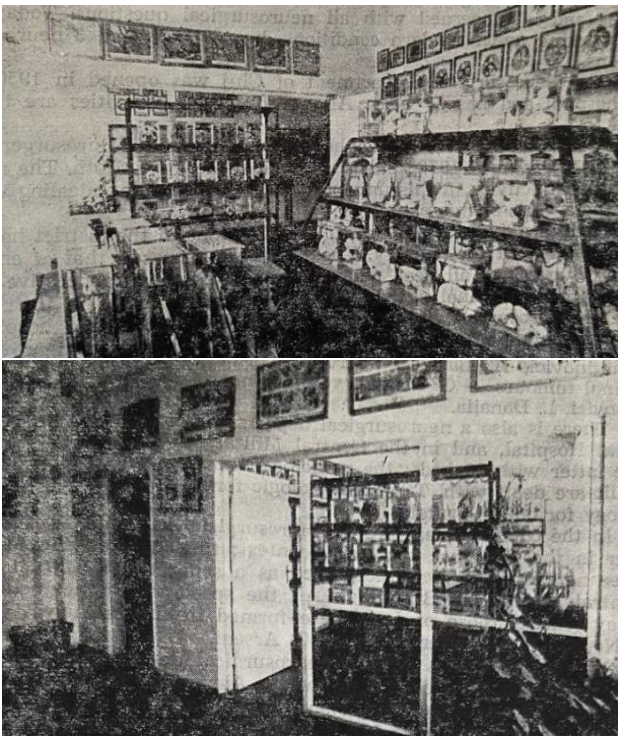


Fig. 6. Museum of Anatomo-Pathological nervous system pieces

SCIENTIFIC ACTIVITY DURING NEUROSURGERY

Scientific activity of Prof. Dr. Constantin Arseni includes the numerous publications, especially those of the cases of Clinic of Neurosurgery from Bucharest. These articles were designed to inform the medical staff in general concerning neurosurgical diseases and to obtain early and competent diagnosis. Among the most interesting publications, some of which have become classical are: "Vertebral sciatica" (1948), "Vascular Diseases of The Brain and Spinal Cord" (1965), "Neuro-ophthalmologic Diagnosis" (1967), "Raised Intracranial Pressure" (1972), "Pathologic Anatomy of Tumors of the Central Nervous System" (1978), "Cerebrospinal Fluid" (1979).

In the domain of pediatric neurosurgery, a self-standing specialty since 1960, the following reference publications were published: "Problems of Neurosurgical Diagnosis in Infantile Pathology" (1978), "Neurosurgical Diseases in Infants and Young Children" (1979), "Infantile Neurosurgical Pathology" (1980) by the cooperation of the teams under C. Arseni, Lenke Horvarth and A. V. Ciurea, on the basis of their experience gained in the Department of Pediatric Neurosurgery.

With the contributions of the entire Clinic of Neurosurgery in the various spheres of this specialty 19 monographs were edited under Prof. Dr. C. Arseni.

An editorial board under C. Arseni published, with the substantial contribution of the medical staff of the Clinic, the "Treatise of Neurology" in 8 volumes, during 1979-1981 period.

The great number of publications are

due not only the constant, assiduous work of the medical staff but also to the numerous up-to-date books (about 7,000) and journals (218 titles) in the library of the Clinic (fig. 7).

Similar, the archives, listed in alphabetical order of subjects are the files of the case cards (150,000) since 1935 and up to date.

The Neurosurgical departments throughout the country have a vast scientific activity, with numerous communications and papers published at home and abroad. The more than 350 specialty publications of Prof. Constantin Arseni are known throughout the world and represent the references on subjects such as tumors on the brain, tuberculomas, parasitic diseases of the nervous system, abscesses of the brain, epilepsy and craniocerebral neuro-traumatology(6).

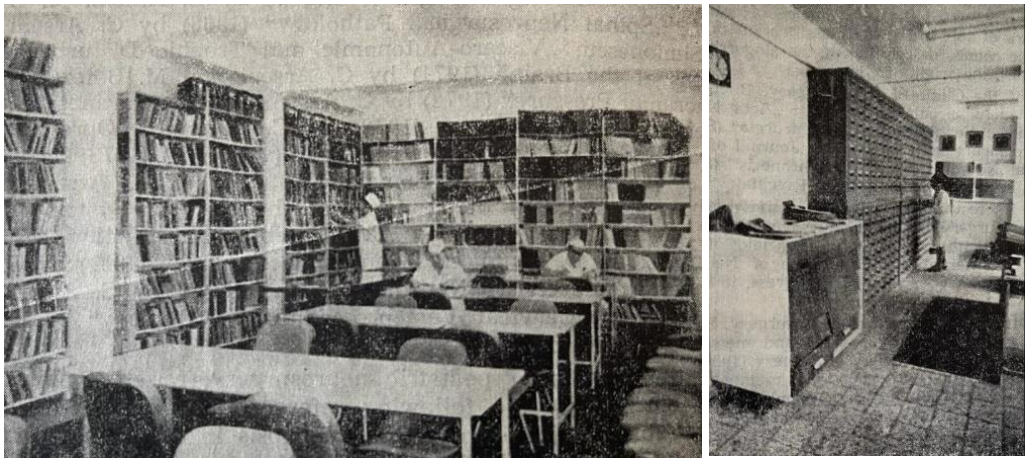


Fig. 7. The Library and the archives files of "Prof. Dr. Bagdasar" Clinical Hospital

RECOGNITION

For his activity in the domain of neurosurgery, Professor C. Arseni has been awarded with numerous titles and membership: Member of International Society of Surgery (1956), Member of the World of Federation of Neurology (1957), Honorary Member of the French Society of Neurosurgery (1959), Member of the Academy of Sciences of New York (1964), Corresponding Member of the R.S.R. Academy (1974), etc.

Along social lines, Prof. C. Arseni was distinguished with medals, orders and prizes for his outstanding merits: The "Victor Babes" prize of the R.S.R. Academy in 1972; the "Meritul Sanitar" in 1970. For his social and professional activity in the field of neurosurgery for more than 45 years, Prof. C. Arseni was elected as Honorary Member of Romanian Academy *post-mortem*.

CONCLUSIONS

Professor Constantin Arseni was a dedicated surgeon, he practiced his job asking from those around him as much as he offered - the maximum. He was a schoolmaster and a teacher in the true sense of the word. Although criticized for his didactic extremism and the pretensions he raises from the aspirations of the title of "Neurosurgeon", Professor Constantin Arseni teaches neurosurgery "in the hard way", in an exhaustive and elitist way. Most of the

heads of the neurosurgical departments in the country are former students of his. As a sign of gratitude for the contributions made to the health of the Romanian people, C. Arseni was elected Member of the Romanian Academy in 1980 and President of the Academy of Medical Sciences in 1984.

Professor Constantin Arseni was an exemplary professional, a model of conduct, which will forever remain in our memory, of the neurosurgeons, and of the Romanian neurosurgery, leaving behind a legacy hard to match for those who followed him.

CONFLICT OF INTEREST AND FUNDING

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FACTORS THAT INFLUENCE THE DESIRE TO AVOID A NEW PREGNANCY. A SINGLE CENTER STUDY

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FACTORS THAT INFLUENCE THE DESIRE TO AVOID A NEW PREGNANCY. A SINGLE CENTER STUDY **(**Abstract). The **aim** of the study was to identify the factors that are related to the women's desire to avoid a new pregnancy. **Material and methods**: A total of 197 mothers were questioned about the pregnancy after giving birth, and the study collected data about their sexual-related lives, the satisfaction with their relationship with partners and medical team, peripartum aspects, and their desire to have a new pregnancy in the next period, using *the Desire to Avoid Pregnancy Scale* (DAP), a psychometric scale. The analysis of the data was done using the SPSS program, version 24. **Results**: The analysis of the data revealed that women declared that they were mostly satisfied with the relationships they had with their gynecologists (M = 5.46 ± 1.59), the medical care they received from the nurses (M = 5.49 ± 1.43), the hospitalization conditions (M = 6.05 ± 1.26), the hospital food (M = 5.86 ± 1.33), and the provision of medical equipment in the hospital (M = 6.04 ± 1.11). The DAP subscales revealed the following results: *Cognitive Desire and Preferences* - M = 20.26 ± 3.79; *Affective Feelings and Attitudes* - M = 10.47 ± 3.56; and *Anticipated Practical Consequences* - M = 7.93 ± 3.22 for a total DAP score of about M = 37.56 ± 10.75 with scores ranging from 13 (0.6%, N = 1) to 56 (2.3%, N = 4). Married women had a significantly lower score on the DAP scale, both compared to women who lived in cohabitation and compared to those who no longer maintained a relationship with the child's father. More results were obtained considering the variables age at first sexual contact, level of education, marital status, intended or unintended pregnancy, and satisfaction with medical care during pregnancy. **Conclusions**: There are a lot of factors that influence the desire of women to avoid a new pregnancy, and ob-gyn doctors and psychologists should take them into consideration when medical care is provided to mothers who have a pregnancy within a short time after giving birth. **Keywords**: PREGNANCY, THE DESIRE TO AVOID PREGNANCY SCALE, ROMANIA, TEENAGE PREGNANCY, SINGLE MOTHER, SATISFACTION WITH PREGNANCY, PAIN SCALE.

The birth rate for 2023 was about 17,464 births per 1,000 people, a 1.15% decline from 2022, and worldwide, fertility fell from an average of 5 births per woman in 1950 to 2.3 births per woman in 2021, with an increased rate in poor countries (1).

More than half of pregnancies are unintended, and some trends can be identified in diverse countries: high rates of pregnancies among teenagers, an increase in the pregnancy rate among women older than 40, the desire to have children after reaching educational and financial stability, children born in a single-parent family (2, 3).

There are a lot of factors that influence the desire of women to have or not have children: the family model, the image of women in the cultural group, the influence of peers, the level of education, the marital status, the medical or sexual education, the type of family, the consumption of drugs, the financial status, the involvement in risky sexual behavior (4-7). Cultural norms related to pregnancy and reproduction can be determined by a variety of values and could be addressed through education about a responsible, healthy sex life, family planning, and emphasizing sexual behavior within marriage. In some cultures, pregnancies resulting out of wedlock may be considered immoral, causing social pressure, while in others, conceiving a child may be considered a cultural responsibility. Depending on a woman's status in a culture, the decision about pregnancy or appropriate family planning can be determined (8).

The quality of sex education is one of the educational factors involved in the incidence of pregnancies. The more this education includes complex information related to notions of anatomy, physiology, sexuality, contraceptive methods, or family planning, the more the ability to make responsible decisions is increased. Education plays an important role in accessing medical services. Research shows that achieving high levels of education discourages teenage pregnancy (9).

Socio-economic factors can contribute to the incidence of pregnancy in adults and minors through the level of education. The higher this is, the greater the ability to make responsible family planning decisions. A limited or increased level of financial resources can contribute to access to medical monitoring and reproductive health services. Socio-economic factors can amplify each other, resulting in a lower or higher incidence of pregnancies depending on them (10, 11).

Additionally, the impact of religious factors on the desire to have children has significant echoes in general, but predominantly on women. The diversity of religious communities can significantly impact this aspect, and the factors that influence this decision can also be diverse. One of them referred to the specific rules of a religious doctrine regarding the onset of sexual life, sexuality, contraception, and family planning. Another aspect is the promotion of patriarchy, where women have a limited role in deciding whether to get pregnant or how many members of a family they have (12). The absence of sex education as determined by religious rules is another factor that affects the decision to get pregnant (13). The occurrence of pregnancies can also be influenced by social pressure on women and couples to have children or by restricting access to family planning and contraception services (14).

The aim of the present study was to identify the factors that are related to the desire to avoid pregnancy in women.

MATERIAL AND METHODS

Participants and data collection

The study was conducted between May 1st-15th, 2023, and the questionnaires were distributed to women hospitalized at the

"Cuza-Voda" Maternity Hospital in Iasi, Romania, after they gave birth. The participants were informed about the purpose of the study, about the fact that they could withdraw from the study whenever they wanted, without consequences, and about the confidentiality of the data. No incentive was given to respondents. The inclusion criteria were confinement after birth and filling out the questionnaire before the deadline. The exclusion criteria were questionnaires that were incomplete or submitted after the deadline. Finally, 179 questionnaires were included in the research.

The online questionnaire was created using the Google Forms application (Alphabet, Mountain View, CA, USA) and was developed to obtain detailed information about satisfaction with everything related to the act of giving birth and the desire to become pregnant.

a. The first part of the questionnaire gathered socio-demographic information (such as age, marital status, living environment, level of studies, number of children, and if they had a stable job).

b. The second part collected information about sex life and pregnancy (at what age the first sexual contact took place, how old they were when they got pregnant for the first time, if there were any miscarriages), as well as detailed information about the current pregnancy (the way they got pregnant, if the pregnancy was planned and wanted, if it was felt as difficult, the type of pregnancy, how many months of pregnancy they had when they consulted the gynecologist, and if the gynecologist counseled them during the pregnancy, the type of birth and if it was considered to be difficult, if they were assisted by a doctor during the birth, and if they were consulted by a psychologist).

c. The third part of the survey aimed at the satisfaction of women who had just given birth with regard to the relationship with their gynecologists from all points of view (communication, how to explain the medical situation, medical care, emotional support, and the time given during consultations). It also aimed at these women's satisfaction regarding different aspects related to the communication of the patients with the nurses and the midwives in the hospital, the medical care received, the conditions of hospitalization, the conditions in the delivery room, the food received, and the provision of medical equipment in the hospital. Furthermore, the participants were asked how they appreciated the attitude of the gynecologist towards them and the extent to which they believed that the gynecologist considered all the aspects presented by the patient. In this section, questions were asked about the degree of women's satisfaction regarding the relationship with the family and the child's father, but also related to the lives they led both during pregnancy and before becoming mothers for the first time.

d. The final part of the questionnaire included one psychological tool - *the Desire to Avoid Pregnancy Scale* (DAP), which is a psychometrically validated measure of pregnancy preferences that was developed in the USA in 2019 (15, 16). The DAP scale covers three conceptual domains: (1) cognitive desires and preferences; (2) affective feelings and attitudes; and (3) anticipated practical consequences. Each item uses a Likert scale, scored 0–4, to ask women how much they agreed or disagreed with a statement about either becoming pregnant in the next 3 months or having a baby in the next year. The re-

sponses were averaged, and a higher score represents a higher DAP (16). The DAP scale showed good reliability, with a Cronbach's α of 0.95 (15, 16).

Statistical analysis

The statistical analyses were performed using *IBM Statistical Package for Social Sciences* (SPSS) for Windows, *version 24* (SPSS Inc., Chicago, IL, USA). The results for descriptive statistics were expressed as means and standard deviations (SD).

The normality of the data distribution was assessed using the Kolmogorov-Smirnoff test. Given the fact that all data are not normally distributed, a bivariate analysis will be performed, and non-parametric tests will be applied. To assess comparative results considering planning and wanting a pregnancy, the way to stay pregnant, the way to give birth to the child, and performing medical tests during the pregnancy, the Mann-Whitney test was performed.

The comparative results considering marital status were assessed using the Kruskal-Wallis H test to determine if there were statistically significant differences between more than two groups of an independent variable on a continuous or ordinal dependent variable.

The Spearman correlation was used to test the relationship between variables. A p-value < 0.05 was considered statistically significant.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee No. 5035/21.04.2023 of "Cuza-Vodă" Obstetrics and Gynecology Hospital, Iasi, Romania.

RESULTS

Socio-demographic data

The women in the study were, on average, M = 28.43 ± 7.50 old (with a minimum of 13 and a maximum of 53 years old). More than half of them came from urban areas (55.5%, N = 96).

The participants of the study had a good level of education, less than half of them had completed university studies (44.5%, N = 77), and approximately one third of them had high school education (27.2%, N = 47). More than half of the women in the present study had a stable job (63.6%, N = 110).

Most of the participants were married (74.6%, N = 129), and less than half of the women had given birth to their first child (45.1%, N = 78). More than a third of the women declared that they had a second birth (33.5%, N = 58), while the rest declared that they still had two (14.5%, N = 25), three (2.3%, N = 4), four (1.7%, N = 3), five (2.3%, N = 4), or six or more children at home (0.6%, N = 1).

Information about sexual life, pregnancy, and birth

The average age at which women had their first sexual contact was M = 17.84 ± 3.27. The average age at which women became pregnant for the first time was M = 23.98 ± 5.93, the youngest mothers being 12 years old (1.2%, N = 2), and the oldest being 41 years old (0.6%, N=1). Less than a third of the women declared that they had miscarriages (27.7%, N = 48).

Regarding the current pregnancy, more than a third of the women did not plan to become pregnant (39.3%, N = 68). Most of the women in the present study got pregnant naturally (91.3%, N = 158), and a small part of them resorted to in vitro ferti-

lization (8.7%, N = 15). Additionally, most of the women had a single pregnancy (93.1%, N = 161), with a few of them having a twin pregnancy (5.8%, N = 10) or even a multiple pregnancy (1.2%, N = 2).

The number of months of pregnancy at which women consulted a gynecologist was on average M = 2.87 ± 2.25. Most of the women in this study considered that they did not have a difficult pregnancy (78.0%, N = 135); instead, almost half declared that the birth was difficult (43.9%, N = 76), approximately one third of the women (28.3%, N = 49) stating that the pain felt throughout the birth and immediately after the birth was the greatest pain they had experienced. More than half of the women were consulted by a psychologist after birth (52.0%, N = 90).

Most of the women (96.0%, N = 166) were assisted by a doctor at birth, and a little more than half of them gave birth naturally (50.9%, N = 88). On average, the Apgar score obtained by the newborns was M = 8.38 ± 1.41.

Satisfaction regarding the doctor-patient relationship, the relationship with the medical staff, and the family

More than half of the women (64.2%, N = 111) who participated in the study declared that they were treated with respect by their gynecologist. Furthermore, more than half of the patients were very satisfied with the conditions in the delivery room (53.2%, N = 92). More than a third (36.4%, N = 63) said that the gynecologist considered all the aspects presented by the patients to a large extent.

On a scale from 1 (*very dissatisfied*) to 7 (*very satisfied*), women were mostly satisfied with the relationship they had with the gynecologist, the average being M = 5.46 ± 1.59. Similarly, women were even more satisfied with the medical care they received from the midwives (M = 5.49 ± 1.43), the hospitalization conditions (M = 6.05 ± 1.26), the hospital food (M = 5.86 ± 1.33), and the provision of medical equipment in the hospital (M = 6.04 ± 1.11). Other detailed results are presented in first table.

TABLE I.

Self-rated items regarding satisfaction with the doctor-patient relationship and the relationship with the medical staff *

How satisfied are you with …	1	2	3	4	5	6	7	M ± SD
the relationship with the gynecologist in the hospital?	1 (0.6)	4 (2.3)	16 (9.2)	40 (23.1)	19 (11.0)	19 (11.0)	74 (42.8)	5.46 ± 1.59
the time the gynecologist at the hospital allotted you during the consultations?	3 (1.7)	24 (13.9)	27 (15.6)	17 (9.8)	11 (6.4)	21 (12.1)	70 (40.5)	5.03 ± 2.00
the communication with the gynecologist in the hospital?	6 (3.5)	23 (13.3)	30 (17.3)	14 (8.1)	10 (5.8)	23 (13.3)	67 (38.7)	4.94 ± 2.05
the way in which the gynecologist clearly explained the details related to the medical situation?	7 (4.0)	29 (16.8)	22 (12.7)	13 (7.5)	9 (5.2)	21 (12.1)	72 (41.6)	4.96 ± 2.13
the medical care that the gynecologist in the hospital gave?	2 (1.2)	11 (6.4)	16 (9.2)	37 (21.4)	16 (9.2)	19 (11.0)	72 (41.6)	5.31 ± 1.74
the emotional support that the gynecologist in the hospital gave you?	41 (23.7)	22 (12.7)	11 (6.4)	8 (4.6)	10 (5.8)	15 (8.7)	66 (38.2)	4.35 ± 2.54
the medical care that the nurses in the hospital gave you?	2 (1.2)	2 (1.2)	9 (5.2)	34 (19.7)	37 (21.4)	27 (15.6)	62 (35.8)	5.49 ± 1.43

How satisfied are you with …	1	2	3	4	5	6	7	M ± SD
the communication with the nurses in the hospital?	1 (0.6)	2 (1.2)	20 (11.6)	41 (23.7)	26 (15.0)	22 (12.7)	61 (35.3)	5.31 ± 1.53
the communication with the midwives in the hospital?	2 (1.2)	11 (6.4)	19 (11.0)	27 (15.6)	24 (13.9)	29 (16.8)	61 (35.3)	5.26 ± 1.69
the medical care you received during your hospitalization?	-	5 (2.9)	8 (4.6)	37 (21.4)	29 (16.8)	27 (15.6)	67 (38.7)	5.54 ± 1.44

* Number of answers (N) and percentage (%), means and standard deviations (M±SD)

Regarding the relationships with family members, around half of the participants declared that they were very satisfied, both with the relationship they had with the children's fathers (52.0%, N = 90), and with the relationships with the families (48.6%, N = 84), the average being M = 5.93 ± 142, respectively M = 5.80 ± 1.52. In terms of their lives before becoming mothers for the first time (M = 5.79 ± 1.44) and during pregnancy (M = 5.66 ± 3.38), women's satisfaction with life as a woman was slightly lower.

Desire to Avoid Pregnancy Scale (DAP)

The Cronbach Alpha score was 0.93 for the total scale, 0.87 for Domain 1 – Cognitive Desire and Preferences, 0.86 for Domain 2 - Affective Feelings and Attitudes and 0.74 for Domain 3 - Anticipated Practical Consequences.

For these DAP subscales, we obtained the following results: *Cognitive Desire and Preferences* - M = 20.26 ± 3.79, *Affective Feelings and Attitudes* - M = 10.47 ± 3.56 and *Anticipated Practical Consequences* - M = 7.93 ± 3.22.

The total DAP score was on average M = 37.56 ± 10.75, scores ranging from 13 (0.6%, N = 1) to 56 (2.3%, N = 4).

Comparative and correlational analysis

A Mann-Whitney U test was run to determine if there were differences in the DAP total score between women who planned the pregnancy and wanted to be pregnant and those who didn't. The DAP total score was statistically significantly higher in women who did not want the pregnancy (Mdn = 42.00) than in women who wanted to be pregnant (Mdn = 32.00), ($p < 0.01$, U = 829.00, z = - 4.080).

The comparative analysis also showed that there were differences in the DAP score, in the variable that refers to the way that women got pregnant ($p = 0.043$, U = 195.00, z = -2.028), in the sense that women who got pregnant naturally had a higher score on this scale (Mdn = 38.00) than women who got pregnant through in vitro fertilization (Mdn = 27.00).

The results showed that there was a significant difference regarding the score obtained on the *Desire to Avoid Pregnancy Scale* and the way in which the women gave birth to their babies ($p = 0.008$, U = 996.50, z = -2.657), in the sense that those who had a natural birth had a higher score on this scale (Mdn = 40.50), compared to those who underwent a caesarean section (Mdn = 33.50).

The results of the Mann-Whitney test ($p = 0.001$, U = 622.50, z = -3.462) showed that there was a significant difference in the total score on the DAP scale and the item that investigated whether women had medical investigations during pregnancy. Thus, the total DAP scores were significantly lower in women who had these analyses (Mdn = 35.00), compared to women

who did not have medical investigations during pregnancy (Mdn = 43.00). Additionally, the results of the Mann-Whitney test showed that there was a significant difference in terms of the total DAP score for the variable that measures whether the women were advised by a gynecologist during pregnancy (p = 0.001, U = 259.00, z = -3.355). Thus, women who were not followed or advised by any doctor during pregnancy had a significantly higher score on the DAP scale (Mdn = 51.50), compared to those who were advised by a gynecologist, regardless of whether this was a woman (Mdn = 35.00) or a man (Mdn = 35.00).

A Mann-Whitney U test was run to determine if there were differences in the DAP total score between women who felt different degrees of pain during pregnancy. Thus, the results (p = 0.042, U = 5.00, z = -2.030) showed that those who felt a little pain during pregnancy obtained a significantly lower score on the DAP scale (Mdn = 24.00), compared to those who felt the highest degree of pain (Mdn = 42.00).

There were significant differences on the DAP scale among women depending on how concerned they were with their health and the fetus status during pregnancy (p = 0.001, U = 622.50, z = -3.462). Thus, women who had had medical investigations during pregnancy had a significantly lower score on the DAP scale (Mdn = 35.00), compared to those who had not (Mdn = 43.00).

A Kruskal-Wallis H test was conducted to determine if there were differences in total DAP scores between the type of relationship that mothers had with the child's father: the "married" (n = 76), "in cohabitation" (n = 29), and "I am no longer in a relationship with the child's father" (n = 5) groups. The mean ranks of the DAP scores were statistically significantly different between groups, $\chi 2(2) = 21.015$, $p < 0.001$. A post hoc Mann-Whitney U test was run to determine which groups were different from which other groups. This post hoc analysis revealed statistically significant differences in the DAP scores between married women (Mdn = 33.50) and women who were cohabiting with the child's father (Mdn = 43.00) (p = 0.001, U = 626.00, z = -3.414); married women (Mdn = 33.50) and mothers that were no longer in a relationship with the child's father (Mdn = 56.00) (p = 0.001, U = 20.50, z = -3.329).

Similarly, the median DAP score for women who were cohabiting with the child's father (Mdn = 43.00) and women who were no longer in a relationship with the child's father (Mdn = 56.00) (p = 0.016, U = 23.00, z = -2.417) was statistically significantly different. In conclusion, married women had a significantly lower score on the DAP scale, both compared to women who lived in cohabitation and to those who no longer maintained a relationship with the child's father.

The Spearman correlation analysis found significant correlations between the age at which women had their first sexual contact and the age at which they had their first pregnancy (r = 0.658, p < 0.001), in the sense that the older the women were when they had their first sexual contact, the later in life their first pregnancy would appear. Our results showed that there was a negative correlation between the total score of the DAP and the age at which women became pregnant for the first time (r = -0.198, p = 0.038), in the sense that the higher the age at which women became mothers for the first time, the lower the score on the DAP scale.

The level of education influences various aspects of sexual life. Thus, the results indicated a significantly positive correlation between the level of education and the age at which women had their first sexual contact ($r = 0.676$, $p < 0.001$), in the sense that the more educated the women were and the more they studied, the older they were when they started a sexual relationship for the first time. Moreover, the level of education correlates with the age at which women became pregnant for the first time, in the sense that the more educated the women were, the older they became pregnant ($r = 0.753$, $p < 0.001$).

A strong positive correlation was identified between the item that measures the degree of satisfaction women had with life during pregnancy and the Apgar score of the child at birth, in the sense that the more satisfied the women were with the way they lived during pregnancy, the higher the child's Apgar score ($r = 0.202$, $p = 0.008$).

The results indicated that the number of children correlated positively with the relationship women had with their families ($r = 0.150$, $p = 0.048$), in the sense that the more satisfied they were with their family relationships, the more children the women had.

At the same time, the number of children correlated negatively with the pain experienced during childbirth, in the sense that the more children the women had, the less pain they experienced during childbirth ($r = -0.164$, $p = 0.031$). In addition, the level of pain felt during childbirth correlated negatively with the age at which women became pregnant ($r = -0.357$, $p< 0.001$), in the sense that the older the women were when they became pregnant, the less pain they felt during childbirth.

Our results showed that there was a negative correlation between the total score of the DAP and the age at which women became pregnant for the first time ($r = -0.198$, $p = 0.038$), in the sense that the higher the age at which women became mothers for the first time, the lower the score on the DAP scale. Similarly, the mother's satisfaction related to the relationship with the child's father, respectively with the gynecologist were strongly negatively correlated with the DAP score, so the more the mothers were satisfied with both the relationships with the child's father ($r = -0.249$, $p = 0.009$) and the gynecologist ($r = -0.269$, $p = 0.005$), the lower the score on the DAP scale.

A strong negative correlation was identified between the total score of DAP and women's satisfaction with the lives they had before becoming mothers for the first time, meaning the higher the women's satisfaction, the lower the DAP score ($r = -0.294$, $p = 0.002$). Similarly, patients' satisfaction with the medical care given to them by the gynecologist in the hospital and satisfaction with the emotional support given by the gynecologist in the hospital were negatively correlated with the total DAP score, in the sense that the more the women were satisfied with the medical care ($r = -0.218$, $p = 0.022$) and emotional support ($r = -0.243$, $p = 0.010$) provided by the gynecologist, the lower the score on the DAP scale.

DISCUSSION

The present study identified the variables that related to the desire of women to avoid a new pregnancy in research conducted in a single center in north-eastern Romania. The results identified that the age, the level of education, the level of

pain, the intention of pregnancy, the type of delivery, and the support offered by the family, partner, or medical staff were very important when it came to wanting a new pregnancy. We found that married women registered a significantly lower score on the DAP scale, both compared to women who lived in cohabitation and compared to those who no longer maintained a relationship with the child's father.

The medical care offered during pregnancy and during hospitalization seemed to influence the DAP scores. Women who had medical investigations during pregnancy had a significantly lower score on the DAP scale compared to those who did not. The more the women were satisfied with the medical care ($r = -0.218$, $p = 0.022$) and emotional support ($r = -0.243$, $p = 0.010$) provided by the gynecologist, the lower the score on the DAP scale.

As the literature proved, the DAP scale was considered to be a very useful tool to identify the desire of women to avoid a new pregnancy. It was shown to be highly predictive of pregnancy, with women with the lowest DAP score having an 80% chance of becoming pregnant within 12 months (16).

One of the most important factors was related to peripartum experiences and self-rated pain during childbirth. Studies showed that women's desire to remain pregnant depends on their previous experience with unwanted pregnancies, the birth of children with comorbidities, and the existence of severe pain and trauma during the previous birth (17, 18).

Our study identified that the more educated the women were, the later they became pregnant again. Some other results from the scientific literature showed that young women in serious relationships, who were depressed, and who were not pursuing postsecondary education had a greater desire for pregnancy (19).

The results proved that the more satisfied the women were with their lives during pregnancy, the lower the score on the DAP scale. The present results are in congruence with some others presented by studies focusing on the desire of female patients to have a new pregnancy; for example, having access to medical care or having support from the partner increases the desire for a new pregnancy. Difficult marital relationships, intimate violence, or low income increase the rate of avoiding pregnancy. Moreover, studies have shown that women with an unintended pregnancy have more children than those with a planned pregnancy (20-23).

The present study has some limitations. First, there is not a large enough number of participants to make the results generalizable. Second, the questionnaire was filled out by women with a minimum level of literacy, and no uneducated women were able to read and fill it out. Third, the study did not take into consideration mental or psychological problems, the religious context, or the ethnicity of respondents that could also influence the decision to become pregnant or avoid pregnancy (24, 25).

CONCLUSIONS

Taking into consideration all the factors that are associated with the desire of women to avoid a new pregnancy, educational, psychological, and medical interventions should be shaped by these variables. Therefore, psychologists, physicians, nurses, and family planning counselors must consider all the challenges that women, teenage or adult, may face when they attempt to have children.

CONFLICT OF INTEREST AND FUNDING

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WEIGHT LOSS AND NUTRITIONAL STATUS IN YOUNG AND MIDDLE-AGED ADULTS: RATIONAL APPROACH AND HEALTH IMPLICATIONS

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WEIGHT LOSS AND NUTRITIONAL STATUS IN YOUNG AND MIDDLE-AGED ADULTS: RATIONAL APPROACH AND HEALTH IMPLICATIONS (Abstract): Obesity is considered a major epidemic of the 21st century in many countries including Romania. Often, obesity is correlated with oxidative stress, metabolic syndrome and cardiovascular problems, which on the long-term increases the risk to diabetes and heart failure. The chances of long-term successful weight loss are enhanced by exercise and the sustained exercise is recommended as a non-invasive approach for the prevention and management of modern civilization diseases. **The aim** of the retrospective study was to monitor the evolution of body weight and some indicators that reflected the weight status of young and middle-aged adults, who exercised in an organized manner in gyms in the city of Iasi (Romania) by following the evolution of anthropometric parameters from the monitoring sheets of subjects who performed physical exercises in two gyms in Iasi. **Material and methods**: Data were extracted on socio-demographic aspects (sex, age, level of education, occupation) and physical status (height, weight, body perimeters) at the beginning of the organized physical exercises regimen and after a certain period of regular exercises. **Results:** Moreover, the respondents followed a caloric restrictive diet, using high protein bars to replace one of their meals. The average weight loss rate was -0.83 ± 0.48 kg/month, higher in men (-1.12 kg/month, maximum 2.9 kg/month) than in women (-0.69 kg/month, maximum 3 kg/month). **Conclusions:** Strict diet and exercise regimen monitorization may have a better impact on weight loss, than allowing a free choice of hypocaloric diet and a standard exercise program. However, all available data shows that reducing overweight has long-term benefits on oxidative burden for obese individuals. **Keywords:** EXERCISE, WEIGHT-LOSS, BMI, WAIST-TO-HIP RATIO, DIET RESTRICTION, OXIDATIVE STRESS.

According to World Health Organization, overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. Obesity is one of the most serious public health problems of the 21st century. Although the causes of obesity are complex, lack of physical activity is considered to be an important causal factor. For example, in a representative sample of American adults, the relative risk

of obesity among physically active people in their free time (≥ 5 rounds of physical activity per week) was ≈ 50% lower than among those who were physically inactive (1-4). Although such an association does not demonstrate a cause-and-effect relationship, scientific reports convincingly suggest that there is a link between the level of physical activity and obesity. Relatively sedentary people are more likely to be obese compared to those who are physically active. The National Weight Control Registry reported results on 3,000 subjects who lost more than 10% of their body weight and maintained it for at least one year: the average loss of > 30 kg (60 lb.) was maintained for another 5.5 years and 81% reported an increase in physical activity with energy consumption of 2445 kcal/week in women and 3289 kcal/week in men (walking, cycling, aerobics, running, climbing stairs) (5).

Despite the relatively modest weight loss associated with structured physical activity programs, the results of large epidemiological studies support the concept that among more active people, regardless of weight loss, the risk of cardiovascular disease, cancer and all-cause mortality are lowered (6-16). The level of physical activity strongly predicted mortality, regardless of body size. Those studies have suggested that it is as important for clinicians to assess the fitness of an overweight or obese subject as it is to assess smoking or measure plasma lipids or fasting blood glucose (17, 18). Similarly, in the Nurses' Health study (which included 116,564 people), a higher level of physical activity in women was associated with a reduced risk of mortality in all body weight categories and regular exercising reduces age-related cognitive impairment along with optimal redox signaling mechanisms (10, 19).

Weight loss has been associated with structured physical activity programs, so there is a current interest in high-energy exercise, especially among cardiac subjects in the recovery phase, as most of them are overweight or obese. A recent trial showed that subjects randomized to an intensive counseling and exercise program designed to determine an energy expenditure of 300-3500 kcal/week, achieved a double weight loss compared to those who followed a classic cardiac recovery program (8.2 ± 4 versus 3.7 ± 5 kg), a greater reduction in abdominal circumference, reduced insulin resistance, improvement in plasma lipid levels and metabolic syndrome (50% versus 31%) (20).

The main objective of the retrospective study was to monitor the evolution of body weight and some indicators that reflect the weight status of young and middle-aged adults who exercised in an organized manner in gyms in the city of Iasi.

Thus, we looked for valid data from the monitoring sheets of the people who frequented gyms for a significant period of time. We wanted to know the socio-demographic characteristics of those subjects and their nutritional status, both initially and their evolution. We were also interested in the elements that contributed to the evolution of body weight in a sustained exercise program, the level of compliance with that method and the pace at which someone could lose weight.

All those elements are meant to establish the effectiveness of exercise in the management of nutritional status. Subsequently, we set out to compare those results with those that used mainly other weight loss methods, especially dietary ones.

MATERIAL AND METHODS

The retrospective research was done by following the evolution of anthropometric parameters from the monitoring sheets of subjects who performed physical exercises in two gyms in Iasi. Data were extracted on socio-demographic aspects (sex, age, level of education, occupation) and physical status (height, weight, body perimeters) at the beginning of the organized physical exercises regimen and after a certain interval of time (minimum 6 weeks) of regular exercises. Additional data, such as on body fat percentage or daily intake of calories and nutrients from ingested foods, were only partially available.

The inclusion criteria for the selection of the monitoring sheets for the study were: adults from Iași county, of professional working age (between 18 and 60 years old), who performed regular exercises in the chosen gyms for at least one hour, at least 3 times a week.

The analysis tools used were:

- in order to assess weight status, BMI was calculated on the basis of height and body weight for the subjects included in the study, both at the beginning of training in the gym and at the end;
- based on weight (w, kg), height (h, cm) and age (a, years) it was possible to estimate the rest energy consumption (Basic Energy Expenditure, BEE) according to the Harris-Benedict equations:

$$BEE\ (women) = 655 + (9.5 \times w) + (1.8 \times h) - (4.7 \times a)$$

$$BEE\ (men) = 66 + (13.7 \times w) + (5 \times h) - (6.8 \times a)$$

- the diagnostic criterion for abdominal obesity was waist perimeter over 94/102 cm for men and 80/88 cm for women;
- the waist-to-hip ratio was calculated;
- the evolution of each parameter was monitored over time;
- links have been established between various parameters.

Applied statistical methods

For statistical analysis, the data were loaded and processed using the functions from *EXCEL, EPIINFO* and *SPSS*. The data obtained were systematized and statistically processed, applying tests of significance corresponding to the type of study. Descriptive statistics used the mean for the parametric characteristics and the median for the non-parametric or ordinal variables.

We used the χ^2 test to compare two or more frequency distributions from the same population, when the expected events were excluded. In the case of low frequencies, we applied the Yates correction of the formula, in order to obtain a higher estimate of the difference. In calculating the significant difference between two averages we used the t-Student test taking into account the measurement of variability and the weight of observations. The correlations were established based on the Pearson (r) and Spearman correlation coefficients, with values between -1 and 1. The closer the value of r is to 1 (direct correlation) or -1 (indirect correlation) parameters are dependent from each other.

The Spearman correlation and the χ^2 test were initially used to discover possible associations between variables. At the end we used the logistic regression (binary, univariate or multivariate) and calculated the odd ratio and the 95% confidence interval (CI) for the significant variables obtained from the initial analysis. A value of $p<0.05$ was considered statistically significant for both analyzes.

The unanimously accepted significance threshold is 95%, i.e., $p=0.05$. The lower

the value of p than that value, the stronger the significance.

RESULTS

Epidemiological characteristics of the studied group

The study group consisted of 143 subjects, 97 women and 46 men in the ratio 2.1:1. The distribution by sex was predominantly female (67.8%).

The structure by age groups was (Fig. 1):

- the mean age of the subjects at study entry was 29.5 ± 7.3 years, ranging from 18 to 50 years;
- the mean age of males was 31 ± 7.9 years with variations from 18 to 50 years;
- the mean age of females was 28.9 ± 6.9 with variations between 18 and 50 years;
- there were no statistically significant differences between the two sexes regarding the average age (T test = 1.556; p = 0.124).

There was an increased distribution of cases that fall in the age group 20-29 years (52.17%), both male and female.

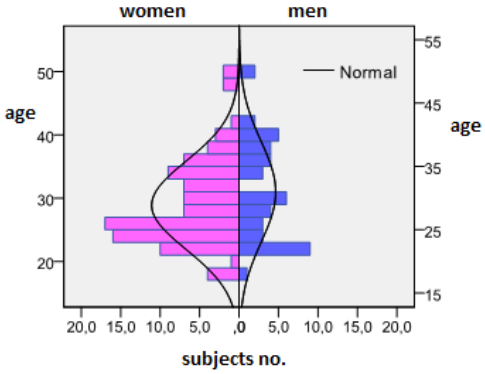

Fig. 1. Distribution by age and sex of the subjects in the study group.

The level of training, as a factor that induces socio-economic conditions as well as the level of education, does not show significant differences between the sexes (X^2 = 1.957; degrees of freedom DF = 2; p = 0.376). The study group recorded 81 cases with higher education (56.6%), 53 cases with higher secondary education (37.1%) and 9 cases with lower secondary education (6.3%) (fig. 2).

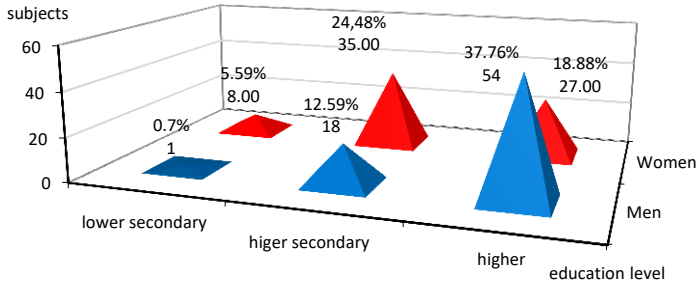

Fig. 2. Distribution by age and sex of the subjects in the study group.

Initial anthropometric characteristics

The subjects from the study group presented the following anthropometric characteristics (tab. I):

- the average waist in males was significantly higher than in females (p<0.001);
- the average weight in men was significantly higher than in females (p<0.001);

- the excess weight over the optimal value was significantly higher in men than in women;
- the average body mass index (BMI) showed statistically significant differences between the sexes in favor of the male sex ($p<0.001$);
- significant differences were found between the two sexes in terms of waist perimeter ($p<0.001$), but not in terms of the hip perimeter ($p> 0.05$).

A comparative view between the male and female groups is given in second table.

TABLE I.

Initial anthropometric characteristics

Characteristics	Total				Women				Men			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Age (years)	18	50	29.6	7.3	18	50	28.9	7.0	18	50	31.0	7.9
Height (cm)	148	188	167.6	7.5	148	176	164.5	6.7	168	188	174.2	4.4
Optimal weight (kg)	44.8	86.0	61.3	8.1	44.8	68.0	56.8	5.0	64.0	86.0	70.9	4.2
Initial weight (kg)	48	135	80.3	19.4	48	122	70.8	13.4	76	135	100.3	14.3
Surplus weight (kg)	-5.6	57.7	18.9	13.7	-5.6	57.7	14.0	11.6	7.3	57.3	29.3	11.8
Surplus weight (%)	-8.2	89.4	29.9	19.7	-8.2	89.4	24.6	19.3	10.5	79.3	41.1	15.6
BMI (kg/m^2)	18.9	42.4	28.3	5.2	18.9	39.5	26.1	4.2	25.1	42.4	33.0	3.8
Waist (cm)	65	135	90.9	17.1	65	126	84.2	14.4	85	135	104.9	13.8
Hips (cm)	78	132	99.2	10.7	78	132	99.5	11.4	83	124	98.6	8.9
Waist-to-hip ratio	0.7	1.4	0.9	0.1	0.7	1.1	0.8	0.1	0.9	1.4	1.1	0.1
Base metabolism (kcal)	1278	2516	1689.6	322.7	1278	2010	1495.1	130.9	1808	2516	2099.7	196.4

TABLE II.

Comparison between the initial anthropometric characteristics of the two sexes.

Characteristics	Women		Men		t-Test for Equal Means				
	Mean	SD	Mean	SD	t-test	p	Difference of means	CI (95%)	
Age (years)	28.9	7.00	31.00	7.90	-1.556	0.124	-2.10	-4.80	0.6
Height (cm)	164.5	6.70	174.2	4.40	-10.349	0.000	-9.70	-11.5	-7.8
Optimal weight (kg)	56.8	5.00	70.90	4.20	-17.632	0.000	-14.1	-15.7	-12.5
Initial weight (kg)	70.8	13.4	100.3	14.3	-11.737	0.000	-29.5	-34.5	-24.5
Surplus weight (kg)	14.0	11.6	29.30	11.8	-7.302	0.000	-15.4	-19.6	-11.2
Surplus weight (%)	24.6	19.3	41.10	15.6	-5.480	0.000	-16.6	-22.6	-10.6
BMI (kg/m^2)	26.1	4.20	33.00	3.80	-9.773	0.000	-6.90	-8.3	-5.5
Waist (cm)	84.2	14.4	104.9	13.8	-8.257	0.000	-20.7	-25.6	-15.7
Hips (cm)	99.5	11.4	98.60	8.90	0.493	0.623	0.90	-2.6	4.3
Waist-to-hip ratio	0.80	0.1	1.100	0.10	-12.059	0.000	-0.20	-0.3	-0.2

It is worth mentioning that that 41 normal-weight women have also done physical exercises at the gym. Of those, 6 weighed less than the optimum and 35 exceeded

their optimal weight (on average by 5.8 kg, ranging between 0.6 and 11.9 kg). The excess weight compared to the ideal weight for females was on average 15.1 kg, varying between 0.6 and 57.7 kg, and for males of 29.4 kg, with variation between 7.3 and 57.5 kg (fig. 3).

The average BMI was 26.1±4.2 kg/m^2 in women, and 33.0±3.8 kg/m^2 in men, with a significant difference between the sexes (p = 0.001) (tab. II, fig. 4).

The classification into different categories of body weight (according to the BMI criterion) showed that less than a third of the participants had a normal weight. Men who entered the gym with the intention of losing weight were overweight (21.7%) or had varying degrees of obesity (78.3%) (fig. 5). In women, the situation was different (χ^2 = 59.79, DF = 2, p <0.001), 42.2% of them being normal weight and only 14.4% being obese.

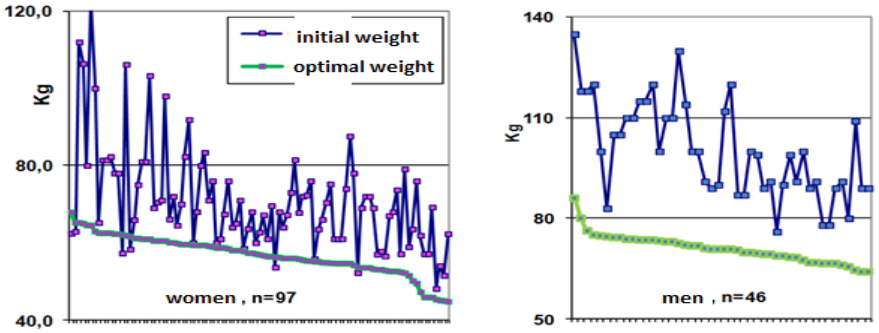

Fig. 3. Differences between the initial weights of the subjects and their ideal weight.

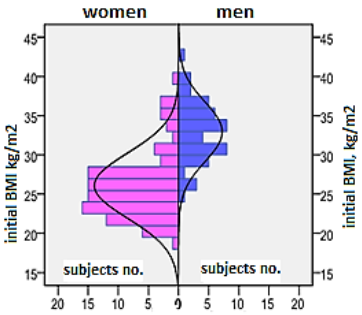

Fig. 4. Distribution of cases according to initial BMI

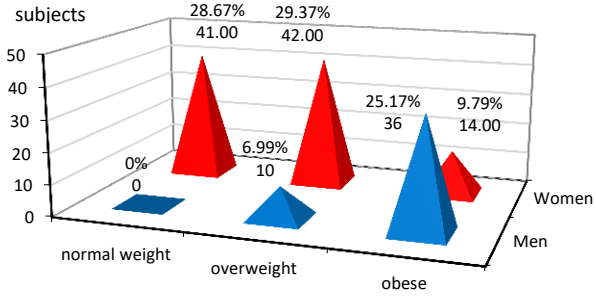

Fig. 5. Initial nutritional status by sex (% of total)

Initial body perimeters

The initial waist perimeter in females varied from 65cm to 126cm, registering an average value of 84.2 ± 14.4 cm, and in males the variation ranged from 85 to 135 cm, with an average of 104.9 ± 13.8 cm, with significant differences between sexes (p <0.001) (fig. 6).

Using the waist perimeter as a diagnostic criterion, abdominal obesity was present in 45.7% of men and 39.2% of women. The abdominal perimeter was normal in only

38.5% of participants, 45.5% of women and 23.9% of men (fig. 7). There were significant differences between the two sexes ($p < 0.05$).

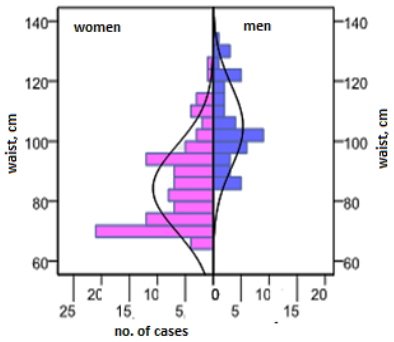

Fig. 6. Distribution of the cases according to the initial waist perimeter (cm).

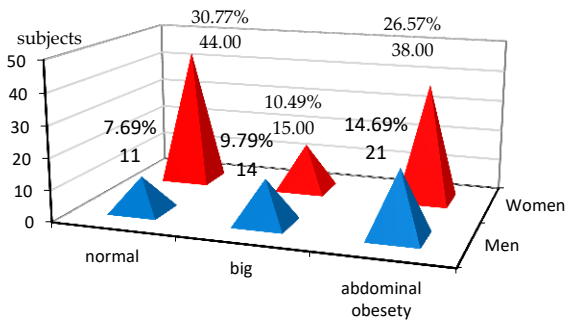

Fig. 7. Abdominal obesity among study participants.

The two diagnostic criteria (abdominal perimeter and BMI) for characterizing the nutritional status were strongly correlated with each other ($p < 0.001$) (fig. 8). The Pearson correlation index had high values in both women ($r = 0.736$) and men ($r = 0.748$).

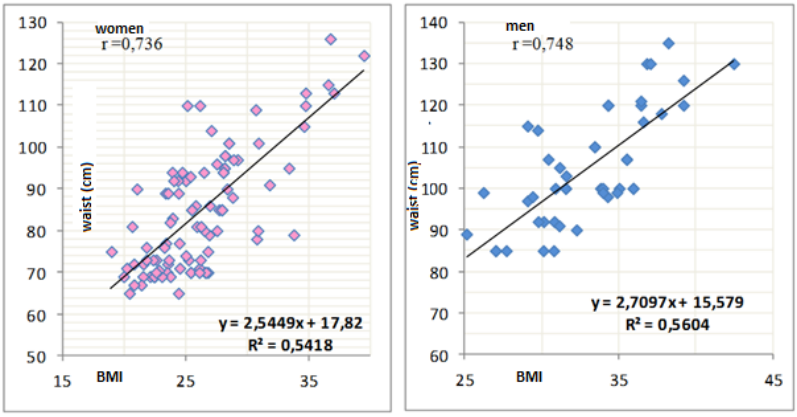

Fig. 8. Correlation between BMI and waist circumference.

The initial hip perimeter registered slightly lower average values in males compared to females, but the difference was not statistically significant ($p > 0.05$). The values of the initial hip perimeter varied in the range of 78-132 cm in females, with an average of 99.5 ± 11.4 cm, while in males the limits were in the range of 83-124 cm, with an average of 98.6 ± 8.9 cm (fig. 9).

The calculated waist-to-hip ratio was 0.85 in women and 1.1 in men ($p < 0.001$).

Basal metabolism. The calculated resting energy consumption was significantly lower in women (1495 kcal/day) than in men.

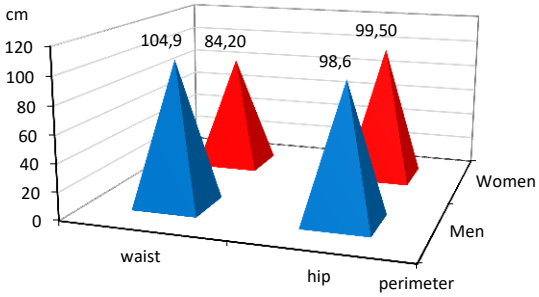

Fig. 9. Average values of body perimeters (cm).

The evolution of the studied parameters during the exercise program

There was a recorded period of 8.98 ± 4.7 months during which the evolution of body weight through physical exercises at the gym was evaluated. It is worth mentioning that the duration of participation in physical exercises in the gym was shorter for women (7.37 ± 4.4 months) than in men (12.38 ± 3.4 months) (p <0.001).

For the whole group, the subjects lost on average -8.1 ± 6.82 kg (-9.2% of body weight) and -7.6 ± 5.3 cm from the abdominal perimeter (tab. III). During the weight-loss period, the evolution of all anthropometric indicators that characterize the nutritional status was statistically significant, with a very high degree of significance (p <0.001), both in men and women (tab. III).

TABLE III.

Evolution of anthropometric parameters

		Beginning				End				Difference	t-Test (p)	r
		Mean	SD	Min	Max	Mean	SD	Min	Max			
Total	Weight (kg)	80.3	19.4	48	135	72.3	15.3	46.8	110.0	8.02	<0.001	0.951
	Surplus weight (kg)	18.9	13.7	-5.6	57.7	10.9	10.6	-8.5	43.1	8.02	<0.001	0.873
	Surplus weight (%)	29.9	19.7	-8.2	89.4	17.5	16.7	-12.4	69.7	12.43	<0.001	0.878
	BMI (kg/m^2)	28.3	5.2	18.9	42.4	25.6	4.2	18.0	36.3	2.75	<0.001	0.914
	Waist (cm)	90.9	17.1	65	135	83.3	14.6	60.0	122.0	7.60	<0.001	0.956
	Hips (cm)	99.2	10.7	78	132	93.9	11.1	69.0	135.0	5.28	<0.001	0.947
	Waist-to-hip ratio	0.92	0.14	0.68	1.38	0.89	0.13	0.65	1.20	0.03	<0.001	0.959
Women	Weight (kg)	70.80	13.4	48	122	65.3	12.1	46.8	106.0	5.46	<0.001	0.925
	Surplus weight (kg)	14.00	11.6	-5.6	57.7	8.5	10.6	-8.5	43.1	5.46	<0.001	0.899
	Surplus weight (%)	24.6	19.3	-8.2	89.4	15.1	17.9	-12.4	69.7	9.51	<0.001	0.900
	BMI (kg/m^2)	26.1	4.2	18.9	39.5	24.1	3.9	18.0	36.3	1.99	<0.001	0.907
	Waist (cm)	84.2	14.4	65	126	78.4	13.5	60.0	118.0	5.85	<0.001	0.963
	Hips (cm)	99.5	11.4	78	132	94.8	12.3	69.0	135.0	4.71	<0.001	0.957
	Waist-to-hip ratio	0.85	0.11	0.68	1.11	0.83	0.11	0.65	1.07	0.02	<0.001	0.963
Men	Weight (kg)	100.3	14.3	76	135	86.9	10.1	70.0	110.0	13.39	<0.001	0.896
	Surplus weight (kg)	29.30	11.8	7.3	57.3	16.0	8.8	-0.5	37.5	13.39	<0.001	0.815
	Surplus weight (%)	41.10	15.6	10.5	79.3	22.6	12.5	-0.7	56.6	18.58	<0.001	0.833
	BMI (kg/m^2)	33.00	3.8	25.1	42.4	28.6	2.9	23.1	35.9	4.35	<0.001	0.843
	Waist (cm)	104.9	13.8	85	135	93.6	11.1	75.0	122.0	11.28	<0.001	0.902
	Hips (cm)	98.60	8.9	83	124	92.1	7.8	79.0	114.0	6.48	<0.001	0.932
	Waist-to-hip ratio	1.060	0.10	0.89	1.38	1.02	0.09	0.82	1.20	0.05	<0.001	0.826

There were also differences between the two sexes - men lost 13.4 kg (reducing their excess weight by 18.6%) and women 5.5 kg (with a difference of -9.5% in terms of excess weight). The reduction of BMI, it was higher in men (-4.35 kg/m^2) than in women (-2 kg/m^2). The waist perimeter, there was a double reduction in men (-11.3 cm) compared to women (-5.8 cm).

Initially, most women were normal or overweight (86.6% of them), while men were in the opposite situation, as the majority (78.3%) were obese (tab IV). Men had significantly higher degrees of obesity, one subject being morbidly obese and 14 were severely obese, compared to only 4 women that were in the category of grade I obesity. The differences in levels of BMI between the two sexes remained significant even at the end of the study, however, the differences in the category of abdominal perimeter disappeared.

TABLE IV.
Characterization of the study group according to the initial and final anthropometric parameters

Parameter	Category	Total n=143		Women n=97; 67.8 %		Men n=46; 32.2 %		χ^2 Test
		n	%	n	%	n	%	
Initial BMI	normal weight	41	28.7	41	28.7	0	0	$\chi^2 = 59.788$ DF = 2 p<0.001
	overweight	52	36.4	42	29.4	10	7.0	
	obese	50	35.0	14	9.8	36	25.2	
Initial BMI	normal weight	41	28.7	41	28.7	0	0	$\chi^2 = 60.681$ DF = 4 p<0.001
	overweight	52	36.4	42	29.4	10	7.0	
	grade I obese	31	21.7	10	7.0	21	14.7	
	grade II obese	18	12.6	4	2.8	14	9.8	
	grade III obese	1	0.7	0	0	1	0.7	
Initial Waist perimeter	normal	55	38.5	44	30.8	11	7.7	$\chi^2 = 7.498$ DF = 2 p = 0.024
	big	29	20.3	15	10.5	14	9.8	
	abdominal obesity	59	41.3	38	26.6	21	14.7	
Final BMI	underweight	2	1.4	2	1.4	0	0.0	$\chi^2 = 35.391$ DF = 3 p<0.001
	normal weight	63	44.1	58	40.6	5	3.5	
	overweight	57	39.9	30	21.0	27	18.9	
	obesity	21	14.7	7	4.9	14	9.8	
Final Abdominal perimeter	normal	86	60.1	57	39.9	29	20.3	$\chi2 = 0.247$ DF = 2 p = 0.884
	big	24	16.8	17	11.9	7	4.9	
	abdominal obesity	33	23.1	23	16.1	10	7.0	

Although the weight loss was significant, the optimal weight was only achieved in a few cases. In the case of some women, a final weight lower than the one considered optimal was reached (fig. 10).

The data obtained (fig. 11) showed that 29.4% of the subjects (17.5% women and 11.9% men) lost between 5 and 10 kg, and 17.5% lost between 10 and 15 kg. Weight loss of over 20 kg (maximum 36.3 kg) was also recorded in 7.7% of subjects (10 men and one woman).

By comparing the weight lost to the initial weight of the subjects, we notice that about a quarter of them lost between 5-10% of their initial weight and a third of women

lost less than 5% of their body weight. In total, 41.3% of subjects lost over 10% of their initial body weight, and 5.6% lost more than 20% (fig. 12).

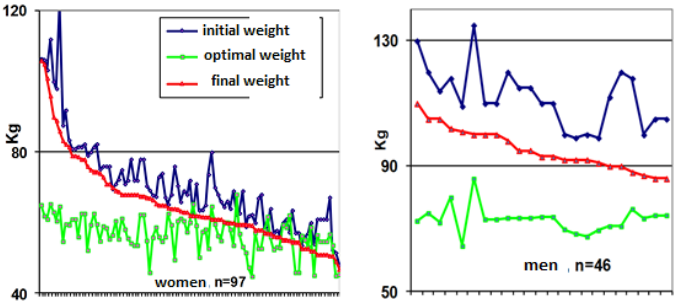

Fig. 10. Evolution of body weight compared to the initial weight of the subjects and to the ideal weight.

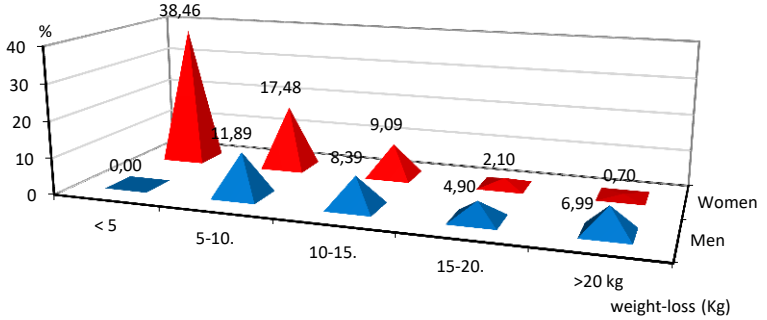

Fig. 11. Distribution of subjects according to weight lost (χ^2 = 54.24; DF = 4; p<0.001).

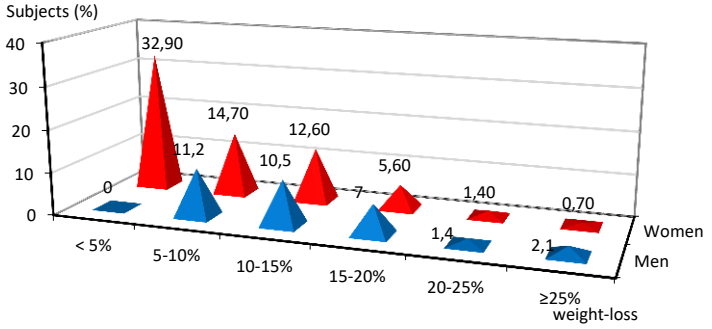

Fig. 12. Distribution of subjects according to the percentage of body weight lost during the weight loss program (χ^2 = 35.50; DF = 5; p = <0.001).

Other indicators of weight loss, used mainly in reporting the results of surgical treatment of obesity (bariatric surgery) (18-22), are the percentage of decreased BMI and the percentage of decreased weight. In our study, the BMI decrease was -9.54% for the whole group, with lower values for women (-7.66%) than for men (-13.33%). That index adjusts the percentage of weight loss, taking into account the person's height,

being more useful in interpreting the degree of excess weight. The percentage of weight loss primarily targets the effect of therapy. In our study, 42.4% was the percentage for the whole group, with higher values in men (45.7%) than in women (39%).

Statistically significant changes ($p < 0.001$) were also registered in the case of body perimeters. The waist perimeter decreased on average by -5.9 ± 3.9 cm for women, and by 11.3 ± 6.1 for men. As a result, the final abdominal perimeter had an average in the optimal range. The waist-to-hip ratio decreased in men from 1.06 to 1.02 and in women from 0.85 to 0.83.

Involvement of various factors on the percentage of weight loss

By using the Spearman coefficient and regression techniques/ANOVA we identified significant correlations between the weight loss percentage (relative to the initial weight) and the factors that influenced the process: program duration ($r = +0.765$, $p < 0.001$) (fig. 13), initial BMI ($r = +0.44$, $p < 0.001$) (fig. 14), initial excess weight (kg), which also influenced the theoretical goal set initially ($r = +0.452$, $p < 0.001$). From the analysis of the obtained data, it was found that the percentage weight loss achieved was not statistically significantly correlated with age ($r = -0.055$, $p = 0.514$).

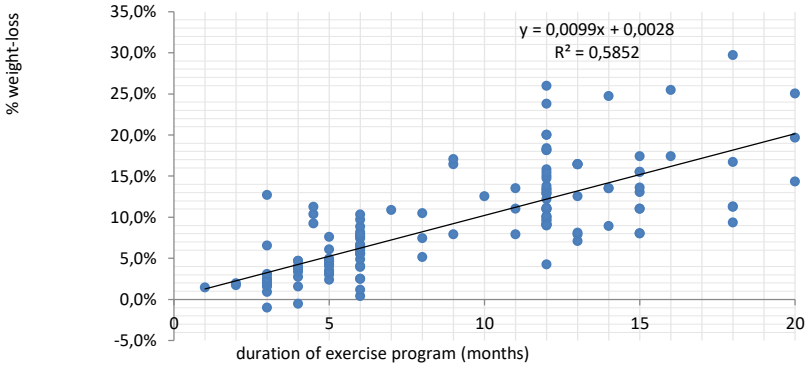

Fig. 13. The influence of the duration of the program on the weight loss percentage.

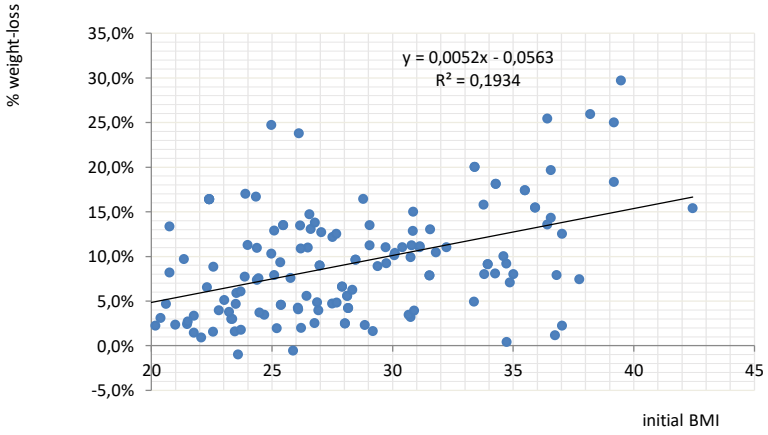

Fig. 14. Influence of initial BMI on weight loss.

The average weight loss rate was -0.83 ± 0.48 kg/month, higher in men (-1.12 kg/month, maximum 2.9 kg/month) than in women (-0.69 kg/month, maximum 3 kg/month), with statistically significant differences between the sexes (t-test = 4.955, $p < 0.001$). We also found that the average monthly rate of weight loss was negatively correlated with age ($r = -0.315$, $p < 0.001$).

DISCUSSION

One of the limiting factors of our study was that the information from the monitoring sheets could only partially characterize the diet of the participants. What we know is that the subjects resorted to caloric restrictions in food intake. Comparing our data to that from the scientific literature, we found that in our study, weight loss was higher than that reported in studies based on exercise without changing the diet but falls between the values obtained in studies that combined diet with exercise or resorted to medication.

From the observation sheets we deduced that the participants also resorted to caloric restrictions in food intake, but we could not characterize the used diet. Using hypocaloric diets remains a desirable approach to enhance the efficacy of the exercise program. Moreover, excess food intake leads to oxygen reactive species supra production at the mitochondrion level, which in turn induces inflammation and prooxidative enzyme activation (e.q. malondialdehyde, NADPH oxidase etc.). Excess nutrients (especially sugar derived products and fat-rich foods) in patients with diabetes and endothelial disfunction or hypertension enhances multiple factors that aggravate metabolic syndrome and cellular processes. Available patients' data indicates that lipid peroxidation and oxidative stress is positively correlated to increased waist circumference and BMI, while adiponectin is negatively correlated to these parameters (26, 27). This also indicates that there is a direct relation between oxidative stress and weight gain, suggesting that losing weight or weight management is a good preventive approach to decrease the high levels of oxygen reactive species in obese and overweight people (28).

The group was homogeneous in terms of epidemiological characteristics. From the point of view of anthropometric and nutritional parameters, some statistically significant differences were found between women and men. The average BMI in men was higher than in women (33.0 ± 3.8 kg/m^2 *vs.* 26.1 ± 4.2 kg/m^2), and the classification in different categories of body weight using the BMI criterion showed significant differences between the sexes. All men who entered the gym with the intention of losing weight were overweight or had varying degrees of obesity, while more than 40% of women were normal weight, and some of them (6.2%) had an initial weight below the optimum.

Those significant differences between the sexes could be explained by different motivation - men resort to weight loss methods when weight is exceeded and health is endangered, but women can also be motivated by aesthetics. Interestingly, motivation is behind the efficacy of most dietary and exercise programs (29-31). Moreover, the physical activity in itself has a positive effect on the mindset of patients (enhancing self-esteem) and on their lifestyle (32). More recent data indicates that type 2 diabetes can be bounced back with the help of a 3-step structured program that includes diet management, restrictions and food reintro-

duction, along with long-term weight loss maintenance and monitorization. Even if the results were promising, some concerns and controversies were noted among the participants and supervising specialists, especially related to taking patients off the anti-hypertensive and anti-diabetic medication (33). Although the voluntary participants in our study did not declare any health problems, the obesogenic lifestyle increases the risk for cardiovascular complication and type 2 diabetes. Habit changes depend mostly on self-motivation when no psychological support is available. This explains the need to include specialized support and advice in regard to proper diet and physical activity approach for long-term weight management.

There was 8.98 ± 4.7 months recorded period for evaluating the evolution of body weight through physical exercises at the gym and for the entire group there was an average loss of 8.01 ± 6.82 kg, i.e., -9.2% of body weight and -7.6 ± 5.3 cm from the waist perimeter. The duration of exercise period in the gym was shorter for women (7.37 ± 4.4 months) than for men (12.38 ± 3.4 months), probably due to the fact that the initial excess weight was higher in men than in women.

The average weight loss rate was -0.83 ± 0.48 kg/month, higher in men (-1.12 kg/month, maximum 2.9 kg/month) than in women (-0.69 kg/month, maximum 3 kg/month) and was negatively correlated with the age of the subjects.

In a 2007 meta-analysis (34), the average weight loss was 5-8.5 kg (5-9%) in the first 6 months of weight-loss regimen involving calorie-restricted diets or weight loss medication. In contrast, groups that only exercised or received advice showed minimal weight loss, regardless of the evaluation period. In interventions that combined diets with caloric restrictions in combination with exercise, there were moderate weight loss after 6 months, and in studies with a much longer duration of 48 months, an average weight loss of 3-6 kg (3-6%) was maintained for a long time.

Although the weight loss following the physical effort in the gym was significant, the final average weight obtained exceeded the calculated optimal weight by 10.9 ± 10.64 kg, i.e., by an average of 17.4 ± 16.7%. However, at the end of our research 16 people had less than the calculated optimal weight and another 8 reached the ideal weight ± 1 kg.

The surplus compared to the ideal weight for females was on average 15.1 kg, and for males 29.4 kg. Abdominal obesity was initially encountered in 41.3% of study participants (39.2% of women and 45.7% of men). The calculated waist-to-hip ratio was 0.85 in women and 1.1 in men. Those aspects confirmed that there is a higher tendency to store adipose tissue in men in the "apple" distribution, which implies an increased metabolic risk.

Some online weight loss program and activity tracker reveal significantly more weight loss at 3 months and a higher weekly moderate-to-vigorous intensity physical activity is associated with greater long-term weight loss and weight maintenance (35, 36).

Another review of the scientific literature on the role of physical activity in bodyweight management (36) pointed out that although weight loss through exercise alone was significantly greater than zero, the absolute and percentage values were modest, but if a diet change is added to the physical exercises, the differences in weight loss become significant. Weight loss depends on the energy difference be-

tween intake and consumption. That difference can be increased by combining low-calorie diets (low intake) with structured exercise programs (high consumption).

However, it was found that there may be other benefits of practicing physical activities for weight loss, such as better weight loss, with mostly fat loss and better long-term maintenance of weight (higher metabolic rate) (37). Exercise practiced three times a week, in combination with replacing one meal a day with a nutritional formula can increase fitness and improve the body's composition, but the intake of thermogenic substances does not bring additional benefits (38). More research is ongoing in terms of evaluation of direct correlations between the oxidative status and weight-loss program. Taking into account all available data, the evaluation of physical activity pattern cand be a good predictor for the health status, early cardiovascular risk and metabolic syndrome outcome.

CONCLUSIONS

Our research indicates once again that physical activity, especially high energy exercises, and small changes in diet without strict restrictions contribute to weight loss. Moreover, the voluntary subjects included in the study did not abandon the exercise program during the observation period. However, a long-term body weight management program should include psychological and motivational support for overweight and obese individuals, thus ensuring better health consequences by maintaining systematic physical activity.

INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical review and approval were waived for this study due to the obtaining of individual consent of all voluntary participants, for which no identification data was used. No clinical testing was required, only retrospective data from the completed data sheets was used.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest. B. Huzum, I. Chirila and Riana Maria Huzum had equal contribution and should be regarded as main authors of the manuscript.

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RETROSPECTIVE STUDY ON THE PREVALENCE OF ORAL SYMPTOMS IN THE SPECIFIC MANIFESTATIONS OF PATHOLOGY CAUSED BY SARS-CoV-2 VIRUS

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RETROSPECTIVE STUDY ON THE PREVALENCE OF ORAL SYMPTOMS IN THE SPECIFIC MANIFESTATIONS OF PATHOLOGY CAUSED BY SARS-CoV-2 VIRUS (Abstract): The aim of this study is to retrospectively analyze the prevalence of oral pathology in the range of symptoms characteristic of the clinical picture caused by the SARS-CoV-2 virus. The study was carried out on 779 patients with COVID-19, hospitalized between September 2020 and April 2021 in CF Hospital from Iasi, Romania. The sample's structure on genders was almost perfectly equilibrated (49.6% men and 50.4% women), with age spread within all age ranges, between 7 and 93 years; around a half of patients were adults between 40-59 years old (45.6% men and 49.4% women). The percentage of asymptomatic patients is significantly higher among those afebrile (27.7%) compared to febrile ones (6.1%). Among the afebrile, 65.2% have 1-4 symptoms, compared to 87.7% of the febrile, and 7.1% have 5-9 symptoms, compared to 6.1% of the febrile. We noticed increased incidences of oral symptoms in patients with aggravated condition (febrile) by comparison with the others, in most cases - with only one exception, gum bleeding, which is slightly more frequent in case of afebrile / sub febrile patients (59.2% cases compared with 55.1% cases of febrile patients). Oral signs including dysgeusia, xerostomia, oral mucosal lesions and periodontal pathology could be associated with SARS-CoV-2 infection or equally could be considered secondary lesions of COVID19 infection, which are extremely useful elements in the early diagnosis of people with COVID19. **Keywords:** COVID-19, SARS-CoV-2, ORAL SYMPTOMS, XEROSTOMIA, DYSGEUSIA, GENERAL STATUS.

The coronavirus pandemic triggered in 2019 affected an impressive number of people, namely 110, million and spread to 224 countries (1-5). The pathology of COVID 19, characterized by a symptomatic polyvalence, has the SARS-CoV-2 virus as a pathogen. The clinical aspects of a patient diagnosed with the SARS-CoV-2 virus is particularly complex, bringing together multiple signs and symptoms, found in the different territories without excluding the oral territory, a particularly important level in the early diagnosis of these pathologies (6-9).The frequent symptomatology for this

type of pathology was represented by fever, cough, dyspnea but also by a series of oral signs that were found in a quantifiable proportion (10-13).The most common oral manifestations in patients with COVID 19 were dysgeusia and xerostomia. Dysgeusia refers to the inability of patients to identify the taste of food or drink, a symptom with profound implications for the quality of life of affected patients (13-16). It is important to note that oral signs associated with coronavirus persisted in patients for three months after the general condition symptoms had subsided to disappearance, which underlines the fact that oral symptoms could materialize into side effects of COVID 19 pathology (17-20). Oral symptoms associated with COVID 19 infection were dysgeusia, xerostomia, oral mucosal lesions, macroglossia, temporomandibular joint disorders and a range of periodontal disease (21-25).

The aim of this study is to retrospectively analyze the prevalence of oral pathology in the range of symptoms characteristic of the clinical picture caused by the SARS-CoV-2 virus.

MATERIALS AND METHODS

The study was carried out on 779 patients with COVID-19, hospitalized between September 2020 and April 2021 in CF Hospital from Iasi, Romania. The sample's structure on genders was almost perfectly equilibrated (49.6% men and 50.4% women), with age spread within all age ranges, between 7 and 93 years; most patients (around a half of them were adults between 40-59 years old (45.6% men and 49.4% women). The detailed demographic features of the studied sample are specified in first table.

TABLE I.

The sample's structure on genders and age groups

		Gender				Total	
		m		F			
		N	%	N	%	N	%
Age range	under 20 years	8	2.1%	7	1.8%	15	1.9%
	20-29 years old	24	6.2%	26	6.6%	50	6.4%
	30-39 years old	50	13.0%	44	11.2%	94	12.1%
	40-49 years old	89	23.1%	90	22.9%	179	23.0%
	50-59 years	87	22.5%	104	26.5%	191	24.5%
	60-69 years	73	18.9%	63	16.0%	136	17.5%
	70-79 years	36	9.3%	36	9.2%	72	9.2%
	over 80 years	19	4.9%	2.3	5.9%	42	5.4%
Total		386	100.0%	393	100.0%	779	100.0%

The statistical analysis was performed in *SPSS 29.0*. The qualitative data were reported as absolute frequencies and percentages, and the quantitative data were reported as averages and standard deviations. The associations between qualitative data were investigated using the Chi-squared test. Binary logistic regression (Forward LR) was used to investigate the cumulative effect of risk factors for aggra-

vated evolution. The ROC analysis was performed in order to identify the cut-off values of quantitative parameters for aggravated evolution (febrile state). The values of $p < 0.05$ were evaluated as statistically significant, and the values of $p < 0.01$ were evaluated as statistically highly significant.

RESULTS

We focused our study on the investigation of patients' evolution, characterized through its severity - febrile patients *vs.* afebrile or sub febrile patients. Most patients had a good evolution, being afebrile 665 - 85.4%), while 65 patients (8.3%) were sub febrile for 1-2 days and 49 patients (6.3%) were febrile 1-3 days. Afebrile and sub febrile patients were included in the same category, febrile patients being investigated separately.

No statistically significant differences are found between febrile and afebrile patients neither in terms of their distribution by gender nor by age ranges - therefore, these two parameters do not function as risk factors for the unfavorable evolution of patients (tab. II).

TABLE II.

Distribution by gender and age range according to evolution

		Evolution							
		Afebrile / Sub febrile		Febrile		p	OR	95% CI	
		n	%	n	%			Lower l.	Upper l.
Gender	male	360	49.3%	26	53.1%	0.612	-	-	-
	female	370	50.7%	2. 3	46.9%				
Age	under 60 years	495	67.8%	34	69.4%	0.819	-	-	-
	over 60 years	235	32.2%	15	30.6%				

Among the investigated symptoms, significantly increased percentages are observed in febrile patients compared to others in the case of cough (59.2% *vs.* 38.9%), fever at onset (40.8% *vs.* 16.3%), loss of appetite (16.3% *vs.* 6.0%), nausea (10.2% *vs.* 2.7%) and diarrhea (10.2% *vs.* 1.9%). The highest risk is recorded for patients with digestive disorders: diarrhea (OR = 5.812) and nausea / vomiting (OR = 4.034) (tab. III).

TABLE III.

Distribution of recorded symptoms according to evolution

	Evolution							
	Afebrile / Sub febrile		Febrile		p	OR	95% CI	
	n	%	n	%			Lower l.	Upper l.
Rhinorrhea	33	4.5%	1	2.0%	0.717	-	-	-
Dyspnea	73	10.0%	7	14.3%	0.339	-	-	-
Chest pains	82	11.2%	7	14.3%	0.515	-	-	-
Cough	284	38.9%	29	59.2%	0.005**	2.277	1.264	4.103
Fever	119	16.3%	20	40.8%	0.000**	3.541	1.938	6.469
Feverish	22	3.0%	0	0.0%	0.390	-	-	-
Chills	48	6.6%	5	10.2%	0.371	-	-	-

	Evolution							
	Afebrile / Sub febrile		Febrile		p	OR	95% CI	
	n	%	n	%			Lower l.	Upper l.
Sweats	31	4.2%	4	8.2%	0.269	-	-	-
myalgia	98	13.4%	7	14.3%	0.864	-	-	-
sore throat	43	5.9%	5	10.2%	0.217	-	-	-
anosmia	80	11.0%	3	6.1%	0.288	-	-	-
ageusia	42	5.8%	1	2.0%	0.512	-	-	-
Physical weakness	156	21.4%	10	20.4%	0.874	-	-	-
dizziness	35	4.8%	3	6.1%	0.727	-	-	-
DISORDERS	157	21.5%	11	22.4%	0.877	-	-	-
loss of appetite	44	6.0%	8	16.3%	0.012*	3.042	1.344	6.883
Nausea / vomiting	20	2.7%	5	10.2%	0.016*	4.034	1.446	11.257
Diarrhea	14	1.9%	5	10.2%	0.005**	5.812	2.002	16.868
Other pains	74	10.1%	8	16.3%	0.172	-	-	-
Other	45	6.2%	4	8.2%	0.540	-	-	-
Asymptomatic	202	27.7%	3	6.1%	0.000**	0.170	0.052	0.554

We also investigated by comparison in these two categories of patients the presence of oral symptoms (tab. IV). We noticed increased incidences of oral symptoms in patients with aggravated condition (febrile) by comparison with the others, in most cases - with only one exception, gum bleeding, which is slightly more frequent in case of afebrile/sub febrile patients (59.2% cases compared with 55.1% cases of febrile patients). The most common oral symptoms met in the case of febrile patients were taste altered (81.6% cases) and Candida (79.6% cases). Despite these specific elements, we didn't report, anyway, statistically significant differences between the two samples investigated.

The percentage of asymptomatic patients is significantly higher among those afebrile (27.7%) compared to febrile ones (6.1%). Among the afebrile, 65.2% have 1-4 symptoms, compared to 87.7% of the febrile, and 7.1% have 5-9 symptoms, compared to 6.1% of the febrile (tab. V).

TABLE IV.

Distribution of recorded oral symptoms according to evolution

	Evolution				
	Afebrile / Sub febrile		Febrile		p
	n	%	n	%	
Xerostomia	324	44.4	28	57.1	0.922
Chewing problems	390	53.4	32	65.3	0.106
Swallowing difficulty	422	57.8	34	69.4	0.111
Mouth ulcerations	387	53.0	32	65.3	0.095
Gum bleeding	432	59.2	27	55.1	0.575
Taste altered	515	70.5	40	81.6	0.097
Candida	520	71.2	39	79.6	0.208

TABLE V.

Distribution of symptoms according to their number, according to evolution

		Evolution, p = .000**			
		Afebrile / Sub febrile		Febrile	
		n	%	n	%
No. symptoms	0	202	27.7%	3	6.1%
	1	82	11.2%	5	10.2%
	2	132	18.1%	3	6.1%
	3	178	24.4%	24	49.0%
	4	84	11.5%	11	22.4%
	5	36	4.9%	2	4.1%
	6	10	1.4%	1	2.0%
	7	5	0.7%	0	0.0%
	9	1	0.1%	0	0.0%
Total		730	100.0%	49	100.0%

The AUC coefficient for the number of symptoms is 0.665, with a confidence interval between 0.599 and 0.731, statistically significant (p = .000**), but with little potential for predicting the evolution of patients. The identified cut - off value is 2.50, with a fairly good sensitivity of 0.776 and a specificity of 0.570 (fig. 1) - so the risk of worsening evolution due to fever increases in patients with at least 3 cumulative symptoms, compared with the others.

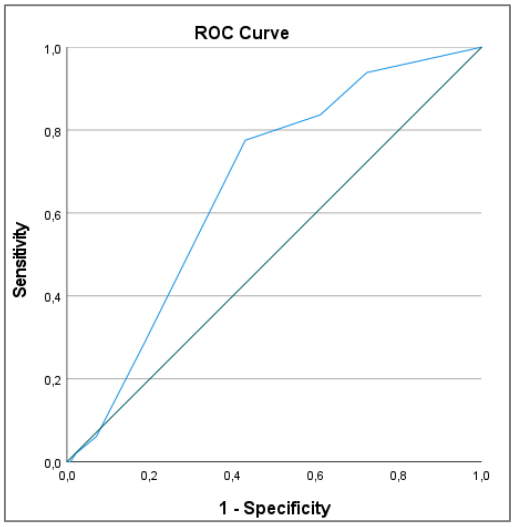

Fig.1. No. symptoms *vs.* evolution - ROC curve

The overall share of patients with comorbidities is slightly higher among those with fever (69.4%) compared to those with afebrile illness (63.8%), but the difference recorded is not statistically significant; the separate analysis on particular comorbidities highlights the same phenomenon; thus, among febrile patients there are

slightly more cases of cardiovascular diseases (63.2% *vs.* 61.4%), liver diseases (10.2% *vs.* 8.2%), kidney diseases (10.2% *vs.* 8.4%), anemia (6.1% *vs.* 2.3%), thyroid disorders (6.1% *vs.* 4.1%) and neoplasm (6.1% *vs.* 3.8%) , but without the threshold of statistical significance being reached (tab. VI).

TABLE VI.
Distribution of registered comorbidities according to evolution

	Evolution							
	Afebrile / Sub febrile		Febrile		p	OR	95% CI	
	n	%	n	%			Lower l.	Upper l.
Comorbidities	466	63.8%	34	69.4%	0.433	-	-	-
HTN	242	33.2%	15	30.6%	0.714	-	-	-
CIC	28	3.8%	0	0.0%	0.249	-	-	-
ICC	27	3.7%	3	6.1%	0.428	-	-	-
AF	43	5.9%	3	6.1%	1.000	-	-	-
IVC	28	3.8%	4	8.2%	0.135	-	-	-
Other cardio-vascular diseases	70	9.6%	5	10.2%	0.804	-	-	-
High CV risk	10	1.4%	1	2.0%	0.513	-	-	-
Stroke	8	1.1%	1	2.0%	0.444	-	-	-
DM type II	100	13.7%	5	10.2%	0.488	-	-	-
Obesity	25	3.4%	1	2.0%	1.000	-	-	-
Bronchial asthma	16	2.2%	0	0.0%	0.616	-	-	-
Respiratory diseases	27	3.7%	0	0.0%	0.406	-	-	-
Digestive diseases	62	8.5%	1	2.0%	0.170	-	-	-
Hepatic diseases	60	8.2%	5	10.2%	0.593	-	-	-
Renal diseases	61	8.4%	5	10.2%	0.597	-	-	-
Anemia	17	2.3%	3	6.1%	0.125	-	-	-
Thrombocytopenia	7	1.0%	1	2.0%	0.407	-	-	-
Dyslipidemia	11	1.5%	0	0.0%	1.000	-	-	-
Thyroid diseases	30	4.1%	3	6.1%	0.456	-	-	-
Genital diseases	19	2.6%	1	2.0%	1.000	-	-	-
Psychiatric diseases	12	1.6%	1	2.0%	0.573	-	-	-
Neoplasm	28	3.8%	3	6.1%	0.437	-	-	-
Other	88	12.1%	4	8.2%	0.414	-	-	-

The number of cumulative comorbidities does not differ statistically significantly in febrile patients compared to the others, although the share of patients without comorbidities is slightly higher among those afebrile (36.2%) compared to febrile ones (30.6%); among febrile patients, both the share of cases with 1-3 cumulative comorbidities (57.2% *vs.* 54.5%), and the share of cases with 4-7 cumulative comorbidities (12.2% *vs.* of 9.2%) (tab. VII).

Multivariate analysis by binary logistic regression identifies 3 significant predictors for the evolution aggravated by febrile state, namely: the presence of fever at the onset ($p = 0.015^{**}$), of diarrhea ($p = 0.026$) and of at least 3 cumulative symptoms ($p = 0.002$) - (tab. VIII).

TABLE VII.
Distribution of comorbidities according to their number, according to evolution

		Evolution, p = .175			
		Afebrile / Sub febrile		Febrile	
		n	%	n	%
No. comorbidities	0	264	36.2%	15	30.6%
	1	176	24.1%	19	38.8%
	2	128	17.5%	7	14.3%
	3	94	12.9%	2	4.1%
	4	47	6.4%	5	10.2%
	5	12	1.6%	0	0.0%
	6	6	0.8%	1	2.0%
	7	3	0.4%	0	0.0%
Total		730	100.0%	49	100.0%

TABLE VIII.
Predictors of worsening evolution - multivariate regression

	Sig. p	OR Ex (B)	95% CI for EXP (B)	
			Lower I.	Upper I.
Fever	0.015	2.227	1.168	4.248
Diarrhea	0.026	3.499	1.164	10.525
No. symptom	0.002	3.237	1.549	6.766

We also investigated the influence of pulmonary changes on the evolution of patients; we found that the changes statistically significantly associated with worsening evolution are ground glass appearance (observed in 76.6% of febrile patients and 58.7% of others) and confluence (observed in 14.9% of febrile patients compared to 6.1 % of others). Other pulmonary features found in higher percentages in febrile patients were condensation (12.8% *vs.* 6.7%), consolidation (4.3% *vs.* 3.2%), and fibronodular sequelae (8.5% *vs.* 7.0%). 19.3% of afebrile patients have normal pulmonary appearance, compared to only 8.5% of febrile ones (tab. IX).

TABLE IX.
Distribution of CT changes according to evolution

	Evolution							
	Afebrile / Sub febrile		Febrile		p	OR	95% CI	
	n	%	n	%			Lower I.	Upper I.
Ground glass	368	58.7%	36	76.6%	0.016*	2.303	1.151	4.609
Condensation	42	6.7%	6	12.8%	0.135	-	-	-
Confluent	38	6.1%	7	14.9%	0.030*	2.713	1.139	6.458
Consolidation	20	3.2%	2	4.3%	0.661	-	-	-
Opacity	25	4.0%	0	0.0%	0.247	-	-	-
Accentuated interstitial design	57	9.1%	3	6.4%	0.790	-	-	-
Fibronodular sequelae	44	7.0%	4	8.5%	0.766	-	-	-
Crazy look paving	5	0.8%	0	0.0%	1.000	-	-	-
Pulmonary nodule	15	2.4%	0	0.0%	0.616	-	-	-
Other changes	50	8.0%	2	4.3%	0.569	-	-	-
Normal appearance	121	19.3%	4	8.5%	0.066	-	-	-

Another element recorded at the admission of the patients was the CRP level. Almost all patients had elevated CRP, the mean value recorded being 28.8944 ± 46.11459 mg/l, with a range of variation between 0.04 mg/l and 293.31 mg/l. It was also found that CRP values are significantly higher in men compared to women, and also in patients over 60 years old, with symptoms and comorbidities (tab. X).

TABLE X.

Batch CRP values (mg/L) - descriptive statistics parameters

		N	Mean	SD	Minimum	Maximum	p
Total		730	28.8944	46.11459	0.04	293.31	
Sex	male	365	34.9502	52.60998	0.04	288.00	0.000**
	female	365	22.8386	37.65097	0.11	293.31	
Age range	under 60 years	493	19.7890	37.87123	0.11	293.31	0.000**
	over 60 years	237	47.8350	55.18540	0.04	288.00	
Asymptomatic	no	538	32.4145	49.42139	0.04	293.31	0.000**
	yes	192	19.0308	33.41817	0.04	193.23	
Comorbidities	no	250	14.8232	27.35627	0.04	165.36	0.000**
	yes	480	36.2231	51.86774	0.04	293.31	

There is a clear association between increased CRP values and the presence of lung changes on CT; thus, patients with altered CT have an average CRP value of 32.6039 ± 46.37504 mg/l, with a maximum reaching 293.31 mg/l, compared to patients with normal CT, with a significantly lower average CRP value at 9.1630 ± 23.70186 mg/l. Significantly increased values for CRP are observed in patients with ground glass appearance and confluence; in the case of other pulmonary changes (condensation, consolidation, opacity, crazy appearance paving , pulmonary nodule), although the CRP values are also higher, the differences do not reach the threshold of statistical significance (tab. XI).

TABLE XI.

Comparative CRP values (mg/L) according to the presence of lung changes

CT results		N	Mean	SD	Minimum	Maximum	p
Changed appearance	no	116	9.1630	23.70186	0.11	200.17	0.000**
	yes	527	32.6039	46.37504	0.04	293.31	
Ground glass	no	253	19.9616	38.11499	0.11	293.31	0.000**
	yes	390	33.8330	46.81263	0.04	285.94	
Condensation	no	599	27.8338	43.18197	0.04	293.31	0.251
	yes	44	35.7436	55.07690	0.12	285.94	
Confluent	no	598	26.9783	43.67900	0.04	293.31	0.003**
	yes	45	46.9356	45.83208	2.30	171.05	
Consolidation	no	622	27.9046	44.09396	0.04	293.31	0.141
	yes	21	42.3086	42.70402	2.03	133.91	

CT results		N	Mean	SD	Minimum	Maximum	p
Opacity	no	618	27.4074	42.73797	0.04	285.94	0.076
	yes	25	52.2960	66.52948	0.71	293.31	
Accentuated interstitial design	no	587	28.9208	45.10893	0.04	293.31	0.310
	yes	56	22.6541	31.23921	1.95	121.48	
Fibronodular sequelae	no	598	29.0326	44.85272	0.04	293.31	0.067
	yes	45	19.6362	31.39343	0.04	148.48	
Crazy look paving	no	639	28.0954	43.86517	0.04	293.31	0.042
	yes	4	73.0400	64.40576	5.01	148.48	
Pulmonary nodule	no	628	28.0402	43.42670	0.04	293.31	0.422
	yes	15	42.3940	66.87906	0.28	203.40	
Other changes	no	593	27.9897	44.39448	0.04	293.31	0.446
	yes	50	32.9448	40.45563	0.71	174.40	

ROC analysis for CRP values at admission reveals an AUC coefficient (Area under Curve) of 0.756, with a confidence interval between 0.711 ÷ 0.802, statistically significant (p = 0.000**) and with moderate efficiency in predicting pulmonary changes - table 11. The identified cut - off value is 7.800, corresponding to a sensitivity of 0.620, quite low, and a specificity of 0.810 - therefore, a CRP value below the limit of 7.800 represents an 81%.

The efficiency of CRP values in predicting the appearance of ground glass is lower (0.633), for the cut - off value of 10.52, with rather poor sensitivity and specificity. Similarly, the effectiveness of CRP values in predicting the appearance of confluence is also relatively low (0.690), but the identified cut - off value of 6.245 has a fairly good sensitivity of 0.889, which means that patients with CRP above the limit of 6.245 will have confluence in 88.9% of cases (tab. XII, figs. 2, 3, 4). The CRP values bigger than 6.245 but smaller than 7.800, which correspond to a risk of confluence of 88.9%, and to a probability of 81% to not have major changes on CT - were identified in a subsample of 43 patients, having the following features: 12 patients (27.9%) have confluence, but only one of them has also changes on CT, being febrile; other 3 patients have changes on CT but are not febrile, and the rest of 8 patients are also afebrile and the pulmonary CT was not achieved.

TABLE XII.

ROC analysis for CRP values - admission

	AUC	p	95% confidence interval of AUC		Cut -off value	Sensitivity	Specificity
			L. inf.	L. sup.			
Modified CT	0.756	0.000**	0.711	0.802	7.800	0.620	0.810
Ground glass appearance	0.633	0.000**	0.593	0.674	10.520	0.556	0.688
Confluent	0.690	0.000**	0.620	0.761	6.245	0.889	0.426
Evolution - febrile state	0.615	0.008**	0.540	0.689	4.540	0.896	0.331

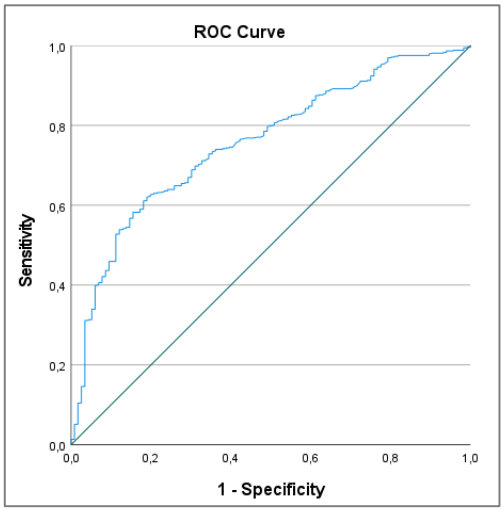

Fig. 2. CRP values *vs.* Modified CT - ROC curve

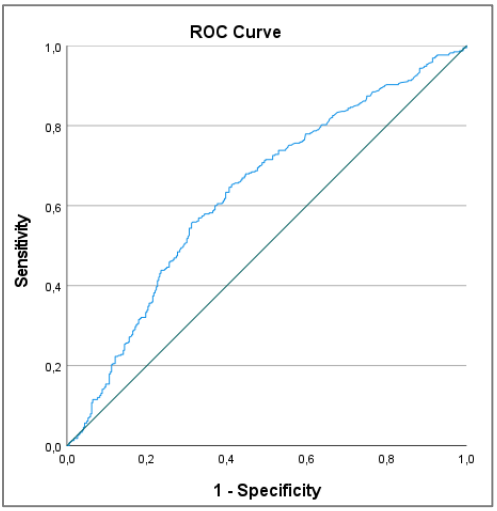

Fig. 3. CRP values *vs.* Ground glass appearance - ROC curve

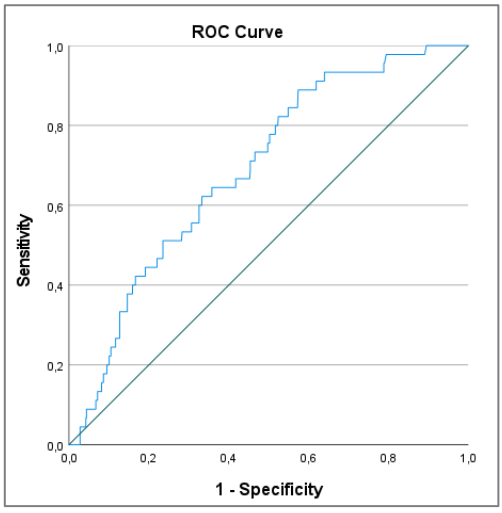

Fig. 4. CRP values *vs.* Confluence – ROC curve

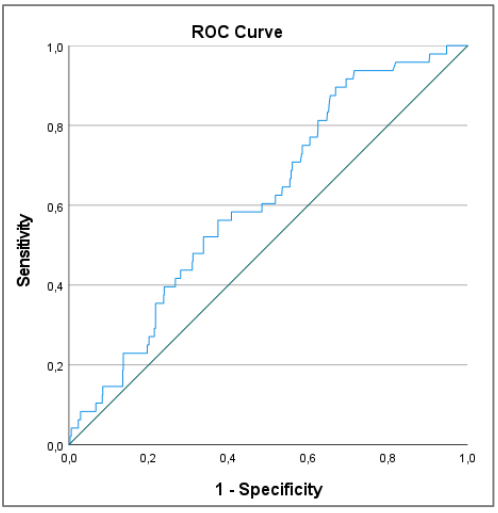

Fig. 5. CRP values *vs.* Evolution – ROC curve

CRP values are almost double in febrile patients (with an average of 41.9288 ± 8.82026) compared to afebrile ones (27.9770 ± 1.71499), the difference being also statistically significant - table 12. The associated AUC coefficient is 0.615, statistically significant but quite reduced, again the cut - off value identified, of 4.540, has a sensitivity of 0.896, which means that patients with CRP above the limit of 4.540 will present a febrile state in 89.6% of cases (tab. XIII, fig. 5).

TABLE XIII.

Comparative CRP values according to the evolution of the patients

		N	Mean	SD	Minimum	Maximum	p
Evolution	Afebrile / Sub febrile	682	27.9770	1.71499	0.04	293.31	0.043*
	Febrile	48	41.9288	8.82026	1.65	285.94	

DISCUSSION

The results of our study are superimposable on those obtained by Amorim dos Santos J, and collaborators (2021), highlighting that xerostomia was a prevalent oral manifestation in these patients diagnosed with SARS-CoV-2 virus (26).

Salivary secretion was frequently affected in patients with COVID 19. There was no significant gender difference but burning sensation and dysphagia was present in these patients. Xerostomia significantly disrupted the comfort of patients with long-term repercussions on dental status. Studies by Gherlone and collaborators in 2021 associated the presence of xerostomia with salivary duct dilatation, which highlights the existence of a significant inflammatory response in the salivary glands (27).

In SARS-CoV- 2 infection oral mucosal lesions were present in a lower proportion than the presence of xerostomia, patients diagnosed with COVID 19 had oral mucosal lesions within 10 days of infection. Geriatric individuals or those who had complications requiring long-term hospitalization with poor hygiene were more susceptible to the presence of oral mucosal lesions.

The results of our study are also consistent with the observations in the study by Amorim dos Santos J and collaborators (2021) regarding the prevalence of fungal infections in patients with SARS-CoV-2 (26). Equally gingivitis and bleeding gums were frequently noted in these patients. Brandão and collaborators in a study conducted in 2021 found the presence of lesions similar to aphthous clinical entities the aspects that individualized them were represented by round or irregular shapes of the presence of an erythematous halo and the entire surface being covered by a white pseudo membrane (28).

Periodontal disease affected a significant proportion of patients who were diagnosed with severe periodontitis. A number of research studies have suggested that periodontal pathogens may aggravate the symptoms of pneumonia or lead to increased levels of systemic inflammatory cytokines.

Almost all patients had elevated CRP at admission, its values being significantly increased in male patients over 60 years of age with symptoms and comorbidities. There is a clear association between increased CRP values and the presence of lung changes on CT, particularly in patients with ground glass appearance and confluence. ROC analysis shows that a CRP value below the cutoff of 7.800 represents an 81% probability that the patient will not have pulmonary complications, and a CRP value above the cutoff of 6.245 is associated with an 88.9% probability that the patient will have confluence. Also, CRP values are almost double in febrile patients compared to afebrile ones, and patients

with CRP above the limit of 4,540 will present a febrile state in 89.6% of cases. The evolution of patients with COVID-19 could be significantly influenced by xerostomia periodontal disease, aspects that required well-managed trajectories of oral health monitoring of these patients.

The limits of the study can be related to the limited therapy of oral pathology in the context of aggravated general condition, the therapeutic solution in the oral area being only for emergencies.

CONCLUSIONS

Most patients had a good evolution, being afebrile or sub febrile, the percentage of febrile patients being 6.3% of the total. Symptoms present in significantly increased percentages in febrile patients compared to others were cough, fever at onset, loss of appetite, nausea/vomiting and diarrhea, without identifying associated comorbidities; multivariate analysis identifies 3 significant predictors for the occurrence of febrile conditions, namely fever at onset, diarrhea and the presence of at least 3 cumulative symptoms.

Pulmonary changes statistically significantly associated with worsening evolution are ground glass appearance and confluence.

Oral signs including dysgeusia, xerostomia, oral mucosal lesions and periodontal pathology could be associated with SARS-CoV-2 infection or equally could be considered secondary lesions of COVID19 infection, which are extremely useful elements in the early diagnosis of people with COVID-19.

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INCREASED ANXIETY AMONG HEMODIALYSIS PATIENTS DURING THE COVID-19 PANDEMIC. A SINGLE CENTER EXPERIENCE

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INCREASED ANXIETY AMONG HEMODIALYSIS PATIENTS DURING THE COVID-19 PANDEMIC. A SINGLE CENTER EXPERIENCE (Abstract): The COVID-19 pandemic affected millions of people worldwide, and the hemodialysis population was one of the most affected. Anxiety, defined as an affective state characterized by psychomotor restlessness, indistinct fear, and no discernible object, is growing worldwide and among hemodialysis patients, as well. The pandemic has extensively worsened social and economic challenges and has further expanded existing psychosocial alterations in this patient category. With a heightened awareness of their susceptibility to contracting COVID-19 and experiencing severe complications, coupled with concerns about financial instability and the loss of loved ones, the imposition of quarantine and isolation measures has intensified levels of anxiety, depression, and sleep disturbances among these patients. **Materials and methods:** In the current study, all 102 patients included were enrolled in the chronic hemodialysis program at AVITUM-BBRAUN Suceava. All patients included in the analysis completed the Kidney Disease and Quality of Life (KDQOL™-36) questionnaire. **Results:** The findings revealed an increase in anxiety, perception of pain, and sleep disturbances, contributing to an overall low quality of life Score among the participants. **Conclusions:** Chronic kidney disease profoundly impacts the daily lives of HD-patients, affecting not only physical health but also emotional well-being and quality of life. COVID-19 pandemic had an additional negative impact. Providing adequate support and care to patients with chronic kidney disease is crucial for effectively managing the condition and improving their quality of life and adherence to treatment. **Keywords:** QUALITY OF LIFE, COVID-19, ANXIETY, PATIENT SELF-ASSESSMENT, DIALYSIS, OUTCOMES.

Quality of life is determined by individuals' perceptions of their social situation in accordance with their own needs, standards, and aspirations (1). It encompasses physical, mental, and social well-being, as well as the ability to perform everyday tasks (2).

The concept of quality of life includes various elements related to the physical, economic, social, health, and disease situations of individuals. It encompasses the content and nature of activities in which individuals engage, the characteristics of social relationships and processes in which they participate, as well as subjective states of satisfaction, dissatisfaction, happiness, and frustration. The concept of quality of life in relation to health sets out various

dimensions or areas that contribute to overall well-being and satisfaction with one's health status. These include symptoms perceived and reported by the patient, physical function, professional and household activities, social interactions, psychological well-being, side effects of treatment, and the patient's perception of the disease itself (3).

Hemodialysis patients face unique challenges in maintaining their quality of life. They not only struggle with chronic health issues associated with kidney failure but also must manage the demanding nature of time-consuming therapy sessions. The increased attention given to quality of life is evident from the growing number of research studies devoted to its exploration, considering it a significant predictor of mortality and morbidity (4, 5).

Despite global concern for improving the quality of life of chronic patients, the prevalence of depression, anxiety, and sleep disorders is constantly increasing compared to the general population (6). The COVID-19 pandemic affected millions of people worldwide, and the hemodialysis population was one of the most affected. Anxiety, defined as an affective state characterized by psychomotor restlessness, indistinct fear, and no discernible object, is growing worldwide and among hemodialysis patients (7). It is important to note that the relationship between anxiety and quality of life is bidirectional. This means that while anxiety can have a negative impact on one's quality of life, factors such as social support, adaptation strategies, and access to mental health resources can also influence the experience and severity of anxiety symptoms (6).

COVID-19 infection, a severe respiratory infection caused by SARS-CoV-2, spread rapidly worldwide, leading the World Health Organization (WHO) to declare it a pandemic in March 2020. The pandemic affected millions of people worldwide, with a mortality rate in patients over 65 years of age of 34.5% (8). COVID-19 infection is caused by SARS-CoV-2, which belongs to the Beta coronaviruses (CoV) and manifests as severe respiratory syndrome. The outbreak originated in the Chinese city of Wuhan in December 2019 as an atypical viral pneumonia that quickly spread worldwide (9). Transmission occurred rapidly through direct contact with infected individuals, contact with contaminated surfaces or biological products (10, 11).

According to the December 2020 COVID-19 Weekly Epidemiological Update released by the World Health Organization (WHO), there were reported 79.2 million confirmed cases of COVID-19 and over 1.7 million deaths worldwide. While some patients were asymptomatic, others experienced a wide spectrum of symptoms such as fever, cough, myalgia, diarrhea, nausea, and anosmia, leading up to severe respiratory failure, septic shock, and multiple organ failure (10, 11).

Organic lesions were associated with a cytokine storm, vascular endothelium injury, and disseminated vascular coagulation, resulting in increased occurrences of strokes, myocardial infarctions, or pulmonary thromboembolisms (12).

The global pandemic has worsened social and economic challenges on a broad scale, and has additionally heightened preexisting psychosocial issues within this specific patient population (13). Displacements between home and dialysis centers, patient influx in confined spaces as well as interactions with medical staff have made hemodialysis patients, who have compromised immune systems, a vulnerable category for contracting COVID-19 (14).

The anxiety triggered by stressors related to COVID-19 includes apprehension regarding the contagious nature of the disease, financial instability, the loss of family members, friends, or colleagues, and the imposition of mandatory quarantine and social isolation. This is especially crucial for patients who already contend with heightened vulnerability due to elevated levels of physical and mental distress. In individuals undergoing dialysis, symptoms of depression and anxiety are prevalent and correlate with adverse clinical outcomes such as diminished quality of life, heightened rates of hospitalization, and increased mortality (15). Previous studies on epidemics have shown that the level of anxiety and depression in a group was much higher than the risk of contracting the disease (9). The attitude of hemodialysis patients towards the disease varies depending on their level of education, personal perception, family and surrounding support. Thus, the perception of the disease, dependence on the dialysis machine, dietary restrictions, comorbidities, and chronic pain are associated with restlessness, insecurity, and a sense of constraint due to limited mobility, all leading to reactive psychological suffering (16).

Anxiety, defined as an affective condition, is the emotion of fear that arises in stressful or threatening circumstances. It is a typical reaction to threat, but if it becomes excessive or lasts for a long time, it may be considered an anxiety disorder (10).

Unlike depression that has been extensively debated, anxiety has been less studied, although previous research showed that over 50% of hemodialysis patients with psychological impairment associate anxiety-depressive ailment (17). Anxiety and depression lead to sleep disorders, which over time develop into chronic fatigue (both mental and physical), deteriorating everyday life and exacerbating symptoms of depression and/or anxiety, thereby entering a vicious circle (18,19). Consequently, anxiety and depression remain associated with demographic, socio-economic and educational factors, with different prevalence rates depending on these factors (10).

Suceava was the first region in Romania seriously affected by COVID-19, with the first and most severe restrictions imposed. The dialysis population was one of the most affected categories of chronic disease patients during the pandemic, which is why we wanted to evaluate the impact of the first wave of COVID-19 among this population.

MATERIALS AND METHODS

In the current study, all 102 patients included were enrolled in the chronic hemodialysis program at AVITUM-BBRAUN Suceava. The study was conducted with the approval of the ethics committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi and the ethics committee of Avitum Romania (since late 2019).

All patients included in the analysis completed the Kidney-Disease and Quality of Life (KDQOL™-36) questionnaire. Dialysis centers currently use the KDQOL-36 tool to evaluate diverse facets of patients' quality of life throughout their dialysis treatment. The insights gleaned from responses to KDQOL-36 scales are subsequently utilized to customize individualized care plans for each dialysis patient. The evaluation questionnaire used in our study aimed to assess the impact of COVID-19 on various areas, including physical Score, pain intensity, daily activity, social functionality, mental health com-

ponent, sleep quality, social support, patient satisfaction with medical care, and anxiety. All patients completed the questionnaire during their dialysis sessions.

Microsoft Office Excel/Word 2013 and IBM *SPSS Statistics 25.0* were used for the statistical analysis. The Shapiro-Wilk test was used to determine the distribution of quantitative data, which were then displayed as means with standard deviations or as medians with interpercentile intervals. Using the Mann-Whitney U test, independent quantitative variables with non-parametric distributions were examined. Depending on the equality of variances shown by the Levene test, independent quantitative variables with normal distribution were evaluated using either the Student' t-test or the Welch's t-test. When assessing correlations between quantitative variables, non-parametric variables were correlated using Spearman's rho correlation coefficients, while normal distribution variables were correlated using Pearson correlation coefficients.

RESULTS

The fist table embodies parameters related to physical functioning, emotional well-being, daily activity performance, and social interactions. Each of the KDQOL-36 kidney-specific scales is Scored by transforming all items linearly to a range of 0–100 and then averaging the Scores. The results are presented with its mean ± standard deviation and median (with interquartile range), minimum and maximum deviation average.

TABLE I.

KDQOL-36 Assessment Scores

Parameter	Mean ± SD	Median (IQR)	Min	Max
Score-PF	50 ± 22.04	55 (35-65)	0	90
Score-RP	46.57 ± 45.6	50 (0-100)	0	100
Score-BP	50.49 ± 21.91	55 (42.5-67.5)	0	100
Score-GH	22.16 ± 10.35	25 (15-30)	0	40
Score-PCS	42.3 ± 19.56	43.75(29.21-60)	0	76.3
Score-VT	37.74 ± 16.17	40 (30-50)	0	65
Score-SF	51.47 ± 21.49	50 (37.5-62.5)	0	100
Score-RE	47.05 ± 42.04	33.3 (0-100)	0	100
Score-MH	51.92 ± 13.7	56 (48-60)	4	68
Score-MCS	47.04 ± 18.91	50.62 (35.14-62.28)	1	80
Score-CKD Symptoms	83.25 ± 13.67	85.41 (79.16-92.18)	29.2	100
Score-CKD Effects	60.43 ± 12.67	60.71 (53.57-71.42)	21.4	82.1
Score-Weight CKD	29.22 ± 13.79	31.25 (18.75-37.5)	0	50
Score-status career	45.09 ± 14.94	50 (50-50)	0	50
Score-Sleep	58.62 ± 15.84	62.5 (55-67.5)	7.5	85
Score-social support	88.88 ± 12.03	83.33 (83.33-100)	66.7	100
Score-dialysis support	94.6 ± 8.15	100 (87.5-100)	75	100
Score-patient satisfaction	95.26 ± 7.91	100 (83.33-100)	66.7	100
Score-KDCS	69.42 ± 6.79	70.71 (65.9-75)	45	79.3

PF- Physical Functioning Score; RP- Role Physical Score; BP- Bodily Pain Score; GH- General Health; PCS- Physical Component Score; VT- Vitality Score; SF- Social Functioning; RE-Role-Emotional; MH-Mental Health; MCS-Mental Component Summary; CKD- Chronic Kidney Disease; KDCS- Kidney Disease Composite Score.

The findings indicate low scores in physical functioning, emotional well-being and increased levels of sleep disturbances and pain. Conversely, the evidences demonstrate strong social support, high level of patient satisfaction with medical care and support (patient satisfaction and dialysis support), and positive mental health.

TABLE II.
Correlation Between Quality of Life and Chronic Kidney Disease

Correlation	p*
Score_BP (p<0.001**) x Score_GH (p<0.001**)	<0.001, R=0.345
Score_BP (p<0.001**) x Score_MH (p<0.001**)	<0.001, R=0.380
Score_BP (p<0.001**) x Score_MCS (p=0.001**)	<0.001, R=0.481
Score_BP (p<0.001**) x Score_Sleep (p<0.001**)	<0.001, R=0.340
Score_BP (p<0.001**) x Score_KDCS (p=0.001**)	0.004, R=0.286
Score_GH (p<0.001**) x Score_MH (p<0.001**)	<0.001, R=0.563
Score_GH (p<0.001**) x Score_MCS (p=0.001**)	<0.001, R=0.500
Score_GH (p<0.001**) x Score_EffectsCKD (p=0.001**)	<0.001, R=0.478
Score_GH (p<0.001**) x Score_Sleep (p<0.001**)	<0.001, R=0.371
Score_GH (p<0.001**) x Score_KDCS (p<0.001**)	<0.001, R=0.525
Score_PCS (p<0.001**) x Score_MH (p<0.001**)	0.002, R=0.296
Score_PCS (p<0.001**) x Score_MCS (p=0.001**)	<0.001, R=0.521
Score_PCS (p<0.001**) x Score_EffectsCKD (p=0.001**)	0.002, R=0.300
Score_PCS (p<0.001**) x Score_Sleep (p<0.001**)	<0.001, R=0.347
Score_PCS (p<0.001**) x Score_KDCS (p<0.001**)	<0.001, R=0.425
Score_MH (p<0.001**) x Score_EffectsCKD (p=0.001**)	<0.001, R=0.415
Score_MH (p<0.001**) x Score_KDCS (p<0.001**)	<0.001, R=0.357
Score_MCS (p=0.001**) x Score_Sleep (p<0.001**)	0.002, R=0.302
Score_MCS (p=0.001**) x Score_KDCS (p<0.001**)	0.004, R=0.282
Score_EffectsCKD (p=0.001**) x Score_Sleep (p<0.001**)	0.003, R=0.288

BP- Bodily Pain Score; GH- General Health; PCS- Physical Component Score; MH-Mental Health; MCS-Mental Component Summary; CKD- Chronic Kidney Disease; KDCS- Kidney Disease Composite Score.

Table II depicts the correlation between various aspects of quality of life and chronic kidney disease. Each correlation is indicated by the Pearson correlation coefficient (R) along with its corresponding p-value. The p-values are denoted as significant (**p<0.001) or marginally significant (*p=0.001). These correlations provide insights into how various aspects of quality of life are interrelated in the context of chronic kidney disease.

The correlation between Score BP (bodily pain) and Score GH (general health) is highly significant (p<0.001) with a correlation coefficient (R) of 0.345, indicating a moderate positive correlation between blood pressure and general health. Similarly, each correlation entry in the table provides insights into the strength and significance of the relationship between different aspects of quality of life and chronic kidney disease.

The strength of the correlation is interpreted based on the magnitude of the correlation coefficient (R), where values closer to 1 indicate a stronger correlation, with positive values indicating a positive relationship and negative values indicating a negative relationship**.**

The correlation between Score GH and Score MH has an R-value of 0.563, indicating a relatively stronger positive association between general health and mental health (tab. II).

On the other hand, correlations such as Score MCS and Score Sleep have R-values of 0.302, suggesting a weaker positive relationship between mental health and sleep quality. The significant positive correlations observed between Score BP and other parameters such as Score GH, Score MH, Score MCS, and Score Sleep indicate an association between higher levels of bodily pain and poorer general health, mental health, and sleep quality.

DISCUSSION

The study investigated the impact of the COVID-19 pandemic on the health-related quality of life and physical activity levels of individuals undergoing hemodialysis. It involved 102 hemodialysis patients surveyed in October 2020. Descriptively, the findings revealed an increase in anxiety, perception of pain, and sleep disturbances, contributing to an overall low quality of life score among the participants. In line with previous research, the study found that physical functioning was significantly reduced in hemodialysis patients compared to the general population. This observation remained consistent with the findings of other studies (20).

The average age of the participants in the examined group was 57.28 years, with a standard deviation of ±13.23 years. Patients undergoing hemodialysis frequently experience fatigue and weakness during and after their dialysis sessions, which can persist between treatments and hinder their ability to engage in daily activities and exercise (21). Additionally, individuals undergoing hemodialysis commonly experience bone mineral disorders, which increase the risk of bone fractures, bone pain, and osteoporosis. These conditions can restrict mobility, elevate the risk of falls and fractures, and consequently impact physical functioning (22). Moreover, peripheral neuropathy, characterized by sensory disturbances (such as numbness or tingling) and motor deficits in the extremities (weakness or loss of coordination), can further compromise balance, coordination, and mobility, thereby affecting physical functioning and increasing the risk of falls.

The current study reveals that the physical condition of hemodialysis patients is reduced by half compared to normal levels, a finding consistent with previous research (19).

Despite variations in prevalence and perception among hemodialysis patients, the intensity of pain remains high, as observed in our survey. Pain experienced by these individuals can arise from various sources, including vascular access sites, musculoskeletal issues, muscle cramps, discomfort from prolonged immobility, and neuropathic conditions. Factors such as diabetes, vitamin deficiencies, osteoporosis (21) and uremic toxins can exacerbate pain symptoms (22). Notably, numerous studies have demonstrated a significant relationship between a low pain index and psycho-emotional balance, indicating similar be-

havioral patterns between the two (23).

The COVID-19 pandemic significantly disrupted the daily activities of the patients under study, aligning with findings from prior research, primarily attributed to imposed isolation and restrictions (24). Among HD subjects, a notable correlation exists between increased mortality rates and self-reported low levels of physical function. This association is compounded by the observation that HD patients are considerably more sedentary compared to other inactive populations.

Literature research indicates that HD patients classified as sedentary, face a more than 60% higher risk of mortality annually than those who engage in regular physical activity. Enhancing muscle strength represents a potentially beneficial factor for HD patients (25), achievable through the incorporation of progressive resistance training into their exercise regimens. Despite the significant link between physical function and outcomes for HD patients, such as mortality, objective measures of physical function are underutilized.

Our results did not indicate an impact upon the enrolled subjects' careers, as the sensitive majority were not employed, consistent with findings from other studies (26). The thrice-weekly dialysis program, along with the lengthy four-hour sessions and the logistics of commuting between home and the dialysis center, influence and often restrict patients' ability to maintain employment; this might be one of the most reasonable explanations regarding their job-free status. Moreover, it is worthy to mention that during the pandemic, patients largely relied on sickness benefits, disability pensions, and social support without experiencing significant income impacts.

The study findings revealed an increase in sleep disorders during the pandemic, in line with previous research (26). Sleep plays a vital role in supporting immune function, metabolism, and cardiovascular health. Insomnia, a common sleep disorder characterized by difficulty in falling or staying asleep, or non-restorative sleep, is closely linked to the onset and exacerbation of mental health issues such as depression, anxiety, and mood disorders. Chronic sleep disturbances can contribute to mood and emotional disorders, resulting in heightened irritability, stress, and emotional volatility. They can significantly diminish overall quality of life by affecting physical health, mental well-being, and social functioning (27).

Insomnia is prevalent among hemodialysis patients, with contributing factors including pain, discomfort during dialysis, restless legs syndrome, anxiety, depression, medication side effects, obesity-related sleep apnea, and fluid overload (28, 29). During the COVID-19 pandemic, sleep disorders among hemodialysis patients have become an even more pressing concern due to added stressors and disruptions in routine healthcare services, including night shifts for COVID - positive patients. Consequently, there has been a disturbance of the circadian rhythm, the internal clock of the body that regulates sleep-wake cycles (30).

The study results indicate an elevated level of patient satisfaction regarding the quality of medical care and support from medical staff during the pandemic, consistent with findings from other studies. (31). Satisfaction is often subjective and influenced by personal preferences, values, and expectations. It reflects an individual's

perception of the extent to which their needs, desires, and goals are met across various aspects of their lives (32).

The increased satisfaction observed in our study can be attributed to the safety measures implemented in dialysis centers, including screening protocols, the use of personal protective equipment, infection control practices, and effective communication between medical staff and patients. These measures, along with the support provided and the individualized approach to meeting each patient's needs by the medical staff from AVITUM-BBRAUN Suceava, Romania, contributed to the augmented satisfaction levels.

In our study, we observed a notable elevation in the level of social support among the participants. This finding suggests a positive trend towards enhanced social networks and interpersonal connections within the studied population. Such an increase in social support may play a crucial role in buffering against the negative effects of stress and adversity, potentially contributing to improved overall well-being and resilience among individuals facing health challenges. Further analysis and exploration of the mechanisms underlying this observed increase in social support could provide valuable insights into the factors influencing social dynamics and support systems within this context. These results align with most studies indicating that social support is crucial for maintaining mental health and a good quality of life. Studies conducted in Ethiopia and the USA among dialysis patients of various races have pointed out that limited social support is associated with reduced quality of life and emotional imbalance (32, 33).

Constantly worrying about the possibility of contracting the disease leads to hypervigilance regarding symptoms, excessive hand washing, and avoidance of situations or places perceived as risky. The overall perceived stress score was found to be significantly higher in the HD patient group. It is possible that the need for regular treatment at a dialysis center, along with the inability to adhere to quarantine or social distancing recommendations, played a role in the high level of anxiety observed among patients undergoing HD. Previous studies have also reported elevated levels of anxiety during the pandemic, which may stem from fears of infection, social isolation, physical distancing measures, and economic challenges (34). In this context, the study's findings revealed an extensive level of anxiety among the participants, which aligns with findings from some previous studies (35, 36).

Death anxiety can be described as a feeling of worry, irritability, or fear stemming from awareness of a real or perceived threat to one's existence. Patients undergoing dialysis, who rely on the treatment to sustain their lives, often experience a shortened quality of life and a reduced life expectancy, which can contribute to death anxiety.

Despite the fact that hemodialysis therapy has improved survival rates, grappling with a serious health condition can exacerbate feelings of death anxiety. Moreover, given its association with various psychological disorders, death anxiety represents a significant psychological symptom that warrants attention.

Anxiety related to the coronavirus pandemic, often referred to as coronavirus-generated anxiety, pertains to instances of dysfunctional anxiety triggered by the

COVID-19 pandemic. Research conducted on the general population during the pandemic has indicated that heightened levels of anxiety related to COVID-19 can trigger severe psychological issues (37, 38, 39).

The region where the study was carried out, Suceava county, was particularly hard-hit by the pandemic, which may account for the expanded degree of anxiety observed among the participants.

Concerns about contracting the infection, especially given their underlying health conditions, as well as apprehensions about virus transmission within the dialysis unit, likely contributed to this heightened anxiety.

The COVID-19 pandemic has introduced significant challenges for hemodialysis patients, exacerbating existing difficulties and introducing new stressors that have had a profound impact on their health, well-being, and quality of life.

The current study revealed that patients experienced worsened sleep disorders and increased pain intensity, both of which had significant implications for their overall quality of life.

Recognizing and addressing sleep disorders through targeted interventions and interdisciplinary collaboration are crucial steps toward improving sleep quality and optimizing outcomes related to insomnia within this population.

Moreover, individuals with higher levels of education may exhibit greater self-confidence and self-control, potentially leading to better treatment adherence. With a more extensive health education, these individuals may possess a better understanding of their treatment and health, which could encourage them to more effectively adhere to medical guidelines.

The connection between educational attainment and psychosocial factors, such as stress and coping skills, alongside treatment adherence, is likely multifaceted and complex.

In conclusion, chronic kidney disease profoundly impacts the daily lives of affected patients, affecting not only physical health but also emotional well-being and quality of life.

Considering that new research indicates that many patients place a higher priority on life-quality outcomes - like physical independence - than on longevity, it is reasonable to conclude that providing adequate support and care to patients with chronic kidney disease is crucial for effectively managing the condition and maximizing their quality of life.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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EVALUATION OF THE QUALITY OF PROFESSIONAL LIFE OF MEDICAL STAFF IN THREE EMERGENCY MEDICINE UNITS FROM ROMANIA

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EVALUATION OF THE QUALITY OF PROFESSIONAL LIFE OF MEDICAL STAFF IN THREE EMERGENCY MEDICINE UNITS FROM ROMANIA (Abstract): Understanding the professional quality of life of medical staff and its determinants is essential for addressing significant issues in this area. A preliminary study is necessary to identify organizational weaknesses and strengths, with the ultimate goal of enhancing healthcare services and their quality. Given its impact on service quality, the professional quality of life of medical staff is a critical concern for healthcare organizations. This study **aimed** to evaluate the professional quality of life of medical staff, presenting and critically assessing the available evidence on the overall professional quality of life of medical personnel in three emergency medical units in Romania. **Materials and methods**: To evaluate the professional quality of life of medical staff in emergency medicine units, a study was conducted from September 1, 2023, to September 30, 2023. The research took place in three emergency units in Romania: "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, "Sf. Ioan cel Nou" County Emergency Hospital from Suceava and the Inspectorates for Emergency Situations (IES) from Iasi and Suceava Counties (IES). **Results**: Three groups of employees representing medical personnel from three emergency medicine units in Romania were analyzed. They are represented by 42 doctors (47.73%), 32 nurses (36.36%) and 14 workers in the Inspectorate for Emergency Situations (IESw) (15.91%). The average age of the study participants was 36.4± 8.41 years. The results indicate the mean values of the sub scores for the ProQOL subscales: compassion satisfaction, burnout and secondary traumatic stress. These were compared between the three groups according to profession (physician, nurse, IES workers). Significant differences in their mean ProQOL subscale scores were noted. **Conclusions**: Although physicians, nurses, and IES workers share many professional experiences, significant differences arise from their specific roles, work environments, and the unique stresses they encounter. Recognizing these differences is crucial for addressing their distinct needs and effectively supporting their well-being. **Keywords**: PROQOL, EMERGENCY MEDICINE UNITS, MEDICAL STAFF, BURNOUT, COMPASSION SATISFACTION, SECONDARY TRAUMATIC STRESS.

INTRODUCTION

Burnout syndrome, recognized by the WHO as a work-related disease, arises from chronic stress (1). It typically develops in high-pressure environments, with unstable work schedules, shifts, and overnight work (2, 3). This condition negatively impacts the mental and physical health of workers, particularly healthcare professionals (4). Burnout is characterized by three main components: emotional exhaustion, depersonalization, and low personal accomplishment (5).

Job satisfaction, perception of professional life, and professional quality of life collectively define the quality of professional life (6). This quality significantly impacts health services and the overall efficacy of any healthcare process. The professional quality of life of healthcare workers directly influences the quality of care provided and patient satisfaction, making it essential to consider the satisfaction of these workers. Assessing professional quality of life helps identify organizational strengths and weaknesses (7). Research indicates that implementing practices to enhance professional quality of life benefits institutions, healthcare workers, and patients alike (8, 9). Therefore, healthcare organizations must measure and address professional quality of life to improve care quality and patient satisfaction (10).

Professional quality of life is a complex, multidimensional concept shaped by personal feelings and perceptions. Factors determining this quality include management practices, relationships with colleagues, demographic characteristics, workload, job promotion opportunities, salary and rewards, and autonomy (11).

The significance of professional quality of life lies in its impact on health services and care quality (12). The relationship between professional quality of life and outcomes in professional practice is well established. While this area has been extensively studied among healthcare workers, research on nurses in emergency medicine units remains limited (13). Factors influencing professional quality of life encompass individual, social, environmental, contextual, operational, and administrative elements, as well as external factors like patient demands, healthcare policies, and the labor market (14, 15). Specific factors for emergency medicine staff include salary, workload, and educational satisfaction, and work environment, relationships with colleagues, demographic characteristics, shift work, and management style (16).

The growing shortage of medical staff has increased the responsibilities of nurses and doctors. Despite their critical role in prevention and health promotion, there is limited evidence regarding their professional quality of life (17). Identifying the professional quality of life of medical staff and the associated determinants can address major issues in this area. A preliminary study is necessary to overcome organizational weaknesses and highlight strengths, ultimately aiming to improve healthcare services and their quality (18).

The professional quality of life of medical staff is a crucial issue that healthcare organizations must examine due to its influence on service quality.

This study aimed to evaluate the professional quality of life of medical staff. It seeks to present and critically assess the available evidence on the overall professional quality of life of medical personnel in three emergency medical units in Romania.

MATERIALS AND METHODS

In order to evaluate the quality of the

professional life of medical staff in emergency medicine units, a study was carried out between September 1st, 2023 and September 30th, 2023. The research was carried out in three emergency units in Romania: "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, "Sf. Ioan cel Nou" County Emergency Hospital from Suceava and the Inspectorates for Emergency Situations (IES) from Iasi and Suceava Counties.

The study used the Professional Quality of Life (ProQoL) questionnaire as a data collection tool (19). This questionnaire (ProQOL) comprises 30 items with a 5-point Likert scale (1 = Never, 2 = Rarely, 3 = Sometimes, 4 = Often, 5 = Very often) and is composed of three subscales: compassion/satisfaction (10 items), burnout (10 items) and secondary traumatic stress (10 items).

Compassion/satisfaction items describe the positive aspects of helping work ("I get satisfaction from being able to help people"), as opposed to burnout ("I feel trapped in my work as a helper") and secondarily traumatic stress ("I think that the traumatic stress of those I help may have affected me as well") (19).

Respondents were instructed to indicate how often they had experienced each item in the past 30 days. Questionnaires were scored by summing item responses for each 10-item subscale, with higher scores indicating higher levels of compassion satisfaction, posttraumatic stress, and exhaustion. The scores from each of the subscales can be classified into compassion satisfaction, burnout and secondary traumatic stress: < 22 low; 23–41 medium; > 42 height.

The Romanian version of the ProQOL for the current study has a Cronbach's alpha coefficient (assessment of the internal consistency of the questionnaire) of 0.82 for compassion satisfaction, 0.78 for secondary traumatic stress and 0.87 for burnout.

The statistical analysis of the data was conducted using the *SPSS version 29.0* software package. Descriptive statistics were calculated for the analyzed variables. Continuous variables were reported as mean values with standard deviation (SD). The Kolmogorov-Smirnov test was used to verify the normal distribution of the variables. Qualitative variables were presented as absolute counts (n) and relative frequencies (%). Group comparisons were performed using the Pearson chi-square test and the Kruskal-Wallis test. A significance level (p-value) of less than 0.05 was considered statistically significant.

RESULTS

Three groups of employees representing medical personnel from three emergency medicine units in Romania were analyzed. They were represented by 42 doctors (47.73%), 32 nurses (36.36%) and 14 workers in the Inspectorate for Emergency Situations (IESw) (15.91%). The average age of the study participants was 36.4± 8.41 years (mean ± standard deviation).

Study participants were informed about the purpose and content of the questionnaire. They were told the following: "The ProQOL questionnaire assesses how you deal with various difficult, stressful situations at work. By difficult situations, we mean the following: exposure to a situation whereby a person is faced with an event involving death or the threat of death, serious injury, injury or a threat to physical well-being. So, we are interested in the extent to which interacting with patients with different problems affects your personal life, positively or negatively. Re-

search shows that a human connection is made between the medical setting and the patient, even if the patient is not a well-known person, and this connection can influence your way of being. Please evaluate each statement in the first part by referring to your experiences at work, not your personal life. Also, when thinking about stressful experiences, consider the definition given above, that is, stress caused by dealing with patient difficulties, not by your collegial relationships or other aspects of the workplace."

The Professional Quality of Life (ProQOL) scale measures the positive and negative aspects of working in helping professions, encompassing compassion satisfaction, burnout, and secondary traumatic stress. The analysis assessed significant differences occurring between physicians, nurses, and Intensive Emergency Services (IES) workers on specific items of the ProQOL questionnaire. Significant differences were noted between the following items 1, 5, 6, 9, 11, 12, 21, 22, 24, 25, 28, 29 and 30 (tab. I).

1. "I am in a good mood most of the time."

Physicians often experience high levels of stress due to their extensive responsibilities and the critical nature of their decisions. Their mood may be more variable compared to nurses and IES workers, influenced by the pressure of making life-or-death decisions. Nurses, while also under stress, may have more direct patient interaction, which can provide emotional rewards that boost mood. However, their mood can still be negatively impacted by the emotional labor of care giving.

IES workers often face the most acute stress due to the unpredictable and high-stakes nature of emergency services. Their mood might fluctuate significantly, often swaying between the adrenaline rush of successful interventions and the lows of witnessing trauma.

5. "I flinch or get scared when I hear different unexpected sounds."

Physicians typically work in more controlled environments where unexpected sounds are less frequent, possibly making them less likely to flinch compared to IES workers. However, the constant beeping of monitors and alarms can still be startling. Nurses are often in bustling environments with frequent alarms and calls, potentially desensitizing them to sudden noises, but prolonged exposure can still lead to heightened startle responses. IES workers are most exposed to unexpected, loud, and often distressing sounds. Their work environment predisposes them to higher startle responses due to the nature of emergencies and the need for rapid, responsive action.

6. "I feel that I still have energy after a day of working with people I help."

Physicians may experience significant mental fatigue due to the intensity of their decision-making processes and long working hours, leaving them with little energy at the end of the day. Nurses might have varying levels of energy post-shift. Physical exhaustion is common due to the demanding nature of their tasks, but emotional fulfillment from patient interaction can sometimes counterbalance this fatigue. IES workers are likely to be the most drained, both physically and mentally, after a day of responding to emergencies. The high-stress environment and physical demands of their job often leave them exhausted.

9. "I think that it is possible to be affected by the stressful situations of the people I work with."

Physicians are often required to main-

tain a level of detachment to make objective decisions, but the cumulative effect of patient stress and critical cases can significantly impact them over time. Nurses are more likely to develop close emotional bonds with patients, making them more susceptible to being affected by the stressful situations their patients endure. IES workers face the rawest and most acute forms of stress. The intensity and immediacy of emergencies can profoundly affect them, given the life-and-death situations they frequently encounter.

11. "Because of the nature of my work, I feel that different things annoy me."

Physicians might experience annoyance stemming from systemic issues, administrative tasks, or the high expectations placed on them. Nurses often face the brunt of operational inefficiencies, staff shortages, and demanding patient care routines, contributing to frequent annoyances. IES workers deal with high-stress situations where any small operational hiccup or miscommunication can lead to significant annoyance due to the potential consequences.

12. "I like the work I do (the situation of providing specialized support)."

Physicians generally have high levels of job satisfaction from providing specialized medical care and making significant impacts on patient health. Nurses often derive satisfaction from their direct patient care roles and the ability to provide comfort and support, which is a core aspect of their job. IES workers find gratification in the immediacy and impact of their interventions, though the high-stress nature of their work can sometimes overshadow this satisfaction.

21. "I feel overwhelmed because the tasks I have seem endless."

Physicians may feel overwhelmed due to the combination of patient care, administrative responsibilities, and continuous professional development requirements. Nurses often experience a similar sense of being overwhelmed due to understaffing, high patient-to-nurse ratios, and extensive care giving duties. IES workers face overwhelming situations due to the unpredictable nature of emergencies and the continuous demand for their services without much respite.

22. "I believe that through my work I am useful to society."

Physicians typically have a strong sense of societal contribution through their ability to treat and cure illnesses. Nurses also feel highly useful to society, given their essential role in patient care and recovery. IES workers often perceive their work as highly impactful, given their role in saving lives and providing critical emergency care.

24. "I am proud of what I can do for those around me."

Physicians have a deep sense of pride in their ability to diagnose and treat complex medical conditions, often reflecting years of specialized training. Nurses take pride in their hands-on care and the personal connections they build with patients, often seeing the direct impact of their work on patient recovery. IES workers have immense pride in their ability to respond effectively in high-pressure situations and make a tangible difference in emergencies.

25. "As a result of work activities, some times I have thoughts that scare me."

Physicians might experience distressing thoughts due to the high-stakes nature of their decisions and the potential for adverse patient outcomes. Nurses can be haunted by the emotional burden of patient suffering and loss, leading to distressing thoughts.

IES workers are the most likely to have frightening thoughts due to the traumatic and often life-threatening situations they encounter regularly.

28. "I can't remember important parts of my professional activity with victims of traumatic events."

Physicians might struggle with memory issues due to cognitive overload and the vast amount of information they must process daily. Nurses can experience memory lapses due to the high emotional and physical demands of their job, especially when dealing with traumatic patient cases. IES workers are prone to memory issues as a coping mechanism to manage the intense stress and trauma associated with their work.

29. "I am a caring person."

Physicians often display care through their dedication to patient outcomes and the extensive effort they put into their work. Nurses are frequently seen as the epitome of caring in the healthcare system, given their close and continuous interaction with patients. IES workers demonstrate care through their rapid, life-saving actions and commitment to providing emergency assistance.

30. "I am satisfied that I chose this profession."

Physicians generally feel satisfied with their profession due to the respect, financial rewards, and the intellectual stimulation it provides. Nurses may find satisfaction in the personal fulfillment derived from patient care and the intrinsic rewards of their care giving role. IES workers often feel a profound sense of purpose and satisfaction from the immediate and visible impact of their work, despite the high stress involved.

TABLE I.

Comparative evaluation of the ProQOL questionnaire according to the profession of study participants

Professional Quality of Life Scale Items			**Physician (n=42)**	**Nurse (n=32)**	**IES workers (n=14)**	**p-value**
Age, years, mean (SD)			33.1 (7.78)	39.8 (9.02)	38.4 (4.48)	0.001
Gender, male / female, n (%)			8/34 (19.05/80.95)	10/22 (31.25/68.75)	14/0 (100/0)	<0.001
	Never	**Rarely**	**Sometimes**	**Often**	**Very often**	**p-value**
1. I am in a good mood most of the time.						
Physician / Nurse / IESw	0/1/0 (0/3.13/0)	1/0/0 (2.38/0/0)	15/3/2 (35.71/9.38/14.29)	14/19/3 (33.33/59.38/21.43)	12/9/9 (28.57/28.13/64.29)	0.0218*
2. I try to help as many people as I can with the time I have.						
Physician / Nurse / IESw	-	1/0/0 (2.38/0/0)	4/1/1 (9.52/3.13/7.14)	16/16/1 (38.1/50/7.14)	21/15/12 (50/46.88/85.71)	0.1261
3. I feel fulfilled by the fact that I am able to help people.						
Physician / Nurse / IESw	-	0/1/0 (0/3.13/0)	4/0/1 (9.52/0/7.14)	20/11/3 47.62/34.38/21.43)	18/20/10 (42.86/62.5/71.43)	0.1754
4. I feel close to the people I interact with.						
Physician / Nurse / IESw	0/1/0 (0/3.13/0)	5/0/1 (11.9/0/7.14)	15/8/1 (35.71/25/7.14)	15/17/7 (35.71/53.13/50)	7/6/5 (16.67/18.75/35.71)	0.1484

Professional Quality of Life Scale Items			Physician (n=42)	Nurse (n=32)	IES workers (n=14)	p-value
5. I flinch or get scared when I hear different unexpected sounds.						
Physician / Nurse / IESw	15/17/7 (35.71/53.13/50)	9/8/2 (21.43/25/14.29)	9/4/0 (21.43/12.5/0)	7/1/5 (16.67/3.13/35.71)	2/2/0 (4.76/6.25/0)	0.0401*
6. I feel that I still have energy after a day of working with people I help.						
Physician / Nurse / IESw	5/6/1 (11.9/18.75/7.14)	14/3/3 (33.33/9.38/21.43)	12/6/2 (28.57/18.75/14.29)	5/15/4 (11.9/46.88/28.57)	6/2/4 (14.29/6.25/28.57)	0.0161*
7. I find it difficult to separate my personal life from my professional life.						
Physician / Nurse / IESw	10/17/6 (23.81/53.13/42.86)	17/6/4 (40.48/18.75/28.57)	9/7/1 (21.43/21.88/7.14)	4/1/1 (9.52/3.13/7.14)	2/1/2 (4.76/3.13/14.29)	0.1634
8. Sometimes I am not very efficient at work because I am affected by the difficult experiences of the people, I help.						
Physician / Nurse / IESw	11/19/8 (26.19/59.38/57.14)	14/4/2 (33.33/12.5/14.29)	12/5/0 (28.57/15.63/0)	4/3/2 (9.52/9.38/14.29)	1/1/2 (2.38/3.13/14.29)	0.2176
9. I think that it is possible to be affected by the stressful situations of the people I work with.						
Physician / Nurse / IESw	7/6/6 (16.67/18.75/42.86)	12/16/3 (28.57/50/21.43)	12/5/0 (28.57/15.63/0)	6/4/5 (14.29/12.5/35.71)	5/1/0 (11.9/3.13/0)	0.0197*
10. I am attracted to my job, to the situations where I can be helpful to others.						
Physician / Nurse / IESw	2/1/1 (4.76/3.13/7.14)	4/0/0 (9.52/0/0)	10/5/1 (23.81/15.63/7.14)	14/17/3 (33.33/53.13/21.43)	12/9/9 (28.57/28.13/64.29)	0.7944
11. Because of the nature of my work, I feel that different things annoy me.						
Physician / Nurse / IESw	3/12/6 (7.14/37.5/42.86)	13/14/3 (30.95/43.75/21.43)	9/4/1 (21.43/12.5/7.14)	11/1/3 (26.19/3.13/21.43)	6/1/1 (14.29/3.13/7.14)	0.0063*
12. I like the work I do (the situation of providing specialized support).						
Physician / Nurse / IESw	0/4/0 (0/12.5/0)	1/0/0 (2.38/0/0)	6/4/0 (14.29/12.5/0)	16/11/2 (38.1/34.38/14.29)	19/13/12 (45.24/40.63/85.71)	0.0396*
13. Sometimes I feel depressed because of the difficult experiences of the people I work with.						
Physician / Nurse / IESw	13/15/9 (30.95/46.88/64.29)	14/6/1 (33.33/18.75/7.14)	6/5/0 (14.29/15.63/0)	4/4/2 (9.52/12.5/14.29)	5/2/2 (11.9/6.25/14.29)	0.2769
14. I feel that sometimes I also experience the trauma that the people I help go through.						
Physician / Nurse / IESw	12/11/5 (28.57/34.38/35.71)	19/13/6 (45.24/40.63/42.86)	7/4/3 (16.67/12.5/21.43)	2/4/0 (4.76/12.5/0)	2/0/0 (4.76/0/0)	0.6691
15. I have certain principles that help me cope with the situations I face at work.						
Physician / Nurse / IES	1/1/0 (2.38/3.13/0)	3/4/0 (7.14/12.5/0)	8/3/1 (19.05/9.38/7.14)	19/23/4 (45.24/71.88/28.57)	11/1/9 (26.19/3.13/64.29)	0.0024*
16. I am satisfied with the way I manage to apply my knowledge and different techniques in order to provide help.						
Physician / Nurse / IESw	-	-	16/1/3 (38.1/3.13/21.43)	15/19/2 (35.71/59.38/14.29)	11/12/9 (26.19/37.5/64.29)	0.0006*
17. I can say that I have achieved what I always wanted.						
Physician / Nurse / IESw	-	2/0/1 (4.76/0/7.14)	11/3/0 (26.19/9.38/0)	14/15/6 (33.33/46.88/42.86)	15/14/7 (35.71/43.75/50)	0.1591

Professional Quality of Life Scale Items			Physician (n=42)	Nurse (n=32)	IES workers (n=14)	p-value
18. The work I do gives me a lot of satisfaction.						
Physician / Nurse / IES	1/0/0 (2.38/0/0)	2/0/0 (4.76/0/0)	13/4/1 (30.95/12.5/7.14)	15/13/3 (35.71/40.63/21.43)	11/15/10 (26.19/46.88/71.43)	0.0704
19. Sometimes, I feel exhausted as a result of interacting with different people in suffering.						
Physician / Nurse / IESw	3/8/7 (7.14/25/50)	12/10/2 (28.57/31.25/14.29)	10/7/1 (23.81/21.88/7.14)	8/3/2 (19.05/9.38/14.29)	9/4/2 (21.43/12.5/14.29)	0.0681
20. I have feelings of kindness and understanding for the people I help.						
Physician / Nurse / IESw	1/1/0 (2.38/3.13/0)	1/0/0 (2.38/0/0)	8/2/1 (19.05/6.25/7.14)	16/16/2 (38.1/50/14.29)	16/13/11 (38.1/40.63/78.57)	0.1727
21. I feel overwhelmed because the tasks I have seem endless.						
Physician / Nurse / IESw	2/14/7 (4.76/43.75/50)	9/9/2 (21.43/28.13/14.29)	14/2/1 (33.33/6.25/7.14)	11/4/3 (26.19/12.5/21.43)	6/3/1 (14.29/9.38/7.14)	0.0012*
22. I believe that through my work I am useful to society.						
Physician / Nurse / IES	0/0/2 (0/0/50)	0/0/10 (0/0/14.29)	18/5/39 (56.25/35.71/7.14)	14/9/36 (43.75/64.29/21.43)	32/14/88 (0/0/7.14)	0.0158*
23. I avoid certain activities or situations because they remind me of the unfortunate experiences of the people I help.						
Physician / Nurse / IESw	19/13/10 (45.24/40.63/71.43)	13/10/2 (30.95/31.25/14.29)	5/5/1 (11.9/15.63/7.14)	3/2/1 (7.14/6.25/7.14)	2/2/0 (4.76/6.25/0)	0.7929
24. I am proud of what I can do for those around me.						
Physician / Nurse / IESw	0/2/0 (0/6.25/0)	1/0/1 (2.38/0/7.14)	10/1/0 (23.81/3.13/0)	18/9/4 (42.86/28.13/28.57)	13/20/9 (30.95/62.5/64.29)	0.0101*
25. As a result of work activities, sometimes I have thoughts that scare me.						
Physician / Nurse / IESw	14/22/11 (33.33/68.75/78.57)	9/8/1 (21.43/25/7.14)	12/1/1 (28.57/3.13/7.14)	6/1/1 (14.29/3.13/7.14)	1/0/0 (2.38/0/0)	0.0117*
26. I feel "stuck" in the system I have entered.						
Physician / Nurse / IES	13/17/9 (30.95/53.13/64.29)	13/8/2 (30.95/25/14.29)	5/2/0 (11.9/6.25/0)	8/3/2 (19.05/9.38/14.29)	3/2/1 (7.14/6.25/7.14)	0.4448
27. I believe I have a successful career.						
Physician / Nurse / IES	3/2/1 (7.14/6.25/7.14)	5/1/0 (11.9/3.13/0)	15/4/2 (35.71/12.5/14.29)	13/13/4 (30.95/40.63/28.57)	6/12/7 (14.29/37.5/50)	0.0636
28. I can't remember important parts of my professional activity with victims of various traumatic events.						
Physician / Nurse / IESw	9/14/8 (21.43/43.75/57.14)	15/10/4 (35.71/31.25/28.57)	16/4/2 (38.1/12.5/14.29)	2/4/0 (4.76/12.5/0)	-	0.0385*
29. I am a caring person.						
Physician / Nurse / IESw	-	-	9/0/0 (21.43/0/0)	19/15/5 (45.24/46.88/35.71)	14/17/9 (33.33/53.13/64.29)	0.0112*
30. I am satisfied that I chose this profession.						
Physician / Nurse / IESw	2/0/0 (4.76/0/0)	1/0/0 (2.38/0/0)	10/0/1 (23.81/0/7.14)	12/4/2 (28.57/12.5/14.29)	17/28/11 (40.48/87.5/78.57)	0.0061*

Continuous variables were expressed as mean (standard deviation), the variables did have a normal distribution.
Categorical variables: number (%); IESw – Inspectorate for Emergency Situations workers
† Pearson chi-square test; § Kruskal-Wallis test / * Marked effects are significant at $p < 0.05$

The results presented in table II indicate the mean values of the subscores for the ProQOL subscales: compassion satisfaction, burnout and secondary traumatic stress. These were compared between the three groups according to profession (physician, nurse, IES workers). Significant differences in their mean ProQOL subscale scores were noted.

The subscore for Compassion Satisfaction was significantly higher for nurses and IES workers (p = 0.0005), and for the physician the subscores for burnout (p = 0.0018) and secondary traumatic stress (p = 0.0185) were significantly higher (tab. II, fig. 1).

TABLE II.

Statistical indicators of subscores for the three ProQOL subscales by profession

Professional Quality of Life Scale SCORE SUBSCALE	Physician (n=42)	Nurse (n=32)	IES workers (n=14)	*p*-value§
Compassion Satisfaction, mean (SD)	38.40 (6.43)	42.25 (3.32)	44.79 (5.66)	0.0005*
Burnout	25.40 (5.17)	22.06 (3.62)	20.64 (6.83)	0.0018*
Secondary Traumatic Stress	25.88 (6.57)	21.91 (5.43)	22.21 (7.15)	0.0185*

Continuous variables were expressed as mean ± standard deviation (SD); the variables did have a normal distribution.

* Marked effects are significant at p < 0.05 / § Kruskal-Wallis test

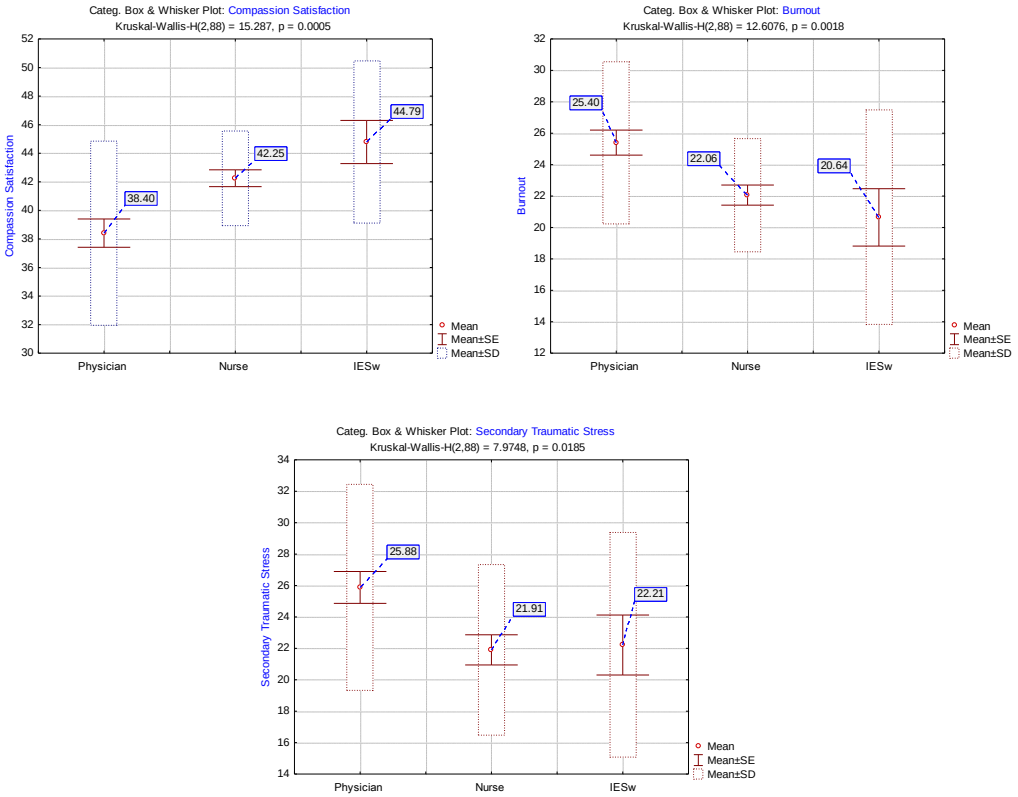

Fig. 1. Box and whisker plot for ProQOL subscores by profession

DISCUSSION

The Professional Quality of Life (ProQOL) scale assesses the positive and negative aspects of work in helping professions, focusing on compassion satisfaction, burnout, and secondary traumatic stress. This synthesis highlights the significant differences between physicians, nurses, and Intensive Emergency Services (IES) workers regarding specific items on the ProQOL questionnaire (19).

In the case of doctors, was noted a great mood variability, they are often mentally fatigued, less frequent startle response but affected by patient stress, they were also annoyed by systemic issues, high satisfaction from specialized care (20), overwhelmed by extensive responsibilities, with a strong sense of societal contribution, and pride in treating complex conditions, distressing thoughts from high-stakes decisions and memory issues from cognitive overload. Dedication to patient outcomes was generally satisfied with profession (21).

The nurses' mood was stimulated by the interaction with the patient despite the stress, there are mentioned a frequent physical exhaustion, some desensitization to sudden noises, but close bonds increase stress susceptibility. They were annoyed by operational inefficiencies, satisfaction from direct care, overwhelmed by high patient ratios, but felt highly useful, and proud of patient recovery. In this case emotional burden leaded to distressing thoughts and memory lapses (22).

Medical staff is often seen as the epitome of caring, finding deep fulfillment in their care giving roles. Their close and continuous interaction with patients allows them to provide comfort and support, which not only benefits the patients but also enhances the nurses' sense of professional satisfaction and personal accomplishment (23).

In the case of IES workers, significant mood changes were observed, being the most physically and mentally exhausted, highest startle response, profoundly impacted by acute stress. IES workers were annoyed by operational hiccups and gratification from impactful interventions. Overwhelmed by constant demand, they perceive work as highly impactful and immense pride in emergency response. IES workers often experience frequent frightening thoughts and may develop memory issues as a coping mechanism. Despite these challenges, they demonstrate their care through life-saving actions and maintain a profound sense of purpose, even under high stress (12, 24).

While all three groups-physicians, nurses, and IES workers-experience stress and derive satisfaction from their roles, the nature of their specific duties leads to significant differences in how they experience mood, energy, stress, job satisfaction, and professional pride. Understanding these differences is essential for providing tailored support to enhance their well-being and effectiveness in their respective roles (25, 26).

Hospital policies can be revised to address the root causes of physicians, nurses, and IES workers burnout syndrome. By allocating resources appropriately and conducting further research on effective prevention methods, hospitals can reduce the incidence of burnout among physicians, nurses, and IES workers. These measures will enhance the quality of workers from emergency medical units and lower turnover rates, which are essential for providing high-quality nursing care.

Workers from emergency medical units face significant challenges due to adverse working conditions. Factors such as workload overload, rotating shifts, low salaries, lack of autonomy, workplace violence, and bullying can lead to high emotional exhaustion, low accomplishment, and high deper-

sonalization (27). Additionally, lacking a partner can also contributes to these issues by depriving nurses of the security and support partners provide (17). These challenges can result in severe emotional problems, including insomnia, irritability, and stress (19). High burnout rates among nurses are associated with increased medication errors, higher infection rates, more patient falls, and lower quality of care (9, 16). Implementing spiritual interventions in the workplace may help mitigate these adverse outcomes (18). Variations in study results across countries may be due to differences in nurse-patient ratios and national medical systems' workloads (11). Furthermore, the imbalance between low salaries and high workloads significantly contributes to burnout (21). Increasing wages and reducing workloads could effectively address this issue. Differences in assessment tools also lead to varying results, as each scale has different criteria. Many nurses in the United States have considered leaving their jobs due to burnout (28). Despite these challenges, workers from emergency medical units are vital for achieving health-related goals, such as patient safety, managing non-communicable diseases, providing people-centered care, and ensuring universal health coverage (1). The prevalence of nursing burnout has been gradually increasing over the past decade, necessitating urgent attention.

CONCLUSIONS

In conclusion, while physicians, nurses, and IES workers share many similarities in their professional experiences, significant differences arise due to the nature of their specific roles, the environments they work in, and the unique stresses they face. Understanding these differences is crucial for addressing their distinct needs and supporting their well-being effectively.

While all three groups-physicians, nurses, and IES workers-experience stress and derive satisfaction from their roles, the nature of their specific duties leads to significant differences in how they experience mood, energy, stress, job satisfaction, and professional pride. Understanding these differences is essential for providing tailored support to enhance their well-being and effectiveness in their respective roles.

CONFLICT OF INTEREST AND FUNDING

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STATIC AND WHOLE-BODY IMAGES *VS*. SPECT: TOGETHER OR SEPARATE FOR AN ACCURATE DETECTION OF TRANSTHYRETIN CARDIAC AMYLOIDOSIS

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STATIC AND WHOLE-BODY IMAGES VS. SPECT: TOGETHER OR SEPARATE FOR AN ACCURATE DETECTION OF TRANSTHYRETIN CARDIAC AMYLOIDOSIS (Abstract): Transthyretin cardiac amyloidosis (ATTR-CA) is a protein deposition disease that represents a challenge to be diagnosed by the practicing physician. As the gold standard method is not widely available correlations between biomarkers, electrocardiography, echocardiography, bone scintigraphy and free light chain dosage has proven to be a reliable alternative. Bone scintigraphy represents the corner stone for ATTR-CA detection; however, some patients cannot undergo all the required images for an easy and accurate diagnosis. Therefore, the aim of this study is to determine the bare minimum images that can be performed for an accurate diagnosis. **Materials and methods:** 80 patients that met the inclusion criteria were evaluated throw bone scintigraphy. Acquired images included: whole body bone scan (early and delayed), static followed by Single Photon Emission Computed Tomography (SPECT), if possible, centered on the thorax. Images were interpreted by visual scoring and semiquantitative methods. **Results**: 20 patients were diagnosed with ATTR cardiac amyloidosis by visual scoring, however not all of them underwent all the necessary images. Semiquantitative methods results were above the established threshold and therefore sustained the visual score. Nevertheless, ratio results differed between static and SPECT images. **Conclusions**: Ideally all type of images should be acquired for this type of patients, however, we determined that a minimum of whole-body ± static centered on the thorax images interpreted throw visual scoring and semiquantitative analysis can be enough if SPECT is not possible for a reliable diagnosis. **Keywords**: WHOLE-BODY, STATIC, SPECT, ATTR-CA, VISUAL SCORING, SEMIQUANTITATIVE ANALYSIS.

Transthyretin cardiac amyloidosis (ATTR-CA) is a rare disease characterized by continuous and progressive deposition of amyloid at the myocardium level resulting in a thickening of both ventricles and inter-atrial-ventricular septum. Undiagnosed and therefore untreated, can be fatal to the patient. The mechanism behind it is based on misfolding the transthyretin protein (a plasma transport protein that circulates as a stable tetramer and transport thyroxin and retinol). ATTR-CA can be

classified as wild-type (ATTRwt) and hereditary or with mutation (ATTRm or ATTRh) (1, 2).

Diagnosing this rare disease has always represented a challenge for the practicing physician. The gold standard method, endomyocardial biopsy, has proven to be invasive and not widely available. Therefore, the European Society of Cardiology (ESC), has presented and alternative to this method, by correlating the electrocardiography, ultrasonography and bone scintigraphy characteristics. Nevertheless, screening for these patients, before reaching the actual methods for detection, is based on the so called "red flags" that offer a possible profile for the most likely ATTR-CA candidate (3). The final diagnosis for ATTR-CA is represented by bone scintigraphy, an imaging investigation technique that studies the biodistribution of a radiotracer at the skeletal level using bisphosphonates such as HDP (hydroxyethylene diphosphonate) which is mainly indicated in oncology, rheumatology, bone and joint infections, orthopedics, sports and traumatology, metabolic bone disease and pediatrics. Nevertheless, it is also indicated for detecting ATTR-CA Standard uptake mechanism for bone scan is based on the absorption to the surface of hydroxyapatite crystals and it is dependent on local bone vascularization and osteoblastic activity. However, in case of ATTR-CA the exact uptake mechanism is not completely elucidated and therefore a few theories that try to explain it have emerged that are based on the calcium level, microcalcification, the amount and type of fibrils involved (3-10).

The acquisition protocol according to the American Society of Nuclear Cardiology (ASNC) and European Association of Nuclear Medicine (EANM) practice points guideline is based on acquiring planar centered on the thorax (anterior, lateral and left anterior oblique) or whole body (WB) images, followed by SPECT centered on the thorax at 1 hour with optional acquisition at 3 hours after the iv administration of the radiotracer. Image processing and interpretation is based on the Perugini visual score that analysis the uptake of the myocardium and a semiquantitative analysis based on comparing the heart uptake with the uptake contralateral side (H/CL) and the rib (H/R). According to the ASNC practice points guide the threshold for H/CL ratio for ATTR positive patients should be over 1.5 on SPECT images at one hour. However, for comparing the heart to the rib uptake, the guide suggests a visual evaluation (11-13).

Therefore, a correct assessment of heart uptake using visual score and semiquantitative methods may represent a solution for a better and more accurate detection. Nevertheless, basing the ratio result only on SPECT images obtained at one hour may prove to be useful in patients that have a referral diagnosis of possible cardiac amyloidosis. For patients that have been accidentally diagnosed (different referral diagnosis), most likely the SPECT at one hour step was missed. As a result, the diagnosis was most likely based on the static images. The aim of this paper is to compare the visual score and the semiquantitative method of both static and SPECT centered on the thorax images in order to determine the bare minimum of images that can be acquired for an accurate diagnosis.

MATERIALS AND METHODS

The lot was comprised of patients from the Nuclear Medicine Laboratory of "Sf. Spiridon" County Clinical Emergency

Hospital Iasi archive (2015-2023) and was based on the following criteria: patients with a referral diagnosis of possible cardiac amyloidosis, ATTR positive patients after bone scintigraphy regardless of the referral diagnosis, ATTR positive patients that underwent both static and SPECT centered on the thorax images. The analysis was focused especially on the patients that had underwent both static and SPECT images.

The patients were required to sign an informed consent. All the protocols were approved by the Ethics Committee of both the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania and "Sf. Spiridon" County Clinical Emergency Hospital of Iasi.

Bone scintigraphy acquisition protocol include early and delayed whole body (WB) images, static centered on the thorax images early (WB unavailable) and delayed followed by SPECT centered in the thorax.

Images processing and interpretation relied on the Perugini visual score and a semiquantitative method based on ratios between different regions of interest (ROI). The ROI for the static images was made initial on the highest uptake region (from a visual point of view) of the heart muscle, without including the ribs and by using the "mirror" function it was projected on the contralateral side and placed consecutively first over one of the ribs and then between the ribs (contralateral). The ROIs made on the SPECT images were made in a similar fashion, with the initial ROI made over the heart and using the "mirror" function it was projected on the contralateral side. The ROIs in this case include the whole left ventricle and was made on the transverse and coronal incidences. However, the initial ROI over the heart was made on the slice which presented the maximum dimension of the heart and was maintained for all of the other slices. The ratio results were analyzed based on the following parameters (tab. I):

TABLE I.
Semiquantitative method results and interpretation (H=heart; R=rib; CL=contralateral)

Ratio	Result	Interpretation
H/R	> 1	Myocardial uptake greater than rib uptake
	< 1	Myocardial uptake lower than rib uptake
	1	Myocardial uptake similar to rib capture
H/CL	0 - 1.49	Absence/slight uptake of the radiopharmaceutical in the myocardium
	1.5 - 3	Moderate/increased uptake of the radiopharmaceutical in the myocardium

RESULTS

Out of 80 patients with referral diagnosis of possible cardiac amyloidosis only 20 were determined to be ATTR positive after bone scintigraphy. Due to the fact that the other patients presented visual scores and ratio result underneath the threshold they were classified as non-ATTR (n-ATTR) and required further investigations. Not all of the patients were able to undergo SPECT imaging, however, almost all underwent static centered on the thorax images and all of them made WB images. The patients that were accidentally diagnosed, presented only with WB images. Nevertheless, H/CL and H/R, were obtained regardless of the acquired images.

Visual Score

Visual score was determined on early and delayed images. Most of the early images were acquired only in patients that

presented a referral diagnosis of cardiac amyloidosis. Therefore, for patients that were accidentally diagnosed, this type of images was not acquired.

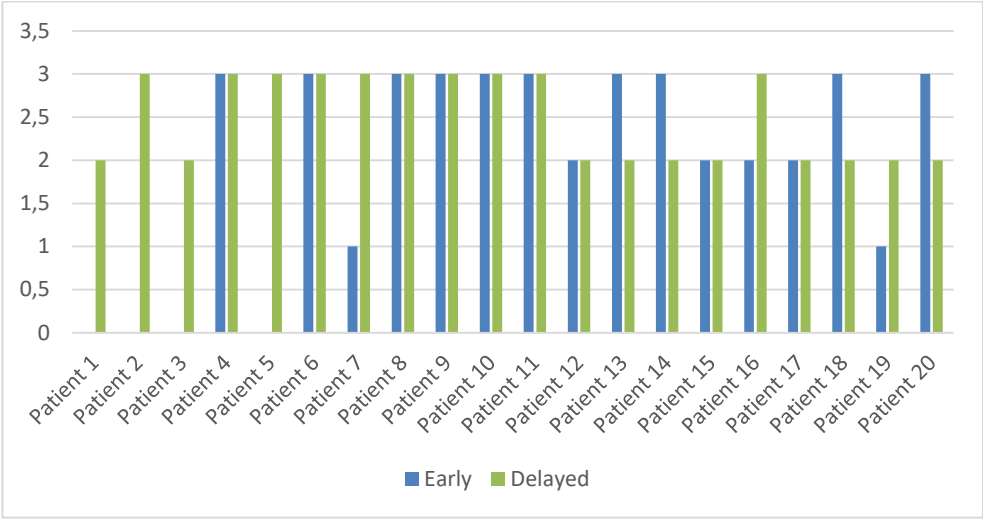
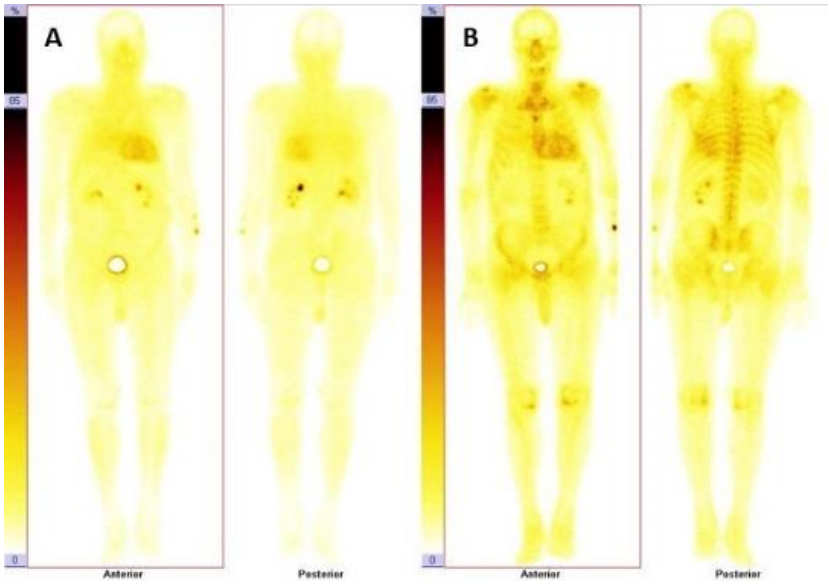
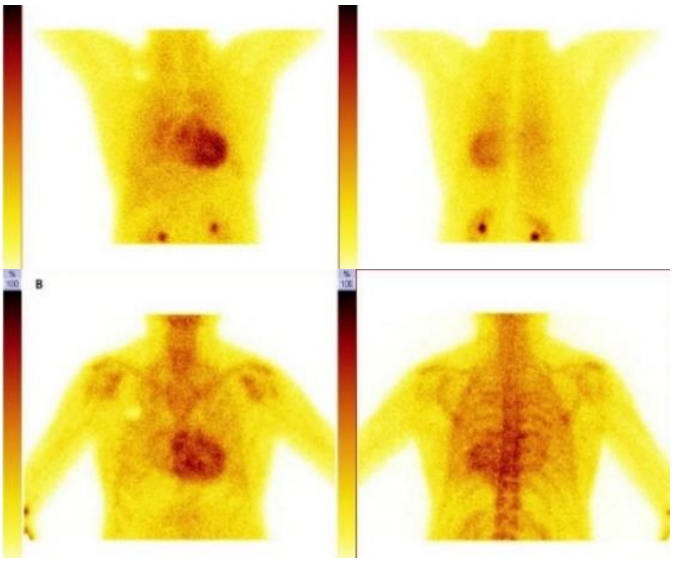


Fig 1. Visual Score of early and delayed images regarding for all 20 patients

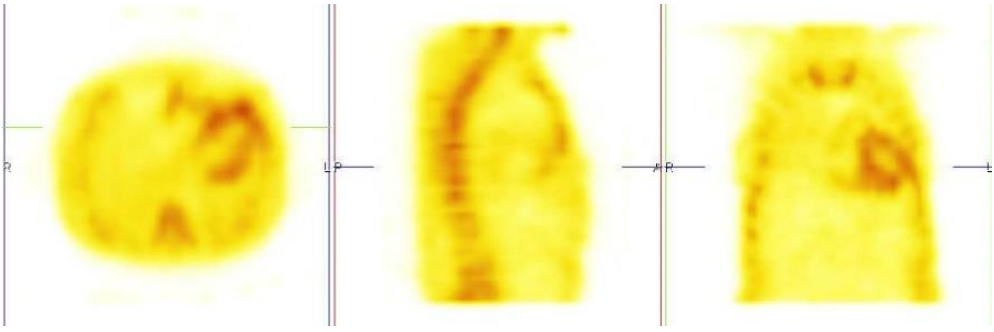
Visual scoring varied on early images, however, the aim of these images was to determine the location and dimension of the heart. Delayed images presented visual scoring typical for ATTR-CA (scores of 2 or 3).



CASE 1: 81 years old patient suspected of cardiac amyloidosis:
A. early WB images - visual score = 3; **B.** delayed WB images - visual score = 3



CASE 2: 52 years old patient suspected of cardiac amyloidosis: **A.** early static centered on the thorax - visual score = 3; **B.** delayed static centered on the thorax - visual score = 3



CASE 3: 82 years old patient suspected of cardiac amyloidosis: SPECT imaging reveals high visual uptake of the myocardium resulting in ATTR-CA

Fig 2. 3 Cases from our database - ATTR-CA positive patients determined by visual scoring Visual Scoring and Semiquantitative method

ATTR positive patients only with WB or Static images

The H/R and H/Cl ratios were performed on all of the patients that underwent bone scintigraphy and the visual score revealed a possible myocardium uptake. In accidental diagnosis in which only WB images were acquired, the ratios were performed only on those images, as static centered on the thorax were not acquired. Ratio results varied as follows: H/R: 0.9-2.08; H/Cl: 1.20-2.71. Nevertheless, in both cases, the ratio results were over the threshold and sustained the visual score.

The average of these ratio, demonstrated that the threshold of 1 for the H/R ratio and 1.5 for the H/Cl ratio represent, in association with the visual score as the bare mini-

mum for a possible diagnosis of ATTR-CA.

ATTR positive patients with WB/static and SPECT centered on the thorax images

The patients that underwent both WB/static and SPECT centered on the thorax, the ratio results differed slightly, but not to significant. In both image types, the H/R and H/Cl ratio results were over the determined threshold, therefore sustaining the visual score.

TABLE II.

Correlation between Delayed Visual Scoring and Semiquantitative Analysis on patients with WB/static and SPECT centered on the thorax images

	Visual scoring	H/R	H/CL		
	Delayed images	Static or WB	Static or WB	SPECT	
				Transvers	Coronal
Patient 4	3	1.68	2.09	2.13	2.50
Patient 6	3	1.56	1.75	1.82	1.82
Patient 7	3	2.06	2.41	2.97	2.98
Patient 11	3	1.67	1.60	2.37	2.98
Patient 13	2	1.64	1.81	2.11	1.79
Patient 14	2	1.88	2.07	2.80	2.94
Patient 15	2	1.66	1.72	1.88	1.57
Patient 16	3	1.77	2.08	2.71	2.25
Patient 17	2	1.42	2.12	1.61	1.75
Patient 18	2	1.08	1.54	2.90	2.11
Patient 19	2	1.19	1.65	2.30	1.80
Patient 20	2	1.18	1.63	1.81	2.06
AVERAGE Semiquantitative method results		1.57	1.87	2.28	2.19

By comparing the two semiquantitative methods it can be surmised that at least one ratio exceeds the established threshold. It can also be stated, that by comparing the H/CL made on the static images to those obtained on SPECT, the ratio results differ, in some cases. An explanation for this difference in the ratio result can be due to the type of images that were acquired. Static images are acquired in 2D, presenting the possibility of overlay, while SPECT presents a 3D image, reducing considerably the overlay factor. H/R ratio can only be made on static (2D) images therefore and therefore the ratio results are much lower compared to H/CL, but over the threshold in almost all of the patients. Furthermore, SPECT images offer a much clearer and with decreased background noise compared to static images. As a result, the ratios made on the SPECT images will present a higher result compared to those obtained on the static images.

Relying only on one ratio to sustain the visual score may prove to be inefficient (e.g. patients 18 - 20), as it can offer an equivocal result at best. By correlating both ratios made on static and SPECT images the additional information can offer a more accurate diagnosis.

DISCUSSION

ATTR-CA is a protein deposition disease located mainly in the myocardium. Diagnosing this rare pathology has proven to be difficult as, the gold standard method (endomyocardial biopsy) is not widely available. Therefore, alternative diagnostic

algorithms based on correlating different investigations have been utilized. As a result, nowadays, the main investigation used for ATTR-CA detection is bone scintigraphy, which has proven to be invaluable for this type of disease. The evaluation is based on myocardium uptake of the radiotracer. Standard acquired images include planar and SPECT centered on the thorax (WB images being considered only as an option) followed by visual evaluation of the uptake.

Our study aimed to determine if ATTR-CA diagnosis can be made on planar or WB images without necessarily using SPECT images even though it is recommended by the guidelines. Interpretation wise, we used visual scoring and semiquantitative methods based on H/CL made on both planar ± WB and SPECT and H/R made on planar ± WB. Due to the fact that some patients were discovered accidentally. As such, we compared the H/CL ratio result made on planar ± WB images to those obtained on SPECT images. In almost all of the cases the threshold on both image types was overcome. Due to the fact that planar images offer a 2D image of the patient, the problem of overlay should be taken into consideration and therefore, the ratio results could be lower than those obtained on SPECT, but still over the threshold. We also recorded cases in which the ratio result was close to the threshold, but didn't pass it on planar images compared to SPECT. In cases such as these, the H/R ration offered an additional parameter to the equation, that presented the possibility, along with the visual score, of ATTR-CA but in an early stage. In cases such as this, SPECT imaging could prove to be invaluable if the additional parameter didn't offer sufficient evidence to determine the diagnosis.

SPECT images offer a 3D image of the patient. The main advantage in these cases is the fact that the H/CL ratio result is much clearer in determining the presence of ATTR-CA than those obtained on planar images. However, in this type of images, H/R cannot be performed and as a consequence the result can be based only on visual scoring and H/CL region. Therefore, an additional parameter that could lighten the decision cannot be obtained on this type of image. Another disadvantage of this images refers to the dimension and position of the myocardium. If the myocardium is too large or if it's located to medial, it may be difficult to determine the H/CL region as the ROI made on the heart cannot be accurately mirrored to another region big enough or that doesn't include the myocardium to obtain a good result. In such cases, planar images may prove invaluable.

Ideally for ATTR-CA planar/WB and SPECT images should be performed and evaluated in the clinical context, visual scoring and semiquantitative analysis as each of them present different advantages and disadvantages. Therefore, in accidental diagnosed cases, if SPECT is unavailable, the ATTR-CA diagnosis can be determined by correlating the visual score, with the H/CL and H/R ratios.

CONCLUSIONS

In an ideal situation for diagnosing ATTR-CA, standard protocol should be followed regardless. Therefore, acquired images should be planar/WB followed by SPECT between 2-4 hours. Early planar/WB images, if acquired, present an approximation of the myocardium size and position, but cannot ascertain the presence of ATTR-CA. If SPECT images cannot be acquired, planar images should be enough to make a correct diagnosis, by analyzing them throw visual scoring and semiquanti-

tative analysis (H/CL and H/R).

To conclude, planar/WB vs SPECT, ideally together, if not possible, at least planar/WB for a correct diagnosis of ATTR-CA.

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CONFLICT OF INTEREST AND FUNDING

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THE PREDICTIVE VALUE OF RED BLOOD CELL DISTRIBUTION WIDTH-COEFFICIENT OF VARIATION IN PREGNANT PATIENTS WITH TREATED THROMBOPHILIA WHO DELIVERED VIA CESAREAN SECTION AT TERM

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THE PREDICTIVE VALUE OF RED BLOOD CELL DISTRIBUTION WIDTH-COEFFICIENT OF VARIATION IN PREGNANT PATIENTS WITH TREATED THROMBOPHILIA WHO DELIVERED VIA CESAREAN SECTION AT TERM (Abstract): RDW-CV has predictive value in many early-pregnancy-related conditions. Research question: Can RDW-CV be utilized predictively in pregnant patients at term with thrombophilia? **Materials and methods:** We compared RDW-CV values, antepartum and postpartum, according to postpartum uterine involution, maternal features, and fetal outcomes in 79 pregnant patients with treated thrombophilia, who formed the study group, and in another 80 patients of a similar age and parity, who formed the control group. Every patient received a sonogram during the first 1–2 days after the cesarean section, and their uterine evaluation was interpreted using the PUUS (Postpartum Uterine Ultrasonographic Scale). **Results:** Our group of PUUS ≥ 1 pregnant and postpartum patients with treated thrombophilia was the only group with an RDW-CV value over 14. An RDW-CV of 14.5 was the highest value in the Rh-negative group of pregnant patients with treated thrombophilia. An RDW-CV of 14.48 was the highest level in the Rh-negative postpartum patients with treated thrombophilia. (4) **Conclusions**: An RDW-CV of ≥14 might predict uterine hematometra (PUUS ≥ 1). The RDW-CV values were higher in the Rh-negative patients. **Keywords:** RDW-CV, THROMBOPHILIA, PREGNANCY, CESAREAN SECTION, ULTRASONOGRAPHY.

INTRODUCTION

Thrombophilia is a medical condition characterized by an increased tendency to form blood clots (1). Previous studies have shown a relationship between thrombophilia and pregnancy-related issues such as recurrent pregnancy loss (RPL), complications with in vitro fertilization, implanta-

tion failure, and ovarian hyperstimulation syndrome (1, 2).

Previous studies also found that the postoperative RDW and inflammatory biomarkers were significantly higher in transfused cesarean section parturients (3-5), which suggests a significant association between the postoperative inflammatory response and transfusion in obstetric patients.

Febra (6) demonstrated a significant association between an early high RDW and the diagnosis of acute unprovoked deep vein thrombosis. An RDW of ≥14% was found to be an independent predictor of unprovoked venous thromboembolism in adult patients.

The aim of this study is to ascertain whether uterine involution, assessed with the help of Postpartum Uterine Ultrasonographic Scale (PUUS) can be predicted by RDW-CV values.

MATERIALS AND METHODS

This is a secondary analysis of a previously published study (7). In this retrospective study with prospective data collection, we included 159 pregnant patients, comprising 79 pregnant patients with treated thrombophilia in the study group and another 80 patients of a similar age and parity. After gathering the study group, consisting of 80 patients, we added by intent a similar number of patients who were age- and para-matched, and then we removed one thrombophilia patient whose data were incomplete (tab. I). The inclusion criteria were as follows: pregnant patients at term, with known thrombophilia, receiving low-molecular-weight heparin treatment, and who were referred to our hospital for delivery at term by means of a cesarean section between the 1st of October 2017 and the 1st of December 2021. Hospital policy required that patients already diagnosed with thrombophilia be provided a cesarean section at 38 weeks' gestational age, and these patients comprised the study group. All of the patients with thrombophilia already had established diagnoses. Treatment with low-molecular-weight heparin was ongoing. Thrombophilia screening tests were not available at the study hospital; thus, the control group had their blood sent to specialized laboratories for screening, and patients were included following a negative result. The exclusion criteria were as follows: patients with diseases that prohibited heparin therapy (n = 0), patients with insufficient data (n = 1), patients suffering from thrombocytopenia (n = 2), patients with deep vein thrombosis (n = 0), and patients with cerebral thrombosis (n = 0) (7).

TABLE I.

Patient characteristics: mean, median, standard deviation, and quartile 1 and 2 values (7).

Patients	Thrombophilia Patients (n = 79)	Non-Thrombophilia Patients (n = 80)	Significance, p
Age (years)	30 (±5) 30 (27–34)	30 (±5) 30 (27–34)	0.944
Gestation (number)	3 (±1) 3 (2–3)	2 (±1) 2 (1–2)	<0.001
Parity (number)	2 (±1) 2 (1–2)	2 (±1) 2 (1–2)	0.213

There were no thrombophilia mutations identified in the control group. The nonparametric Mann–Whitney test was used for comparisons.

Every patient received a sonogram during the first 1–2 days after cesarean section, and the uterine evaluation was interpreted with the PUUS (Postpartum Uterine Ultrasonographic Scale). This scale (7, 8) counts the quarters of missing uterine vacuum lines, which could be due to the presence of blood or debris as follows:

- In grade 0, the uterine cavity is completely empty.
- In grade 1, there is a small amount of blood or debris occupying less than one-quarter of the vacuum line.
- In grade 2, there is a slightly larger amount of blood or debris occupying less than two-quarters of the vacuum line.
- In grade 3, there is a large amount of blood or debris occupying less than three-quarters of the vacuum line.
- In grade 4, there is a large amount of blood or debris occupying more than three-quarters of the vacuum line (7, 8).

We further referred to this debris as "uterine hematometra". In a previous study (7) on the same group of patients, we presented the demographic, maternal, and fetal outcomes.

The values and characteristics of the patients' blood following analysis were extracted from the hospital's medical records. For this study, the complete blood count values-the first count was obtained antepartum, and the last count was obtained postpartum-were considered. Hospital policy required blood analyses both 24 hours before and after labor.

We performed a blood analysis using MAN-HEMATO laboratory equipment.

We performed a data analysis using *SPSS version 18.0* (PASW Statistics for Windows, Chicago: SPSS Inc., Chicago, IL, USA). We determined the mean and median values, standard deviations, and quartiles. We also used the nonparametric Mann-Whitney U test and Spearman's correlation. We considered $p < 0.05$ to indicate significance (7).

We conducted this study in accordance with the Declaration of Helsinki and approval No. 9/17.09.2017 of the Ethics Committee of "Elena-Doamna" Clinical Hospital of Obstetrics and Gynecology Iasi.

RESULTS

First, we aimed to elucidate whether there was any difference in the involution of the uterine cavity postpartum using the PUUS. Because we had few patients with PUUS grades 1–4, we considered PUUS $\geq$ 1 to indicate any uterine cavity that did not close during the first 48 h postpartum, and we compared these with uterine cavities that did close, designated PUUS = 0, to simplify the comparison.

There was no significant difference between the RDW-CV values in the patients with PUUS = 0 as compared to those with PUUS $\geq$ 1, neither in pregnant patients with treated thrombophilia nor in the non-thrombophilia patients (tab. II). However, the PUUS $\geq$ 1 pregnant and postpartum patients with treated thrombophilia were the only group with RDW-CV $\geq$ 14.

There was a weak negative correlation between RDW-CV and age in the non-thrombophilia pregnant and postpartum patients (tab. III): RDW-CV decreased with increasing age among the non-thrombophilia patients.

TABLE II.

RDW-CV values in treated thrombophilia patients and non-thrombophilia patients, with a closed uterine cavity within 24–48 hrs. (PUUS = 0) or not (PUUS ≥ 1).

The upper line represents the mean values with the standard deviation; the lower line represents the median values with quartiles 1 and 3.

RDW-CV in Pregnant Patients with	PUUS = 0	PUUS ≥ 1	Significance, p
Treated thrombophilia	13.77 ± 1.60 13.51 (12.73; 14.65)	14.06 ± 1.77 14.02 (13.27; 14.26)	0.546
Non-thrombophilia	13.77 ± 1.30 13.47 (12.84; 14.38)	13.43 ± 1.15 13.59 (13.19; 14.23)	0.917
RDW-CV in Postpartum Patients with	**PUUS = 0**	**PUUS ≥ 1**	**Significance, p**
Treated thrombophilia	13.96 ± 1.80 13.53 (12.72; 15.08)	14.04 ± 1.76 13.72 (13.11; 14.59)	0.765
Non-thrombophilia	13.80 ± 1.37 13.26 (12.87; 14.55)	13.60 ± 1.13 13.67 (13.20; 14.40)	0.742

PUUS: Postpartum Uterine Ultrasonographic Scale.

TABLE III.

The correlation coefficients between RDW-CV and patient age.

Pregnant Patients	Correlation Coefficient	Significance, p
Treated thrombophilia	−0.110	0.300
Non-thrombophilia	−0.288	0.009
Postpartum Patients	**Correlation Coefficient**	**Significance, p**
Treated thrombophilia	−0.110	0.330
Non-thrombophilia	−0.350	0.002

There was no correlation between RDW-CV and the number of gestations of the patients. There were no significant differences in the RDW-CV values of the four ABO blood groups. There was no correlation between RDW-CV and the ABO blood group.

There was no significant difference between RDW-CV in the two Rh blood groups (tab. IV). There was no correlation between RDW-CV and the Rh blood groups. However, the Rh-negative pregnant patients with treated thrombophilia had the highest RDW value at 14.5, and postpartum the same group had the highest value, at 14.48.

There was a weak correlation between RDW-CV and BMI in the pregnant and postpartum patients with treated thrombophilia (tab. V), indicating that RDW-CV increased with increasing BMI among the patients with treated thrombophilia.

There was no correlation between the fetal outcomes and RDW-CV values.

TABLE IV.

RDW-CV values in treated thrombophilia patients and non-thrombophilia patients according to the Rh blood factor.

The upper line represents the mean values with standard deviations; the lower line represents the median values with quartiles 1 and 3.

RDW-CV in Pregnant Patients with	Rh-Positive	Rh-Negative	Significance, p
Treated thrombophilia	13.73 ± 1.56 13.58 (12.76; 14.45)	14.50 ± 1.96 14.54 (12.77; 16.17)	0.291
Non-thrombophilia	13.70 ± 1.29 13.48 (12.85; 14.30)	13.90 ± 1.17 13.68 (12.83; 14.84)	0.671
RDW-CV in Postpartum Patients with	**Rh-Positive**	**Rh-Negative**	**Significance, p**
Treated thrombophilia	13.91 ± 1.74 13.61 (12.80; 14.67)	14.48 ± 2.18 14.09 (12.42; 16.59)	0.566
Non-thrombophilia	13.76 ± 1.35 13.47 (12.86; 14.53)	14.01 ± 1.25 13.78 (12.98; 14.81)	0.577

TABLE V.

The correlation coefficients between RDW-CV and maternal features.

Pregnant Patients	Feature	Correlation Coefficient	Significance, p
Treated thrombophilia	Height	−0.094	0.409
	Weight	0.168	0.139
	BMI	0.236	0.037
Non-thrombophilia	Height	0.140	0.215
	Weight	0.117	0.302
	BMI	0.039	0.731
Postpartum Patients	**Feature**	**Correlation Coefficient**	**Significance, p**
Treated thrombophilia	Height	−0.052	0.656
	Weight	0.217	0.061
	BMI	0.279	0.015
Non-thrombophilia	Height	0.108	0.365
	Weight	0.132	0.269
	BMI	0.076	0.525

BMI = body mass index.

DISCUSSION

RDW-CV demonstrates anisocytosis (9). The higher the RDW-CV value, the more serious the anisocytosis. RDW-CV increases in many diseases. A decreased value means that the cells are mostly uniform in size.

Febra (6) demonstrated a significant association between an early high RDW and the diagnosis of acute unprovoked deep vein thrombosis. An RDW of ≥14% was found to be an independent predictor of unprovoked venous thromboembolism in adult patients. Our group of PUUS ≥ 1,

pregnant patients with treated thrombophilia had an average RDW of 14.06 (tab. II) and was the only group with an RDW value over 14. The postpartum group, like the postpartum patients with treated thrombophilia, had an RDW-CV of 14.04 and remained the only group with an RDW-CV over 14, even after delivery. Nevertheless, this group showed no deep vein thrombosis during hospitalization, suggesting that the anticoagulant treatment was necessary and effective.

Here is an important clinical implication: since only the treated thrombophilia patients whose uterine cavities did not involute had an RDW-CV over 14%, they were also at risk of venous thromboembolism. This makes sense. RDW-CV reflects anisocytosis, which generates thrombosis. Thrombosis did not occur due to the anticoagulant treatment, but anisocytosis remained. This means that thrombophilia patients with an RDW-CV over 14% are at risk of having a slower postpartum uterine involution, and they will require extra medical attention.

An RDW-CV of 14.5, the highest value, occurred in the Rh-negative pregnant patients with treated thrombophilia, indicating that a Rh-negative status is a potential predictor of deep vein thrombosis in pregnant patients with thrombophilia. An RDW-CV of 14.48 was the highest value in the postpartum group, occurring specifically in the Rh-negative postpartum patients with treated thrombophilia, and an RDW-CV of 14.01 was the highest value in the Rh-negative non-thrombophilia postpartum patients.

There was a weak negative correlation between RDW-CV and age in the non-thrombophilia pregnant patients; RDW-CV decreased with increasing age among the pregnant patients.

In pregnant women with obesity, RDW is lower, indicating that a larger maternal body size during pregnancy generates more and smaller erythrocytes (10). In our study, RDW-CV increased with increasing BMI among the pregnant patients with treated thrombophilia and was not influenced by BMI in the non-thrombophilia pregnant patients, showing that either the condition or treatment of thrombophilia, associated with a higher BMI, generated anisocytosis.

There was no correlation between fetal outcomes and postpartum RDW-CV values. This is in accordance with a study in Croatia, which demonstrated that asymptomatic carriers of inherited thrombophilic polymorphisms do not have an increased risk of adverse perinatal outcomes (11).

The strength of this work is that, to the best of our knowledge, it is the first to correlate RDW-CV and uterine postpartum involution, and it is also the first to do this in thrombophilia patients. The weakness of this work is that the number of patients was small, so further studies including more patients, and eventually more medical centers, are required in order to confirm these results.

CONCLUSIONS

In treated thrombophilia pregnant patients, an RDW-CV value over 14% might be a predictor of late postpartum uterine involution (PUUS grade ≥ 1).

The RDW-CV values were higher in the Rh-negative patients.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflicts of interest, and that this research received no external funding.

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EVALUATING THROMBOTIC RISK IN PATIENTS WITH COLORECTAL AND GASTRIC CANCER

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EVALUATING THROMBOTIC RISK IN PATIENTS WITH COLORECTAL AND GASTRIC CANCER (Abstract): We decided to study the computed tomography appearance and staging of gastric and colorectal cancers in relationship with vein thrombosis and pulmonary embolism because these tumors and their thrombotic complications are leading causes of morbidity and mortality in adult patients. **Materials and methods:** We retrospectively searched the databases of "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, and we discovered 102 patients with gastric and colorectal cancer who underwent computed tomography (CT) with a 64 detectors Incisive Philips CT scan between January and December 2022. The study was focused on the CT appearance of the tumor (location, length and thickness, local invasion), lymphadenopathies, metastases, and the correlation between tumor characteristics and the tendency for vein thrombosis. **Results:** We studied 102 patients (77 with colorectal and 25 with gastric cancer), mean age: 68.85±13.13 years, and among them we discovered 19 (18.62%) cases with vein thrombosis. Cancer-associated thrombosis has a weak negative correlations tumor thickness and length, weak positive correlation with tumor extension outside the digestive wall, number of loco-regional lymphadenopathies, and metastases. **Conclusions:** We found a strong association between vein thrombosis and gastric and colorectal malignant tumors, there is a weak correlation with tumor extension outside the digestive wall, the number of loco-regional lymphadenopathies and the presence of metastases, so we cannot confidently predict thrombosis by studying these tumor characteristics. **Keywords:** GASTRIC CANCER, COLORECTAL CANCER, THROMBOSIS.

INTRODUCTION

Malignancy leads to a hypercoagulable state associating vein thrombosis and / or pulmonary embolism (1), cancer patients having a 4 times higher risk of thromboembolism than general population (2, 3). Cancer-associated thrombosis is the second mortality cause, following the mortality by cancer itself, and preceding the mortality determined by various infections (4); mortality is lower in cancer patients without deep vein thrombosis and / or pulmonary embolism and significantly higher in case of those associating thrombosis (5, 6). Gastric cancer and colorectal cancer are leading causes of morbidity and mortality in

adult patients, many authors studied the frequency of associated venous thromboembolism, and created strategies for prophylaxis and treatment (7). Most patients with gastric cancer and colorectal cancer have no obvious clinical symptoms in the early stage, are diagnosed at an advanced stage, when pain, fatigue, and even jaundice can appear, related to lymphadenopathy or liver metastases (8). Thrombosis can be an early symptom of cancer, but the incidence of varies with cancer type, stage, patient characteristics, and anticancer-associated treatment (9, 10). Computed tomography has a high sensitivity for gastric cancer detection (11) and is the gold standard method for evaluating tumor invasion, regional and distant lymphadenopathy, liver and peritoneal metastases (which are associated with poor prognosis) (12, 13, 14). For colorectal cancer, computed tomography is also the standard method for determining disease stage but staging is improved in case of rectal cancer by using MRI which has been documented to be more accurate than CT (15). Current therapeutic agents of digestive tract cancer-associated thrombosis mainly comprise low weight molecular heparins, and eventually inferior vena cava filters, because oral anticoagulants, and vitamin K antagonists are contraindicated (5, 7, 10).

MATERIALS AND METHODS

We retrospectively searched the databases of "Sf. Spiridon" County Clinical Emergency Hospital Iaşi to identify patients who underwent abdominal and pelvic contrast enhanced CT (CECT) for suspicion of gastric and colorectal cancer during 2022 and we discovered 102 patients with imaging suspicion for these malignancies; among them 24 had also suspicion of vein thrombosis or pulmonary embolism (PE) and computed tomography pulmonary angiography (CTPA) was also performed.

Computed tomography was performed with a 64 detectors Incisive Philips CT scan; for those patients with PE suspicion, CTPA was performed, followed by abdominal and pelvic CT examination, for the other patients only abdominal and pelvic CT was done. All patients with suspicion of gastric cancer ingested immediately prior the investigation 500 mL water to distend the stomach and those with colorectal cancer underwent a colonic cleansing preparation followed by a 1500-2000 mL water enema before the procedure.

A noncontrast enhanced scan was performed on the regions of interest. We injected intravenously up to 100 mL of 400 mg I/mL Iomeprol (Iomeron 400 mg/mL) for CTPA and up to 120 mL of 370 I/mL Iomeprol (Iopamiro 370 mg/mL) for abdominal and pelvic CECT, with an automatic injector, with the speed of 3-4 mL/second, followed by 50 mL of saline chaser; we used bolus tracking technique to choose the moment of arterial phase scanning for CTPA: the scan started with a delay of 22-25 seconds when the density in the pulmonary trunk was over 150-180 HU.

For abdominal and pelvic CT scan we measured the CT densities during contrast injection in the descending aorta just above the diaphragm; when the density was over 120 HU, the scanning started with an injection-to-scan delay for the arterial phase 15-20 seconds, for portal venous phase 60-70 seconds, and 4 minutes for delayed phase.

Gastric cancer and colorectal cancer staging are based on the 8th edition of the AJCC TNM classification of malignant tumors (16, 17).

The study was focused on the following

features: CT appearance of the tumor (location, length and thickness, local invasion), lymphadenopathies, metastases, and the correlation between tumor characteristics and the tendency for vein thrombosis and PE.

RESULTS

We studied 102 patients (77 with colorectal and 25 with gastric cancer), and among them there were 64 (62.75%) males and 38 (37.25%) females, with mean age: 68.85±13.13 years, age range: 18 to 93 years, the majority of patients concentrated in the 60-80 age range (57-55.88% patients), with the highest concentration around the age of 70. We encountered only five sincrone or metacrone large bowel tumors and two associations between colonic and gastric cancer.

The study of the tumor length (fig. 1) and thickness in correlation with age group, shows a wide range of tumor dimensions, with an ascending trend from 20 to 80 years, the 60-80 years group having the highest variability in tumor dimensions, with the median being higher than the previous age groups suggesting that older patients might have tumors detected at later stages.

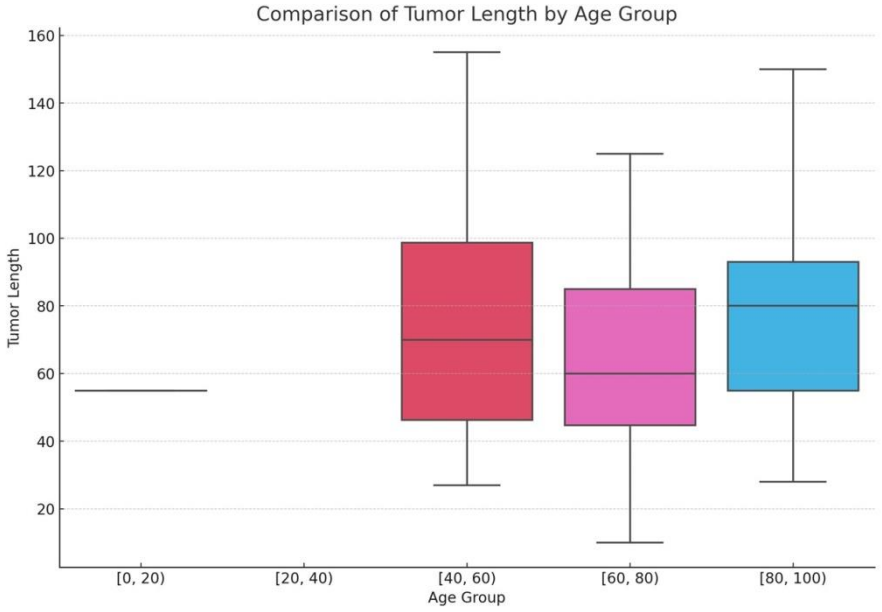

Fig. 1. Relationship between tumor length and age group

We used Pearson correlation coefficient (r) and p-value to measure the strength and direction of the linear relationship between two variables; r ranges from -1 (perfect negative linear relationship) to 1 (perfect positive linear relationship) and p-value is significant between $0.001 \leq$ and < 0.05.

Correlations between tumor characteristics and loco-regional tumor extension

The localization of the tumors influences treatment strategies, so it is important to notice the most frequent locations: in case of the large bowel, rectum and sigmoid have a higher prevalence of tumors (37-48.05%

cases), followed by ascending colon (14-18.18%). Gastric cancer has an equal distribution between antrum: 8 cases (32%), fornix and body (7-28% cases each), other localizations being rarer.

Among all the patients we had studied the highest number of invasive tumors are present in the rectum and sigmoid colon (18-17.64% cases), cecum and ascending colon (10-9.80% cases), and indicating that the lesions with these locations have a higher tendency for local invasion; even if there were only 7 invasive gastric tumors, they represent a significant percentage (6.86% of all lesions and 28% of gastric cancers).

The r values we calculated for tumor thickness reveal that is moderately correlated with both tumor length (0.526) and the extension of the tumor outside the digestive walls (0.325), indicating that as tumors become thicker, they tend to grow larger and invade surrounding tissues more extensively; while there is some relationship between tumor thickness and the number of loco-regional lymphadenopathies, it is relatively weak (0.225), and age appears to have a minor inverse relationship with tumor thickness, but this correlation is also weak (-0.188).

Pearson correlation coefficient and p-values show the significance of tumor thickness correlations with tumor length, extension of the tumor outside the digestive walls, number of loco-regional lymphadenopathies, concluding that they are not likely due to random chance (tab. I).

TABLE I.

Correlations between tumor thickness and other variables

Tumor thickness	Pearson correlation coefficient	p-value	Classification
Tumor length	0.526	<0.001	Highly Significant
Thickness Outside the Digestive Walls	0.325	<0.001	Highly Significant
Number of loco-regional adenopathy	0.225	<0.05	Significant
Age	-0.188	<0.1	Marginally Significant

Tumor length shows a moderate positive correlation with thickness outside the digestive walls (0.470) and a weak positive correlation with locoregional adenopathy number (0.238). Thickness outside the digestive wall shows a moderate positive correlation with locoregional adenopathy number (0.490).

The most frequent metastasis are the liver ones (21-20.58%) (fig. 2), followed by peritoneal and non-regional lymphadenopathies (10-9.80%), pulmonary (8-7.84%), splenic, renal, and bone secondary lesions (1-0.98%). Age distribution of liver metastases ranges from 50 to 89 years, common age group is over 70 years (13-61.90% cases) and the prevalence concerning the gender is in favor of male patients (15-71.42%). The correlation between liver and other metastatic sites is the following:

- with the peritoneal and non-regional lymph node metastases is moderate positive: patients with hepatic metastasis are likely to have peritoneal and non-regional lymph nodes metastasis as well;
- with pulmonary metastasis there is a weak correlation, suggesting a lower likelihood of co-occurrence with liver metastasis;
- splenic, renal, and bone metastasis exhibit a very weak or no correlation with liver metastasis, so they are rarely found together with hepatic metastasis.

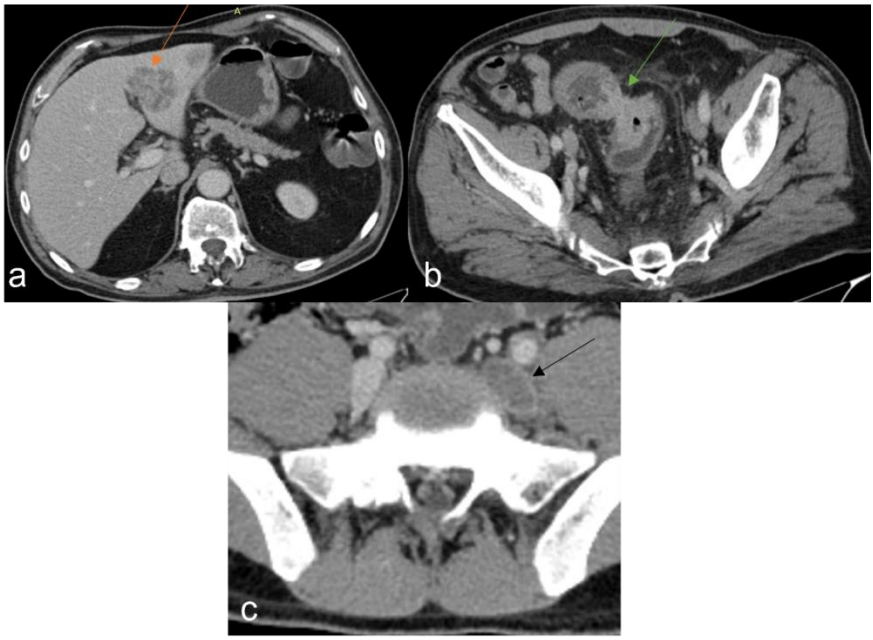

Fig. 2. Abdominal and pelvic CT, portal venous phase: a. liver metastases (orange arrow); b. sigmoid colon cancer (green arrow); c. left common iliac vein thrombosis (black arrow)

Correlations between tumor characteristics and vein thrombosis

Among the patients we had studied we found 19 (18.62%) cases with vein thrombosis associated with pulmonary embolism in 7 (6.86%) cases, most of them (15-78.94%) being males. This study discovered the following correlations between vein thrombosis and tumor characteristics (fig. 3):

- with tumor thickness and length: weak negative correlations; for example, the tendency of vein thrombosis is not increasing with tumor thickness (coefficient is -0.0017), indicating a slight negative relationship with thrombosis, also this relationship is not statistically significant ($p = 0.061$);
- with tumor extension outside the digestive wall: weak positive correlation;
- with number of loco-regional lymphadenopathies: weak positive correlation (0.306), suggesting that higher loco-regional adenopathy numbers are slightly associated with an increased likelihood of thrombosis;
- portal vein thrombosis: weak correlations with tumor thickness (-0.084), tumor length (-0.035), and thickness outside the digestive walls (-0.071), and moderate correlations with other thrombosis types such as iliac vein thrombosis (0.219) and inferior vena cava thrombosis (0.243).

Studying the TNM staging we found only 10 cases of T1-T2 lesions, and most of the patients had locally advanced tumors in 92 (90.19%) cases (T3: 42 cases, T4: 50 cases). The majority of patients (86 cases-84.31%) had lymph node involvement (N1: 52 cases, N2: 22 cases, N3: 12 cases), but distant metastases were present in 37 (36.27%) cases. The correlation of TNM categories and the tendency for vein thrombosis are presented in second table.

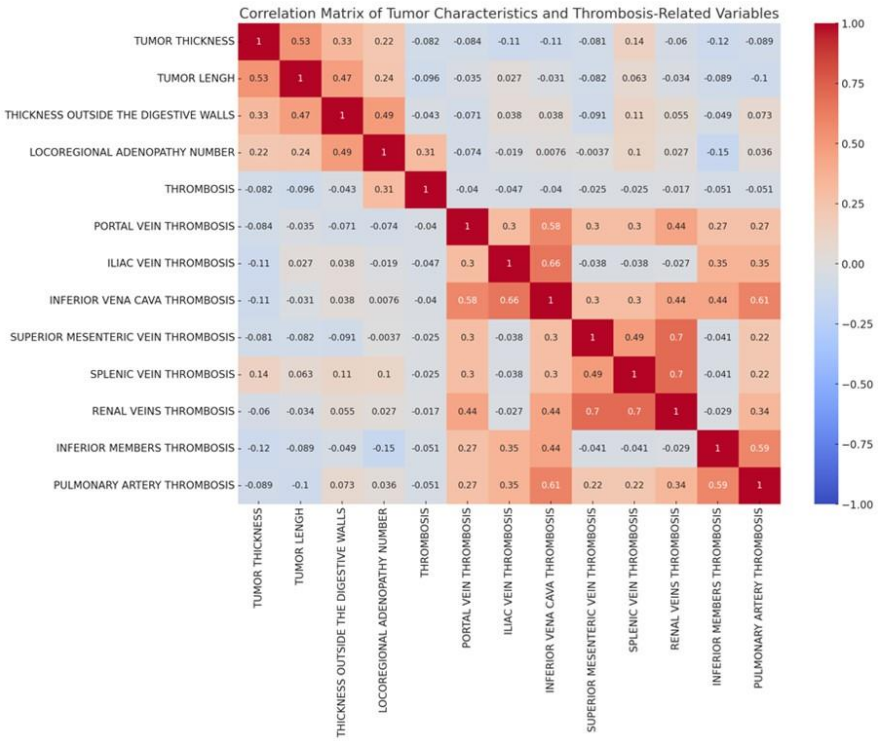

Fig. 3. Correlations between tumor characteristics and thrombosis:
red = positive, blue = negative; the intensity of the color = the strength of the correlation

TABLE II.

Correlation between TNM categories and the tendency for thrombosis

Thrombosis	Pearson correlation coefficient	p-value	Classification
T	-0.327	0.040	Significant
N	0.306	p < 0.001	Highly Significant
M	0.178	0.040	Significant

Tumoral local invasion (T) has the coefficient -0.327, indicating a negative relationship with thrombosis, which is statistically significant (p = 0.040). However, there is a weak correlation between the number of loco-regional lymphadenopathies and thrombosis (r= 0.306), for each additional locoregional adenopathy, the thrombosis score increases; this relationship is statistically significant (p < 0.001), suggesting that patients with more loco-regional lymphadenopathies might have a higher likelihood of developing thrombosis. The presence of metastases has a weak positive(r=0.178), statistically significant (p = 0.040) correlation with vein thrombosis.

DISCUSSION

We studied 102 patients and among them only 25 had gastric cancer, determining an inaccurate statistic for this particular group, so we studied the correlations between tumor characteristics and the rela-

tionship with cancer-associated thrombosis for the whole group of patients.

Gastric and colorectal cancers were frequent (62.75%) in case of male patients, and liver metastases have a higher prevalence in males (71.42%), suggesting the need for gender-specific strategies in early detection and follow-up for this demographic.

The majority of patients are concentrated in the 60-80 age range (55.88% patients), and liver metastases are frequent in case of patients over 70 years old (61.90%) so the surveillance for gastric and colorectal cancers has to be extended also for these age groups.

Although the association between gastric tumors (including neuroendocrine ones) and breast cancer (mainly lobular type) is cited in the literature, we found this association only in 1 case, without any correlation with vein thrombosis (18, 19, 20). Among the patients we had studied we found a significant number (19-18.62%) of vein thrombosis, most of them (15-78.94%) being males; this tendency suggests that the appearance of thrombosis in case of a male subject to ring a bell for the presence of a malignant tumor.

The correlations between thrombosis and tumor thickness and length are weak negative correlations; tumor extension outside the digestive wall has a weak positive correlation with thrombosis.

Tumoral local invasion in other organs has a statistically significant, negative relationship with thrombosis, but between the number of loco-regional lymphadenopathies and the presence of metastases there is a weak positive correlation with vein thrombosis.

For further studies we think about correlating malignant gastric, colonic and pancreatic lesions with vein thrombosis, heart conditions and COVID-19 infection, using artificial intelligence programs (21, 22).

CONCLUSIONS

Most of the patients with metastatic gastric and colorectal cancers from our study were old males, who had also cancer associated thrombosis, so the surveillance for these malignancies has to be focused on them and also extended for older age groups.

Even if we found a strong association between vein thrombosis and gastric and colorectal malignant tumors, there is a weak correlation with tumor extension outside the digestive wall, the number of loco-regional lymphadenopathies and the presence of metastases, so we cannot confidently predict thrombosis by studying these tumor characteristics.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SELF-EVALUATION OF NURSING STUDENTS REGARDING THEIR COMMUNICATION SKILLS

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SELF-EVALUATION OF NURSING STUDENTS REGARDING THEIR COMMUNICATION SKILLS (Abstract): **The objective of the study** was to evaluate the confidence of nursing students regarding their communication skills and how prepared they felt working with different kinds of patients. **Materials and methods**: 160 students enrolled in nursing studies from all years of study were included in the research. An online questionnaire was constructed for the present study to gather sociodemographic and academic data. Additionally, self-rated items were created to collect information about students' confidence in their communication skills. Two instruments were used to evaluate their learning communication skills and interpersonal communication competence: *Interpersonal Communication Competence Scale* and *Communication Skills Attitude Scale*. **Results**: Nursing students who scored high on the *Communication Skills Attitude Scale* demonstrated greater engagement in social interactions and exhibited supportiveness and promptness in their relationships with others. Only 7.5% of participants reported receiving additional training in communication skills. Freshman students perceived communication with elderly patients more challenging compared to students in advanced years. **Conclusions**: Theoretical and practical stages of continuing education after graduating from nursing schools are essential requirements for nurses. This ensures that they can effectively apply their knowledge and skills across various healthcare settings and cater to different categories of patients. Direct feedback during practical stages and moral support from academic teachers and clinical trainers help students to feel more confident about their communication skills. **Keywords:** NURSING STUDENTS, COMMUNICATION SKILLS, ACADEMIC TRAINING, EMPATHY.

INTRODUCTION

The nursing profession is one of the most challenging professions because it involves working with people in a vulnerable situation, both physically and mentally. This situation has a negative impact on the personal and professional life, but also on the family and social life of the patient. Therefore, nurses need to develop effective communication skills with patients and undergo thorough theoretical and practical training during their academic years. This preparation allows them to work competently with different categories of patients, including pediatric, elderly, chronic, palliative, and those with disabilities. Poor

nurse-patient communication can lead to lower satisfaction, reduced adherence to treatment recommendations, and poorer health outcomes (1).

Nursing students must focus on curricular and extracurricular training to develop their skills in therapeutic communication (TC). TC is defined as the process of using verbal and nonverbal communication to connect with patients (2). In the medical context, nurse-patient communication represents not only an exchange of objective information, but also addresses physiological, psychological, environmental and spiritual care. It also includes beliefs about recovery, life perspectives, relevant concerns about disease and personal needs. (3) Students must be encouraged to develop their desire for lifelong learning and acquisition of communication skills, in relationship with their permanent needs and job-related context. In academic nursing training, scholars recommend incorporating video-training and practical sessions based on scenarios that allow students to analyse and practice their skills. Fioravanti, *et al.* (4) and Kaplonyi *et al.* (5) identified that realistic situations provided good opportunities to improve the competencies of nursing students through human interaction and immediate feedback, while Moscaritolo (6) recommended peer instruction and mentoring as effective methods for improving nursing students' skills. Cannity *et al.* (7) identified three directions for efficient training: responding empathically to patients; discussing death, dying, and end-of-life goals of care; and responding to challenging family interactions. In the light of recent trends in providing medical care, many researchers also recommend training nurses in communication skills applicable to intercultural environments. This recommendation is due to the increasing rate of migration of nurses for work and patients seeking better care abroad. For example, Didgson, *et al.* (8) identified the necessity to enhance nurses' cultural competency in the context of globalization, while Demelius (9) mentioned the importance of providing opportunities for nurses to experience different cultures and interact with people from different cultural backgrounds. In their study, Szpak and Kameg (10) identified that numerous nursing students reported anxiety or lack of confidence in their ability to communicate with patients as barriers to effective communication. These barriers included reluctance to engage with patients or families, difficulty initiating or maintaining conversations, feeling underappreciated and anxious about engaging in complex discussions such as those involving death and dying. Additionally, concerns about their professional performance was cited as a source of job-related stress. The present study aimed at the self-evaluation of nursing students enrolled in nursing studies about their confidence when communicating with patients during their practical stages in hospitals.

MATERIALS AND METHODS

Participants

The study was conducted from October to December 2024 among nursing students. The participants were students enrolled in all years of study at the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi / Faculty of Medicine, the participants were informed about the purpose of the study and the confidentiality of data, and about the fact that they could withdraw from the study whenever they wanted, without consequences. No incentives were given to participants. The instrument was

distributed to approximately 430 students. Inclusion criteria comprised students enrolled in Nursing from years of study 1-4. Exclusion criteria: questionnaires filled in after the deadline. Finally, a total of 160 participants were included in the present study, representing 37.21% of the targeted participants.

Study Instruments

The questionnaire was constructed using Google Forms and consisted of three sections:

The first part of the questionnaire collected information about socio-demographic and academic data such as age, marital status, gender, background, year of study, and the employment status.

The second part of the questionnaire collected self-reported data regarding students' opinions on their satisfaction with the communication skills developed during their academic years. It assessed how comfortable they felt when communicating with patients during internships, their perception of the importance of communication skills in their practice, and whether they had attended extracurricular courses focused on developing communication skills. (e.g, *How comfortable do you feel when communicating with patients during your practical stages? Do you consider that communication skills are important for your profession? How difficult do you think the communication is when communicating with patients, during your practical stages as a student? How difficult do you think communication is when working with palliative, paediatric, geriatric, chronically ill patients etc.* Further items collected data about students' experiences and feelings when communicating with family members. Responses were provided using *yes/no* options, or rated such as *very difficult/difficult/not very difficult/not at all difficult*.

The third part of the questionnaire aimed to identify learning communication skills and interpersonal communication competence. To evaluate students' attitudes towards learning communication skills during medical courses, *The Communication Skills Attitude Scale* (CSAS) consists of 26 items, 13 of which are written in the form of positive statements and 13 negative statements regarding communication skills learning. This instrument was developed by Ress *et al.* in 2002 (11). Each item is accompanied by a 5-point Likert scale, ranging from 1 (strongly disagree) to 5 (strongly agree). *The Interpersonal Communication Competence Scale,* developed by Rubbin in 1994 assesses the ten dimensions of interpersonal communication competence (self-disclosure, empathy, social relaxation, assertiveness, altruism, interaction management, expressiveness, support, promptness, and control of the environment) and establishes the quality of the communication process. (12), This scale is composed of 23 items with answers given according to a five-point Likert-type scale (from 1 = *never* to 5 = *very often*). The alpha-Cronbach score for the "Communication Skills Attitude Scale was 0.71 and 0.76 for "Interpersonal Communication Competence Scale", indicating good reliability.

Statistical Analysis

All analyses for the present study were performed using *IBM Statistical Package for Social Sciences (SPSS) Statistics (SPSS Inc, Chicago, IL, USA).* For descriptive data, such socio-demographic and academic information, means and standard deviations (SD) were calculated. The Spearman correlation was used to test the relationship

between variables with a $p < 0.05$ considered statistically significant.

RESULTS

Socio-Demographic and Academic Data

Students from all years of study responded to the questionnaire. The data analysis showed that the majority of students were female from rural area, having no prior experience as nurses and being into a relationship (tab. I).

TABLE I.

Sociodemographic characteristics

Sociodemographic data	n (%), M ± SD
Sex	
Male	11 (6.9)
Female	149 (93.1)
Age	21.16 ± 2.64
Area of origin	
Urban	62 (38.8)
Rural	98 (61.3)
Educational level	
1st year	39 (24.4)
2nd year	32 (20.0)
3rd year	28 (17.5)
4th year	61 (38.1)
Experience as nurse (employed)	3 (1.9)
Marital status	
single	74 (46.3)
in a relationship	86 (53.8)

Self-evaluation of communication skills

Students were asked about their comfort level when communicating with patients during their practical stages. Findings showed that 19 % of the participants felt very comfortable (N = 31), 61.9% declared that they felt comfortable (N = 99), 18.1% felt a little comfortable (N = 29), and only 0.6% felt not comfortable at all (N = 1). The survey revealed that all participants (100%, N = 160) considered communication skills as being an important skill for practicing the nursing profession. Only 7.5% of the participants reported additional training in communication skills. A strong majority of participants declared that empathy was always necessary for nursing (86.3%). A total of 21.9% of the participants (N = 35) sustained that they felt well-prepared to communicate with patients, while 50.6% (N = 81) felt well prepared. When asked about their ability to communicate through non-verbal language (such as gestures, facial expressions, or tone of voice), 7.5% of the participants (N = 12) rated their ability as excellent, and 81.3% (N = 130) rated it as very good. The analysis of data indicated that students found the communication skills training received during university to be moderately to significantly beneficial (M = 3.43), suggesting that most of the nursing students considered that their education played a meaningful role in the development of their communication skills. A total of 62.5% (N = 100) of students reported that the nurses they met during their internships served as role models in terms of communication skills (tab. II).

The questionnaire assessed students' perception of their ability to communicate with patients with chronic illness or other categories such as pediatric or geriatric ones. The results indicate significant differences between years of study in terms of perceived difficulty in communicating with elderly patients ($F(3,156) = 8.073$, $p = 0.000$). Thus, first-year students perceived communication with elderly patients more challenging compared to students in advanced years (MY1 = 1.79, MY4 = 1.40). Similar differences were identified in communication with children: ($F(3,156) = 5.968$, $p = 0.001$), with first-year students perceiving greater challeng-

es than their senior peers (MY1 = 1.41, MY4 = 1.13). Regarding palliative care patients (p = 0.108), no significant differences were found among the four years of study. The perception of difficulty remained consistent regardless of the year of study (F (3,156) = 2.057, p = 0.108). Additionally, no significant differences were found between years of study regarding the perceived difficulty of communication with patients with disabilities (F (3,156) = 1.046, p = 0.374).

TABLE II.
Profile of the nursing student

ITEMS	n (%) M ± SD
How comfortable do you generally feel when communicating with patients during your internships?	
• Very comfortable	31 (19.4)
• Comfortable	99 (61.9)
• A little comfortable	29 (18.1)
• Not comfortable at all	1 (0.6)
Do you think communication skills are important in nursing practice?	
• Yes	160 (100)
Have you taken any extracurricular training to develop your communication skills?	
• Yes	12 (7.5)
How necessary is empathy in the process of communicating with patients?	
• It is always necessary	138 (86.3)
• It is necessary in certain situations	22 (13.7)
• It is not necessary	0 (0)
How well prepared do you feel in terms of communication skills?	
• I feel quite prepared	35 (21.9)
• I feel well prepared	81 (50.6)
• I don't feel sufficiently prepared	44 (27.5)
• I don't feel prepared at all	0 (0)
As a nursing student, how difficult has it been for you to communicate with patients?	
• Very difficult	0 (0)
• Difficult	29 (18.1)
• A little difficult	107 (66.9)
• Not difficult	24 (15)
How do you manage to communicate through non-verbal language (gestures/ facial expressions/ tone of voice) with patients?	
• Excellent	12 (7.5)
• Good	130 (81.3)
• Not at all	18 (11.3)

ITEMS	n (%) M ± SD
Do you feel understood when you are having a conversation with the patient?	
• Yes	151 (94.4)
How difficult is it for you to remain calm and empathetic when the patient is stressed and agitated?	
• Very hard	3 (1.9)
• Heavy	48 (30)
• Easy	98 (61.3)
• Very easy	11 (6.9)
Do you consider that the attitudes of professional nurses are always a model to be followed?	
• Yes	100 (62.5)
Do you find it difficult communicating with multidisciplinary team?	
• Yes	45 (28.1)
On a scale of 1 to 5, how much do you think the information you received during academic years helped you develop your communication skills?	
• 1	4 (2.5)
• 2	22 (13.8)
• 3	63 (39.4)
• 4	43 (26.9)
• 5	28 (17.5)

As some results indicated significant differences between groups. The findings showed that freshman students perceived communication with elderly patients as more difficult than second-year students ($p = 0.002$, [0.13, 0.71]). First-year students significantly perceived communication with elderly patients as more challenging compared to second-year students. Additionally, first-year students significantly perceived communication with elderly patients as more difficult compared to fourth-year students ($p = 0.000$, [0.20, 0.67]). There were no significant differences regarding the perceived difficulty of communication between second-year and third-year students, or between second year and fourth-year students.

The study also explored the level of comfort nursing students experienced when communicating with patients during their practical stages. Students in Y4 reported the highest level of comfort in communicating with patients ($M = 2.02$), followed by students in Y2 ($M = 1.97$). Similar findings were identified for students from Y3 ($M = 1.82$) and Y1 ($M = 1.87$). To explore the relationships between variables regarding the CSAS scale and subsequent dimensions of ICCS, Pearson correlation analysis

was conducted. The results highlighted several significant associations between the variables included in the study. Firstly, the overall CSAS score showed significant positive correlations with self-disclosure ($r = 0.174$, $p = 0.028$), interactions ($r = 0.207$, $p = 0.009$), supportiveness ($r = 0.211$, $p = 0.007$) and promptness ($r = 0.290$, $p<0.001$). These results suggest that nursing students with a high CSAS scale score tended to be more involved in social interactions and showed supportiveness and promptness in their relationships with others. Regarding self-disclosure, it showed significant and positive correlations with relaxation ($r = 0.368$, $p < 0.001$), assertiveness ($r = 0.439$, $p < 0.001$), and expressiveness ($r = 0.424$, $p < 0.001$). These correlations indicate that nursing students who self-disclose were more relaxed, assertive, and expressive in social interactions. Relaxation was also positively associated with assertiveness ($r = 0.494$, $p < 0.001$), supportiveness ($r = 0.337$, $p < 0.001$), and promptness ($r = 0.497$, $p < 0.001$). This relationship emphasizes that nursing students who were relaxed in social contexts tended to be assertive, supportive, and prompt in their responses. A strong correlation was identified between promiscuity and control ($r = 0.513$, $p < 0.001$), suggesting that individuals exhibiting promiscuous behavior tended to have high control over their actions. Furthermore, promptness showed a positive correlation with supportiveness ($r = 0.464$, $p < 0.001$), underlining a link between promptness and supportive attitudes in social interactions. Conversely, variables such as alter centrism and empathy had weak or insignificant correlations with most other variables, suggesting that they were not closely related to the constructs assessed in this study.

DISCUSSION

Our study proved that a significant majority of nursing students (94%) felt well prepared regarding their communication skills, although a small number of students (7%) sustained that they participated to extracurricular training in order to increase their abilities. Increased confidence over the academic years is related to the adequate topics, and their practical stages supervised by their teachers in clinics or hospitals, where nursing students can directly experience professional relationships with patients and family members. Many respondents mentioned that they easily succeeded in remaining calm when facing a conversation with an aggressive or nervous patient. All surveyed students agreed that empathy was an important skill in nursing practice.

Our study demonstrated that nursing students feel comfortable about their verbal abilities but less confident in their non-verbal skills. Nearly 7% of students sustained that they felt they use non-verbal language appropriately. These results proved that students are more focused on verbal communication, which is easier to control, over non-verbal communication, such as body language and intonation. The analysis of results revealed different perspectives on working with pediatric, geriatric, palliative, or patients with disabilities.

These findings suggest that university curricula should be more focused on training students to work with these patient groups. Our results are congruent to those identified in the scientific literature, indicating a requirement for a good theoretical and practical training of students regarding the nurse-patient relationship based on communication skills.

Ahmady *et al.* (2018) emphasised that direct supervision is essential during their

practical stages, making the presence and regular supervision provided by a clinical instructor key factors in facilitating a positive learning experience. Some other studies identified that practical stages decrease students' levels of fear, lack of confidence, and fear of mistake, suggesting increased exposure to practical situations results in decreased stress in nurse-patient communication (13).

Baraz *et al.* (2015) (14) and Panda *et al.* (2021) found that support from clinical staff and teachers supervising practical stages who provide support, supervision and guidance were described as essential and motivating factors in the learning process, with a positive reinforcement for nursing students. The study highlighted the relationships between self-rated ability, satisfaction, and actual performance (15).

Limitations of the study: the first limitation of the research is due to its small number of male subjects. This situation is a common one, considering that there are fewer males enrolled in nursing studies and also working in nursing field, making a comparative analysis based on sex unfeasible. Studies have attempted to identify differences between male and female nurses. For example, Buthelezi *et al.* (2015) showed that male students described themselves as being vulnerable due to cultural perspectives and societal expectations since nursing is viewed as a female-dominated profession in health-care area. Another limitation of the study is related to the fact that the research was conducted in a single center, which means the results cannot be generalized (16).

CONCLUSIONS

Effective communication between patients and healthcare providers is essential for patient care and recovery. Therefore, nursing students must be very well prepared to develop meaningful and adequate relationships with both patients and their families. Communication skills are developed throughout the academic year through theoretical and practical stages. However, continuing education remains a mandatory requirement for nurses to better adapt their knowledge and practical skills to meet the demands of the health organizations and the category of patients they work with. The professional and moral support from academic teachers and clinical trainers empower nursing students to feel more confident about their communication skills.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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A FINITE ELEMENT ANALYSIS OF MINI-IMPLANT EFFECTS ON THE PERIODONTIUM: FROM BIOMECHANICAL SIMULATION TO ORAL HEALTH AND MENTAL WELLBEING

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A FINITE ELEMENT ANALYSIS OF MINI-IMPLANT EFFECTS ON THE PERIODONTIUM: FROM BIOMECHANICAL SIMULATION TO ORAL HEALTH AND MENTAL WELLBEING (Abstract): Mini implants are widely used as temporary anchorage devices (TADs) in orthodontics, providing stable and minimally invasive anchorage. However, excessive or misdirected stresses at the bone-implant interface may compromise periodontal integrity and long-term success. To assess stress distribution and bone deformation around orthodontic mini implants under varying insertion angles and load magnitudes using finite element analysis (FEA), and to explore their clinical and wellbeing implications. **Materials and methods:** A 3D FEA model was developed in SolidWorks, based on CBCT data and manufacturer specifications for a titanium mini implant (8×1.7 mm). The bone model consisted of 2 mm cortical and 8 mm trabecular bone layers. Simulations were performed for insertion angles of 30°, 45°, 60°, and 90° under para-axial forces of 1 N and 5 N. Stress and deformation were evaluated using the von Mises criterion. **Results:** The highest stress values were recorded at the implant-cortical bone interface, particularly at 90° insertion, and increased with load magnitude. Stress rose from 27 MPa at 1 N to 266 MPa at 5 N, while bone deformation ranged from a few to several tens of micrometers. Insertion geometry and loading intensity significantly influence the biomechanical behavior of peri-implant bone. Moderate insertion angles (45-60°) ensured a more balanced stress distribution and greater stability. **Conclusions:** Finite element modelling supports personalized, safe, and psychologically comfortable orthodontic treatments, linking biomechanical precision with oral and mental wellbeing. **Keywords:** FINITE ELEMENT ANALYSIS, MINI-IMPLANTS, STRESS DISTRIBUTION, CORTICAL BONE, PERIODONTAL BIOMECHANICS, INSERTION ANGLE, ORAL AND MENTAL WELLBEING.

INTRODUCTION

Mini implants, also known as temporary anchorage devices (TADs), have revolutionized modern orthodontic and periodontal therapy by providing stable, minimally invasive, and cost-effective anchorage for tooth movement and skeletal correction. Their clinical success relies not only on the mechanical design of the implant but also on the complex biomechanical interactions with the surrounding periodontal tissues, including the cortical and trabecular bone, periodontal ligament, and gingival interface (1-4).

Despite their widespread use, the pre-

cise distribution of stress and strain within the peri-implant region remains a critical determinant of biological response and long-term stability. Inappropriate insertion angles or excessive loading may cause microdamage, localized bone resorption, or even implant failure. Conversely, controlled stress levels within physiological limits can stimulate bone remodeling and strengthen osseointegration (5-7).

Finite Element Analysis (FEA) has emerged as a powerful computational method for simulating these interactions in a non-invasive and highly reproducible manner. By combining imaging data (such as CBCT) with accurate material modeling, FEA enables the visualization and quantification of stress patterns within the implant-bone-periodontium complex. This allows clinicians to anticipate biological outcomes under various clinical conditions before treatment is initiated (8-11).

However, current literature has often emphasized mechanical optimization alone, overlooking the broader implications for oral and mental wellbeing. The stability of anchorage systems has direct effects on treatment duration, procedural discomfort, and aesthetic outcomes - all of which influence patient confidence, adherence, and emotional state during orthodontic therapy (12-14). The integration of biomechanical predictability with patient-centered psychological comfort thus represents a new paradigm in contemporary dental care.

Accordingly, the present study aims to perform a finite element analysis of stress distribution and bone deformation around orthodontic mini-implants under different insertion angles and loading conditions. Beyond quantifying mechanical performance, the study seeks to link these biomechanical outcomes with their potential clinical and psychosocial significance, providing a holistic view of mini-implant therapy as a bridge between functional precision and overall wellbeing.

MATERIAL AND METHODS

In this study, a finite element model was developed to determine the stress distribution at the interface between the bone and the orthodontic mini-implant, depending on the insertion angle and the applied force.

The analytical model was generated using imaging data (CBCT) and the characteristics of the mini-implant (8 × 1.7 mm) as specified by the manufacturer. A CBCT scan of the mini-implant inserted into a mandibular mould was performed to accurately observe and reproduce the contact established between the two structures.

For the construction of the analytical model, it was also necessary to determine the characteristics of the mini-implant, namely its dimensions and geometry (morphology). The following algorithm was applied:

I. Determination of the Thread Geometry

Determination of the length of segment (CD), expressed in pixels:

$$CD = \sqrt{(710 - 650)^2 + (565 - 395)^2} = 180 \text{ px}$$

Given that CD = 1.7 mm, we define the ratio:

$$\alpha = 180/1.7 = 106 \text{ px/mm}$$

Verification of the accuracy of the calculated ratio:

$$OB = \sqrt{(1333 - 680)^2 + (730 - 480)^2} = 700 \text{ px}$$

$$700/180 = 3.(8)$$

$$8/1.7 = 4.7$$

Since the ratios are not equal, another possible reference point was sought to

define the 8 mm length (fig. 1)

$$EB = \sqrt{(1333 - 565)^2 + (730 - 395)^2} = 840 \text{ px}$$

$$840/180 = 4.6 \approx 4.7 = 8/1.7$$

In this context, the ratio is corrected to α = 840 / 8 = 105 px/mm.

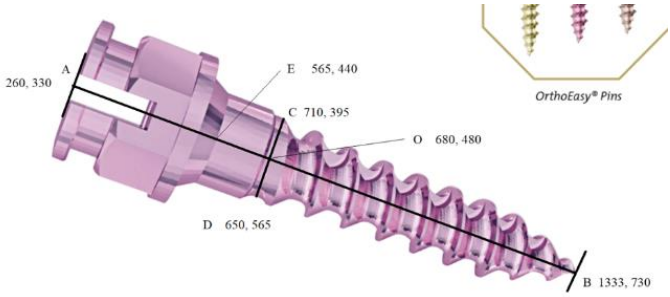

Fig. 1. Determination of the thread geometry using another reference point

II. Approximation of the Screw Geometry

The coordinates of the points are: A(915, 660), B(980, 680), C(980, 655), D(950, 649).Thus, the quadrilateral shown in the image has the following dimensions: AB = 68 px = 0.65 mm, BC = 25 px = 0.24 mm, CD = 30.59 px = 0.29 mm, DA = 46.32 px = 0.44 mm. Determination of the unthreaded segment length: OE = 121.76 px = 1.15 mm s (fig 2).

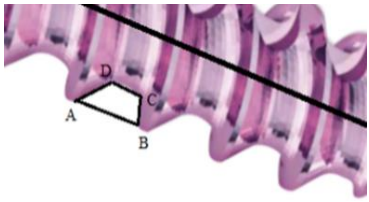

Fig 2. Approximation of the screw geometry

Once the dimensions and morphology of the mini-implant were calculated and analyzed, the 3D model could be created. Using the obtained data, the parameters were transferred into the SolidWorks software, where the analysis proposed in this study was performed using the Finite Element Method (FEM).

In this study, the bone sample was modeled as a cylinder (fig. 3) with a diameter of 10 mm and a height of 10 mm, of which 2 mm represented the cortical bone and the remaining 8 mm corresponded to the trabecular portion. For this geometric body representing the bone, the following material properties were assigned: Young's modulus of 1.5×10^{10} N/mm² (MPa) for the cortical layer and 1.5×10^{9} N/mm² (MPa) for the trabecular component, both with a Poisson's ratio of 0.3.

For the mini-implant, material properties corresponding to titanium were applied, with a Young's modulus of 1.05×10^{5} N/mm² (MPa) and a Poisson's ratio of 0.37. The materials were considered homogeneous, linearly elastic, and isotropic. Subsequently, the two bodies were brought into contact, thereby defining the interface between the mini-implant and the bone.

A total of 16 geometric models were developed according to the objectives of this study, each incorporating the proposed

variable specifically, the insertion angle of the mini-implant (90°, 60°, 45°, and 30°) and the para-axial force applied to it (1 N and 5 N). The stress generated by each applied force on both the mini-implant and the surrounding bone was evaluated, and the results were expressed according to the von Mises stress criterion.

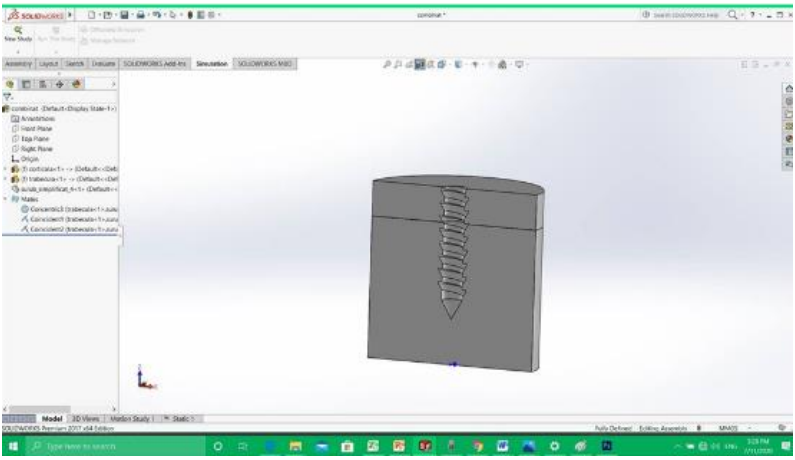

Fig. 3. Raw bone block (section view)

RESULTS

The stress evaluated for the mini-implant with constant dimensions of 8 × 1.7 mm, under different insertion angles (30°, 45°, 60°, and 90°) and applied forces (1 N and 5 N), increased proportionally with both the angle and the magnitude of the applied force. A similar trend was also observed for the stress distributed within the bone.

TABLE I.

Results of the static analysis under a 1 N load

Force value	Inclination relative to the screw axis	Inclination with respect to the screw axis	Maximum bone stress
1N	90 deg	27MPa	13MPa
	60 deg	24MPa	12MPa
	45 deg	18MPa	10MPa
	30 deg	10MPa	6MPa

TABLE II -

Results of the static analysis under a 5 N load

Force value	Inclination relative to the screw axis	Inclination with respect to the screw axis	Maximum bone stress
5N	90 deg	266MPa	70MPa
	60 deg	200MPa	55MPa
	45 deg	145MPa	40MPa
	30 deg	78MPa	23MPa

The maximum stress values on the mini-implant and on the bone were observed, for each applied force, at an insertion angle of 90°. The area subjected to the highest stress was located at the interface between the mini-implant (screw) and the cortical bone. This region exhibited the greatest concentration of stress, which was distributed across both the implant and the bone in all simulations.

The stress gradually decreased within the trabecular bone, where the lowest values were recorded. The bone underwent deformation in the micrometre range under a 1 N load, while at a 5 N load, the deformation increased, reaching values in the tens of micrometres.

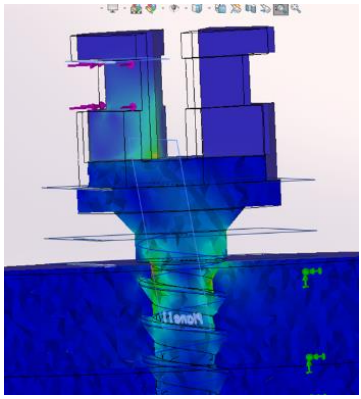

Fig. 4. Determination at 1 N, 90°

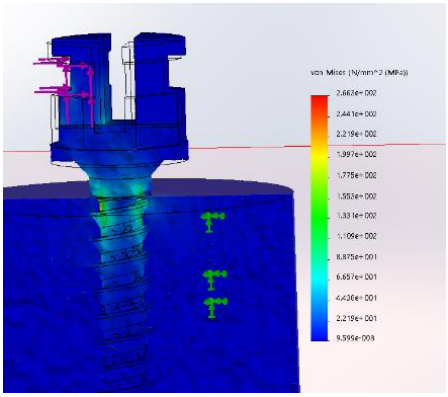

Fig. 5. Determination at 5 N, 90°

DISCUSSION

The current finite element analysis (FEA) offers a detailed exploration of the biomechanical interactions between orthodontic mini-implants and the periodontal support structures. The simulation revealed that both insertion angle and applied load significantly influence the magnitude and distribution of stress at the implant-bone interface. The highest von Mises stress values occurred at 90° insertion, increasing proportionally with force intensity (1N-5N). These results are consistent with previous studies indicating that axial loading amplifies stress transfer to the cortical bone and periodontal ligament, which are the primary structures responsible for implant anchorage and biological adaptation (1, 5, 8, 11, 15).

From a periodontal perspective, the findings highlight the delicate biomechanical balance required to preserve tissue integrity. The cortical layer, with its higher elastic modulus, absorbed the greatest load, while stress values decreased progressively toward the trabecular bone. This gradient is critical for maintaining physiological bone remodeling and preventing microdamage or localized resorption. Excessive compressive stress near the cortical interface can impair microcirculation, limit osteoblastic activity, and initiate inflammatory responses, potentially compromising periodontal health and long-term mini-implant stability (6-10).

In contrast, moderate insertion angles (45°- 60°) produced a more uniform stress distribution, reducing localized peaks within the cortical bone and promoting more favorable load sharing across the periodontium.

This supports the clinical rationale that proper angulation minimizes mechanical trauma to the alveolar crest and reduces the risk of microcracks or cortical perforation - factors that have been correlated with implant loosening or failure in vivo (15-17).

At the tissue level, bone deformation ranged from a few micrometers at 1 N to several tens of micrometers at 5 N, remaining within the physiological tolerance of the periodontal ligament (PDL) and alveolar bone. However, repeated loading, particularly at high forces, may induce micro strain accumulation and activate bone remodeling pathways through mechanic transduction mechanisms. Studies have shown that controlled stress within this range stimulates adaptive bone remodeling, while excessive mechanical input may trigger osteoclastic resorption and connective tissue damage (16-18).

Beyond the mechanical domain, this study aligns with the emerging concept of oral and mental wellbeing in modern orthodontics. The predictable stability of mini-implants contributes to functional efficiency, shorter treatment duration, and reduced anxiety, factors that significantly impact patient satisfaction and psychological comfort. Clinical research has shown that predictable biomechanical control and minimally invasive procedures improve the patient's emotional perception of therapy and enhance treatment adherence (12-14, 19-21). The integration of biomechanical precision with psychological safety thus represents a paradigm shift toward holistic orthodontic care, where success is measured not only by mechanical outcomes but also by quality of life and emotional balance (21).

While the FEA method offers valuable predictive insights, certain limitations must be acknowledged. The model assumes homogeneous, isotropic, and linearly elastic materials, which differ from the anisotropic and viscoelastic behavior of real biological tissues. Additionally, static loading cannot fully replicate the complex dynamic forces of mastication and orthodontic movement. Future research should incorporate patient-specific anatomical models, nonlinear material properties, and in vivo validation to strengthen the translational potential of simulation data (8, 10, 22-24).

In summary, the present study bridges the gap between biomechanical simulation and clinical reality, demonstrating that optimal stress management at the mini-implant-periodontium interface supports both tissue health and patient psychological wellbeing. Such an integrated perspective underscores the future direction of orthodontic innovation: the alignment of engineering precision with human-centered care.

Nevertheless, the present study has limitations inherent to computational simulations. The materials were considered homogeneous, isotropic, and linearly elastic, whereas real biological tissues exhibit anisotropy and viscoelasticity. Moreover, the static loading conditions do not fully replicate the dynamic forces present in vivo. Future research should combine patient-specific 3D imaging, nonlinear material models, and longitudinal clinical validation to establish more precise correlations between simulated stress values and biological outcomes.

CONCLUSIONS

The present finite element analysis demonstrated that both insertion angle and applied load play a crucial role in determin-

ing stress distribution at the mini-implant-bone interface. The highest von Mises stress values were observed at a 90° insertion angle and increased proportionally with load intensity, localizing predominantly in the cortical bone. These findings confirm the cortical layer's key role in load transfer and underline the importance of selecting optimal angulation to minimize concentration zones and prevent microdamage.

From a mechanical standpoint, moderate insertion angles (45°-60°) produced more favorable stress distributions and may contribute to enhanced primary stability, particularly in regions with reduced cortical thickness.

Although the observed bone deformation remained within the physiological range, sustained or excessive loading could initiate localized remodeling or resorption if not properly controlled.

Clinically, the integration of finite element modeling provides a predictive framework for optimizing orthodontic anchorage design, improving safety margins, and individualizing treatment according to bone density and anatomical morphology.

Beyond mechanical optimization, the stable and minimally invasive use of mini implants has positive implications for oral function, aesthetics, and patient mental wellbeing, contributing to reduced anxiety and improved overall quality of life.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding.

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ARE WOMEN LESS WILLING TO CHANGE? THE INFLUENCE OF STRESS, ANXIETY AND DEPRESSION IN GASTROINTESTINAL SYMPTOMS

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ARE WOMEN LESS WILLING TO CHANGE? THE INFLUENCE OF STRESS, ANXIETY AND DEPRESSION IN GASTROINTESTINAL SYMPTOMS. (Abstract) The nutritional profile of an individual constitutes one of the main regulators of gastrointestinal physiology and psychological well-being. This study **aimed** to identify the presence of gastrointestinal symptoms in medical students, health-related behaviors, and their relationship with the presence of stress, anxiety, and depression. **Materials and methods**: a total of 152 students from all years of study were included in the study. The respondents had to fill in an online questionnaire. Sociodemographic and medical related data as well as information about diet, nutritional status, quality of life and health-related behaviors were gathered. Psychological status was assessed using the *DASS-21*. Eating habits were evaluated using the *Rapid Eating Assessment for Participants*, while gastrointestinal symptom severity and impact on daily life were measured using the *Visual Analogue Scale for Irritable Bowel Syndrome* (VAS-IBS). Statistical analyses were performed using *IBM Statistical Package for Social Sciences* (*SPSS*) Statistics for Windows, version 24 (SPSS Inc., Chicago, IL, USA). **Results**: The mean age of participants was 21.28 ± 1.93 years (range: 18-25). More than half stated that they noticed changes in digestion when experiencing fatigue or poor sleep, with frequent changes observed in 27.6% of participants (N = 42). Most participants showed normal levels of stress (88.2%), depression (75%), or anxiety (74.3%). The results on the Food Sufficiency subscale revealed that most participants (71.71%, N = 109) did not eat enough, and did not have an adequate intake of daily meals, proteins, and calcium-rich foods. Additionally, the results of the Healthy Eating subscale showed that most participants (93.4%, N = 142) had insufficient consumption of healthy foods. In contrast, the results on the Low Nutrient Density Foods subscale showed that most participants (88.81%, N = 135) reported limited consumption of foods with low nutritional density, and therefore a more favorable eating behavior. Women demonstrated significantly lower results in terms of willingness to change (M = 1.71 ± 0.68) compared to men (M = 2.00 ± 0.75). **Conclusions**: The data obtained indicated that stress is the main predictor of the severity of gastrointestinal symptoms, while food sufficiency exerted a protective effect. A more adequate diet was associated with reduced symptoms of Irritable Bowel Syndrome. These results show the importance of psychological factors and the quality of food in the quality of life of the young population. **Keywords:** STRESS, ANXIETY, DEPRESSION, MEDICAL STUDENTS, GASTROINTESTINAL SYMPTOMS, SLEEP, DIET, NUTRITIONAL STATUS, PHYSICAL ACTIVITY.

INTRODUCTION

Contemporary psychobiological research has increasingly explored the bidirectional relationship between mental health and somatic functioning, with particular emphasis on the mechanisms underlying the gut-brain axis (1-3). This axis represents a neurohormonal network that connects the enteric nervous system with the central nervous system and provides a psychological framework for the recurrent comorbidity identified between psychological distress and gastrointestinal dysfunction (4). The prevalence of mental health issues is rising in the young adult population, and the mechanisms by which these psychological factors manifest as physical symptoms remain multifactorial. Psychological distress is most commonly operationalized through stress, anxiety, and depression, each exerting specific physiological effects on gastrointestinal homeostasis. Stress, characterized as an integrated physiological and psychological response to actual or perceived threats, involves the central nervous system response. Chronic or excessive activation of key brain regions contributes to emotional dysregulation, excessive visceral sensitivity and disturbances in gastrointestinal function (5).

Although anxiety is a natural adaptive response to stress, when it becomes persistent, excessive, or disproportionate, it may develop into an anxiety disorder, one of the most prevalent classes of mental health conditions worldwide (6). In 2021, it affected more than 359 million people, with a notably higher prevalence in women than in men. Similarly, depression affected approximately 332 million people in the same year, being 1.5 times more common in women than in men (7). The development of both depression and anxiety disorders is influenced by a multifactorial interplay between biological, psychological, social, and environmental factors. Consequently, these affective disturbances interact with the gastrointestinal system through specific neuroimmune pathways, linking psychological dysregulation to somatic impairments.

Growing evidence indicates that this association is modulated by modifiable lifestyle factors such as nutritional habits, sleep quality, and physical activity (8-10). The nutritional profile of an individual represents one of the main regulators of gastrointestinal physiology and psychological well-being. Contemporary dietary patterns among young adults frequently exhibit a "Westernized" profile, characterized by high intake of processed foods, with limited nutrient density (11). Despite high caloric consumption, these dietary patterns remain deficient in essential macro- and micronutrients required for proper nervous system functioning. Furthermore, the consumption of vegetables and cereal products rich in dietary fiber and essential nutrients remains insufficient (12). Increasing evidence suggests that adequate nutrient intake exerts a protective effect against systemic inflammation while preserving gastrointestinal motility and function (13).

In contrast, the consumption of low-quality food has been associated not only with the exacerbation of functional gastrointestinal symptoms but also with the intensification of anxiety and depressive states through neuroimmune pathways. Consequently, diet may function as a critical mediator whereby psychological distress may promote maladaptive eating behaviors, ultimately undermining gastrointestinal health and creating a vicious cyclic

pattern (14). However, nutrition must be considered within the broader framework of lifestyle-related health determinants. The prevalence of physical inactivity and sleep disturbances has increased in recent decades, with both factors demonstrating independent and codependent associations with obesity and alterations in gut microbiota composition (15, 16). According to Zhu and Huang (17), a physiological state of sleep improves metabolism and immunity, whereas sleep deprivation promotes hyperalgesia, affecting neurobiological systems and mediators that simultaneously impair emotional regulation.

Similarly, physical activity modulates autonomic nervous system balance and the endocrine responses. Sedentarism has been associated with increased sympathetic activity, anxiety, depression and gastrointestinal dysfunction vulnerability (18). Despite extensive research examining these factors individually, integrative approaches that evaluate the synergistic effects of sleep, diet, and physical activity in relation to stress, anxiety, and depression-and their cumulative impact on gastrointestinal health in non-clinical populations-remain underdeveloped.

In recent years, dysregulation of the bidirectional brain-gut axis has been increasingly recognized as a central framework for understanding the pathophysiology of Irritable Bowel Syndrome (IBS), which is characterized by abnormal functioning of the enteric, autonomic, and central nervous systems (19). The comorbidity between IBS and psychiatric diseases, such as anxiety and depression, has been individualized in up to 60% of patients (20). While the connection between Irritable Bowel Syndrome and stress is well documented through its impact on the intestinal sensitivity, secretion, motility, and permeability, the behavioral pathways underpinning this association require further elucidation. The extent to which negative emotional states affect gastrointestinal health directly or through mediated changes in lifestyle habits remains insufficiently understood. A thorough understanding of these dynamics is essential for the development of holistic health strategies aimed at the 18 to 25-year-old population. The present study aimed to characterize the multidimensional interplay between lifestyle behaviors, psychological functioning and gastrointestinal health in a young adult population. The central aim was to evaluate the extent to which dietary behaviors, defined by both nutrient sufficiency and dietary quality, mediate the impact of psychological distress on the intensity of gastrointestinal symptoms.

MATERIALS AND METHODS

Study Population. The participants were selected using the snowball method between. The study was developed during October and November 2025. The inclusion criterion in the study was the completion of the questionnaire before the submission deadline. Questionnaires were distributed to medical science students, from all years of study. The respondents were informed about the purpose of the research and the use of the collected data. No incentives were offered. Finally, 152 questionnaires were included in the study.

Data collection. The questionnaire was constructed using Google Forms (Alphabet, Mountain View, CA, USA).

a. The first part of the questionnaire collected socio-demographic and lifestyle-related data (such as age, gender, environment of origin, Body Mass Index - BMI, religion, adherence to religious fasting

practices, smoker/non-smoker status, consumption of psychoactive substances, number of hours of sleep per night, self-assessment of sleep quality, and engagement in physical activities).

b. The second section of the questionnaire included information about the participants' health status (diagnosis of gastroesophageal reflux disease, irritable bowel syndrome, and respiratory, gastric, or intestinal infections, as well as the frequency of medication use. Information was also collected regarding the use of medication or dietary supplements, such as antibiotics, magnesium, zinc, vitamin E, supplements, B vitamins, and omega-3/omega-6 supplements. Additionally, the participants reported the type of diet they followed, including Mediterranean, DASH, Nordic, Japanese, traditional Romanian, keto, oligoantigenic, or vegetarian/vegan diet.

c. The third section included a psychological questionnaire designed to measure negative emotional states which can have a major influence on the health status of the participants - *DASS-21* (21). The questionnaire was a set of three self-rating scales that indicated the severity of core symptoms in the domains of depression, anxiety, and stress. The DASS score was calculated by summing the responses to the items of each subscale, with each item rated on a Likert scale from 0 (“Not at all”) to 3 (“Applied very much or almost all of the time”).

d. The next part of the questionnaire used a tool that quickly assessed the participants' eating habits - *Rapid Eating Assessment for Participants, 21 questions - REAP v.2* (22). This instrument is an updated and validated dietary screener designed to quickly assess a patient's eating behaviors, dietary patterns, and readiness to change. The questionnaire included five subscales: Food Sufficiency, Healthy Eating, Intake of Low-Nutrient Foods, Exercise and Willingness to Change. The response options were "Every day", "More than two times a week" or "More than three times a week" (depending on the item), "Two or fewer times a week" or "Three or fewer times a week" and "Never". The score assigned to the response options ranged from 0 to 3 points. The highest possible score for REAP v.2 is 60 points. Although higher numbers generally represent healthier habits, a given person might be high on one subscale and low on another.

e. The final part of the survey included a self-report tool designed to measure the severity of gastrointestinal symptoms and their impact on a patient's life - *The Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS)* (23). It consists of seven visual analogue scales assessing pain, diarrhea, constipation, bloating, vomiting/nausea, psychological well-being, and the influence of intestinal symptoms on daily life. Each item received a score from 1 (*maximum perceived severity*) to 10 (*absence of symptoms*). Two additional items, if the subject experienced defecation urgency or a sensation of incomplete evacuation when defecating (*yes* or *no*).

In addition, at the end of the questionnaire, several items were added to assess whether certain foods had a perceived negative effect on the diet, and which ones, whether changes in digestion were observed when participants were tired or slept poorly, as well as digestive symptoms that occurred during stressful periods.

Statistical analysis. All statistical analyses for this research were performed using *IBM Statistical Package for Social*

Sciences (SPSS) Statistics for Windows, version 24 (SPSS Inc., Chicago, IL, USA). Results for descriptive statistics were expressed as means and standard deviations (SD).

The normality of data distribution was tested using the Kolmogorov-Smirnov test. Given that the data were partially normally distributed, parametric tests were applied. To assess the comparative results considering gender and diagnosis of a chronic disease, the Independent Sample t-Test was performed.

Pearson's correlation coefficient was used to test the relationship between variables. A p-value < 0.05 was considered statistically significant. A multiple mediation analysis was also conducted to explore the role of Food Sufficiency and Low-Density Nutrient Foods as mediators between stress and gastrointestinal symptoms. The PROCESS procedure for SPSS, developed by Andrew F. Hayes, was used to test this model. The statistical significance threshold was set at $p < 0.05$, which ensured a rigorous interpretation of the results.

Ethical approval. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee of Prolife Clinics Iași, Romania, No.74/03.07.2025.

RESULTS

Socio-demographic and lifestyle-related data

The participants aged M = 21.28 ± 1.93, with ages ranging from 18 to 25 years. Most participants were women (76.3%, N = 116) and more than a half originated from urban areas (66.4%, N = 101).

Regarding weight status calculated according to Body Mass Index (BMI), more than half of the participants had a normal weight (63.8%, N = 97), less than one quarter were overweight (23%, N = 35), and a smaller proportion (9,9%, N = 15) were underweight, with BMI values below the WHO limit of 18.5 kg/m^2. The results showed that most participants were Orthodox Christians (78.9%, N = 120). More than half said they did not fast (63.8%, N = 97), while one quarter reported adherence to Orthodox Christian fasting periods (25.7%, N = 39). More than half of the participants were non-smokers (56.6%, N = 86), whereas one quarter declared that they smoked daily (25%, N = 38). Most of the participants did not consume psychoactive substances (90.8%, N = 138), while a small proportion consumed them occasionally (8.6%, N = 13). More than half of the participants reported sleeping between 5 and 7 hours per night (63.8%, N = 97) and more than a quarter slept between 7 and 9 hours per night (25.7%, N = 39). The quality of sleep during the night described by most participants was: "I sleep well, but sometimes I wake up" (50%, N = 76), "I sleep very well, without interruptions" (25%, N = 38), "I am a light sleeper and wake up often" (11.8%, N = 18).

Regarding physical activity, nearly half of the participants reported engaging in light physical activity occasionally (48.7%, N = 74), while one third reported performing moderate physical activity several days a week (30.3%, N = 46).

Participants' health, digestion and diet status

The participants were asked about whether they had ever been diagnosed with any diseases related to the digestive system. Most participants reported no such diagnoses. However, a small proportion had been diagnosed with gastroesophageal

reflux disease (7.2%, or 11 people) or irritable bowel syndrome (3.9%, or 6 people). Additionally, over one quarter of the participants (27.6%, N = 42) reported at least one episode of respiratory, gastric, or intestinal infection.

Regarding the use of medications and supplements, less than half of the participants (47.4%, N = 72) reported that they rarely used medications for any condition, while over one quarter reported monthly use (26.3%, N = 40). Within the previous 12 months, less than half of the participants reported antibiotic use (46.1%, N = 70). More than half of the participants used Magnesium supplements (52.6%, N = 80), and one third of the participants used Zinc supplements (33.6%, N = 51), B vitamins (35.5%, N = 54) or omega-3/omega-6 supplements (31.6%, N = 48). Vitamin E supplements were consumed by a small part of the participants (17.1%, N = 26).

Approximately one third of the participants reported following a traditional Romanian diet (31.6%, N = 48). Smaller proportions reported adherence to other diets: 9.9% (N = 15) followed a Vegetarian or Vegan diet, 7.9% (N = 12) chose a Mediterranean diet, and 3.9% (N = 6) consumed a Nordic diet.

Participants were asked whether there were certain foods that clearly affected their digestion. Responses were distributed as follows: about one third said “never” (31.6%, N = 48), more than one quarter answered “yes, definitely” (25.7%, N = 39), and less than one quarter answered “sometimes” (23.7%, N = 36). Foods that triggered digestive disorders included dairy products (9.2%, N = 14), fast food and pastries (8.55%, N = 13), some fruits and vegetables such as bananas, tomatoes, peas, beans (6.57%, N = 10), but also sweetened or fizzy drinks (3.28%, N = 5).

Over half of the participants reported experiencing noticeable changes in digestion when they were fatigued or had insufficient sleep, with such changes occurring frequently (27.6%, N = 42) or occasionally (29.6%, N = 45). In addition, during stressful periods, participants manifested various digestive symptoms, with abdominal pain (33.6%, N = 51) or bloating (26.3%, N = 40) being the most commonly reported.

Depression, Anxiety, Stress Scale (DASS-21)

The scale demonstrated very good internal consistency, as determined by Cronbach’s alpha of 0.96. Analysis of the distribution of scores on the DASS-21 showed that approximately half of the participants had a normal level of stress (54.6%), depression (49.3%), or anxiety (44.1%) (tab. I).

TABLE I.
DASS-21 Severity Classification

	Normal	Mild	Moderate	Severe	Extremely severe
Stress	83 (54.6%)	21 (13.8%)	21 (13.8%)	14 (9.2%)	13 (8.6%)
Depression	75 (49.3%)	7 (4.6%)	40 (26.3%)	8 (5.3%)	22 (14.5%)
Anxiety	67 (44.1%)	16 (10.5%)	30 (19.7%)	11 (7.2%)	28 (18.4%)

Frequency and percentages (%)

Rapid Eating Assessment for Participants (REAP)

The Cronbach Alpha score was 0.54 for the total scale, 0.48 for *Food Sufficiency subscale*, 0.56 for *Healthy Eating Pattern subscale* and 0.66 for *Low Nutrient Density Foods subscale*. For these REAP subscales, as well as for the items related to *Physical Activity* and *Disponibility to Change* we obtained the following results:

a. food sufficiency - M = 6.24 ± 2.45,
b. healthy eating pattern - M = 9.16 ± 3.22,
c. low nutrient density foods - M = 21.93 ± 3.66,
d. physical activity - M = 2.30 ± 0.92,
e. disponibility to change - M = 1.78 ± 0.71.

The total REAP score was on average M = 39.61 ± 5.98, with scores ranging from 21 (1.3%, N = 2) to 57 (0.7%, N = 1).

The results on the Food Sufficiency subscale indicated that most participants (71.71%, N = 109) did not eat enough, and did not have an adequate intake of daily meals, proteins and calcium-rich foods. Similarly, results on the Healthy Eating subscale showed that most participants (93.4%, N = 142) reported insufficient consumption of healthy foods. In contrast, the results on the Low Nutrient Density Foods subscale showed that most participants (88.81%, N = 135) demonstrated reduced consumption of foods with low nutritional density, indicating a more favorable eating behavior.

The Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS). The total score for the VAS-IBS scale was M = 53.59 ± 14.44, with values ranging from 10 (0.7%, N = 1) to 70 (10.5%, N = 16). Thus, the participants reported a low severity of gastrointestinal symptoms and a relatively good quality of life.

Regarding the response to the last two items of the instrument, less than one quarter of the participants (23.7%, N = 36) stated that they had felt an urgency to defecate in the previous 2 weeks, and less than half of the participants (43.4%, N = 66) reported a sensation of incomplete bowel evacuation. The scale had good internal consistency, as determined by Cronbach's alpha of 0.86.

Comparative and correlational analysis. The independent samples t-test revealed the existence of significant gender differences in Body Mass Index of the participants ($t(150) = 4.15$, $p < 0.001$), with women exhibiting significantly lower BMI values (M = 21.98 ± 3.74) compared to men (M = 24.99 ± 3.96). In addition, significant differences were also identified in terms of the participants' willingness to change ($t(150) = 1.51$, $p = 0.03$). Women had significantly lower results in terms of willingness to change (M = 1.71 ± 0.68) compared to men (M = 2.00 ± 0.75).

Significant differences were identified between participants who received a positive diagnosis and those who did not have a diagnosis of irritable bowel syndrome in terms of physical activity levels ($t(150) = -2.17$, $p = 0.03$). Thus, those who were diagnosed with irritable bowel syndrome had a lower physical activity score (M = 1.50 ± 1.04), compared to those who did not have this condition (M = 2.33 ± 0.91).

The results of the comparative analysis showed that there were significant differences for the variable "positive/negative diagnosis for irritable bowel syndrome" in terms of stress ($t(150) = 3.84$, $p < 0.001$), depression ($t(150) = 4.27$, $p < 0.001$) and anxiety levels ($t(150) = 4.36$, $p < 0.001$),

meaning that those with a positive diagnosis had a significantly higher score on all these psychological scales (M = 31.00 ± 11.91 for stress, M = 35.66 ± 18.39 for depression, respectively 26.66 ± 14.62 for anxiety) compared to those without this diagnosis (M = 14.53 ± 10.22, M = 13.19 ± 12.37, respectively 9.89 ± 8.98). The independent samples t-test indicated the existence of significant differences between participants who received a positive diagnosis and those who did not have a diagnosis of irritable bowel syndrome in terms of VAS-IBS scale results ($t(150) = -2.17$, $p = 0.03$). Thus, those who were diagnosed with irritable bowel syndrome had a lower VAS-IBS score (M = 33.67 ± 12.48), compared to those who did not have this condition (M = 54.40 ± 13.96).

Significant differences were identified between participants who consumed and those who did not consume antibiotics in terms of their willingness to change ($t(150) = 2.48$, $p = 0.01$). Those who reported antibiotic use in the previous 12 months demonstrated a significantly higher score on readiness for change (M = 1.93 ± 0.80) than those who did not report antibiotic use (M = 1.65 ± 0.59).

The results of the comparative analysis showed that there were significant differences for the variable “consumption of antibiotics" in terms of depression ($t(150) = 2.09$, $p = 0.03$) and VAS-IBS score ($t(150) = - 2.76$, $p = 0.007$). Thus, those who had taken antibiotics in the previous 12 months had a significantly higher score on depression (M = 14.65 ± 12.86) than those who had not ((M = 10.75 ± 10.10).

Moreover, those who had taken antibiotics in the previous 12 months had a lower score on the VAS-IBS scale and therefore, a greater severity of irritable bowel syndrome symptoms (M =50.06 ± 16.60) compared to those who had not taken antibiotics (M = 56.60 ± 11.52).

The Pearson correlational analysis found a significant positive association between age and number of hours slept per night ($r = 0.17$, $p = 0.03$), indicating that older participants tended to report longer sleep duration.

The number of hours of sleep correlated negatively with the level of depression ($r = -0.19$, $p = 0.01$), meaning that increased nighttime sleep was associated with lower reported depression. Additionally, there were positive correlations between the number of hours of sleep per night and the scores on the REAP instrument subscale for Low Density Nutrient Foods ($r = 0.17$, $p = 0.03$), suggesting that longer sleep duration was linked to a greater tendency to consume foods low in nutritional density, such as fatty meats, solid fats, sweetened beverages, sweets, and processed snacks. The REAP section on Food Sufficiency correlated negatively with levels of stress ($r = -0.40$, $p < 0.001$), depression ($r = -0.43$, $p < 0.001$), and anxiety ($r = -0.42$, $p < 0.001$), meaning that the more satisfactory the diet, the lower the levels of stress, depression, and anxiety, respectively. Furthermore, Food Sufficiency correlated positively with gastrointestinal symptoms - as measured by VAS-IBS ($r = 0.36$, $p < 0.001$), indicating that the more satisfactory the diet, the less severe the gastrointestinal symptoms and the better the quality of life.

The REAP section on Low Nutrient Density Foods correlated negatively with levels of stress ($r = -0.22$, $p = 0.006$), depression ($r = -0.20$, $p = 0.10$), and anxiety ($r = -0.23$, $p = 0.003$), meaning that the more the participants consumed foods with low nutritional density, the lower the levels

of stress, depression, and anxiety, respectively. In addition, Low Nutrient Density Foods correlated positively with gastrointestinal symptoms ($r = 0.21$, $p = 0.007$), meaning that the more the participants consumed foods with low nutritional density, the less severe the gastrointestinal symptoms.

There was a negative correlation between the level of physical activity measured using the REAP instrument and the level of anxiety ($r = -0.20$, $p = 0.01$), indicating that the higher the level of physical activity, the lower the anxiety. The level of physical activity correlated positively with gastrointestinal symptoms ($r = 0.22$, $p = 0.005$), meaning that the more the participants exercised, the less severe the gastrointestinal symptoms were.

Readiness to change within the REAP instrument correlated negatively with gastrointestinal symptoms ($r = -0.21$, $p = 0.009$), meaning that the greater the participants' willingness to change their eating behavior, the greater the tendency for the severity of gastrointestinal symptoms to increase. A negative correlation existed between willingness to change and Food Sufficiency ($r = -0.21$, $p = 0.009$), meaning that the more interested the participants were in changing their eating behavior, the more their diet was less satisfying. In addition, willingness to change correlated positively with anxiety levels ($r = 0.24$, $p = 0.003$), meaning that the more the participants were interested in changing their eating behavior, the higher their anxiety levels.

A negative correlation was identified between anxiety levels and gastrointestinal symptoms ($r = -0.66$, $p < 0.001$), meaning that the higher the anxiety level, the greater the severity of gastrointestinal symptoms. In addition, the level of stress ($r = -0.59$, $p < 0.001$) and depression ($r = -0.60$, $p < 0.001$) correlated negatively with gastrointestinal symptoms, indicating that the higher the stress and depression levels, the greater the severity of gastrointestinal symptoms.

Mediation analysis. A mediation analysis was conducted to test the mechanisms by which stress level influences the severity of digestive symptoms, considering several potential mediators, represented by the REAP subscales (Food Sufficiency, Low Nutrient Density Foods). The associations between the variables involved have already been analyzed. Thus, significant correlations were found between the two variables:

a. food sufficiency was negatively associated with stress levels ($r = -0.40$) and positively associated with the severity of digestive symptoms ($r = 0.36$);
b. stress level was negatively associated with gastrointestinal symptomatology ($r = -0.59$);
c. low nutrient density foods was positively associated with gastrointestinal symptomatology ($r = 0.21$) and negatively with stress level ($r = -0.22$).

Linear regression analysis revealed the following results:

a. stress significantly predicted digestive symptoms ($\beta = -0.10$, $p < 0.001$);
b. stress significantly predicted food sufficiency ($\beta = -0.09$, $p < 0.001$);
c. stress predicted low nutrient density foods ($\beta = -0.05$, $p = 0.048$);
d. food sufficiency did not predict low nutrient density foods ($\beta = 0.19$, $p = 0.13$);

e. low Nutrient Density Foods did not predict digestive symptoms (β = 0.29, p = 0.27).

The data obtained indicated that stress was the main predictor of the severity of gastrointestinal symptoms. Food Sufficiency had a protective effect: a more adequate diet was associated with reduced symptoms of Irritable Bowel Syndrome (IBS). Low Nutrient Density Foods did not significantly contribute to gastrointestinal symptomatology in the presence of other variables.

In conclusion, the model did not support full serial mediation, but clearly supported simple mediation through Food Sufficiency. Although stress had a strong direct effect on IBS symptomatology, part of this effect was explained by changes in basic eating behavior.

While Food Sufficiency was found to mediate the effects of stress, similar analyses for anxiety and depression did not reveal any notable indirect effects. However, both anxiety and depression were significantly associated with gastrointestinal symptom severity, supporting their relevant role in the pathogenesis of IBS, through predominantly direct mechanisms. This suggests the involvement of distinct psychobiological mechanisms, independent of diet, in the relationship between anxiety, depression and gastrointestinal symptom severity.

DISCUSSION

This study investigated the multifactorial correlation between lifestyle factors, with particular emphasis on nutritional habits, psychological well-being, gut health and mental health. The results characterize a vulnerability profile in which psychological distress functions as a central catalyst for both maladaptive eating behaviors and functional somatic symptomatology.

Data analysis revealed that different psychological factors exert a distinct influence on gastrointestinal health. While stress was identified as a direct predictor of gastrointestinal symptom severity, mediation analysis indicated that a portion of this effect was accounted for by changes in eating behavior, specifically Food Sufficiency. This implies that stress-related changes in nutrition can play a role in exacerbating gastrointestinal symptoms. Similar patterns have been observed in broader mental health studies. Madison and Kiecolt-Glaser (2019) reported that stress and depression affect gastrointestinal function both directly and indirectly through diet related behaviors (24). However, in our study, an indirect dietary influence, especially food insufficiency, was observed exclusively for stress, with no significant mediation identified for depression.

In contrast, anxiety and depression were predominantly associated with gastrointestinal symptoms through direct psychological mechanisms. These findings underscore the potential role of the neural and endocrine pathways of the gut-brain axis. Rathore *et al. (*2025) also showed in their comprehensive review that anxiety and depression affect gastrointestinal function directly through gut-brain axis mechanisms, highlighting processes beyond dietary modulation (25). Moreover, this result is consistent with the Biopsychosocial Model of IBS, suggesting that anxiety, particularly, may induce direct physiological changes, including visceral hypersensitivity and vagus nerve mediated motility alterations. (26).

Diet quality has been found to be associated with mental disorder severity

through different pathways. Participants with adequate nutrient intake reported lower levels of stress, anxiety, and depression while also having less severe gastrointestinal symptoms, demonstrating the protective effect of Food Sufficiency. This result may reflect the contribution of essential nutrients in regulating neurotransmitter pathways and systemic inflammation, both central to maintaining emotional regulation and gut homeostasis. In contrast, increased consumption of low-density foods corresponded with lower stress levels and decreased anxiety and depression symptomatology in our sample. This particular finding diverges from the results of Sparling *et al.* (2025) and Solomou *et al*. (2023) who reported that both the consumption of low nutrient quality foods and food insufficiency are associated with higher levels of stress, anxiety and depressive symptoms (27, 28).

This discrepancy can be explained by the study design and outcome assessment. Prior studies have focused on long-term dietary patterns, whereas this study reflects a short-term emotional regulation through palatable, calorie-dense foods, illustrating the correlation with the "comfort food" phenomenon. According to Gemesi *et al.* (29), most stress-eaters are particularly enjoying low nutrient foods such as cookies, fast-food, chocolate, while almost 70% of them are overeaters.

A comparison by sex revealed that women exhibit a lower Body Mass Index and lower reported willingness to change than men. The significant gender differences in terms of BMI are consistent with the Tack SHS survey across 12 European countries, which reported a 25.7 kg/m² BMI for men compared with 25.0 kg/m² for women (30). Our finding regarding higher willingness to change among men is in contrast with previous results, which suggests that women are generally more concerned with diet and weight control (31-32). The observed pattern may be explained by the fact that the women in the sample may view their current eating behaviors as adequate by correlating them with a lower BMI. On the other hand, men may be more inclined to acknowledge the need for lifestyle changes associated with elevated BMI values. The result can be determined by several factors: socio-demographic (the young women included in the study are 18-25 years old, which favors a good level of health), medical (the BMI level is comfortable) and socio-cultural (the young women value health, declared that they diet and are concerned about a healthy lifestyle, a fact proven by the researched variables, such as sleep, nutrition, physical activity, lifestyle, etc.) that make them less willing to change because they fill comfortable and satisfied with their body-image.

A key finding of the current study is the strong correlation between irritable bowel syndrome (IBS) and increased psychological burden. Participants with IBS scored significantly higher on the measures of stress, anxiety, and depression. These findings are concordant with the established concept of a “vicious cycle” of functional gastrointestinal disorders. This concept suggests that the unpredictability of symptoms exacerbates psychological distress and perpetuates condition severity (33).

Furthermore, patients with IBS reported lower VAS-IBS scores, reflecting both greater symptom severity and lower perceived quality of life in individuals without a clinical diagnosis. Both the participants with or without IBS stated that they manifested digestive symptoms such as ab-

dominal pain and bloating during stressful periods, which reinforces the idea of an immediate correlation between stress and intestinal function.

Individuals from our study who reported antibiotic consumption in the last 12 months revealed greater depressive symptoms and more severe gastrointestinal issues than non-users. This result confirms the causal link between antibiotic use and adverse mental health outcomes. These findings support the microbiota-gut-brain axis hypothesis, suggesting that antibiotic-induced alterations in the gut microbiome can disrupt neurochemical signaling between the gut and brain (34). Consistent with our results, this group also showed a higher willingness to change their dietary habits, possibly reflecting a response to the symptoms associated with post-antibiotic dysbiosis.

While our research individualizes a negative relationship between mental health and antibiotic use, a study by Dinan and Dinan (35) presents a nuanced perspective on these effects. They highlighted that certain antibiotics may have antidepressant effects by modulating neuroinflammation, microglial activity, and monoamine levels, whereas other antibiotics can negatively influence mental health by reducing microbial diversity, compromising intestinal barrier integrity, and promoting inflammation. This discrepancy can be attributed to differences in antibiotic type, dosage, and context of administration, as our sample primarily included participants using antibiotics for common short-term infections, whereas the positive antidepressant effects described in their review were associated with specific antibiotics administered in controlled clinical or experimental contexts.

Sleep is an important modulator of the gut-brain axis, and our study revealed a negative correlation between sleep duration and depression, confirming prior research on sleep deprivation and mood dysregulation. Sleep disturbances may disrupt the hypothalamic-pituitary-adrenal axis, resulting in a higher stress level, increased intestinal permeability, and intestinal dysbiosis, all leading to higher susceptibility to depressive symptoms (36).

The positive correlation between Low Nutrient Density Foods and longer sleep hours is a paradoxical finding from our study that can be explained by the lifestyle patterns of young adults. Participants might be sleeping more hours in total, but the irregularity of the sleep pattern might contribute to poorer nutritional choices through a specific craving for high-sugar, high-fat foods to provide a quick energy boost, implying that a disrupted circadian rhythm can influence the brain and conduct to an increased activation in reward centres. Our results are congruent with those in the scientific literature, which generally highlights a complex bidirectional relationship (37-38). Moreover, a vast majority of the participants stated that their sleep affected their digestion, which may reflect interconnected effects involving sleep, gastrointestinal function, mental health, and dietary habits.

Another indispensable lifestyle factor, physical activity was associated with lower anxiety levels and gastrointestinal symptoms. This can be explained by both the enhancement of vagal tone, reducing the level of stress and the release of anti-inflammatory cytokines during activity, decreasing systemic inflammation associated with anxiety and IBS (39). This observation was further supported by the finding

that participants diagnosed with IBS had significantly lower levels of physical activity. Sedentarily deprives IBS patients of stress-relieving and motility-regulating benefits, creating a detrimental cycle and aggravating their condition.

Strengths and limitations of the study. This is one of the few studies that conducted an integrative analysis of the synergistic effects of lifestyle factors in relation to the gut-brain axis in a young Romanian adult population. Certain limitations of this study need to be acknowledged. First, the sample consisted predominantly of young women and the results were not fully generalizable to the male population or to other age groups. Second, this research has a cross-sectional design, which prevents the establishment of a causal relationship between the factors and the somatic response. Additionally, reliance on self-reporting can lead to overestimated or underestimated correlation, recall bias and social desirability bias. Another limitation is the unmeasured confounders, such as genetic predisposition, early childhood trauma, or the specificity of the antibiotics used by the participants. Furthermore, the REAP v.2 scale and its subscales had a low internal consistency in this study, with Cronbach's alpha ranging from 0.48 to 0.66.

This could be attributed to the relatively modest sample size (N = 152) or nuanced dietary patterns in this demographic group. For a more comprehensive understanding, future studies should extend this research to clinical populations and investigate whether the observed mediation effects persist over time.

CONCLUSIONS

This study points the importance of healthy eating habits and behaviors in the young population. Stress management, depression anxiety and the adoption of a healthy diet were highlighted as important at this age. These results reflect the importance of educating young people about healthy practices and highlight the impact of psychological balance on physical health.

CONFLICT OF INTEREST AND FUNDING

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EXPLORING THE EFFECTS OF PEPSIN ON SALIVARY PEPTIDOME: INSIGHTS FROM A PROOF-OF-CONCEPT PROTEOMIC PROFILING STUDY USING MALDI-ToF MASS SPECTROMETRY

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EXPLORING THE EFFECTS OF PEPSIN ON SALIVARY PEPTIDOME: INSIGHTS FROM A PROOF-OF-CONCEPT PROTEOMIC PROFILING STUDY USING MALDI-ToF MASS SPECTROMETRY (Abstract): Pepsin, a component of gastric refluxate, has been investigated as a potential salivary biomarker for gastro-esophageal reflux disease (GERD), although its diagnostic accuracy remains uncertain. This proof-of-concept study aimed to characterize the effects of pepsin on the human salivary peptidome using matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF) mass spectrometry. **Materials and methods:** Whole saliva samples were collected from ten healthy adult volunteers under fasting conditions and divided into untreated controls and aliquots digested with pepsin at acidic pH. MALDI-ToF MS was used to profile digestion-induced changes in peptide mass patterns. Spectral data were analyzed using multivariate statistical approaches, including principal component analysis (PCA), linear discriminant analysis (LDA), and hierarchical clustering. **Results:** Pepsin digestion increased peptide signal intensity and spectral complexity compared with controls. PCA demonstrated clear separation between native and digested samples along the first principal component. Ten peptide m/z features showed the strongest association with pepsin exposure based on PCA loadings. LDA and hierarchical clustering further supported this distinction, with the top 15 discriminative m/z features showing consistent enrichment in digested samples despite inter-individual variability. **Conclusions:** Pepsin exposure induces reproducible remodeling of the salivary peptidome detectable by MALDI-ToF MS. Although this peptide-level approach cannot resolve the full diversity of salivary proteoforms, the resulting signatures support the feasibility of identifying markers of reflux-associated enzymatic activity and provide a basis for future validation in clinical GERD cohorts. **Keywords:** SALIVARY PEPTIDOME, MALDI-ToF MASS SPECTROMETRY, PEPSIN DIGESTION, GASTRO-ESOPHAGEAL REFLUX DISEASE, PEPTIDE BIOMARKERS.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most commonly encountered gastrointestinal disorders in clinical practice, with a notable global prevalence estimated to exceed 20% in Western

countries (1). Studies have shown that individuals with reflux disease experience a significant reduction in health-related quality of life compared to the general population, irrespective of the severity of endoscopic findings (2). It poses a significant burden on healthcare systems worldwide, requiring substantial resources in order to manage and treat effectively (3). Currently, ascertaining a GERD diagnosis often relies on performing a series of invasive and expensive procedures, such as upper gastrointestinal endoscopy or ambulatory pH-impedance monitoring (4). However, these methods are hindered by relatively high costs, limited accessibility in some settings, and the requirement for specialized expertise to accurately interpret the findings (5). In this context, there is a growing need for reliable non-invasive diagnostic biomarkers that could aid in the diagnosis of this condition.

Saliva, a readily accessible biofluid, has been proposed as a potential source of biomarkers for GERD. To that end, many studies investigated the use of salivary pepsin as a diagnostic test, a digestive enzyme secreted by gastric chief cells and key factor in tissue injury (6). However, currently there is a lack of consensus regarding optimal collection methods, processing techniques, and timing for measuring salivary pepsin, with varying reports regarding its diagnostic accuracy (7). While the presence of salivary pepsin has been investigated as a diagnostic biomarker in itself, less focus has been placed on its biological effects on the salivary proteome (8). Given pepsin's potent proteolytic activity under acidic conditions, reflux events may be associated with a characteristic salivary peptide pattern that persists beyond the transient presence of the enzyme itself.

The rapid advances in proteomic technologies provide an opportunity to examine these effects in more detail, broadening the avenues of non-invasive diagnostics (8, 9). Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-ToF) mass spectrometry is a robust analytical technique widely used for rapid and precise profiling of peptide and protein mass patterns. Owing to its sensitivity and reproducibility, it represents an appropriate approach for characterizing the salivary peptidome and for exploring potential biomarkers relevant to GERD.

In this proof-of-concept study, we investigated the effects of pepsin exposure under acidic conditions on the human salivary peptidome using MALDI-ToF mass spectrometry. Rather than focusing on the detection of salivary pepsin itself, our approach aimed to characterize the proteolytic peptide signatures generated by *pepsin* activity. Identifying reproducible peptide patterns may provide insights into reflux-related enzymatic effects and advance the discovery of potential non-invasive diagnostic tools for GERD. To complement the experimental digestion, an in-silico simulation of pepsin-mediated cleavage was carried out using MS Digest, a freely available online tool of Protein Prospector program (10), providing a theoretical peptide map to support interpretation of the MALDI-ToF results.

MATERIALS AND METHODS

The study protocol received ethical approval from the Grigore T. Popa University of Medicine and Pharmacy Iași Institutional Review Board (232/13.10.2022). An overview of the study design is presented in first figure. Saliva samples were collected and divided into two preparation groups.

In the control group, samples were acidified only. In the experimental group, acidified saliva was incubated with pepsin to generate digested peptide products. In silico pepsin digestion was performed to support peptide identification. All samples were analyzed using MALDI-ToF mass spectrometry. Spectral data were interpreted and subjected to comparative analysis and pattern identification to evaluate differences between control and pepsin-digested samples (fig. 1).

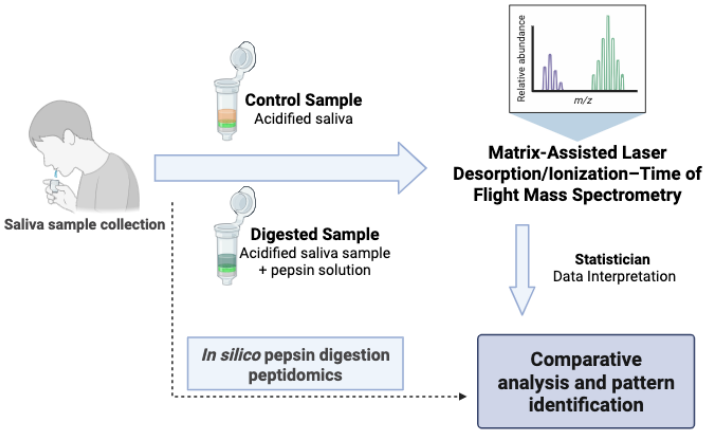

Fig. 1. Detailed workflow for salivary peptidomics analysis using MALDI-ToF mass spectrometry in silico digestion and statistical interpretation of mass spectra (created with BioRender.com).

Saliva Sample Collection

Whole saliva samples were collected from healthy volunteers (n=10) aged 20 to 30 years. Sample collection was performed in the morning following an overnight fast, in the first two hours after waking. To minimize potential contamination, participants were instructed to brush their teeth 30 minutes prior to collection, without consuming any food, beverages, or smoking afterwards. Approximately 2-3 mL of unstimulated saliva was collected in sterile tubes.

Control Samples

Control samples consisted of acidified whole saliva without the addition of pepsin, processed in parallel with the experimental samples under identical conditions, including incubation, centrifugation, desalination, and mass spectrometry analysis. This allowed for comparison to identify pepsin-specific proteolytic effects. These samples were labeled C1 through C10 to ensure consistent tracking throughout the workflow. For the subsequent digestion experiments, the saliva aliquots exposed to pepsin were labeled D1 through D10, following the same numbering scheme as their corresponding control samples.

Pepsin Treatment and Acidification

Pepsin solution (Sigma-Aldrich) was combined with saliva samples in a 1:20 ratio (pepsin to saliva). The total protein concentration in unstimulated whole saliva ranged from 2.9 to 3.1 µg/µL, consistent with values previously reported in the literature (11). Protein concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific) based

on UV-Vis absorbance measurements. The instrument quantified protein levels by measuring absorbance at 280 nm, where aromatic amino acids exhibit characteristic absorption. A sample volume of 1-2 µL was used, and the Nano Drop's short-path optical system allowed direct measurement without cuvettes. Background correction was automatically applied using a reference wavelength (typically 260 nm) to ensure accurate readings for complex biological matrices such as saliva. Acidification of the samples was performed to achieve a pH range of 1-2, ensuring optimal proteolytic activity of pepsin. pH was measured using pH indicator paper, and adjustments were made using hydrochloric acid (0.1 N).

Digestion Process and Supernatant Collection

The saliva-pepsin mixtures were incubated at 37°C for 30 minutes at 450 rotations per minute (rpm) using a shaking incubator. This process was used to maximize pepsin enzymatic activity. All saliva digestion reactions were performed in technical duplicate to ensure reproducibility of the pepsin-mediated proteolysis workflow. Following digestion, samples were centrifuged at 12,000 rpm for 10 minutes and supernatant was collected.

Sample Desalination, Concentration, and Purification

Supernatant samples were desalted, concentrated, and purified using ZipTip C18 pipette tips (Millipore), following the manufacturer's protocol(12). First, the C18 tips were activated by wetting with acetonitrile (100 % ACN). Next, the tips were equilibrated with an aqueous solution containing trifluoroacetic acid (0.1% TFA). The samples were then repeatedly aspirated through the tips, allowing peptides and small proteins to bind to the C18 resin while salts and other impurities were left unbound. After binding, the tips were washed with an acidic solution (0.1% TFA) to remove residual salts and contaminants. Finally, the purified analytes were eluted (50 % ACN in 0.1% TFA), producing a desalted, concentrated solution of analytes ready for analysis by mass spectrometry. The same procedure of purification was applied to the control saliva samples.

MALDI-ToF Mass Spectrometry

MALDI-ToF MS was performed on a Bruker Ultraflex MALDI-ToF / ToF mass spectrometer equipped with a pulsed nitrogen UV laser. The instrument was operated in positive ion mode, and spectra were acquired in reflectron mode. Prior to spotting, peptide samples were mixed with super-DHB (sDHB), a matrix consisting of a 1:1 mixture of 2,5-dihydroxybenzoic acid and 2-hydroxy-5-methoxybenzoic acid, commonly used to enhance peptide ionization and improve signal homogeneity in MALDI analyses. Samples were applied onto a 384-spot stainless-steel target plate using the dried-droplet method by depositing 0.8 µL of matrix solution and 1.0 µL of sample solution, mixed directly on the target and allowed to dry at room temperature. The laser attenuation was set to 80%, and spectra were acquired using the following parameters: 20 kV acceleration voltage, 40% grid voltage, 140 ns delay, low-mass gate of 500 Da, and an acquisition mass range of 450-5000 Da. For each final spectrum, 500 laser shots were accumulated. External calibration was performed using the monoisotopic (M+H)+ ions of the following peptide standards: Bradykinin (1-7) (m/z 757.4), Angiotensin II (m/z 1046.5), Angiotensin I (m/z 1296.7), Substance P (m/z 1347.7), Bombesin (m/z 1619.8),

Renin Substrate (m/z 1758.9), ACTH (1-17) (m/z 2093.1), ACTH (18-39) (m/z 2465.2), and Somatostatin (m/z 3147.5). Theoretical monoisotopic masses were calculated using the ChemCalc online tool. Spectra were processed using Bruker FlexAnalysis 3.4 software. The MALDI ToF mass spectrometry data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (13) with the dataset identifier PXD076267.

Statistical Analysis and Data Processing

Data processing and statistical analyses were performed using Python (version 3.9.x). Raw MALDI-ToF mass spectra were exported as CSV files and processed using a custom computational pipeline. Unsupervised Principal Component Analysis (PCA) was conducted to visualize the global variance and identify inherent clusters between the native and digested peptide profiles. To specifically identify the peptide features that best discriminate between the two conditions, a supervised Linear Discriminant Analysis (LDA) was employed. The predictive performance of the LDA model was validated using Leave-One-Out (LOO) cross-validation. The following Python libraries were utilized: (1) Pandas (v1.3.5) and NumPy (v1.21.6) for data manipulation and numerical operations; (2) Scikit-learn (v1.0.2) for PCA, LDA, and cross-validation procedures; (3) SciPy (v1.7.3) for t-test calculations; (4) Matplotlib (v3.5.2) and Seaborn (v0.11.2) for the generation of high-resolution mirror plots, PCA score plots, and heatmaps.

RESULTS

To evaluate the proteolytic effects of pepsin on the human salivary proteome, whole saliva samples from ten healthy individuals were subjected to pepsin digestion under acidic conditions and analyzed using MALDI-ToF mass spectrometry. For each participant, one control sample and one digested aliquot were compared. Analysis of the acidified saliva samples provided a stable proteomic baseline. Following exposure to pepsin, distinct pepsin-induced proteomic changes were observed in the salivary samples. To assess the reproducibility of the digestion workflow, all pepsin-treated saliva samples were processed in technical duplicate. The duplicate digestions consistently produced comparable peptide profiles and signal intensities, confirming the robustness of the proteolytic procedure.

From the ten saliva samples analyzed, we identified three distinct salivary peptide profiles. Each profile combines a set of shared peptide signals with additional, profile-specific features that differ in intensity, even though the MALDI-ToF analytical conditions were kept constant. These reproducible patterns, observed across individuals, indicate inherent variability in the salivary peptide composition. Such variability is expected, as the salivary peptidome is influenced by physiological factors including individual protease activity, oral microbiota, hydration status, and subtle differences in sample handling. Consequently, the presence of distinct peptide profiles among healthy participants is consistent with previously reported inter-individual diversity in the human salivary proteome(14). Within these three categories, sample C1 displayed a peptide profile highly similar to that of sample C6, while the profile of sample C2 closely resembled that of sample C9. In contrast, samples C3-C5, C7, C8, and C10 formed a larger group characterized by markedly similar peptide patterns. This clustering further supports the presence of inherent inter-individual variability in salivary peptide composition under identical MAL-

DI-ToF analytical conditions.

To exemplify the three peptide profile types identified among the ten saliva samples, we present representative MALDI-ToF spectra corresponding to each category. Figure 2 illustrates the first profile type, using sample C1 as a representative example. Panel A shows the peptide mass fingerprint of the untreated saliva sample (C1), characterized by a series of well-defined signals spanning the 450-5000 m/z range, reflecting the endogenous salivary peptide distribution under acidic conditions. Panel B displays the corresponding spectrum of the same sample following pepsin digestion (D1), where shifts in peak intensities and the appearance of additional lower-molecular-weight ions indicate pepsin-mediated proteolysis. Figure 3 illustrates the second peptide profile type identified among the ten saliva samples, using sample C2 as a representative example. Panel A shows the MALDI-ToF spectrum of the untreated saliva sample C2, characterized by a distinct distribution of endogenous peptide signals that differentiates this profile from the one shown in figure 2. Panel B presents the corresponding spectrum of the same sample following pepsin digestion (D2). As observed for the first profile type, pepsin exposure results in the appearance of additional low-molecular-weight ions and modifications in peak intensities; however, the fragmentation pattern and relative signal distribution are characteristic of this second profile category.

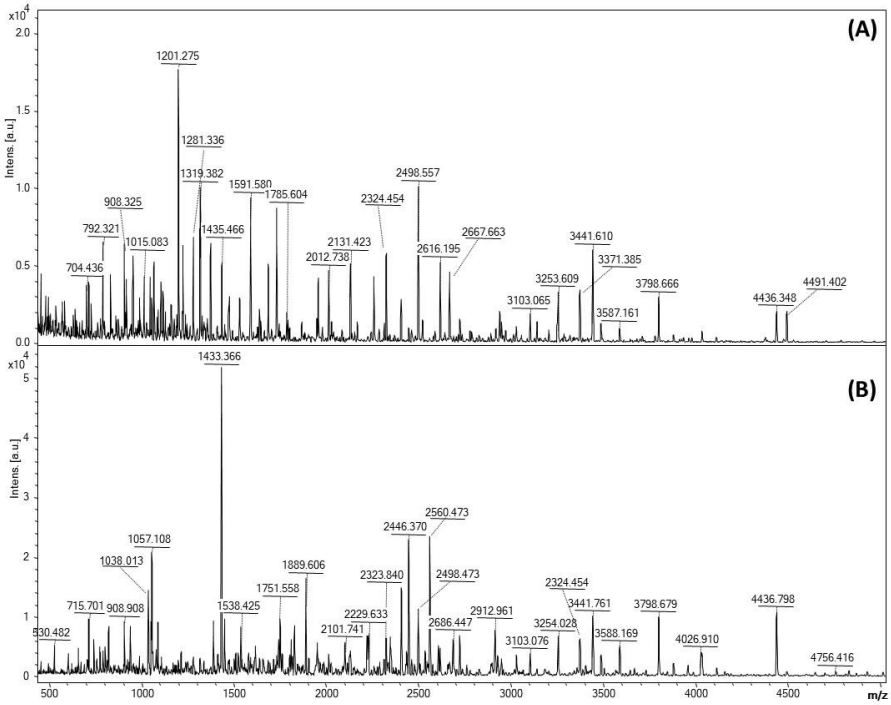

Fig. 2. Mass spectrometric peptide mass fingerprints illustrating one of the three salivary peptide profile types identified among the ten saliva samples. (A) MALDI-ToF spectrum of the untreated saliva sample C1 and (B) Corresponding MALDI-ToF spectrum of the same sample following pepsin digestion (D1)

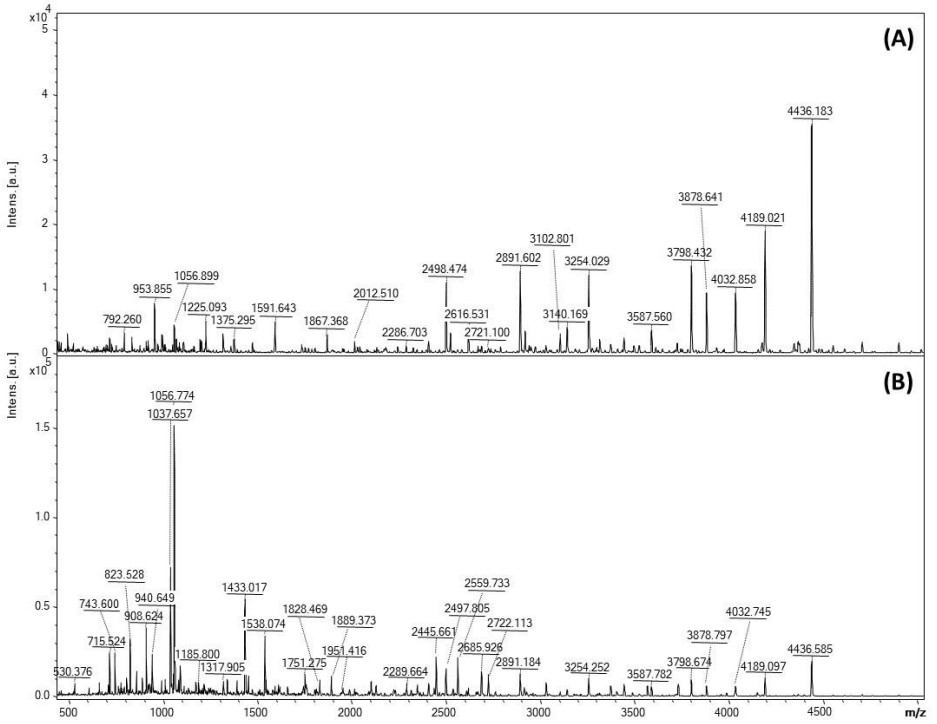

Fig. 3. Mass spectrometric peptide mass fingerprints illustrating a second salivary peptide profile type identified among the ten saliva samples. (A) MALDI-ToF spectrum of the untreated saliva sample C2, and (B) Corresponding MALDI-ToF spectrum of the same sample after pepsin digestion (D2).

Figure 4 illustrates the third peptide profile type identified among the ten saliva samples, using sample C7 as a representative example. Panel A shows the MALDI-ToF spectrum of the untreated saliva sample C7, characterized by a peptide distribution that is distinct from the patterns observed in figures 2 and 3. This profile type is defined by a specific arrangement of endogenous peptide signals and relative peak intensities that consistently grouped samples C3-C5, C7, C8, and C10 within the same category. Panel B presents the corresponding spectrum of the same sample following pepsin digestion (D7). As with the other profile types, pepsin exposure results in the appearance of additional low-molecular-weight ions and notable shifts in peak intensities. However, the fragmentation pattern observed for C7/D7 is characteristic of this third profile category and differs from the proteolytic signatures (figs. 2, 3).

For the in-silico component of the study, a dedicated salivary protein database was first constructed from the UniProt Knowledgebase (UniProtKB). A keyword search using the term “salivary” was performed and restricted to the organism Homo sapiens (Human). This query yielded 638 protein entries, which were exported and compiled into a custom

FASTA database for subsequent computational analysis.

In silico proteolysis was carried out using the MS-Digest online tool available through the Protein Prospector suite (https://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msdigest).

Pepsin (porcine gastric) was selected as the proteolytic enzyme to replicate the experimental digestion conditions. Default digestion parameters were applied, including fully specific enzymatic cleavage according to pepsin specificity rules and allowance for up to two missed cleavages. No fixed or variable post-translational modifications were introduced, so the generated peptide list reflected cleavage based solely on primary amino acid sequence.

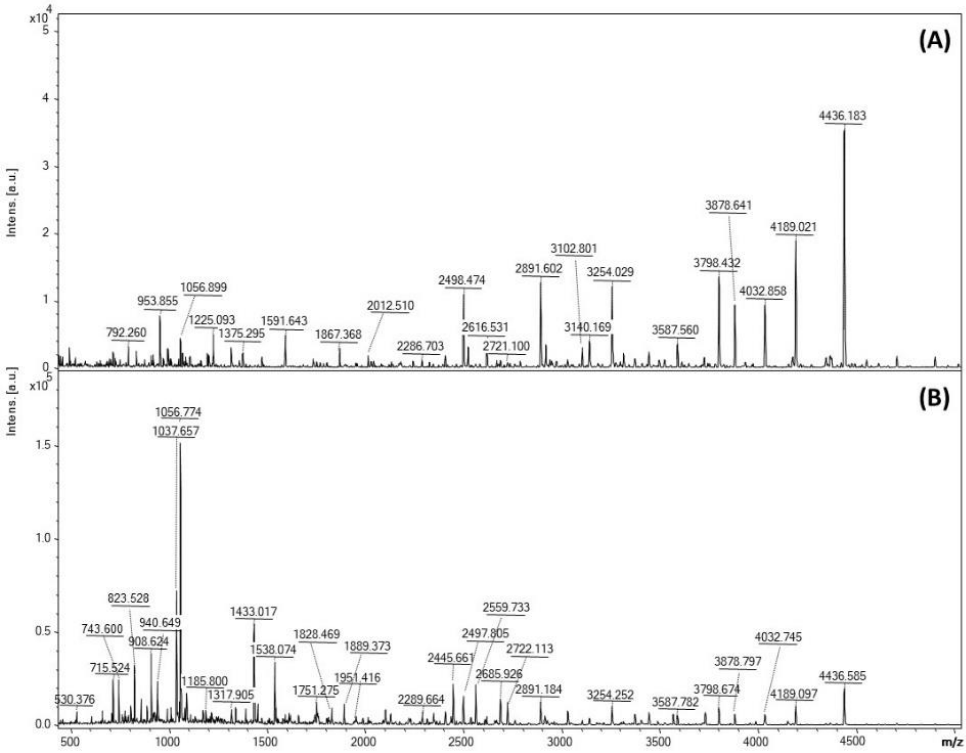

Fig. 4. Representative MALDI-ToF peptide mass fingerprints illustrating the third salivary peptide profile type identified among the ten saliva samples. (A) MALDI-ToF spectrum of the untreated saliva sample C7, and (B) Corresponding MALDI-ToF spectrum of the same sample following pepsin digestion (D7).

Application of these parameters to the 638 salivary protein entries resulted in 25009 theoretical peptide fragments. Because MALDI-MS analysis primarily provides peptide mass information without complete sequence coverage for all detected ions, the in-silico digestion served to generate a list of putative peptide sequences corresponding to experimentally observed m/z values (assuming a +1-ion charge). Therefore, fragment assignment was based on mass matching and represents

predicted candidate sequences rather than unequivocal sequence confirmation in all cases. This approach enabled a systematic comparison between experimentally detected pepsin-derived fragments and theoretically expected cleavage products within the human salivary proteome.

To further explore the variability observed across the salivary peptide profiles and to quantify the proteolytic effects of pepsin, all MALDI-ToF spectra from the control and digested samples were subjected to comprehensive statistical analysis. After peak alignment and intensity normalization, the dataset was evaluated using multiple multivariate approaches, including Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and hierarchical clustering with heatmap visualization. These complementary methods enabled the identification of sample groupings, the discrimination between control and pepsin-digested spectra, and the detection of m/z features contributing most strongly to sample separation.

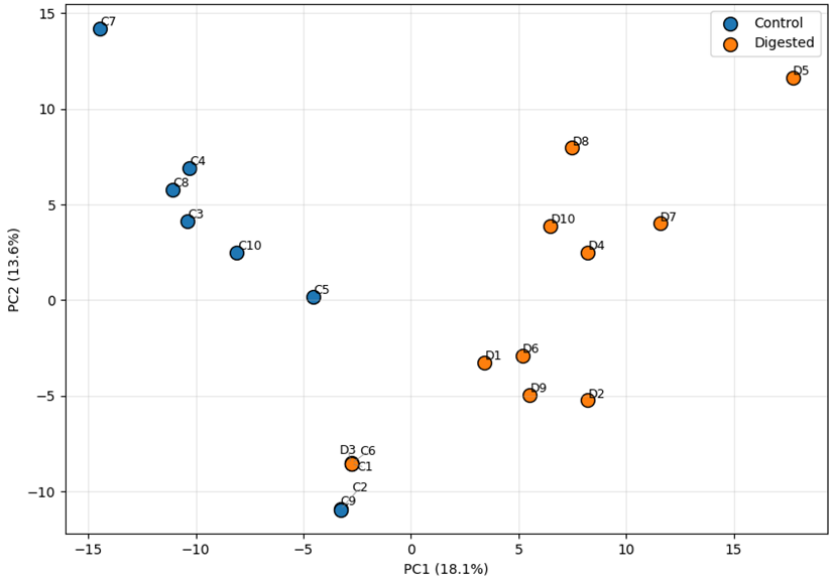

Fig. 5. PCA score plot illustrating the separation between control and pepsin-digested saliva samples (variance explained: 31.7%).

The PCA plot illustrates the impact of pepsin digestion on the salivary peptide composition (fig. 5) (15). The first principal component (PC1) captures the dominant source of variation in the dataset and clearly separates the digested samples (orange circles) from the native saliva samples (blue circles). This distinct clustering demonstrates that pepsin digestion consistently produces a characteristic peptide pattern that is markedly different from the natural inter-individual variability observed in untreated saliva. The separation along PC1 is driven by a specific subset of peptide ions whose intensities increase substantially following pepsin exposure. These ions correspond to the loadings of the first principal component and represent the features most

strongly contributing to the discrimination between the two sample groups.

TABLE I.

Top m/z ions ranked by their PC1 loading values, highlighting the peptide features most strongly associated with pepsin-induced spectral changes.

m/z (Ref)	PC1 Loading Value
530.34	+0.1076
743.49	+0.1075
940.5	+0.1074
1088.82	+0.1072
1750.99	+0.1070
1432.76	+0.1062
908.46	+0.1043
2289.27	+0.1036
1537.94	+0.0998
2406.36	+0.0992

In addition, hierarchical clustering of the most discriminative m/z features revealed a distinct grouping of ions that were consistently enriched in the pepsin-digested samples (tab. I). Based on the PCA loadings, the m/z features contributing most strongly to PC1 were ranked in descending order according to their loading values. The ions with the highest positive influence on PC1 were m/z 530.34, 743.49, 940.50, 1088.82, and 1750.99, followed by m/z 1432.76, 908.46, 2289.27, 1537.94, and 2406.36. Importantly, all top contributors exhibited positive loading values, indicating that their intensities increase systematically in the digested samples and drive the rightward shift of these samples along the PC1 axis. Notably, the peptide masses (tab. I) are also found among the theoretical peptides generated in the in-silico digestion, further supporting their biological relevance. These reproducible features may represent candidate biomarkers of pepsin - mediated proteolysis, pending confirmation through targeted MS/MS identification and validation in independent sample sets.

Building on the PCA findings and the discriminative m/z features (tab. I), the Linear Discriminant Analysis (LDA) provides a supervised dimensionality-reduction approach designed to identify the peptide signals that best separate the two predefined groups(16). Unlike PCA, which captures the directions of maximum variance without using class information, LDA explicitly incorporates the sample labels and learns the optimal combination of m/z features that distinguishes the Control from the Digested spectra (17). The resulting discriminant axis (LD1) positions most native saliva samples on the left and the pepsin-digested samples on the right, reflecting the consistent biochemical shift induced by proteolysis. The central red line represents the decision boundary used for classification. Two samples (C1 and D3) appear near this threshold, indicating intermediate peptide profiles that partially overlap with the opposite group, a finding consistent with the natural inter-individual variability of the salivary peptidome and with the PCA-derived loadings. Model performance was evaluated through Leave-One-Out cross-validation (LOO)(18), yielding an accuracy of 60%, further supporting the conclusion that pepsin digestion introduces systematic and detectable changes in the salivary peptide patterns (fig. 6).

To further extend the multivariate analysis and to visualize sample-level differences across the most relevant peptide markers, a heatmap with hierarchical clustering was generated using the top 15 m/z

features that most strongly distinguished the Control and Digested groups (fig. 7). These peptides ions correspond to the statistically significant markers identified in the PCA loadings and summarized in first table, and their intensity patterns provide a high-density over-view of the proteolytic changes induced by pepsin digestion.

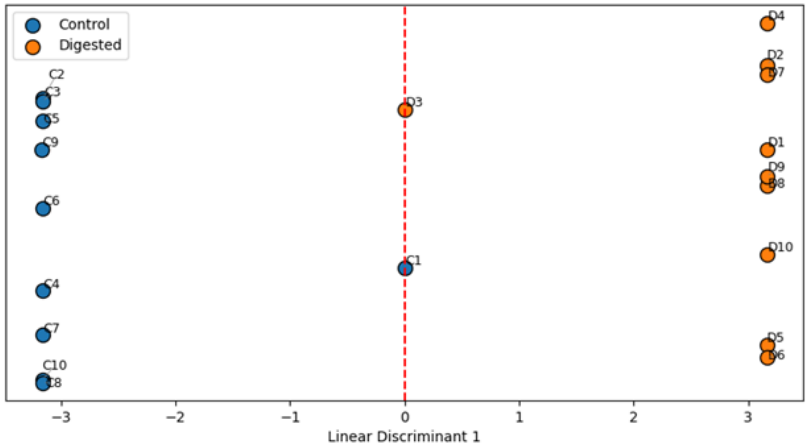

Fig. 6. Linear Discriminant Analysis (LDA) of control and pepsin-digested saliva samples, showing group separation along the LD1 axis and the decision boundary used for classification (LOO accuracy: 60%).

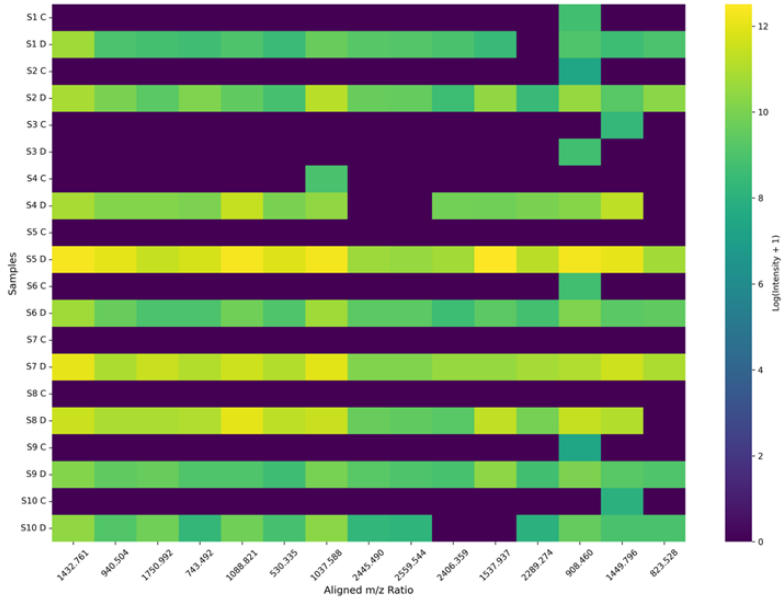

Fig. 7. Heatmap with hierarchical clustering of the top 15 discriminative m/z features, illustrating distinct intensity patterns between control and pepsin-digested saliva samples and highlighting sample-level variability across the dataset.

The heatmap reveals two clearly separated clusters, with control samples displaying consistently lower intensities for these m/z values, while digested samples exhibit marked increases, in agreement with the PCA and LDA results. Notably, Sample 3 shows an atypical pattern: although internally variable, it lacks several of the discriminative m/z features present in the other samples. This explains its divergent position in the clustering and reflects natural inter-individual variability rather than a computational artifact. Overall, the heatmap reinforces the biological relevance of the peptide markers enriched in the digested samples and supports their potential as candidate indicators of pepsin-mediated proteolysis, pending MS/MS validation.

DISCUSSION

GERD is one of the most prevalent gastroenterological disorders encountered in clinical practice, and there is a pressing need for non-invasive diagnostic tools to improve diagnostic accuracy. Saliva has emerged as an attractive and easily accessible diagnostic medium, capable of reflecting both systemic and local pathological processes (19, 20). Salivary pepsin has been investigated as a potential biomarker for GERD; however, reported sensitivity and specificity vary widely across studies, likely due to substantial methodological heterogeneity (21, 22). These limitations suggest that measuring pepsin concentration alone may not fully capture the complexity of reflux-related pathophysiology.

Pepsin is not merely a passive marker of reflux but an active proteolytic enzyme that remains biologically functional under acidic conditions and has been implicated in mucosal injury and inflammation in reflux disease (23, 24). Salivary proteins are more susceptible to proteolytic degradation than serum proteins (25, 26), making the salivary peptidome particularly responsive to pepsin-mediated cleavage.

Disease- or exposure-related alterations can reshape the proteome by generating distinct peptide patterns that reflect functional proteome states, rendering peptide-level analysis especially informative in dynamic biofluids such as saliva (27).

Recent advancements in proteomic technologies have expanded the potential of saliva as a diagnostic medium for a wide range of conditions, including head-and-neck and oral cancer, diabetes, gastric cancer, Sjögren's syndrome, primary sclerosing cholangitis, and COVID-19 infection (8, 28-30). As a proof-of-concept, this study employed MALDI-ToF mass spectrometry to characterize pepsin-induced alterations in the salivary peptidome of healthy volunteers, with the aim of identifying digestion-associated peptide patterns that may serve as indicators of reflux-related enzymatic activity. MALDI-ToF enables rapid and accurate analysis of complex biological samples by determining the mass-to-charge (m/z) ratios of ionized peptides based on their time-of-flight, making it a promising tool for medical diagnostics and biomarker discovery (31, 32).

Previous studies have shown that individuals with GERD exhibit lower oral pH values, which may influence the oral environment (33-35). Proteomic analyses of the acquired enamel pellicle in GERD patients have revealed profound alterations compared to controls, both in individuals with and without erosive tooth wear (36, 37). Moreover, clinical studies of saliva in GERD have demonstrated reflux-associated

remodeling of the salivary proteome, particularly affecting proteins involved in acid resistance and oral tissue protection (38). These findings collectively support the rationale for exploring pepsin-induced peptide signatures as potential biomarkers of reflux activity.

Our results demonstrate that pepsin induces marked and reproducible alterations in the salivary peptidome, reflected by both quantitative and qualitative changes across multiple analytical levels. MALDI-ToF profiling revealed a significant increase in the number of detectable peptide signals following enzymatic digestion, together with the appearance of novel m/z peaks absent in untreated saliva. These findings were further supported by multivariate statistical analyses. PCA showed a clear separation between native and digested samples along PC1, driven by a subset of peptide ions with strongly positive loading values, indicating systematic increases in their intensities after pepsin exposure. The most influential of these features are summarized in first table and overlap with peptides predicted through in silico digestion, reinforcing their biological plausibility.

LDA provided complementary evidence by identifying the optimal combination of discriminative m/z features that maximized group separation. Despite natural inter - individual variability, most samples were correctly classified, and the model achieved a 60% LOO accuracy. Samples positioned near the decision boundary (e.g., C1 and D3) displayed intermediate peptide profiles, consistent with the variability observed in the PCA and heatmap analyses. The hierarchical clustering heatmap of the top 15 discriminative m/z features further confirmed the presence of two distinct sample clusters, with digestion-specific peptides showing consistently elevated intensities in the digested group. One sample (Sample 3) exhibited an atypical pattern due to the absence of several discriminative m/z values, reflecting internal variability rather than analytical error. Together, these converging lines of evidence highlight robust pepsin-induced remodeling of the salivary peptidome. The reproducible detection of digestion-specific peptides across multiple individuals, combined with their statistical relevance and alignment with in silico predictions, supports their potential as candidate biomarkers of reflux-associated pepsin activity. As a proof-of-concept, this study demonstrates the feasibility of using salivary peptidomics to detect enzymatic signatures associated with gastric reflux.

To advance this line of research, we will perform high-resolution ESI-MS/MS analysis of the salivary samples to determine the precise amino-acid sequences and protein origins of the candidate biomarker peptides. This step is essential for confirming their biological relevance and for establishing mechanistic links between pepsin activity and specific proteolytic cleavage events. In parallel, we will expand the study to include clinical cohorts, particularly patients with confirmed GERD, as well as individuals with oral or upper gastrointestinal cancers and patients undergoing chemotherapy, in whom reflux is a frequent adverse effect. Such cohorts will allow us to evaluate whether the digestion-associated peptide signatures identified here are detectable in real-world pathological conditions and whether they correlate with disease severity, symptom burden, or treatment-related reflux.

By integrating controlled experimental data with clinical validation, future studies

will determine the diagnostic robustness, specificity, and translational potential of these salivary peptide markers. Ultimately, this research may contribute to the development of rapid, non-invasive, saliva-based diagnostic tools capable of complementing or reducing reliance on invasive procedures such as pH-monitoring or endoscopy.

CONCLUSIONS

This proof-of-concept study demonstrates that pepsin exposure under acidic conditions induces significant and reproducible alterations in the salivary peptidome, generating distinct peptide signatures that differentiate digested from native saliva. These findings provide foundational evidence supporting the feasibility of saliva-based, non-invasive diagnostics for reflux-associated enzymatic activity. However, several limitations of this proof-of-concept study should be considered. In particular, the human salivary proteome exhibits substantial proteome complexity, consisting of multiple proteoforms generated through proteolytic processing, post-translational modifications, and sequence variation. While MALDI-ToF peptide profiling enables rapid detection of digestion-associated peptide patterns, this approach does not resolve intact protein species and therefore cannot fully capture the diversity of proteoforms present in saliva. Consequently, the peptide signals reported here represent inferred fragments of the salivary proteome, and their identities remain putative until confirmed by tandem MS analysis. In addition, the relatively small sample size and the use of experimentally digested saliva from healthy volunteers limit the generalizability of the findings. Future studies will focus on structural identification of the candidate peptides through ESI-MS/MS and on validating these biomarkers in clinical cohorts, including patients with GERD and individuals with reflux-related symptoms secondary to oncologic therapies.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflicts of interest.

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EPIDEMIOLOGICAL ASPECTS OF PEDIATRIC TUBERCULOSIS IN EASTERN PART OF ROMANIA

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EPIDEMIOLOGICAL ASPECTS OF TUBERCULOSIS IN EAST ROMANIA (Abstract): Tuberculosis continues to represent an extremely major problem of public health worldwide, with global economic and social implications. **Aim** of the study was to evaluate some variables in children admitted with *Mycobacterium tuberculosis* infections. **Material and methods**: The descriptive study performed on a group of 1,849 patients aged between 0-18 years, during 2010 and 2014. **Results**: Distribution of cases highlighted that pediatric patients were more frequently girls (M/F = 0.97), from urban areas (U/R= 1.29), aged of 10-14 (33.04% of total cases), then 5-9 (28.18%). Children of 0-4 age group represented 17.18% of total cases. **Conclusions**: The present study represented the second part of a larger surveillance study started in 1999, with first results published in 2007. The study will be continued next years, taking into consideration also other variables with clinical importance in pediatric patients. **Keywords**: TUBERCULOSIS, CHILDREN, EPIDEMIOLOGY, PNEUMOLOGY.

It is estimated that one-third of the globe population is infected with *Mycobacterium tuberculosis*, and over 10 million cases of tuberculosis (TB) occur every year. Although the disease is curable, there are millions of deaths annually. TB is one of the most important causes of death due to a microorganism and considering the causes of avoidable death, the most important cause of death in the adult population group and an important burden at childhood age. TB continues to represent an extremely major problem of public health worldwide, with global economic and social implications, because over 80% of total deaths occur in active social and demographical age group people of 15-59 years old. TB was considered too one of the main causes of DALY worldwide, after lower respiratory tract infections, diarrheal diseases, perinatal disorders, major unipolar depression, ischemic heart disease and

cerebrovascular accidents). In 2020, it is estimated that there will be a reversal of disease on this scale, meaning that ischemic heart disease will be on the first place, major unipolar depressions on second, road accidents on third, but TB will be on the spot. Another major problem involving TB is the drug-resistant cases. In 2016, 600,000 new cases resistant to rifampicin, the most effective first-line drug, were reported, according to Global tuberculosis report published by WHO experts. The multidrug-resistance was reported for 490,000 cases (MDR-TB) and 47% of these cases were in India, China and the Russian Federation (1, 2, 3, 4, 5).

Specific TB reporting indicators are well defined at the global and national level. Since 1995 standardized TB cases and treatment outcomes have been reported, with incidence and mortality estimates published. WHO has developed a TB-MDR monitoring program with 14 indicators, including the state of well-being and health of the individual, coverage of essential health services, per capita health expenditure, and smoking, diabetes, alcohol consumption. Other indicators are related to the proportion of the population living below the poverty level, the proportion of the health insurance population, the social protection systems, the prevalence of malnutrition, the gross domestic product, etc. To these, the WHO adds the Gini index to income inequality and the proportion of urban population but living in poverty (1, 2, 3).

The implications of TB infection should be analyzed by looking at some specific examples of TB evolution at global and regional level. A first example is the situation in Romania where the measures taken in a certain period led to the decrease of the incidence of TB to 56%ooo in 1985, the incidence in 2000 being more than twice as high. A second example relates to the evolution of TB in the US, where the target for elimination was established by 2010 but cannot be achieved. Due to an erroneous interpretation of the global situation in the 1970s, the attitude towards MT in the US has changed dramatically. In this context, the continued incidence of TB during the period 1985-1992 (with 26,673 cases reported in 1992) was possible, mainly due to: the deterioration of public health infrastructure (especially in the field of infectious diseases and in particular TB) (after 1972), the emergence of the HIV/AIDS epidemic, the reduction of TB training, the decrease of research activities, immigration from endemic countries, the reduction of social programs, transmission of infection in certain units (hospitals, hostels, prisons, etc.) the emergence of infections with resistant and multi-resistant strains of *M. tuberculosis*. The "American" model is very suggestive and can be extrapolated to what has happened in other industrialized countries over the same period and the above-mentioned causes must be considered when developing an effective TB prevention and control strategy (1, 2, 5).

The present study represented the second part of a larger surveillance study started in 1999, with first results published in 2007 (6). The same specific aim was considered to identify and describe demographic variables with clinical and epidemiological importance in pediatric patients with *M. tuberculosis* infections.

MATERIAL AND METHODS

The study was performed between 2010 and 2014 on group of 1,849 patients admitted to a Clinical Emergency Hospital for Children in Galati County, a region situated

in the Eastern part of Romania.

Mycobacterium tuberculosis evidence in their pathological isolates was mandatory. The inclusion criteria were pediatric age groups and the presence of *M. tuberculosis* in pathological isolates. The exclusion criteria did not involve residence area, nor gender or other comorbidities. Data were collected from patients' recordings and hospital statistical reports, and processed using *MS Excel* 2010.

RESULTS

Distribution by year of study described an increased trend from 2010 to 2014 in incidence of *M. tuberculosis* isolates, with a minimum value during 2012 (344 cases) and a maximum in 2014 (399 cases). Calculated average = 369.8; median - 373.6. (fig. 1).

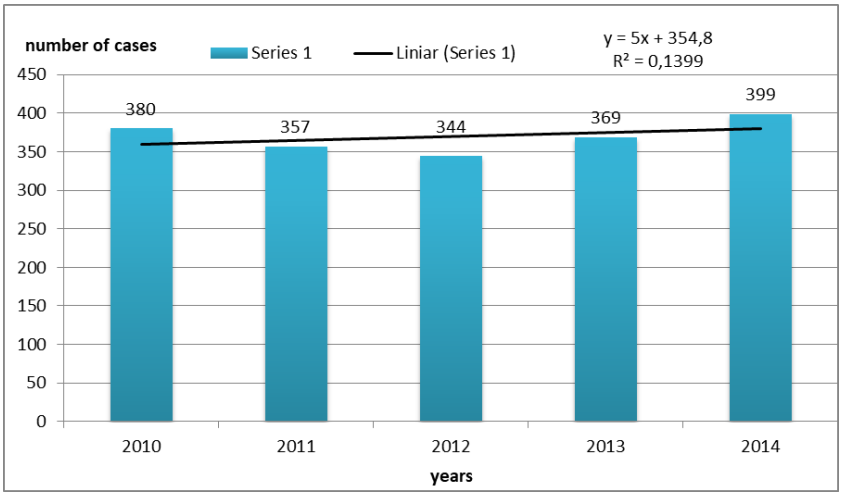

Fig. 1. Distribution of cases by year of study

Distribution by gender showed that girls were more frequent diagnosed with a *M. tuberculosis* infection than boys, with a total M/F = 0.97, but with no statistical significant difference in male / female in every year of study (tab. I).

TABLE I

Distribution of cases by gender

Year of study	Distribution by gender				Total
	Female		Male		
	No.	%	No.	%	
2010	188	49.47	192	50.53	380
2011	184	51.54	173	48.46	357
2012	159	46.22	185	53.78	344
2013	192	52.03	177	47.97	369
2014	213	53.38	186	46,62	399
Total	**936**	**50.62**	**913**	**49,38**	**1849**

The next demographic variable considered was the residence area, showing there was a statistical significant difference between urban and rural patients in 2011 and 2012 ($p<0.05$) and with U/R = 1.29 (tab. II).

TABLE II
Distribution of cases by residence area

Year of study	Distribution by area				Total
	Urban		Rural		
	No.	%	No.	%	
2010	215	56.57	165	43.43	380
2011	221	61.91	136	38.09	357
2012	212	61.62	132	38.38	344
2013	187	50.68	182	49.32	369
2014	208	52.13	191	47.87	399
Total	**1043**	**56.41**	**806**	**43.59**	**1849**

Calculated trends during the studied years highlighted that female cases had an up-trend, but patients from urban area a down trend (fig. 2).

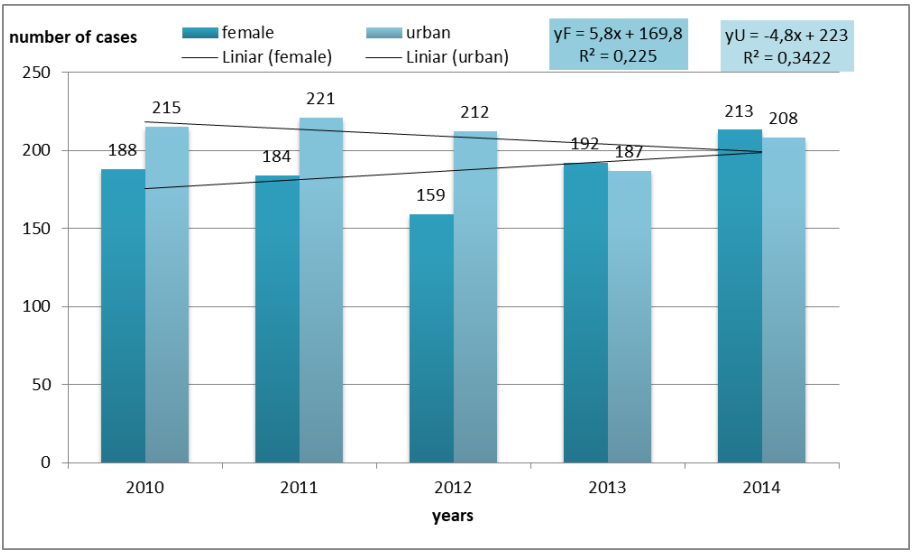

Fig. 2. Comparative distribution of cases by the most frequent gender and area variables

Pediatric age group distribution showed that children studied group aged of 10-14 years were the most frequently affected by infections with *M. tuberculosis*, with a total of 611 cases or 33.04%, followed by 5-9 aged patients (521 cases meaning 28.18% of total).

Children aged between 0 and 4 years represented only 17.19% of cases, but with an important clinical and epidemiological significance (tab III, fig. 3).

TABLE III

Distribution of cases by age group

Year of study	Distribution by age group (years)								Total
	0-4		5-9		10-14		15-18		
	No.	%	No.	%	No.	%	No.	%	
2010	92	28.93	112	21.49	89	14.56	87	21.81	380
2011	52	16.35	87	16.69	154	25.21	64	16.04	357
2012	38	11.95	84	16.12	151	24.71	71	17.79	344
2013	63	19.81	134	25.72	93	15.21	79	19.79	369
2014	73	22.96	104	19.98	124	20.31	98	24.57	399
Total	**318**	**17.19**	**521**	**28.18**	**611**	**33.04**	**399**	**21.59**	**1849**

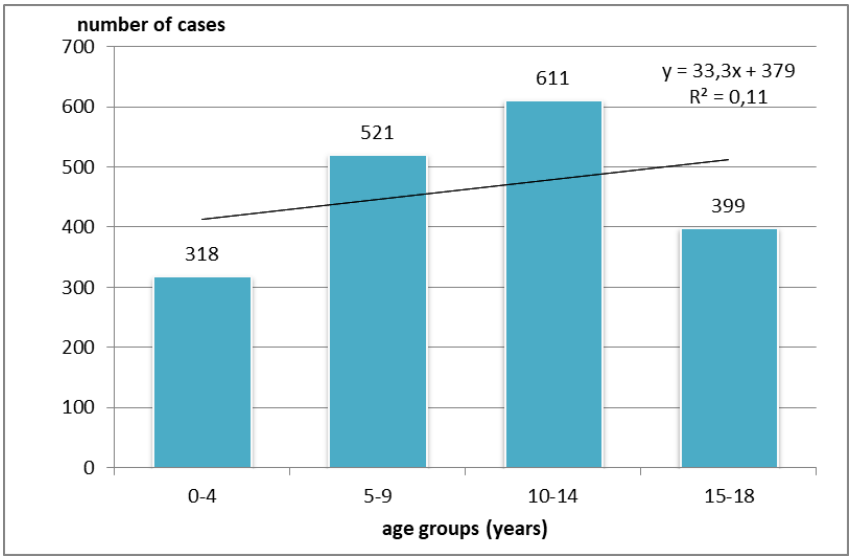

Fig. 3. Age histogram

DISCUSSION

Tuberculosis accounts for more than 20% of all TB cases in countries with high incidence of the disease and 5% in high developed regions. TB in children is a neglected pathology that remains in the adult shadow because of negative smears often detected so that the burden of this disease in pediatric age is underreported. WHO highlighted that positive TB in children under the age of 14 represented 0.6-3.6% of total cases. Most of children under 12 could have negative smears. Countries with high rates of TB rarely performed specific smears in children. Children with TB are often the result of contamination from adult sources. Infants are at risk of serious clinical forms because of immune system immaturity. Nonspecific clinical and radiological signs and variability of them in children are sometimes ignored and make the diagnosis diffuse and imprecise. Treatment can involve multidrug resistant strains, resulting in a reserved prognosis. All these

obstacles, including an increased prevalence of HIV co-infection, require the development of an effective anti-TB vaccine and immunosuppressed patients and a better knowledge of epidemiological features in these age groups (7, 8, 9, 10).

TB has an unequal distribution in all populations, affecting risk groups and being considered a disease of "poverty". Favoring and aggravating factors would be malnutrition, poor living conditions, low income and unemployment, limited access to healthcare services, unfavorable psychosocial influences may affect patients and their families. These population groups at risk have a higher prevalence of TB that often remains undiagnosed for a long time and thus individuals become unknown sources of pathogen for the entire community. Delay in diagnosis due to all the above-mentioned factors leads to aggravation of symptomatology. These patients difficult adhere to treatment and therefore therapy has unfavorable results. Such groups are extremely fragile and must be identified as early as possible to implement properly the management of global control of communicable diseases in populations in which social factors, with the marginalization of these individuals, play an important role (11, 12, 13).

The WHO Millennium goal of eliminating TB by 2050 requires both healthcare and population efforts to improve cooperation on hospital admission and therapy compliance. However, there are several obstacles that seem to impede the achievement of this goal, such as the still high prevalence of TB. Increasing numbers of injecting-drug users in countries with numerous populations and at-risk groups such as India and China, the co-infection with HIV, are difficult to solve and require high costs (14).

CONCLUSIONS

Our descriptive study had its importance in assessing the incidence of *M. tuberculosis* infections in children, and highlighting certain demographic variables up-trended or down-trended during a half-decade. The study will be continued next years, taking into consideration other variables, with clinical importance in pediatric patients.

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NEWS

EVALUATION OF SERUM C-REACTIVE PROTEIN LEVELS IN SUBJECTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS IN COMPARISON WITH HEALTHY CONTROLS

The aim of a study realized by a group of Indian researchers was to evaluate and to compare the serum C-reactive protein levels in subjects diagnosed with chronic periodontitis and aggressive periodontitis. Forty-five subjects were included in the study. The subjects were divided into three groups: *group I* (subjects with clinically healthy periodontium), *group II* (subjects with generalized aggressive periodontitis), and *group III* (subjects with chronic periodontitis). For the measurement of serum C-reactive protein, blood samples were collected from all subjects. Periodontal parameters (plaque index, bleeding index, gingival index, probing pocket depth, clinical attachment loss) were assessed. Serum C-reactive protein levels were assessed by means of a commercially available high sensitivity- serum C-reactive protein enzyme immunoassay kit. The results showed that serum C-reactive protein levels were increased in group III (6.0671 ± 3.15639 mg/L) and group II subjects (4.5453 ± 2.88116 mg/L) compared to the group I (1.0180 ± 0.94069 mg/L). Serum C-reactive protein levels showed a positive correlation with all clinical parameters in group I subjects. Bleeding index (r = 0.073), plaque index (r = 0.120) showed a positive correlation with serum C-reactive protein level in group II and a positive correlation was also seen for plaque index (r = 0.492), probing pocket depth (r = 0.340), clinical attachment loss (r = 0.160), and serum C-reactive protein level in group III subjects. In conclusion, the mean serum C-reactive protein levels were found to be greater in patients with chronic periodontitis compared to those with generalized aggressive periodontitis, but there was no statistically significant difference. (Bolla V, Kumari PS, Munnangi SR, Kumar DS, Durgabai Y, Koppolu P. Evaluation of Serum C-reactive Protein Levels in Subjects with Aggressive and Chronic Periodontitis in Comparison with Healthy Controls: A Clinico-biochemical Study. *Int J Appl Basic Med Res* 2017; 7(2): 121-124)

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SINGLE LARGE SACCULATED DUODENOJEJUNAL FLEXURE DIVERTICULUM. CASE REPORT AND CLINICAL IMPACT

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SINGLE LARGE SACCULATED DUODENOJEJUNAL FLEXURE DIVERTICULUM. CASE REPORT AND CLINICAL IMPACT (Abstract): A diverticulum can develop in any portion of the intestine. Jejunal diverticulum is a rare congenital malformation and refers to the presence of a sac-like mucosal herniation through vulnerable points of the intestinal wall. We herein report a case of an unusual finding of a single giant sacculated jejunal diverticulum located at the first part of the jejunum just after the duodenojejunal flexure in a cadaver. Diverticula usually remain entirely asymptomatic and commonly present with bloating and nonspecific symptoms. In many cases diagnosis is incidentally reached during surgery, endoscopy or imaging study for other reason. However, serious complications may develop, such as bowel obstruction and acute perforation, diverticulitis, bleeding, volvulus, anemia, malabsorption. Related clinical and surgical implications are discussed as well. Knowledge and familiarity with this rare entity is valuable to gastroenterologists when assessing abdominal symptoms and general surgeons, as well, when performing exploratory laparotomy. Although jejunal diverticular disease is a rare condition, potential complications could be fatal. **Keywords:** JEJUNUM, DIVERTICULUM, DIVERTICULITIS.

Jejunal diverticula (JD) constitute a rare entity of the small-bowel with a reported incidence of 0.5 to 2.3% (1) in enteroclysis studies, and a varying incidence of 0.26 (2) to 4.6% (3) in autopsy studies, predominantly affecting male gender (4). Gastroenterologists and colorectal surgeons should be familiar with this entity because they may deal with it in many different clinical settings, as a JD could be the incidental finding during the pre-operative work-up of a colorectal disease patient or radiographically discovered during diagnostic abdominal imaging studies. Intraoperatively, JD may become evident on routine abdominal exploration, or could be the cause of an acute abdomen (5). Some acute presentations of that jejunal malformation include hemorrhage, anemia, malabsorption, volvulus, intussusception, perforation, small-bowel obstruction (1).

We herein report a case of an unusually large, single diverticulum of the first part of the jejunum, just after the duodenojejunal flexure near the ligament of Trietz. Solid understanding of the underlying anatomy is fundamental to offer the best possible treatment to patients suffering from these lesions.

CASE PRESENTATION

During the routine dissection course for teaching and research purposes in the Department of Anatomy of Aristotle University of Thessaloniki, in the small-bowel of a formalin-alcohol fixed and embalmed 72-year-old Greek male cadaver we came across an unusual finding of a single giant JD adjacent to the ligament of Treitz arising from the mesenteric border and extending into the posterior wall of the jejunum. The JD displayed a broad base with a diameter of 4.85 cm and sacculated outer surface (fig. 1A) Precise shape and dimensions of that anatomical finding were determined after the inflation of the gut's lumen with water through a catheter placed at the fourth part of the duodenum, just proximal to the diverticulum. No abdominal symptoms or diseases were retrieved from the medical records of the body donor (fig. 2B). No other diverticula or a Meckel's diverticulum were found after a careful investigation of the rest of the bowel. The diverticulum was found at the initial portion of the jejunum just distal to Treitz ligament. The cause of death was unrelated to the current study. An uneventful cholecystectomy was performed 18 years ago because of an acute cholecystitis. No other concomitant anatomical variations in the abdomen existed. For documentation purposes, repeated photographs of this rare finding were taken.

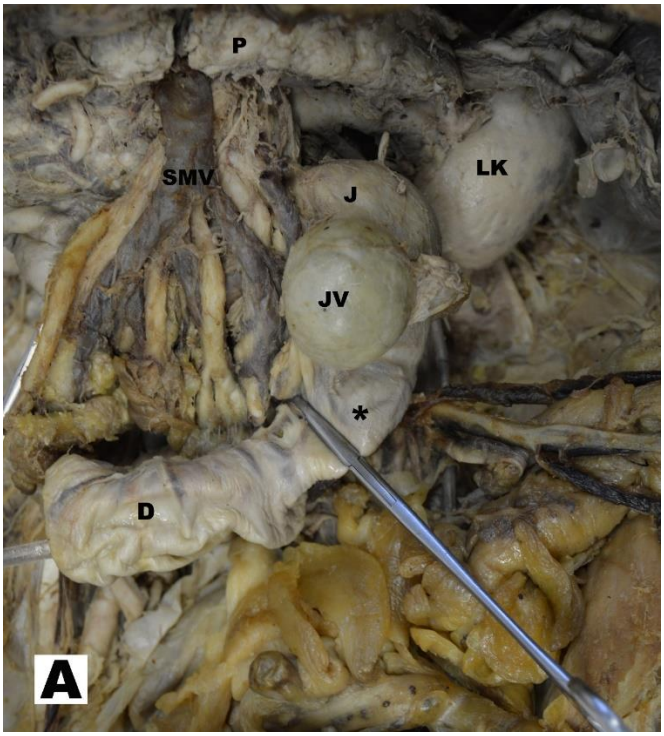

Fig. 1. A. The abdominal organs and vessels of the retroperitoneal space are demonstrated along with the first portion of the jejunum (J), where at the site of the duodenojejunal flexure (asterisk), a jejunal diverticulum (JV) is seen. The fourth portion of the duodenum (D) has been retracted distally. (P: pancreas, LK: left kidney, SMV: superior mesenteric vein).

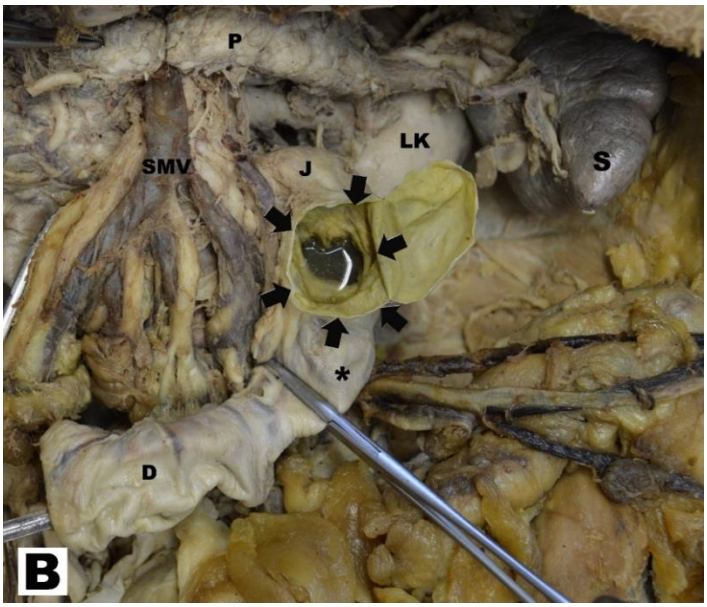

Fig. 1. B. The posterior wall of the jejunal diverticulum has been opened (arrows) to expose its lumen.

DISCUSSION

Although Sir Asley Cooper in 1807 mentioned the presence of JD in his treatise *"The anatomy and surgical treatment of crural and umbilical hernia"* (6), historically the first description of small-bowel diverticula (SBD) in autopsy studies is attributed to Sommering and Baillie in 1794 (7). The first clinical observation, however, was reported by Osler in 1881 (8), whereas the first operation for small-bowel diverticula was undertaken in 1906 by Gordinier and Sampson (9).

The incidence of JD on autopsy studies ranges from 0.26% to 4.6% (10). Noer inflated the small bowel with water, in a large autopsy series and concluded that SBV are more commonly located in the jejunum than in the ileum with an incidence of 8.3%, where twice as many men as women were found affected. In view of the nature of the diverticular wall the perforation cases are surprising low, and should be attributed to the considerable compliance of the submucosa which prevents rupture of the intestinal wall (3). About their precise location, they are most frequent at the proximal jejunum (75%), followed by the distal jejunum (20%) and the ileum (5%) (11). SBV to be more and larger in the proximal jejunum and fewer caudally (12). SBD may co-exist with diverticula of esophagus, stomach, duodenum, colon and urinary bladder, in most cases with that of the colon (11). Benson et al found that 57.65% of their patients with jejunal or ileal diverticula had also diverticula elsewhere in the alimentary tract, mainly in the colon (30 over 85%) (13). SBD are more common in males (13, 14). SBD are usually multiple, as many as 400 reported in one patient (15) SBD are rare congenital malformations of the intestine in children and young adults, most commonly diagnosed over the sixth decade of life, while various other alimentary tract diseas-

es, such as peptic ulcer, cholelithiasis and hiatal hernia may co-exist (13,14).

Non Meckelian SBD are pseudodiverticula, since they are outpouchings containing mucosa and submucosa only. Although etiology remains uncertain it is widely accepted that they develop as a result from high intraluminal pressure, through the muscular layer of the bowel at sites of low resistance, which are usually the points where blood vessels enter the bowel wall. Interestingly, another theory suggests that SBD are the product of intestinal dyskinesia, caused by smooth muscle discoordination (16) or abnormal peristalsis (17).

JD are rare congenital malformations of the small-bowel and most patients are asymptomatic, being the underlying cause of non-specific abdominal symptoms.

Even though clear majority of JD remain clinically silent, 20%- 30% of patients become symptomatic (1, 18). When symptoms become evident they may initially include mild epigastric pain, flatulence and malabsorption. Most commonly encountered acute complications involve inflammation, hemorrhage, perforation, abscess formation and intestinal obstruction (1, 19). Focusing on the location, SBD are statistically more prone to develop acute complications than duodenal ones (17). JD are nearly four times more likely to develop complications compared to duodenal diverticula and nearly 18 times more likely to perforate and develop abscesses (20).

Since most cases of JD remain asymptomatic or appear with vague symptoms, consequently diagnosis is usually delayed. On top of it no reliable diagnostic tests exist for this entity. The diagnosis is made incidentally either by radiographic examination or by laparotomy undertaken for complications (21). Diagnostic methods involved in JD diagnosis include abdominal radiographs where free air in the abdominal cavity may become evident. Computed tomography may reveal an abscess formation or pneumoperitoneum, while barium enema may show the corkscrew sign (22). The triad of obscure pain, anemia and dilated loops of small-bowel on barium radiographs is suggestive of JD presence (23). Diagnostic laparoscopy is very useful in investigating patients with a complicated symptomatology, avoiding, thus, an unnecessary laparotomy. In complicated cases of JD resection of the diseased bowel and a primary end to end anastomosis is required (5). In asymptomatic patients in whom the JD observed incidentally on computed tomography or intraoperatively, no action needs to be taken (24).

CONCLUSIONS

Since most cases of JD remain asymptomatic, general surgeons, gastroenterologists and other physicians involved in the abdominal pain assessment, should be aware and include it in their differential diagnosis. Jejunal diverticular disease may present with non-specific intermittent abdominal symptoms or rarely as acute abdomen with complicated symptomatology which may be fatal, unless prompt diagnosis is made and the appropriate surgical repair is offered to the patient.

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MANAGEMENT OF DYSLIPIDEMIA IN CHILDHOOD MAY PREVENT CARDIOVASCULAR EVENTS IN ADULTS

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MANAGEMENT OF DYSLIPIDEMIA IN CHILDHOOD MAY PREVENT CARDIOVASCULAR EVENTS IN ADULTS (Abstract): Risk factors that accelerate the atherosclerosis development are met since childhood and there are growing evidences which states that reducing them leads to a delay in clinical progression. The presence of cardiovascular risk factors, such as dyslipidemia at an early age, is associated with the presence of cardiovascular disease in adulthood. The ability to identify risk factors in children leads to an opportunity to prevent future cardiovascular events through an effective management of genetic and acquired risk factors. Incidence and prevalence of dyslipidemia among the children and teenagers are increasing from day to day. Identifying children with dyslipidemia, a high risk for an early and accelerated atherosclerosis, must include an extensive assessment of serum lipids and lipoproteins. The screening for dyslipidemia involves family history of hypercholesterolemia, cardiovascular incidents associated with dyslipidemia in relatives of first or second degree and elevated lipid profile for at least two determinations in children 2 to 8 years old and adolescents aged 12 to 16 years. Therapeutic intervention depends primarily on age. Initial approach will always include changes in diet and lifestyle. When necessary, drug treatment will be used in accordance with the recommendations for the different groups of ages. **Keywords:** DYSLIPIDEMIA, CARDIOVASCULAR RISK FACTORS, CHILDHOOD.

Dyslipidemia is associated with significant comorbidities, such as obesity, type 2 diabetes mellitus (DM) and essential hypertension (HBP). Obesity and diabetes are increasing among children and teenagers. Cardiovascular diseases are among the leading causes of death in developed countries and in Romania. Primary prevention aims to extend the range of time from identifying cardiovascular risk factors and cardiovascular disease expression (1).

It is well known that serum levels of lipids and lipoproteins vary depending on age, gender and race, normal values in children under 19 years being different compared to those of adults (2, 3). In childhood, the predominant pattern of dyslipidemia is combined, they can associate moderate to severe elevated values of triglycerides (TG), normal to slightly increased values of LDL- cholesterol (LDL-c) and reduced values of HDL-cholesterol

(HDL-c). Both patterns of dyslipidemia have been shown to be associated with the onset and progression of atherosclerotic lesions in children (4).

It has been found that during puberty lipid levels are lower (total and LDL-cholesterol levels can be reduced by 10% to 20% or more), and after this period, they increase again. Also, after the onset of puberty, increased values of total and LDL-cholesterol was found in girls (3).

Generally, there are four classes of dyslipidemia in pediatric patients known as: medication-related dyslipidemia, dyslipidemia caused by lifestyle factors, genetic dyslipidemia and dyslipidemia secondary to other diseases. Also, there have been described 3 types of genetic dyslipidemias:

- familial hypercholesterolemia is an autosomal dominant disorder caused by severe elevations in total cholesterol and LDLc.
- familial combined hyperlipidemia caused by high LDL-C and triglycerides (TG) levels.
- familial severe hypertriglyceridemia caused by severe hypertriglyceridemia (>885 mg/dL) (5).

The screening of dyslipidemia

The age of 10 (age range 9-11 years) is an ideal time for screening all children (universal screening) (4). The screening is based on a standard lipid panel, taken after fasting, that includes total cholesterol, triglycerides, HDL and LDL-cholesterol. If the child is not fasting it will be investigated non-HDL-c (calculated as: Total Cholesterol - HDL-c) is recommended in the initial screening (which is not influenced by food or drink), unlike triglycerides or LDL-c. Calculated LDL-c is based to the Friedewald formula: Total Cholesterol - triglycerides / 5 + HDL-c (which are influenced by food or drink). Significant increased triglyceride (e.g.>400 mg/dL) excludes LDL-c calculation rule using the Friedewald equation (commonly used by most laboratories). If non-HDL-c is increased (> 145 mg/dL), will have to obtain two lipids profiles *a jèun* for 3 months, with at least 3 months break between assessments (2, 6).

According to the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents 2011 the screening of dyslipidemia is recommended in the following cases:

- children aged over 2 years old with a family history of hypercholesterolemia - one or both parents with total cholesterol ≥ 240 mg/dL or with known dyslipidemia;
- children aged 2 to 8 years and adolescents aged 12 and 16 years, with two modified lipid profiles (every 2 weeks and repeated at 3 months) to detect risk factors;
- in the presence of cardiovascular risk factors;
- in the presence of a significant cardiovascular family history: parents/grandparents, < 55 years for men/< 65 years for women, who have suffered an acute myocardial infarction or sudden death, with atherosclerotic coronary disease, cerebrovascular or peripheral vascular disease;
- universal screening in children aged between 9 and 11 years (before the onset of puberty) with/without cardiovascular risk factors, repeated after the age of 17 years (4).

The results of screening are considered significant if LDL-c is higher than 130 mg/dL, non-HDL-c > 145 mg/dL, HDL c < 40 mg/dL and TG > 100 mg/dL (children under 10 years) or 130 mg/dL (children over 10 years); lipidic profile must be repeated over 14 days (6) (tab. I).

TABLE I

Plasmatic distribution of lipids in children and teenagers

	Normal	Borderline	High
Total cholesterol	< 170 mg/dL	170−199 mg/dL	≥ 200 mg/dL
LDL- cholesterol	< 110 mg/dL	110−129 mg/dL	≥130 mg/dL
TG	0−9 yrs. <75 mg/dL 10−19 yrs. < 90 mg/dL	75−99 mg/dL 90−129 mg/dL	≥100 mg/dL ≥130 mg/dL
HDL- cholesterol	> 45 mg/dL	40−45 mg/dL	< 40 mg/dL
ApoB	< 90 mg/dL	90-109 mg/dL	> 110 mg/dL
ApoA1	>120 mg/dL	115-120 mg/dL	< 115 mg/dL

Cardiovascular risk factors and other causes of dyslipidemia

When cardiovascular risk factors are present at a younger age, their persistence into adulthood was associated with an increased risk for future cardiovascular disease. Identifying vulnerable populations allows the early prevention of CV risk factors progression and future cardiovascular events, with an effective management of genetic and acquired risk factors (7) (tab. II).

TABLE II

Criteria for cardiovascular risk factors and associated diseases

Criteria	Moderate risk	Increased risk
IMC	> 95 percentile but < 97 percentile	> 97 percentiles
Hypertension	HTA without treatment	HTA with treatment
HDL-c	< 40mg/dL	-
Smoking		Active smoker
Diseases	- Kawasaki disease with coronary aneurysms regression - Chronic inflammatory diseases (LES, juvenile inflammatory arthritis) - Nephrotic syndrome	- Kawasaki disease with coronary aneurysms progressive - type 1 and 2 diabetes - Chronic kidney disease/end-stage renal impairment/ post renal transplant status

Other causes of dyslipidemia should also be considered and evaluation should include a complete metabolic panel (liver tests, kidney tests, electrolytes) and specific tests according to secondary causes of dyslipidemia (6, 8) (tab. III).

TABLE III

Screening tests for others causes of dyslipidemia

Disease	Screening tests
Liver damage	Complete metabolic panel
Renal impairment	Complete metabolic panel, urine analysis
Hypothyroidism	TSH, FT4
Diabetes	Complete metabolic panel, urine analysis, postprandial glucose, HbA1c
Obesity / Insulin resistance	Complete metabolic panel, postprandial glucose, insulin
Medication	Steroids, contraceptives, retinoids, protease inhibitors

Management of dyslipidemia

The goal of dyslipidemia treatment is to maintain LDL-c < 30mg/dL, TG <150 mg/dL and HDL-c>35 mg/dL. Lifestyle improvement is the cornerstone of dyslipidemia management. Starting pharmacotherapy is recommended only in case of high or very high cardiovascular risk or if lifestyle changes do not improve lipid levels. The main classes of drugs are statins and omega-3 fatty acids (9).

Nutritional recommendations

Nutritional recommendations during the first 2 years of life

Natural feeding should be encouraged in all infants for the first 6 to 12 months of life. Subsequently, complementary feeding will be progressively introduced after the first 6 months of life. Lipid intake in children under 1 year of age should not be restricted without medical indication and for children over 1 year low saturated fat diet is recommended. It is indicated to avoid sweet juices and to limit the undiluted fruit juice to 100 mL/day. The child will be encouraged to drink water, eat fruits and vegetables, cereals and limit the salt intake. Lipid intake cannot represent more than 30% of the daily ration (saturated fat 8-10% of caloric intake, mono-unsaturated and poly-unsaturated 20% of the total fat intake) and avoiding trans-unsaturated fatty acids (6, 9).

Nutritional recommendations after the age of 2-years-old

It is recommended the consumption of low (2%) or without fat milk. Encourage children to drink water and avoid beverages with added sugar. The total content of fats in the diet of children with dyslipidemia should be 25% to 30%, with saturated fat lower than 10%, mono- and poly-unsaturated fat up to 20%, while avoiding the saturated and trans-unsaturated fatty acids as much as possible. The other 70% calories in the diet should be 15-20% from proteins and 50-55% from carbs. Portions must agree with the caloric intake recommendation for age and sex. Fiber intake is recommended (5 g + age in years/day; in adolescents - 14 g/1000 kcal) and salt intake should be limited. For children with hypercholesterolemia and high LDL-c it's recommended a saturated fat restricted diet (to ensure less than 7% of calorie needs) and limited dietary cholesterol to 200mg /day, with trans fats less than 1%. In high TG levels, the recommendations include the reducing of simple carbohydrates intake, associated with increased consumption of complex carbohydrates, lowering fat intake. If obesity is associated with increased TG. it is recommended to reduce caloric intake and increase the physical activity (6, 10, 11).

Physical activity and lifestyle

Physical activity has a beneficial effect on the children health. Like dietary choices, physical activity patterns are established in childhood and perpetuated further in adulthood. Therefore, the family is primarily responsible for encouraging children to adopt a healthy lifestyle. Physical activity is associated with lower blood pressure, body fat reduction, decreased BMI, decreased TC, LDL-c and TG, increased HDL-c, decreasing insulin resistance. Moderate physical activity is recommended daily or at least one hour a day for 3 days a week of intense physical activity. Spending free time on TV should not exceed two hours per day. In children aged 1 to 5 years, watching TV with the family will be discouraged. Family activities should be undertaken at least once a week, encouraged especially in children aged 3 to 10 years (3, 6, 12).

Drug therapy

Starting lipid-lowering therapy is based on fasting LDL-c levels and the presence/absence of cardiovascular risk factors. Children and adolescents with LDLc ≥ 250 mg/dL and/or TG ≥ 500 mg/dL must be evaluated by a specialist to a proper management (11).

Drug therapy for high LDL-cholesterol

Patients with mildly elevated LDL-c, but with significant family history of premature cardiovascular disease (LDL-c ≥ 160 mg/dL, values older than 10 years, associated with risk factors) or with LDL-c ≥190 mg/dL, with no risk factors, might benefit from drug intervention (12).

The recommended treatment for reducing LDL-c is statins (13). As a rule, statins' therapy is not recommended before the age of 10 years, except for a high-risk family history, cardiovascular disease and/or premature events, the presence of one or more high risk factors or associated risk factors (6) (tab. IV).

TABLE IV

Treatment interventions for elevated LDL-c

LDL-c 130-189 mg/dL	Negative family history Without risk factors	Diet and lifestyle advice
LDL-c 130-159 mg/dL	2 high risk factors or 1 high risk factor + ≥ 2 moderate risk factors or BCV	Diet and medical therapy (statins)
LDL-c 160-189 mg/dL	1 very high-risk factor or ≥ 2 moderate risk factors or Positive family history	Diet and medical therapy (statins)
LDL-c ≥ 190 mg/dL	-	Diet and medical therapy (statins)

The treatment should start with the lowest available dose of statin, administered once daily (14). All statins, except Pitavastatin were approved by the Food and Drug Administration (FDA) in the USA in children with familial hypercholesterolemia. Pravastatin is used in children over 8 years, and all other statins (Simvastatin, Fluvastatin, Lovastatin, Atorvastatin, Rosuvastatin) were administered after the age of ten (1, 6) (tab. V).

The goal is to achieve a reduction in LDL-c levels below 130 mg/dL, ideally < 110 mg/dL. If the target is not reached, then the dose of statins should be increased (usually 10 mg), but with monitoring of liver transaminases (to be repeated every 4 weeks). If the LDL-c level remains high, the dose of statin may be increased. It must be considered that each doubling of the statins dose only leads to a reduction of about 6% of the LDL-c levels.

TABLE V

Indication for use of statins by age

Statins	Age	Dose
Atorvastatin	10-17 years	10-20 mg
Fluvastatin	10-16 years	20-80 mg
Lovastatin	10-17 years	10-40 mg
Pravastatin	8-13 years 14-18 years	20 mg 40 mg
Rosuvastatin	10-17 years	5-20 mg
Simvastatin	10-17 years	10-40 mg

Statin therapy monitoring requires a careful evaluation which will focus on

child growth and pubertal development (height, weight, BMI, Tanner staging), blood pressure and possible adverse effects. Laboratory investigations include lipid levels, liver function tests (ALT, TGO) and creatinkinase each month in the first year, twice a year in the coming years. In case of side effects, medication should be discontinued at least two weeks; later rechecked biological investigations, and if the side effects are solved, medication may be resumed with careful monitoring (4, 9).

If lifestyle changes combined with statin therapy was not successful, it may be added other lipid-lowering drugs. Ezetimibe is an inhibitor of cholesterol absorption, used in children with hypercholesterolemia over 10 years (5, 11, 15).

Drug therapy for elevated triglycerides

Children with elevated TG up to 500 mg/dL are at increased risk for pancreatitis. There is no consensus to prevent pancreatitis in people under 18 years of age with a significant risk. The European Experts Group recommends that administration of fibric acid derivatives and omega 3 fatty acids should be considered in association with nutritional counselling. There is data supporting that pre-prandial addition of Orlistat (Xenical), an inhibitor of pancreatic lipase, associated with a very low-fat diet is an effective way to prevent pancreatitis in people with familial lipoprotein lipase deficit. Adding omega 3 fatty acids can be useful to lower slightly elevated TG, but it has been not established an effective dose. The addition of 1g of omega-3 fatty acids is recommended for adults with cardiovascular disorders, but with minimal effect on reducing TG. Higher doses of omega-3 fatty acids may increase LDL-c levels despite the drop in total cholesterol (result of reducing triglyceride-rich lipoprotein cholesterol) (6, 16-18).

Drug therapy for mixed dyslipidemia

Although the major focus was on identifying and treating children with elevated LDL-C, the most commonly encountered dyslipidemia in clinical practice is hypertriglyceridemia associated with low HDL-c. This model of "atherogenic dyslipidemia" may be genetic and it is most commonly seen in children with obesity (BMI $\geq$ 95 percentile) and insulin resistance (manifested clinically by the presence of acanthosis nigricans, prediabetes, hypertension). It is noted that obesity may exacerbate genetic dyslipidemia and the atherosclerosis process. The safest and most effective treatment for acquired dyslipidemia consists in lifestyle changes, encouraging healthy eating habits, daily moderate to intense physical activity and weight reduction. Tobacco cessation and/or eliminating passive exposure to it can be a strategy for lowering cardiovascular risk in adolescents (17, 18).

Drug therapy for low HDL-cholesterol

Low HDL-c increase the risk of cardiovascular disease. Decreased HDL-c, frequent in obese children who have insulin resistance, is almost always associated with increased TG. There are no approved medical interventions in children with low HDL-c, others than mitigate the standard risk factors through lifestyle changes. In adult people, drugs aimed for raising HDL-c (e.g. transport cholesterol inhibitors or niacin) have not shown clinical efficacy (6-8).

CONCLUSIONS

According to actual guidelines, we recommend universal screening of dyslipidemia for all children aged 9-11 years, regardless of the general condition or the presence/absence of cardiovascular risk factors. The screening will allow us identifying the children with less severely elevated risk factors, in which intervention

will be more effective. Indications for treatment are based on the best available evidence and may be successfully on appropriate follow-up. Given the impact of early atherosclerosis onset in childhood and further cardiovascular complication, it is important that such recommendations to be consistently implemented by primary care providers and pediatrician. Continued implementation of public health programs is needed for the identification and treatment of cardiovascular risk factors in childhood.

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NEWS

THE SPECTRUM OF HISTOLOGIC FINDINGS IN HEPATIC OUTFLOW OBSTRUCTION

Budd-Chiari syndrome and cardiac (congestive) hepatopathy are the 2 most well-known diagnoses in the category of hepatic venous outflow obstruction. Although the 2 diseases differ in their clinical presentations, etiologies, and points of occlusion along the hepatic outflow tract, they show similar morphologic findings on sampling of patient liver tissue. Budd-Chiari syndrome refers to obstruction of the hepatic outflow tract, typically by thrombosis, anywhere from the hepatic venules to the junction of the inferior vena cava and right atrium. It occurs in approximately 0.001% of the population and has numerous potential etiologies, including inherited and acquired prothrombotic states, myeloproliferative diseases, and oral contraceptive use. Patients may present with acute, subacute, or chronic disease. The diagnosis can often be made clinically by radiologic demonstration of outflow obstruction, though patients with obstruction of intrahepatic veins may have normal findings on imaging. Pathologic changes in biopsied or explanted livers of patients with Budd-Chiari characteristically include perivenular necrosis, sinusoidal dilation, and occasionally formation of macronodules resembling focal nodular hyperplasia. In cardiac hepatopathy, right-sided heart failure of any cause prevents proper blood flow into the heart. This leads to a backlog of blood in the venae cavae and ultimately within the liver, causing intrahepatic congestion. This process causes the classic gross appearance of nutmeg liver, and longstanding congestion can lead to extensive fibrosis (termed either cardiac sclerosis or cardiac cirrhosis). The degree of cardiac dysfunction and severity of clinical presentation can vary widely, and as a result, cardiac hepatopathy may not be suspected clinically and therefore could progress undiagnosed. Patients under consideration for heart transplant may undergo liver biopsy to assess for the presence and severity of cardiac hepatopathy, as preoperative hepatic disease has been linked to postoperative morbidity. Biopsy findings include centrilobular and sinusoidal dilation and centrilobular necrosis, depending on the severity of the cardiac disease. A third type of hepatic outflow impairment, sinusoidal obstruction syndrome (previously termed veno-occlusive disease), occurs when the hepatic sinusoids or central veins become obstructed. Though originally described in Jamaican children with alkaloid-induced hepatotoxicity, it is most commonly seen in the setting of myeloablative therapy for hematopoietic stem cell transplantation. It demonstrates a characteristic histologic pattern, with acute disease typified by marked sinusoidal dilation and congestion, with loss of sinusoidal endothelium, and chronic disease demonstrating central vein occlusion. These clinicopathologic findings help distinguish it from Budd-Chiari syndrome and cardiac hepatopathy. Although Budd-Chiari and cardiac hepatopathy are typically diagnosed clinically, patients with occult disease may undergo liver biopsy for nonspecific findings, such as elevated liver enzymes. Given the overlap in histologic pattern in the 2 conditions, the study aimed to determine whether certain microscopic changes were significantly more common in either, allowing for distinction between their otherwise-similar microscopic appearances and facilitating identification of the diseases in patients without a definitive clinical diagnosis (Gonzalez RS,. Gilger MA, Huh WJ. The Spectrum of Histologic Findings in Hepatic Outflow Obstruction. Arch Pathol Lab Med. 2017; 141:98–103).

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PLEUROPERICARDIAL CYST – CASE REPORT

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PLEUROPERICARDIAL CYST-CASE REPORT (Abstract). Cystic lesions within the pericardial space are rare and comprise 7% of the mediastinal masses. We present the case of a 71-year-old woman admitted to the Cardiovascular Rehabilitation Clinic of the Iasi Rehabilitation Hospital for palpitations and vertigo. A routine abdominal ultrasound was performed, which revealed in the 4th segment of the liver a 12-mm hyperechoic nodule suggestive for hemangioma and a round, well-defined 3cm lesion with a possible topography on the diaphragm. An echocardiography has been requested and the cardiac origin was refuted. The barium test showed that the lesion was not located in the gastrointestinal tract. Anteroposterior and latero-lateral chest radiographs were performed, but they did not reveal the tumor. Thus, we decided to visualize it by chest computer tomography with contrast that showed an oval, well-defined lesion of 30/25 mm with homogenous structure, without contrast medium uptake and signs of local aggressiveness in the middle mediastinum, retrocardiac, on the posterior aspect of right atrium, in relation to inferior vena cava and esophagus. The lesions most frequently met within this area are the pleuropericardial ones. Short-term and long-term prognosis is good. **Keywords:** PLEUROPERICARDIAL CYST, MEDIASTINAL TUMOR, PALPITATIONS.

Cystic lesions within the pericardial space are a rare entity and comprise 7% of the mediastinal masses (1). They are more frequently located in the right costophrenic angle (in 70% of the cases) than in the left costophrenic angle (in 10-40%) (2). The most frequently met are the pleuropericardial lesions. The first to describe the mesothelial cyst were Churchill and Mallory in 1937 and later, in 1940, Lambert reported a similar case, described as pericardial cyst (3). They are usually found in the third or fourth decades of life and males and females are equally affected (4).

CASE REPORT

We present the case of a 71-year-old woman without medical history of cardiovascular disease, who was admitted to the Cardiovascular Rehabilitation Clinic of the Iasi Rehabilitation Hospital for palpitations and vertigo. When admitted to the hospital, the patient presented: impaired general status, altered mental status, amnesia, blood pressure of 105/60 mmHg and a heart rate of 60/min, normal heart rhythm but with numerous extrasystoles. Because of this, Holter-ECG monitoring was recommended that revealed 3325 supraventricular ectopies

with 286 supraventricular bigeminy events and 3 supraventricular trigeminy events. Also, there have been observed 622 ventricular ectopies with 7 ventricular bigeminy events and 24 ventricular trigeminy events. An antiarrhythmic treatment was initiated with favorable outcome.

A routine abdominal ultrasound was performed, which revealed in the 4th segment of the liver a 12mm hyperechoic nodule suggestive of hemangioma and a round and well-defined 3 cm lesion with a possible topography on the diaphragm. Because its location could not be established, an echocardiography was requested and the cardiac origin was refuted. Then a barium test was performed that showed that the lesion was not located in the gastrointestinal tract. We have also done an antero-posterior and latero-lateral thoracic radiography, but neither this investigation showed the tumor. Thus, we decided to visualize it by chest computer tomography with contrast that showed an oval, well-defined lesion of 30/25 mm with homogenous structure, without contrast medium uptake and signs of local aggressiveness in the middle mediastinum, retrocardiac, on the posterior aspect of right atrium, in relation to inferior vena cava and esophagus. The lesion had benign characters (pleuropericardial cyst).

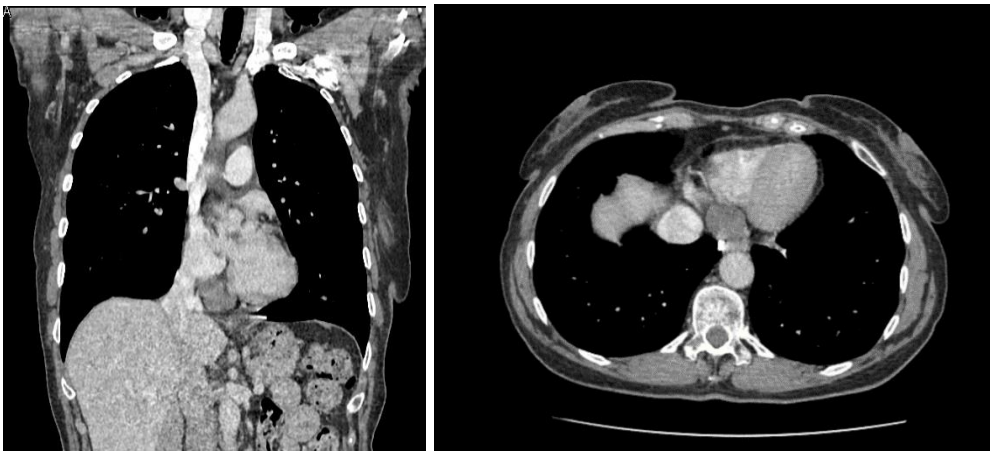

Fig. 1. Pleuropericardial cyst

DISCUSSION

From a theoretical point of view, the mediastinum is divided into 9 regions by 2 vertical planes and 2 horizontal planes into superior, middle and inferior and anterior, middle and posterior. In our patient's case, we had to make the differential diagnosis of the anteroinferior region in which there can be found the endothoracic fascia, inferior pericardial ligament, phrenopericardial ligaments, heart and pericardium (5). It is known that the mediastinum contains numerous vital structures in a relatively small area and has a limited accessibility. At least half of the patients with mediastinal tumors are asymptomatic and the lesions are usually detected incidentally on chest radiographs or other routine laboratory investigations. The asymptomatic lesions are usually benign. The symptoms commonly appear after the compression or invasion of the adjacent organs (6).

The main symptoms that can be observed after the compression or invasion of the bronchial tree are: pain, cough, dyspnea, hemoptysis, dysphonia, wheezing; also, the venous compression determines the superior vena cava syndrome, azygos vein syndrome, thoracic duct syndrome, Menetrier syndrome, bilateral pleural effusion or lower limb edema. There can also appear signs of nervous compression expressed by dysphonia, hemidiaphragm paralysis, hiccups, intercostal neuralgia, Claude Bernard Horner syndrome, Pancoast Tobias syndrome. In addition, we can find tachycardia and palpitations as circulatory signs and, last but not least, dysphagia. Occasionally, mediastinal tumors may manifest clinically as paraneoplastic syndromes, most frequently endocrine (7).

In our patient's case, we considered the following differential diagnosis:

- Pleuropericardial cyst - a mesothelial cyst that grows from the parietal pericardium. It is asymptomatic, being frequently discovered incidentally. It is in the anterior costophrenic angle, predominantly right sided. Chest radiograph reveals a well-defined homogenous opacity, moving with respiration;
- Diaphragmatic relaxation or eventration, determined by the deficient development of the phrenic nerves that reach the muscles and are often located in the antero-median region of the right hemidiaphragm; this was refuted in our case by computer tomography;
- Morgagni hernia that is determined by a defect in the parasternal region of the diaphragm, usually on the right side. Chest radiograph shows well-defined right paracardiac opacity of variable intensity depending on the content (8), negative in our case.

Short- and long-term prognosis is good. Among the most frequent complications we should mention compression, inflammation, rupture and hemorrhage. Intracystic hemorrhage may be spontaneous or post-traumatic. Hemorrhage may determine a quick expansion of the cyst and may culminate with severe pain, cardiac tamponade or congestive heart failure (9, 10, 11).

CONCLUSIONS

Our case is a useful opportunity for clinical remarks firstly given its atypical onset - this cyst was detected incidentally in the seventh decade of life, it was localized on the left side, contrary to most the cases in the literature. In addition, we should take into consideration the fact that in our patient we could not see anything on the chest radiograph, even if in the literature many cases are discovered through this investigation.

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NEWS

LONGER CORONARY ANASTOMOSIS PROVIDES LOWER ENERGY LOSS IN CORONARY ARTERY BYPASS GRAFTING

The first successful CABG intervention was performed in 1964 by Michael DeBakey who bypassed a coronary stenosis by interposing a saphenous vein graft between the ascending aorta and the diseased coronary artery distal to the lesion. Since then, the technique evolved and nowadays the intervention can be performed off-pump and minimally invasive. Despite all progress, clinical studies did not manage to identify the ideal grafting technique in terms of grafting configuration, graft type, graft harvesting, graft preparation, anastomoses, and optimal long-term patency. Distal anastomosis technique has been proved to affect long-term graft patency by numerous studies. However, the appropriate length of distal anastomosis has not been investigated until the research performed by dr. Hiroyuki Tsukui and his collaborators. Their study aimed to evaluate whether longer distal anastomosis provides higher quality of distal anastomosis and better hemodynamic patterns. For this purpose they used a CABG training simulator for building distal anastomosis models. The authors prepared two end-to-side distal anastomosis models of different lengths (10 versus 4 mm) and used computed tomography (CT) to obtain a 3D reconstruction of the inner shape of distal anastomosis. Afterwards, computational flow dynamics (CFD) was used to analyze hemodynamic patterns. The 3D CT reconstruction showed that the quality of distal anastomosis in the 10 mm model was more uniform without vessel wall inversion or kinking compared to 4 mm model. Anastomotic flow area proved to be significantly larger in the 10 mm model compared to the 4 mm one. Also, the anastomotic angle was smaller in 10 mm model compared to 4 mm model, a condition associated with lesser flow turbulences. CFD analysis demonstrated that the 10 mm model had streamlined flow with smooth graft curvature, whereas 4 mm model had abrupt blood flow with directional changes and separation at the toe. Subsequently, the 10 mm model registered a lower energy loss than the 4 mm model. The authors concluded that longer distal anastomosis provided a larger anastomotic flow area, a smaller anastomotic angle, and smoother graft curvatures, factors associated with a higher flow quality and better long-term patency. (Tsukui H, Shinke M, Park YK, Yamazaki K. Longer coronary anastomosis provides lower energy loss in coronary artery bypass grafting. *Heart Vessels*. 2016; 32(1): 83-89).

Cristina Furnică, Grigore Tinică

RARE CAUSE OF RHABDOMYOLYSIS – CASE REPORT

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RARE CAUSE OF RHABDOMYOLYSIS-CASE REPORT (Abstract): Rhabdomyolysis, a syndrome caused by injury to the skeletal muscles, is a condition associated with various signs ranging in from asymptomatic elevation of serum creatine-kinase to acute kidney injury, abnormal heart rate and rhythm, coma and even death. We report the case of a 52-year-old woman who developed rhabdomyolysis following an epileptic seizure. Because a single epileptic seizure does not commonly cause rhabdomyolysis, we tried to identify other contributing factors. The only finding was an electrolyte imbalance due to vomiting. This case had a favorable outcome. Our report highlights the importance of considering rhabdomyolysis in a patient with a recent epileptic seizure, especially when several trigger factors for rhabdomyolysis act simultaneously in the same patient. **Keywords:** RHABDOMYOLYSIS, EPILEPSY, SEIZURE.

Rhabdomyolysis is a syndrome caused by injury to the skeletal muscles. It is a potentially life-threatening condition because the breakdown products of damaged muscle cells are released into the bloodstream. Myoglobin is potentially harmful to the kidneys and within 12 to 24 hours after the initial muscle damage may lead to acute kidney injury (AKI). The risk of developing AKI can be as high as 40% (1). Rhabdomyolysis is believed to be responsible for up to 15% of all cases of AKI (2). Myoglobin has a direct nephrotoxic effect due to its activity as a peroxidase-like enzyme. By interacting with Tamm-Horsfall protein it creates casts that are obstructing the tubule (3). The electrolyte imbalance is responsible for nausea and vomiting, altered mental status ranging from confusion to coma, abnormal heart rate and rhythm. Hyperpotassemia can lead to cardiac conduction impairment, which can cause ventricular fibrillation, abnormally slow heart rhythms and asystole.

CASE REPORT

We report the case of a 52-year-old woman who developed rhabdomyolysis following an epileptic seizure.

The patient had a history of high blood pressure. Over several days, she had acute nausea and vomiting due to inappropriate diet. She was brought to hospital by ambulance after experiencing an episode of tonic-clonic seizure at home. No signs of meningeal irritation or focal neurological

deficit were present. The CT scan excluded any posttraumatic cranio-cerebral injuries, stroke and intracranial expansive processes. Neurological examination made a clear diagnosis of epilepsy.

Chest X-ray ruled out pleural and lung lesions. EKG was normal and transthoracic echocardiography showed only hypertension-induced changes. Abdominal ultrasound did not reveal anything significant.

Patient's initial laboratory assessment showed very high levels of creatine-kinase (45852 U/L), hypokalemia (K=2,7 mmol/l) and sodium, calcium and chlorine levels slightly below the limit values. The highest level of TGO was 606 U/L, of TGP 136 U/L and LDH 1259 U/L. Renal function and uric acid levels were within normal range. Tests for hepatitis virus B and C turned out negative. Thyroid function was normal and no signs of primary hyperaldosteronism were found. The patient denied both alcohol consumption and drug administration at home.

The patient was well hydrated with oral and iv fluids and the electrolyte imbalance was restored. She was closely monitored clinically and by laboratory tests. The case had a favorable outcome, the creatine-kinase (CK) levels gradually decreased, the renal function remained unaltered and there were no consequences of electrolyte abnormalities.

DISCUSSION

Rhabdomyolysis can have many causes and may take various forms, ranging from asymptomatic elevations of serum muscle enzymes to a life-threatening condition.

Causes of rhabdomyolysis can be: *trauma* („Crush syndrome"), *prolonged immobilization* (in elderly people or after stroke or surgery), *exertion* (extreme physical exercise, delirium tremens, tetanus, prolonged seizures or status epilepticus), *extreme body temperature* (malignant hyperthermia, severe hypothermia), *drugs* (statins, fibrates, diuretics, antipsychotic medications, neuromuscular blocking agents used in anesthesia, selective serotonin reuptake inhibitors, abuse of alcohol, amphetamine, cocaine, heroin, LSD, ecstasy), *toxins* (heavy metals, venom from insects or snakes, poisonous plants), *muscle hipoxia* (blocked arterial flow), *metabolic imbalances* (hyperglycemic hyperosmolar state, hyper- and hyponatremia, hypokalemia, hypocalcemia, hypophosphatemia, ketoacidosis, hyperaldosteronism, hypo thyroidism), *autoimmune muscle damage* (polymyositis, dermatomyositis), *infections*, *genetic defects* (4).

Plasma myoglobin increases rapidly after muscle injury, but is quickly cleared by kidneys and a normal level is reached within 24 hours. Because of its short half-life, plasma myoglobin is not as sensitive as CK for diagnosis. The CK levels increase within the first 12 hours after muscle injury, remain elevated for 3–5 days and then gradually fall over the subsequent 6–10 days (5). Therefore, serum CK level is the most reliable test in the diagnosis of rhabdomyolysis. CK levels 5 times higher than the upper limit (i.e. 1.000 IU/L) clearly indicate rhabdomyolysis. The risk of AKI has a linear relationship with the initial and peak CK levels. The higher the CK level, the more likely the AKI will occur, but there is no specific CK level above which AKI occurs (6). Usually levels below 20.000 U/l are unlikely to induce kidney impairment, unless there are other contributing risk factors.

A single epileptic seizure does not commonly cause rhabdomyolysis (7), most

of the cases reported in the literature being due to status epilepticus. However, the muscle forces generated during tonic-clonic seizures alone can cause severe muscle injury. Rhabdomyolysis is also a result of fall during seizures as most of the time falls lead to muscle trauma. Please note that our patient did not show any evidence of direct trauma.

It is important to observe that rhabdomyolysis has been associated with a variety of electrolyte disorders, particularly hypokalemia. Severe electrolyte abnormalities disrupt cell membrane homeostasis, mainly by disturbing the Na/K ATPase pump activity. Hyponatremia (8), hypernatremia (9), hypokalemia (10) and hypophosphatemia (11) may result in rhabdomyolysis. Potassium plays a major role in regulating the skeletal muscle blood flow. Potassium released from muscle cells during exercise normally mediates vasodilation and appropriately increases the blood flow to the muscles. Decreased potassium release due to hypokalemia may promote the development of rhabdomyolysis by decreasing blood flow to the muscles (12, 13). In addition to hypo perfusion, hypokalemia-induced impairment in muscle metabolism may also contribute to muscle dysfunction.

In the here reported case, we strongly believe that the highly-elevated levels of CK (230 times higher than the upper limit) are explained by the combined action of two trigger factors for rhabdomyolysis: seizure and the preexisting electrolyte imbalance, mainly hypokalemia (due to vomiting). In the literature, we found only one case reporting hypokalemia due to vomiting as the single cause of rhabdomyolysis (13).

The prognosis of rhabdomyolysis is heavily dependent on the underlying etiology and associated comorbidities. Despite the lack of any well-organized prospective studies, the available evidence suggests that rhabdomyolysis has an excellent prognosis when treated early and aggressively (14). Moreover, when kidney function is impaired, the prognosis for the full renal function recovery is excellent (3). Fluid resuscitation must be initiated as soon as possible as the fundamental therapeutic measure to prevent AKI.

CONCLUSIONS

Our case report highlights the importance of considering rhabdomyolysis in a patient with a recent epileptic seizure, especially when several trigger factors for rhabdomyolysis act simultaneously in the same patient. Early evidence of this phenomenon provides the chance of preventing irreversible kidney damage by appropriate therapeutic measures, which are simple and worldwide available.

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NEWS

COULD CBCT REPLACE CLASSIC CT IMAGING IN PATIENTS WITH OTOSCLEROSIS?

Otosclerosis is a primary disease of the otic capsule, characterized by alternating phases of absorption of compact bony tissue and replacement with spongy bone. A study of 43 patients with stapes ankilosis, explored by CBCT and HRCT was made with the aim of comparing the diagnostic value of CBCT and HRCT in the preoperative evaluation of otosclerosis. Histologically active otosclerosis (n=23) was detected equally by both HRCT and CBCT. However, CBCT was unable to detected histologically inactive otosclerosis (n=12). In conclusion, HRCT remains the first choice in the imaging diagnosis of otosclerosis. However, CBCT is also a reliable diagnostic tool, continuously evolving by introduction of more powerfull analyser softwares and has the advantage of providing high quality images, with exclusively scanning the examed zone, at a considerably lower radiation dose than HRCT (Révész, P., Liktor, B., Liktor, B. et al. Could cbct replace classic ct imaging in patients with otosclerosis? *Eur Arch Otorhinolaryngol* (2016) 273: 63. doi:10.1007/s00405-015-3490-3).

Ariadna Mocrei

EXCESSIVE PRIMARY POSTPARTUM HEMORRHAGE

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EXCESSIVE PRIMARY POSTPARTUM HEMORRHAGE (Abstract). The diagnosis and treatment of excessive bleeding within the first 24 hours after delivery and its main causes, namely uterine atony, retained placental fragments, vascular lesions by local tissue rupture, and blood clotting disorder are presented. Of great interest is the iconography, which is suggestive and original. **Keywords**: EXCESSIVE, OBSTETRIC, HEMORRHAGE, IMMEDIATE POSTPARTUM.

Excessive primary postpartum hemorrhage (after vaginal or C-section delivery) is often accompanied by an abnormal prenatal coagulogram (specific for obstetrics), which can be a warning sign. It has four major causes: uterine atony (in the case of malformed uterus, fibromyomatous uterus, over-distended uterus due to multiple pregnancy, polyhydramnios or fetal macrosomia, uterine muscle exhaustion after prolonged labor or uterus of an obese mother); retained placental or membrane fragments; uterine rupture, tears of the cervix, vagina, vulva, pelvic floor; coagulation disorders, most commonly disseminated intravascular coagulation (DIC) and secondary fibrinolysis in: uteroplacental apoplexy, dead fetus in utero, amniotic fluid embolism, chorioamnionitis, septic shock, even a severe acute hemorrhage, hemorrhagic shock, *placenta previa*, *placenta accreta*, preeclampsia-eclampsia etc. Bleeding can be external, internal or mixed.

Clinical causal assessment:

- uterus greater than 20 cm/10 cm; low uterine tone – examination of placenta and membranes (evidence of missing placental fragments, membranes);
- coagulability or non-coagulability of blood in the renal pelvis;
- examination of the birth canal: manual exploration of the uterine cavity (for the presence or absence of retained placental fragments and uterine rupture);
- speculum exam for cervical tears; inspection of the external genitalia and perineum.

Simple tests that can quickly pinpoint the diagnosis (1):

- Low platelet count: 15-20,000 indicate a DIC;
- Collection of blood in a 3.5 ml test tube without anticoagulant:
- a small clot with a red blood cells deposit formed at bottom of the tube suggests DIC;
- a clotting time (CT) exceeding 10 minutes means a drop of fibrinogen level below 100 mg%;
- clot formation and its resolution within 30 minutes denotes secondary fibrinolysis;
- the total non-coagulability of blood is indicative of primary fibrinolysis. These tests require laboratory confirmation (1).

Management of uterine atonia:

- compression of abdominal aorta for 5-15 minutes, uterine massage;
- in the already installed IV infusion set introduce 10-15 U oxytocin and/or ergometrine or methergin 0.2 mg or intramuscularly 15-methyl PGF_2; bleeding persists or recurs.

The uterine cavity is explored manually (for removing the retained placental fragments and checking for uterine tears, especially after manual detachment and removal of placenta or other obstetrical procedure or maneuver. If the uterus is intact, introduce your right hand into the uterine cavity, clench your fist and use the other hand to massage the uterus through the abdominal wall. If the bleeding persists inject ergomet or prostaglandins PGE_2 or PGF_2 into the cervix (1).

Prostaglandins PGE_2, PGF_2, and PG_{2a} can be injected most effectively intramyometrially, into the cervix or intravenously and prostaglandin analogue sulprostone (15-methyl PG_{2a}) intramuscularly (1).

It is preferable that intramyometrial (and into the cervix) or intravenous injections to be made after the manual inspection of the uterine cavity. Bleeding persists. Grasp the cervix with forceps (with six claws) into the four cardinal points and use them to strongly pull it down (thus an angle bend of the uterine artery that diminishes the bleeding is obtained). In modern obstetrics, intrauterine message is no longer indicated (2).

In addition to these procedures, Luca (1) mentions the placement of hemostatic threads in "X" on the cervicoisthimic junctions or Henckel forceps on commissures.

In parallel the following are done: correction of volemia, the necessary volemic expansion with blood transfusions (blood collected within the last 6 hours), fresh frozen plasma (thawed), macromolecular solutions, isotonic NaCl or glucose solutions, and correction of coagulability.

Inspection of the uterine cavity is also indicated in case of tears of cervical commissures extending to the vaginal fornix in order to detect a possible commissural tear on the lateral aspect of the segment. If the uterus is not intact, a median sub umbilical laparotomy is performed.

Recent linear segmental cervical ruptures, without infiltration of the myometrial walls, with vaginal wall disinsertion and without broad ligament hematoma are managed by segmentoraphy plus suture of the intravaginal part of the cervix, followed by reinsertion of the detached portion of the vagina and abdominal drainage (plastic tube).

Uterine rupture accompanied by ileal pelvic hematoma increasing in size requires the identification of ureteral tract, external iliac artery and hypogastric artery with ligation of the latter to control the bleeding vessel responsible for causing hematoma. Identification of this vessel when pelvic anatomy is distorted by disease is very challenging, very bloody and very dangerous for ureter. For these reasons *bilateral hypogastric artery ligation* (BHAL) should be performed (3).

In extensive repairs, after protective hypogastric artery ligation, in case of an anfractuous rupture with wide myometrial serohematic infiltration or signs of infection, degenerative lesions (apoplectic), *placenta previa* (frequently accreta) or *placenta accreta*, total hemostatic hysterectomy is performed followed by adnexectomy on hematoma side only in case of massive hemorrhagic infiltration of adnexa. The next step is hematoma removal and hemostatic drainage of hematoma area and pelvis with externalization of the surgical dressing through vaginal section with visceral peritonization (4).

In case of rupture of the uterine cervix, vagina, vulva and pelvic perineal-floor a hemostatic suture is performed.

Blood clotting disorders

The risk of excessive postpartum bleeding is higher in mothers with preexisting clotting disorders (such as idiopathic thrombocytopenic purpura, Willebrand disease, prothrombin complex deficiency) or most commonly in those presenting a disease at risk of hemorrhagic events by disseminated intravascular coagulation and secondary fibrinolysis, such as: uteroplacental apoplexy, fetal death and retention of dead fetus in utero, chorioamnionitis, septic shock, placenta previa or accreta, amniotic embolism, preeclampsia, eclampsia or even the associated severe bleeding, hemorrhagic shock, etc.

Thus, if after delivery mother's blood hemostasis is within normal range, then prompt surgical hemostasis (as soon as possible after the occurrence of bleeding) is the essential therapeutic factor and must be associated with volemic restoration (expansion) with crystalloid solutions, macromolecular solutions, fresh blood (preferably collected within the last 6 hours), fresh frozen plasma (thawed), cryoprecipitate (containing fibrinogen, factor VIII, accelerators), platelet concentrates etc. Thus, hemorrhagic shock can be prevented or reversed (5).

Abundant bleeding with clotting disorders is approached in the same manner. The essential part of therapy is prompt surgical hemostasis ("save blood") concomitant with the replacement of the lost circulating blood with fresh blood, fresh frozen plasma (thawed) in a higher proportion to the volume lost; fresh blood and fresh frozen plasma (thawed) provide coagulation factors (fibrinogen, factors VIII, V, etc.), macromolecular solutions, cryoprecipitate (administered if fibrinogen level is less than 100 mg%), platelet concentrates (administered if platelet count is below 60,000/mm). These are the primary and basic therapy of the hemorrhage syndrome.

In addition to these, in excessive secondary fibrinolysis (strongly confirmed by very short ECLT-euglobulin clot lysis time), Merger et al. (6) recommend the administration antifibryonolitic drugs, such as Iniprol or Trasylol (50-100,000 U). Arias (7) suggested that "there is no place for the use of heparin in obstetric hemorrhages". Luca (2000) showed that in trans catheter embolization of hypogastric arteries, the procedure time of selective catheterization is 1-2 hours (to be well done) and this time is often fatal. EACA (*Epsilon-aminocaproic acid*) is contraindicated (1).

In severe cases when even on correct treatment bleeding persists, bilateral uterine artery ligation (on the lateral margins of uterus), the bilateral hypogastric artery ligation, total hysterectomy and the pelvic excavation packed tightly with gauze should be performed (8).

Statistics show that of hysterectomies, the subtotal one is obviously most frequently performed (especially for uterine atonia). However, total hysterectomy is performed in cases of uteroplacental apoplexy, placenta previa, placenta accreta, irregular uterine rupture propagated towards the cervix, signs of infection and wide areas of serohematic infiltration within the myometrium.

BHAL results in a decrease in blood pressure in the hypogastric artery branches. During an operation for cervical cancer and pregnancy, in which we performed a preventive hypogastric artery ligation, we found that there was blood flow through the sectioned uterine artery, but pressure was much lower.

Figure 1 shows the trajectory of parietal peritoneal incision (dotted) for the surgical approach of hypogastric artery (uterovesical trunk, peritoneal incision, hypogastric artery external iliac artery, ureter, suspensory ligament of ovary).

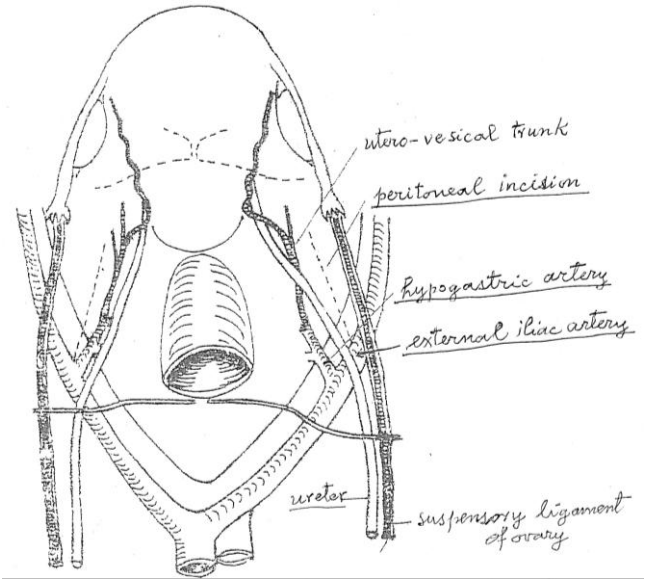

Fig. 1. Trajectory of parietal peritoneal incision (dotted) for the surgical approach of hypogastric artery

Figure 2 is illustrative for median sub umbilical laparotomy. The uterus is inverted over symphysis pubis and compresses it and the hands of the surgeon assistant simultaneously compress the lateral margins of the uterus (inferior side) thus blocking the flow in the ascending branches of the uterine arteries and reducing uterine bleeding.

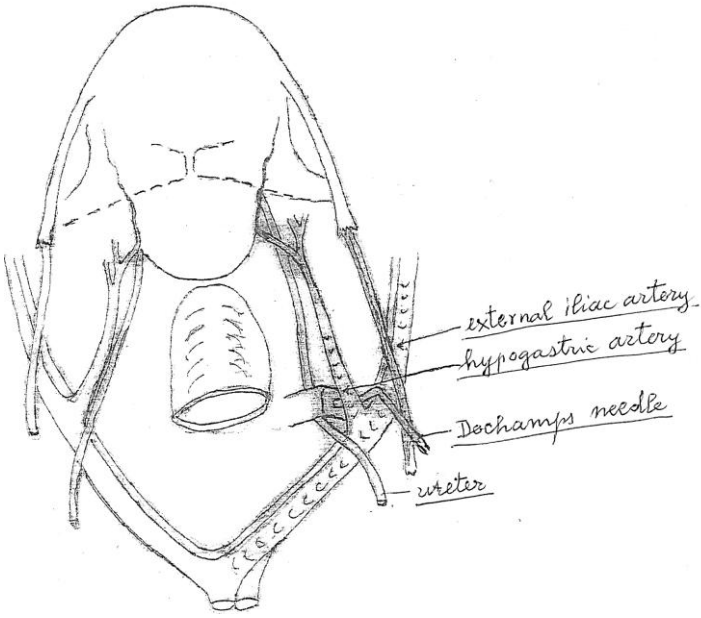

Fig. 2. Hypogastric artery ligation

This way, a provisional uterine hemostasis necessary for performing BHAL is achieved and the pelvis is released (to make BHAL possible). The surgeon will check for surgical hemostasis and clotting at normal blood pressure at the end of surgery.

It should be recalled that in case of a clotting disorder, hemostatic hysterectomy produces new and multiple dangerous hemorrhagic foci (6). That is why it is imperative the clotting disorder to be corrected before hysterectomy.

If hemostasis is uncertain and patient's condition allows it BHAL can be performed before closing the abdominal wall to ensure hemostasis in the postoperative period and avoid reoperation (when BHAL is the only option) (9).

By decreasing blood pressure in the arteries, a clot forming at the level of a vascular continuity solution or in small vessels, or even a platelet plug cannot be grabbed and removed.

CONCLUSIONS

Bilateral hypogastric artery ligation is sometimes effective in achieving hemostasis when performed earlier, reducing intraoperative bleeding and making hemostatic hysterectomy unnecessary. But when the mother is at immediate vital risk, hemostasis can be achieved only by hysterectomy, while by BHAL hemostasis it can be achieved only sometimes.

BHAL reduces bleeding during hemostatic hysterectomy. Thus, BHAL is performed earlier so that in case of failure to have time to perform a hysterectomy

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CLASIC VERSUS NOVEL IN REDUCTION OF ACUTE ANTERIOR DISLOCATION OF THE SHOULDER: A COMPARISON OF FOUR REDUCTION TECHNIQUES

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CLASIC VERSUS NOVEL IN REDUCTION OF ACUTE ANTERIOR DISLOCATION OF THE SHOULDER: A COMPARISON OF FOUR REDUCTION TECHNIQUES (Abstract) **Aim**: The purpose of our study is to compare four different techniques for reduction shoulder dislocation in term of efficacy, duration until reduction and pain felt by patient during the procedure. **Material and methods:** During 2015 year, we conducted a study on 50 chronological patients with anterior shoulder dislocation. After exclusion of four patients, the remaining 46 (74% males, mean age 44.6±20.03 years, range 18 to 89) were randomly assigned in four groups and reduced by Cunningham (C), Kocher (K), Mothes (M) and Hipocrat (H) techniques. All the patients were sedated using Midazolam (2 mg) and Fentanyl (1µg/kgbw) except patients who underwent Cunningham technique. **Results:** The success rate was 76.9% in C group, 98.4% in K group, 90.2% in M group and 87.96% in H group ($p>0.05$). The level of pain in C group was similar to the other groups in the absence of pain medication ($p<0.05$) **Conclusions:** Cunningham technique is a simple, single person technique, drug free, less painful and safer, comparable with other "classical" methods. **Keywords:** ANTERIOR SHOULDER DISLOCATION, CLOSED REDUCTION, SCAPULAR MANIPULATION.

Due to particularly high mobility, shoulder is the most dislocated joint, with a prevalence of 17 per 100,000 individuals; approximately 95% of these dislocations are anterior (1). Various reduction techniques have been described with different rates of success and complications. Most of them are variants of what we consider "classic techniques" described by Kocher (2, 3), Mothes (4), Hipocrat (5, 6, 7) which include traction-counter traction and leverage maneuvers, some are considered "novel techniques" such scapular manipulation (8) and Cunningham (9) based principles of static and dynamic obstruction on the humeral head. The ideal technique should be quick, effective, painless and not cause further injury (10).

Although not all the dislocated shoulder need pain medication, for ethical and legal reasons, the reduction technique should be performed under local or general anesthesia, as soon as possible (11, 12). In the Emergency Department, intravenous administration of opioids and benzodiazepine is commonly used. Although it ensure painless reduction, these drugs have important side effects such as respiratory distress,

hypotension, bronchospasm, which need close respiratory and cardiac monitoring of patients.

There are some clinical trials regarding reduction of scapular dislocation especially in the United States but none of our knowledge about compared efficacy of these four reduction techniques.

The aim of our study is to compare the efficacy of four reduction techniques for anterior shoulder dislocation: Cunningham, Kocher, Mothes and Hipocrat, in terms of time until reduction, post reduction pain and complications.

MATERIAL AND METHODS

We conducted a study in the Emergency Department of "Saint Spyridon" Clinical Emergency Hospital during 2015, according to the guidelines of "Grigore T. Popa" University Committee for Research and Ethical Issues, on 50 consecutive patients with anterior shoulder dislocation. Two polytrauma patients and two Neer III fracture–dislocation patients were excluded.

All the patients were examined before and after the reduction for vascular and neurological lesions. After clinical and radiological diagnosis (anteroposterior view), patients were randomized by drawing envelopes to undergo reduction of the dislocation with one of the four methods used in the study by a single team of orthopedic surgeons.

Inclusion criteria: anterior shoulder dislocation with or without fracture of the great tuberosity, same reduction team, age above 18 and below 90 years.

Exclusion criteria: polytrauma patients, three or four part fracture dislocation, admitting more than 24 hours after the trauma, hypersensitivity to Midazolam or Fentanyl, severe cardiovascular or pulmonary disease, intake of food or fluid during last 4 hours; presence of airway difficulty; sensory problems; motor problems, vascular problems, and consumption of alcohol or other recreational drugs.

The patients undergoing Kocher (K), Mothes (M) and Hipocrat (H) techniques were sedated using Midazolam (2 mg) and Fentanyl (1μg/kgc). For Cunningham (C) technique, all the patients were informed about the procedure and express their consent for reduction. All subjects remained verbally responsive during the procedure.

All methods of reduction were performed as described by their authors. The unsuccessful cases were treated by Kocher reduction. In case of failure, patients were treated close reduction in operating room under general anesthesia.

All patients were cardiac and oxygen saturation monitored during the procedure. After reduction all the patients were immobilized in Velpeau bandage.

The quality of reduction was radiological evaluated (anteroposterior view). The patients were assessed by term of pain during reduction by visual analog scale (VAS) after they became fully oriented.

The patients were discharged after they had fully recovered orientation of time and space with vital signs within the normal range.

Statistical analyses were performed on *SPSS 20.0* version (SPSS Inc., Chicago, Illinois, USA). Patient characteristics were expressed as the mean standard deviation or as a number and percentage. Variables were compared by paired t test. A p value of less than 0.05 was considered statistically significant.

RESULTS

There were no statistically significant

differences between the four groups in terms of age, sex, primary-to-recurrent dislocations ratio, the mechanism of dislocation, the presence or absence of a coexisting fracture of the greater tuberosity, the mean time interval between the injury and the first attempt at reduction, and the reduction time (tab. I)

TABLE I
Clinical characteristic of patients in the four groups

Groups		K (n=13)	M (n=8)	H (n=12)	C (n=13)
Age (years) (Mean±SD)		35.8 ±18.7	44.6±20	47.9±40.6	44.2±19.8
Sex (%)	M	92.3	75	58.4	69,3
	F	7.7	25	41.6	30,7
Origin	Urban	46	37.5	25	38,4
	Rural	54	62.5	75	61,6
Mechanism	Sport activity	85	75	25	38
	Fall	15	25	75	62
Type	Primary	92.3	87.5	75	84,7
	Recurrent	7.7	12.5	25	15,3
Associated fracture		1	0	3	0

There was no vascular or neurological deficits pre or post reduction. Four patients (8,69%) had a great tuberosity fracture which was reduced orthopedic and did not required surgical reduction and fixation. One patient necessitates reduction under general anesthesia in operating room.

The duration of reduction was significantly different between the four groups (tab. II).

TABLE II
Efficacy, time needed for reduction and level of pain felt during the reduction

Groups	Reduction success rate		Time until reduction	Level of pain
	n	%		
K	12	98.4	116.3±41.7	3.38±1.04
M	7	87.5	181.8±57.9	3.25±1.03
H	11	91.66	69.91±15.8	2.33±2.21
C	11	76.92	287.9±126.4	3.07±1.11
p value			0.68	<0.01

DISCUSSION

To our knowledge this is the first study that compares these four techniques. Although it is traditionally belief that the reduction of shoulder dislocation necessitates traction and counter traction in term of force to overcome muscle contracture, there are techniques that do not imply force with

similar success rates (9, 13, 14, 15, 17, 19).

The lower success rate in our study with Cunningham technique and longer time until reduction compared with other technique, it`s probably motivated by a longer learning curve.

While the "classical" techniques of shoulder reduction require pain medication for the reduction to be achieved in terms of patient safety, we obtained significant comparative pain levels without sedation. Cunningham technique has the advantage of being less painful without the health risks (9).

In our study we did not evaluate time spent in the emergency department but it`s evidently shorter in Cunningham group.

Our study has some limitations. We included a small number of patients and the study was unblinded. We must consider that Midazolam has the side effect of anterograde amnesia and it becomes difficult to assess lever of pain on these patients.

Compared to others "classical" techniques, Cunningham have lower rates of mechanical complications (9). Although Kocher technique had a 98, 4% success rate in our study it`s known as unphysiological, brutal, and dangerous because of its association with fractures of the surgical neck of the humerus and neurovascular complications (11, 17, 18).

CONCLUSIONS

Although larger patient studies are necessary for confirmation, Cunningham technique should be considered because it's single person, drug free, less painful and safer, comparable with other "classical" methods.

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NEWS

IS THERE ANY SUCH THING AS "NO EVIDENCE OF DISEASE ACTIVITY IN MULTIPLE SCLEROSIS"?

No evidence of disease activity (NEDA) in Multiple Sclerosis (MS) is a concept that has emerged throughout the last years as a result to new and effective therapies. The concept includes parameters such as freedom of relapse, stability on the Expanded Disability Status Scale (EDSS) – no sign of clinically worsening, as well as a stop of dissemination in time and space of demyelinating lesions on the MRI. Nevertheless, this is not enough to appreciate NEDA, as bedside relapse rate, disability progression and neuropsychological aspects such as cognitive status, depression, fatigue and quality of life are parameters also to be considered in the attempt of a better definition of the phenomenon. For all that, a multiple sclerosis decision model (MSDM) that would include all of these items to be evaluated, promises to support early treatment decisions. However, prospective studies are still required (Stangel M, Penner IK, Kallmann BA et al, Towards the implementation of 'no evidence of disease activity' in multiple sclerosis treatment: the multiple sclerosis decision model, *The Adv Neurol Disord.* 2015 Jan; 8(1): 3–13).

Dan Trofin

COMPLEX MANAGEMENT OF FRAILTY FRACTURES – CASE REPORT

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COMPLEX MANAGEMENT OF FRAILTY FRACTURES – CASE REPORT (Abstract): Frailty fractures encompass pelvic ring fractures in the elderly - a condition more frequently encountered in women, usually with pre-existing osteoporosis. The incidence of these fractures is increasing, following the increase in average life expectancy. Most of the times, they are the result of a minor injury, of which the patient has little or no recollection; the clinical signs and symptoms, radiological changes and the degree of fracture stability vary greatly. As such, there is a wide range of therapeutic options and one of the most important decisions for the physician is choosing between surgery and conservative treatment. We present the case of an elderly female patient with multiple pelvic ring fractures, and a medical history of cardiovascular disease and osteoporosis; the orthopedic examination recommended conservative treatment which was carried out in the Geriatric Clinic of the "Dr. C.I. Parhon" Hospital. Following the complete physical examination and through geriatric evaluation, a complex therapeutic plan was devised, which included: recovery through physiotherapy, adequate nutrition, psychological support, treatment of the cardiovascular comorbidities and prevention of the consequences of extended bed rest. The treatment lasted for 60 days and the outcome was favorable: decreased bone frailty, mainly through preserving muscle tone and osteoarticular function, correction of malnutrition, improvement of depression and partial recovery of patient's mobility; the results of our therapy made it possible for the patient to return home, where she leaves alone. She will need help with daily household chores, but she intends to continue the recovery therapy as means of increasing her quality of life and independence. **Keywords:** FRAILTY FRACTURES, SENIOR PATIENTS, RECOVERY THERAPY.

As the elderly segment of the population increases, so does the incidence of geriatric syndromes. Consequently, their impact on the medical services is more and more taxing, especially in the case of osteoarticular conditions that are associated with an increased risk of falling. Most frequently, patients with osteoporosis experience either hip or pelvic ring fractures (1). Pelvic ring fractures in the elderly are considered among frailty fractures (2) and are usually encountered in women over the age of 80. The gender-specific incidence has a different pattern according to age - from 0.54/0.38 ‰ for patients aged 65-69 to 9.35/4.45 ‰ for ages over 90; also, inci-

dence is higher for institutionalized or moderately disabled persons (3). Most often, pelvic ring fractures in the elderly are the consequence of a minor injury - either same-level fall at home or during the bed to chair transfer. Frequently, these fractures occur on an osteoporotic bone. Both pre-existing comorbidities and the clinical consequences of the fracture (significant pain, total immobilization, and dependency) darken the prognosis and require a thorough assessment prior to a therapeutic decision (3). There is no consensus regarding the orthopedic approach: surgery versus conservative treatment, both being associated with considerable risks (4, 5).

We present the case of an elderly female patient with multiple pelvic ring fractures, for whom a conservative treatment was recommended. She was subsequently transferred to the Geriatric Clinic of the "Dr. C.I. Parhon" Hospital for a concomitant cardiovascular complication. The comprehensive geriatric assessment and the therapeutic plan, which were managed by a multidisciplinary team, led to the satisfactory recovery of the patient and allowed her to return to her own home.

CASE REPORT

We present the case of an 80 year-old female patient, living in an urban house, who was admitted to the Orthopedics Department with multiple pelvic ring fractures (fracture of the anterior left acetabular wall without displacement, left ischiopubic fracture, sacral wing fracture) following a minor fall injury at home (fig. 1). She had a recent medical history of arterial hypertension (for which she was prescribed treatment with ACE inhibitors) and osteoporosis. During hospitalization, a routine cardiology consultation revealed an episode of tachyarrhythmia (high frequency atrial fibrillation).

Fig. 1. The X-ray of the patient showing multiple pelvic ring fractures (fracture of the anterior left acetabular wall without displacement, left ischiopubic fracture, sacral wing fracture).

Due to the recently-diagnosed cardiovascular comorbidities and because the fractures were stable (no associated displacement), the orthopedic surgeon opted for conservative treatment, despite the fact that the fractures had occurred on a pathologic bone. The patient was transferred to the Geriatric Clinic for a thorough assessment prior to her discharge at home.

On admission, the patient had significant pain in the lumbosacral and pelvic areas, with partial bed immobilization (she could turn from one side to the other), mildly elevated blood pressure, psychomotor agitation, anxiety, and depression. Additional investigations did not identify other medical conditions except for the previously diagnosed arterial hypertension. Comprehensive geriatric assessment revealed: malnutrition (21/30 MNA score), complete dependency (assessed by ADL and IADL scales), moderate depression (assessed by the GDS scale), clinical frailty (assessed by the Freid scale) and the absence of cognitive disorders

(assessed by the MMSE scale). Both a physiotherapist and psychologist consulted the patient and participated in developing the management plan.

The therapeutic approach of the multidisciplinary team focused on the following points: ensuring careful monitoring of the cardiovascular status, providing an appropriate diet for malnutrition, osteoporosis and heart disease, securing an immediate, suitable and continuous functional recovery, offering psychological counseling and qualified care to prevent complications of prolonged bed rest. Special attention was given to functional recovery, due to the complexity of the case. The treatment plan had to take into account the fact that fractures occurred on osteoporotic bones (6), that the orthopedic treatment consisted in relative bed rest (no cast immobilization and no stepping on the affected leg for 60 days), and that the patient wanted to return as soon as possible to her home, where she lived alone.

One of the most important roles was played by the physiotherapist, who had to attempt meeting the following aims:

a. decrease the pain that occurred mainly during the recovery sessions, but also while performing the smallest movements in the bed;

b. recovery, restoration and consolidation of the affected motor functions, correct and full recovery of force and joint mobility, muscle strength recovery in both upper and lower limbs (7);

c. restoration of stability, controlled movement and ability for body support when the patient began to walk using the frame, crutches and, at the end, the cane, with gradual and correct resumption of independent walking;

The procedures used to accomplish the set goals were:

- electro acupuncture at acupoints in the painful area for instant pain relief, resulting in greater amplitude of the movements during passive, active-passive and active physiotherapy;

- sedative and relaxing therapeutic massage, which resulted in a significant decrease of pain. This procedure also had a positive mental effect on the patient, who's compliance during treatment increased; active involvement of the patient augmented the beneficial effects of physiotherapy, with excellent results despite osteoporosis and old age (which, usually, is a hindrance in the recovery dynamic);

- massage and reflexotherapy, which determined a growth in basal metabolic rate, stimulating respiratory and circulatory apparatus functions, improving sleep quality and the person's general state;

- breathing exercises and bilateral posterior chest massages in order to avoid bedsores and, more importantly, to prevent decubitus pneumonia.

Recovery treatment was gradually more complex. As the fracture consolidated, the pelvic member on the fracture's side was loaded progressively and the use of the thoracic limb was encouraged (8). The patient's relative bed rest lasted for 3 weeks as per orthopedic recommendations for stable fractures. At the beginning of the third week, the patient sat up at the side of the bed, and, by the fourth week, she managed standing up and performing short movements (without pressuring the affected limb), with the help of an attendant and the walking frame, which enables good control of body weight distribution and center of gravity adjustment. At the same time, improvement of the nutritional status, decreased anxiety and depression, and

efficient control of blood pressure values were achieved. 60 days after the injury, the patient was discharged with a relative dependency status (walking by cane) and need of in-home help.

DISCUSSIONS

Given the improved life expectancy, the rising incidence of osteoporosis and frailty inevitably led to an increase in pelvic ring fracture rates, which are considered frailty fractures. They typically involve elderly females (over 80 years old), are diagnosed after a minor injury (sometimes the patient does not remember when the trauma occurred) and have disastrous consequences for the patient and their caregivers: prolonged bed rest with severe deterioration of the muscles, joints and bones associated with increased dependency level (in some cases, institutionalization is needed due to the inability to return home). This represents an important health care burden both from a social and an economic point of view (9).

Conservative treatment, which is preferred in the stable fractures of patients with clinical frailty, cardiovascular comorbidities, accelerated osteoporosis and malnutrition is associated with a long period of immobilization and bed rest, and, inevitably, all its complications. The complex treatment (physiotherapy, psychotherapy, proper nutrition and continuous medical monitoring), performed in a specialized medical unit, is the main step towards therapeutic success, allowing complex patient recovery and restoring quality of life.

Because the Romanian health care system lacks specialized centers for complex recovery of the elderly, this program was implemented at the Geriatric Clinic, where constant supervision and medical care, nutritional counseling, psychological assistance and a thorough physiotherapy schedule, together with patient's compliance and her wish to return home, led to the recovery of walking ability, with a significant decrease of the dependency level, and allowed for a quality of life status similar to that prior to the fracture.

CONCLUSION

Pelvic ring fractures in the elderly are considered among frailty fractures because they are usually encountered in women over the age of 80. There is no consensus regarding the orthopedic approach: surgery versus conservative treatment, both being associated with considerable risks. The particularities of old and very old age (frailty and osteoporosis, the tendency to malnutrition, depression and cognitive impairment, the concomitant chronic diseases) need a comprehensive geriatric assessment performed by a multidisciplinary team who is well trained to devise a complex recovery plan, performed in a specialized clinic and which would allow the patient to return, as much as possible, to the way of life that existed prior to fracture.

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NEWS

GINGIVAL, PLASMA AND SALIVARY LEVELS OF MELATONIN IN PERIODONTALLY HEALTHY INDIVIDUALS AND CHRONIC PERIODONTITIS PATIENTS: A PILOT STUDY

Melatonin (N-Acetyl-5-hydroxytryptamine) is an indole amine hormone synthesized predominantly by the pineal gland. Extrapineal synthesis and release of melatonin can occur from organs like ovaries, retina, gastrointestinal tract and also from cells such as human lymphocytes. Melatonin regulates circadian rhythm and the biological clock in the human body. In addition, it performs numerous other functions like immunomodulation and osteopromotion. Melatonin has potent antioxidant action against reactive oxygen species and can mitigate deleterious effects of free radical damage in the human body. It is well known that chronic periodontitis is an inflammatory condition of the tooth supporting structures with a microbial aetiology, in which dysregulated immune response and a jeopardized oxidant - anti oxidant balance play a major role in causing tissue destruction and tooth loss. Previous studies have shown increased oxidative stress markers and lowered antioxidant status in periodontal disease compared to health. In this context, the presence of melatonin as an antioxidant in saliva and plasma and its association with the pathogenesis of chronic periodontitis has been researched. It has been hypothesized that salivary melatonin can act as a potent local antioxidant boosting the defence mechanism against periodontal pathology. Till date no study has investigated, melatonin levels in the gingival tissue. This study demonstrates the presence of melatonin in gingival tissue. Furthermore, melatonin levels are lowered in gingival tissues of chronic periodontitis patients (Balaji TM, Vasanthi HV, Rao SR, et al. Gingival, Plasma and Salivary Levels of Melatonin in Periodontally Healthy Individuals and Chronic Periodontitis Patients: A Pilot Study. Journal of Clinical and Diagnostic Research. 2015, Vol-9(3): 23-25).

Doina Butcovan

PREDISPOSANT FACTORS FOR INTIMAL HYPERPLASIA AND THEIR MECHANISMS OF ACTION IN OPEN VASCULAR SURGERY. A REVIEW

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PREDISPOSANT FACTORS FOR INTIMAL HYPERPLASIA AND THEIR MECHANISMS OF ACTION IN OPEN VASCULAR SURGERY. A REVIEW (Abstract): **Background:** Intimal hyperplasia is a multifactorial process that determines local stenosis and subsequent ischemia after revascularisation procedures. It is controlled by a plethora of cytokines, growth factors and proteases that interact through multiple mechanisms, empowering or inhibiting their hyperplasic action. Although the elements involved are the same, the resulted degree of vascular stenosis varies. **Methods:** To highlight the predisposing conditions which enhance the vascular response to injury we consulted articles from Pubmed database, searching for the keywords "intimal hyperplasia", "neointimal hyperplasia" and "risk factor". **Results:** 101 articles were included in the study. Risk factors analyzed are classified into local and systemic and the mechanisms by which they augment the process are explained. **Conclusion:** Knowledge of risk factors and their control when possible is a key element in increasing revascularization patency. **Keywords:** VASCULAR INJURY; INTIMAL HYPERPLASIA; RISK FACTORS.

Intimal hyperplasia, a process triggered by vascular injury, is the proliferation of vascular smooth muscle cells in the media, followed by their migration to the intima, the transformation from contractile in secretory phenotype and secretion of extracellular matrix constituents, end result being a narrowing of the vascular lumen and subsequent ischemia in the irrigated territory (1). The process is controlled by a plethora of cytokines, growth factors and proteases that interact through multiple mechanisms, empowering or inhibiting their hyperplasic action (1). Although the elements involved are the same, the resulted degree of vascular stenosis varies. This raises the question whether there are predisposing conditions which is thought to enhance the vascular response to injury and can be controlled in order to increase revascularization patency.

METHOD

Consulting articles from the Pubmed database, we conducted a search for the combination of keywords "intimal hyperplasia" AND "risk factor", “neointimal hyperplasia” AND "risk factor", until september 2014. The search yielded 430 articles. The two authors individually screened the abstracts and 101 were found relevant for this study. From this articles, the fol-

lowing variables were colected: paper first author, year of publication, risk factor described, and method of determination (animal experiments, clinical trials).

RESULTS

Although there is a great heterogeneity of the determined risk factors, for a better understanding we decided to classify into local and systemic factors. For every risk factor both experimental and clinical trials results are reviewed and a short description of the mecanism involved is provided.

LOCAL FACTORS

Local hemodynamic factors have a major influence on vascular remodeling process. A basic conditions for an increased patency in arterial revascularization is the existence of a quality runoff. Christoph Hehrlein conducted an experiment on dogs, ligating the femoral artery proximal to its trifurcation and thus altering runoff and then producing laser thermal injury and balloon endothelial denudation. He found an average increase of 32% of the area of intimal hyperplasia in animals with altered runoff compared to control, $p < 0.01$ (2), independently of the severity of the lesion. The trend of development of neointimal tissue in conditions of poor runoff was explained by Dobrin. The author has quantified the effect of each force acting on the vascular wall in subsequent histological changes and found that intimal hyperplasia occurs under low shear stress conditions (3). Shear stress is directly proportional to the speed of blood flow, which in turn is directly influenced by the runoff. Bassiouny showed that at the level of an end-to-side anastomosis there are two areas of intimal hyperplasia: at the suture line and at the receiving vessel floor. Realizing an artificial model which simulated the conditions of geometry and pulsatility of an end-to-side anastomosis, he showed that at the floor level there is an area of stagnant flow compared to the cuff, where the flow rate is high and it correlates with the lack of cuff hyperplasia experimentally demonstrated (4). Another way in which an altered runoff promote intimal hyperplasia is due to a low local concentration of vasodilator endothelium released factors (NO, prostacyclin). As Rubanyi proved, the release of this factors is directly proportional to the flow rate (5). These substances have a powerful antimitogenic effect. Under conditions of poor runoff, their low concentration allows the unduly development of neointimal tissue. In this regard, Morinaga showed that intimal hyperplasia areas shrinks if the vessel is reimplanted into a normal flow parameters system (6). All of this experimental studies were confirmed by clinical trials. In a series of 191 femoropopliteal and femorodistal reconstruction, it was determined that the rate of patency at 6 months dropped from 88.2% in patients with good runoff to 21.8% in those with altered runoff ($p<0.01$) (7). Scali shown that a low distal graft end-diastolic velocity determined by duplex ultrasound at the end of the procedure predicted poor outcome in terms of patency (8).

Characteristics of autologous grafts.

Vein grafts used for myocardial and peripheral revascularization often show areas with altered histological structure, either **fibrous or varicose**. Histological studies showed in these areas a severe medial disruption, with smooth muscle cells transformation from contractile to secretory phenotype, an increase in the number of vasa vasorum and altered extracellular matrix metabolism (9). Transplantation of

venous grafts in the arterial circulation submit them to a tensile and shear stress much higher than those normally acting on the vein wall. Normal physiological response to the augmentation of these forces is the process of "arterialisation", characterized by panmural hypertrophy, both cellular and matrix (9). This process is triggered by endothelial denudation caused by shear stress and muscle cell proliferation and subsequent apoptosis caused by tensile stress (10). Both in stenotic and varicose dilated areas laminar flow is affected, there are major turbulence and tangential deformation which causes major endothelial and medial damage (11). These lesions, appeared on a vascular wall with altered histological structure, have the effect of an exacerbated intimal and medial hyperplasia. It was shown experimentally that external stenting of venous grafts resulted in uniform flow, attenuation of tangential distension and limited intimal proliferative response (12). This procedure was adopted in clinical practice. A randomized trial of external stenting for saphenous vein grafts in coronary artery bypass grafting found that mean intimal hyperplasia area was significantly reduced in the stented group versus nonstented group and determined marginally significant improvement in lumen uniformity and less ectasia in the stented group (13).

Vascular trauma occurred during the process of harvesting the graft is another factor contributing to the development of neointima. It has two components. First, the dilatation has the effect of physical trauma - endothelial denudation. In these areas there is adherence of platelets and leukocytes that release cytokines and growth factors (14). Bare areas are endotelized in the first 14 days by proliferation and migration of adjacent endothelial cells (15), but the result is a dysfunctional neoendotelium, with a low capacity in production and secretion of nitric oxide and prostacyclin which are antihyperplasic factors (16). The second component is the ischemic trauma, related to the time elapsed until reimplantation. It should be noted that oxygen used by endothelial cells and the internal media comes through diffusion from the bloodstream, while external media and adventitia are oxygenated by vasa vasorum network. Initially, after harvest, the graft undergo a panmural ischemia, being affected all three tunics. Once inserted into circulation, endothelium and internal media resume their aerobic metabolism, whereas ischemia persists in external media and adventitia until the completion of vasa vasorum neovascularization. Ischemic injury promote intimal hyperplasia in two ways. Superoxide radicals synthesized in anaerobic metabolism (17) has a potent mitogenic effect (18). Second, it has been demonstrated a trend of migration of smooth muscle cells from the external media to intima, due to higher oxygen concentration at this level, regardless of the presence of endothelial injury when vasa vasorum are damaged (19).

SYSTEMIC FACTORS

Metabolic alterations.

As in the case of atherosclerosis, **diabetes** plays an important role in neointimal proliferation. The augmented rate of coronary stenosis after angioplasty and stenting in diabetic patients compared to nondiabetics, having as substrate intimal hyperplasia, has been documented in several studies (20,21). Transient hyperinsulinaemia appears to be the main cause and occur both in patients treated with oral anti-diabetic drugs

who have insulin resistance as well in patients receiving intermittent subcutaneous insulin (22). Insulin stimulates intimal hyperplasia by accelerating the production of growth factors and by direct mitogenic effect (23). The second cause is represented by the advanced glycation end products (AGEs), which accumulate in vascular tissue with age (24). The vascular smooth muscle cells have specific receptors (RAGE) for them and the AGE - RAGE interaction results in stimulation of inflammation, smooth muscle cell proliferation and increased secretion of extracellular matrix components (25). In a murine model, insulin-resistant Zucker fatty rats who recived long acting insuline theraphy for 4 weeks, carotid intima/media ratio was significantly reduced after balloon catheter injury compared to control (26). A study performed on 14,788 patients who underwent infrainguinal lower extremity arterial bypasses found that diabetes mellitus had a negative association with early graft failure (95% confidence interval, 0.58-0.89, p=0.002), determined by intimal hyperplasia and not by progression of atherosclerosis (27).

Additive effect of **hypercholesterolemia** in the development of postlesional intimal hyperplasia is known since 20 years ago and is based on exacerbating local inflammatory process (28). Under conditions of hypercholesterolemia, monocyte chemoattractant protein (MCP-1), also known as chemokine ligand 2 (CCL2) is expressed on vascular smooth muscle cells in large numbers (29) and its receptor (CCR2) on the surface of monocytes. CCL2-CCR2 interaction is the main way by which monocytes are recruited in increased number at the site of an endothelial injury (30), maintaining the inflammatory process and stimulating the development of neointimal tissue. The inhibition of this interaction by administration of CCL2 monoclonal antibodies has resulted in a marked reduction in neointimal area (31). In animal experiments it was found that under hypercholesterolemia, there is a growing mRNA expression of type I collagen, increased secretion of this type of collagen in the extracellular matrix, together with a significant reduction of matrix metalloprotease activity (32). A third negative effect induced by hypercholesterolemia, closely linked to oxidative stress, is the slowdown in postlesional reendotelisation process (33). In a study on patients with carotid restenosis after endarterectomy, it was found that levels of plasma cholesterol, total triglycerides and low density lipoprotein apoprotein B were higher than control (34).

Homocysteine. One of the first studies that have shown a link between increased blood levels of homocysteine and postendarterectomy progression of carotid intimal hyperplasia was carried out on a animal model by Frederick Southern in 1998 (35). Since then, many of the mechanisms by which hyperhomocysteinemia causes vascular injury and promotes neointimal formation were elucidated and are represented by chronic inflammation and subsequent endothelial damage, modulation of gene expression by altering methylation, inducing a procoagulant status, induction of apoptosis and extracellular matrix remodeling (36). The posibility of hyperhomocysteinaemia beeing a risk factor for vein grafts stenosis was examinated in a case-control study, that revealed a direct connection between elevated plasma levels of homocysteine and low patency rates (37).

Coagulation disorders.

Malfunctions of elements involved in the coagulation process can result in aug-

mented vascular hyperplasia. Platelet adhesion to vascular lesion and parietal thrombus formation are the triggers for the vascular healing process. It is known the role of platelets in the process, mainly through PDGF release, the most powerful mitogen for cells of mesenchymal origin and a potent chemoattractant for smooth muscle cells, macrophages and neutrophils (38). An exacerbated response to injury when the platelet count is pathological increase was documented in chronic myeloproliferative disorders, especially polycythemia vera and essential thrombocythemia and is manifested both in the medium vessels - the coronary and pedal arteries (39) and the smaller vessels - arteries dermis, causing generalized racemose livedo (40).

Factor Xa forms a complex with factor Va and cleaves prothrombin to active thrombin, which converts fibrinogen to fibrin (41). Thrombin also stimulates platelet aggregation and degranulation by cleaving protease activated receptors on the platelet membrane. It is a powerful smooth muscle cells mitogen and promotes their migration in the intima (42). Increased thrombin generation conditions, like neoplasic diseases, prothrombin thrombophilia or postmenopausal hormone replacement therapy were associated with an accelerated intimal hyperplastic response (43,44). Procoagulant activity of thrombin is specifically inhibited by antithrombin III in plasma and heparin cofactor II (HCII) in the subendothelial layer of the vascular wall, in the presence of dermatan sulfate. Because heparin cofactor II / dermatan sulfate complex effectively inactivates surface-bound thrombin, it inhibit proliferation of vascular smooth muscle cells and fibroblasts. A genetic deficiency of this factor is highly associated with an exacerbated response to injury (45). Takamori demonstrated on a group of 134 patients that a high HCII plasma level is a protective factor against in-stent restenosis (46).

Hyperplasia is also promoted when there is an increase in fibrinolysis. Plasmin is formed from plasminogen under the action of a series of activators (PA), most important being urokinase type – PA (u-PA) in the serum and tissue type – PA (t-PA) bound to fibrin (47). The role of plasmin is to remove the cloth by degrading fibrin, but it promotes hyperplasia by mediating the migration of smooth muscle and inflammatory cells within the blood vessel wall, increasing collagen deposition and cholesterol accumulation (48). Plasminogen activator inhibitor type 1 (PAI-1), a protein synthesized by vascular endothelial and smooth muscle cells, is the primary inhibitor of t-PA and urokinase (49). Although increased levels of this inhibitor correlates with myocardial infarction (50), decreased plasma levels of PAI-1 is documented to be a risk factor for stent restenosis (51) and experimental animal models have shown to reduce the area of hyperplasia when the production of this protein is stimulated (52). These apparently paradoxical results have a common denominator, namely reduced production of plasmin. A low level of plasmin prevent fibrinolysis, thus promoting clot progression, but also this low level involves a decreased prohiperplastic effect.

Cigarette smoking

Known as the main risk factor in the progression of atherosclerosis, smoking plays an important role in initiating and augmenting intimal hyperplasia. Exposure to cigarette smoke increases the number of platelets and their ability to adhere to damaged vessel wall and inhibit their mito-

chondrial activity (53). Smoking also cause neutrophilia and the activation of neutrophils by the complement system. Although neutrophils do not secrete growth factors, they contribute to tissue injury through the release of oxygen radicals and proteases (54). Chronic cigarette smoking increases monocyte adherence to cultured human umbilical vein endothelial cells in vitro, promoting inflammation (55). Endothelial cells are also affected, smoking disrupting normal regulatory properties, increasing cell wall permeability and the likelihood of detachment with injury and decreasing endothelial cell DNA synthesis . The smooth muscle cells are direct affected by cigarettes via polycyclic aromatic hydrocarbons found in the smoke, which are increasing their proliferation rate and viability (56). Experiments performed on murine models had shown that smoking accelerates intimal hyperplasia in a dose-dependent manner (57).

Endothelial progenitor cells

The vascular endothelium is a dynamic organ, formed by a continuous layer of cells that cover the luminal surface of blood vessels and react to physical forces and chemical signals, affecting vasomotion, thrombosis, platelet aggregation and inflammation. Endothelial progenitor cells (EPCs) isolated from peripheral blood are originating from the bone marrow. Their role is to migrate, differentiate into endothelial cells and cover intimal defects (58). It has been demonstrated a direct correlation between the decreased number of EPCs in peripheral blood and progression of coronary artery disease (59). Likewise, in laboratory experiments, autologous EPCs transplantation enhanced the vasculoprotective properties of the reconstituted endothelium resulting in inhibition of neointimal formation (60). Another study showed that anti - vascular endothelial cadherin antibody-coated stents captured circulating EPCs, thus resulting an accelerated endothelial recovery on stent and reduced intimal hyperplasia (61). The number of circulating EPCs falls in patients with hypercholesterolemia, vitamin D deficit, low plasma estrogen level and is stimulated by exercise training, administration of erythropoietin and statins.

Discussion

Although the progression of atherosclerosis in some cases is causing stenosis after open surgical revascularization, most frequently the low patency of these procedures is due to a compounded vascular healing process after injury. Therefore the vascular surgeon must know the intimate mechanisms by which the process unfolds and especially the causes that exacerbate it. The most important contribution that the doctor can have in limiting the negative effects caused by hyperplasia is represented by run-off selection, when this option exists. Whether it is coronary or peripheral revascularization, a poor run-off have catastrophic effects on intervention patency. For infragenicular bypasses, American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines recommend that the tibial or pedal artery that is capable of providing continuous and uncompromised outflow to the foot should be used as the site of distal anastomosis (62). This recommendation points out the importance of the outflow artery status throughout its length on limb salvage, patency and pacient survival. There are also numerous angiographic scores that quantify the quality of runoff, the most used being Rutherford revised score, a method validated by clinical stud-

ies to predict the evolution of revascularization in patients with critical limb ischemia (63).

Vein graft harvesting, preparation and ischemia time also influence the degree of progression of intimal hyperplasia. Increasingly more surgeons are using the „no touch" technique in venous graft preparation. This procedure involves that the vein is neither stripped nor distended, but harvested with a fat pedicle. The PATENT study compared markers of vascular injury and smooth muscle cells activation in veins harvested using this technique vs the conventional one and found that „no touch" preserved intimal, medial and adventitial architecture, with similar leg recovery in both groups. (64). Distention during preparation is an important element in future graft evolution. A distention pressure lower than 140 mmHg prevents endothelial denudation, reduction of endothelial-dependent relaxation and intimal thickening and limiting intraluminal pressure can be obtained by using a pressure release valve (65). Preparing the arteries before vein harvesting and making the anastomoses simultaneously by two teams of surgeons decreases graft hipoxia, simple gestures with a huge impact on long term patency. Many vascular surgeons refuse to use varicose veins in arterial reconstructions, preferring synthetic materials, but new external scaffolding technologies are emerging with encouraging results, especially in coronary surgery. External stenting stabilizes flow parameters, particularly shear stress, downregulating the molecular processes involved in neointimal formation (66).

Although local factors outweigh the process, should not be forgotten the importance of systemic factors, which are most often controllable. The antidiabetic and lipid-lowering medication should be carefully determined and adjusted by the diabetologist prior to surgery and a close collaboration between the vascular surgeon and the haematologist is necessary in patients with myeloproliferative disease requiring revascularization procedures. New evidences emerge in favor of short-term dietary restriction immediately before surgery to reduce intimal hyperplasia and to protect from ischemia-reperfusion injury (67). Also, animal studies and in vitro experiments suggests that a short anti-inflammatory treatement with dexamethasone decreases vein graft thickening and levels of proinflammatory cytokines associated with hypercholesterolemia, but there is a lack of bed-side evidence (68). The beneficial effect of smoking cessation on reducing neointimal formation has been proven by numerous studies, so the patient should be directed to specialized antismoking centers prior to surgery. One study showed that stopping smoking rapidly increases circulating levels of endothelial progenitor cells and the magnitude of increase was greater in light smokers than in heavy smokers (69). Another study determined that heavy smoking before surgery significantly increase matrix metalloproteinase subtypes MMP-2 and MMP-9 gene expression, in the same time decreasing the gene expression of tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 (70).

All these therapeutic measures conducted at the right time can significantly increase the patency of revascularization procedures.

CONCLUSIONS

Intimal hyperplasia is a multifactorial process that determines local stenosis and subsequent ischemia. In addition to drugs

that have proven useful in inhibiting the process, knowledge of risk factors and their control when possible is a key element in increasing revascularization patency.

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OXIDATIVE STRESS IMPLICATIONS IN VARIOUS LUNG DISEASES. FOCUSING ON ONE LUNG VENTILATION

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OXIDATIVE STRESS IMPLICATIONS IN VARIOUS LUNG DISEASES. FOCUSING ON ONE LUNG VENTILATION (Abstract): One the most intriguing biomedical fact nowadays is that many of the existent diseases exhibit a certain molecular component expressed through oxidative damage. In this way, it seems also that lung diseases have an oxidative stress component which is clearly shown by impairment in oxidant/antioxidant mechanisms balance. In several pathological cases, each and every cell present in lung tissue may be capable to produce reactive oxygen species (ROS) as a part of the inflammation signaling. ROS are also produced in normal conditions, but a strong antioxidant defence system acts in order to transfer the impaired electrons of the ROS reducing them to neutral species. In this way, in one lung ventilation (OLV) surgical maneuver an increase in the ROS production occurs mainly due to 100% use of oxygen during artificial ventilation. Unfortunately, the only way in which the oxidative damage can be minimized is by carefully and correctly executing this maneuver. Moreover, no antioxidant therapy can be considered working or safe yet, although some animal modeling showed promising results. **Keywords**: REACTIVE OXYGEN SPECIES, THORACIC SURGERY, LUNG RESECTION, COMPLICATIONS, ANTIOXIDANT THERAPY.

Nowadays, it is generally known that living organisms on Earth can survive only due an appropriate concentration of oxygen in the breathing air. Both plants and animals' metabolic pathways are based on a continuing exchange of atomic particles between reactive species and pathway partners. At the same time, it seems that these reactive species also play an important part in the process that make living bodies age and finally die. As the oxygen species formed in respiration chain participate in all homeostatic reactions, energy production, enzymatic activation, pathways regulation and so on, it seems that the same species can also easily participate in order to end cellular activity by activating the well-known self-destructive mechanism called apoptosis. This paradox can be well observed in lung diseases (LD) where the oxygen privation leads to serious tissue damage (1). It was only currently that we understood that reactive oxygen species (ROS) can also play a crucial role in LD and that the lung tissue may be extremely vulnerable when exposed to oxidative

stress during a LD, a surgical procedure, or after specific post-operatory maneuvers such as lung-ventilation. Therefore it seems that oxidative stress damage may be of great importance when it comes on LD due to lung vulnerability to the toxic effects of oxygen and the oxygen damage can occur and radically change pathologies features.

The pathology of oxidative stress begins when an imbalance in oxidant-antioxidant systems occurs. The accumulation of ROS can cause damage to DNA, lipids, proteins, and carbohydrates. There are methods to measure oxidative stress shown for example by increased lipid peroxidation products, or the DNA oxidation, and protein carbonyl formation in lung tissue. As the lipid peroxidation can be an excessive ROS production marker, the oxidized lipids may possess signaling potential. It is the case of the isoprostanes, by-products of membrane lipid peroxidation involved in bronchoconstriction occurrence and airway hyperresponsiveness in asthma that can also be powerful vasoconstrictors in pulmonary arterial hypertension or acute lung injury (2, 3).

One lung ventilation (OLV) is currently used in video-assisted surgery and also in open lung surgery. OLV purpose is to decrease the blood flow through pulmonary circulation that could lead to temporary ischemia followed by reperfusion after restoring ventilation, in combination with iatrogenic hyperinflation, all of which can imply in ROS production (4).

It is interesting that both common lung diseases and artificial lung ventilation involve oxidative stress damage to lung tissue, but no comparative or cumulative study was made. This review aims to present several aspects considering the oxidative stress occurring generally in the common lung diseases and particularly during or after surgeries that require the assistance of artificial lung ventilation. Surprisingly, it seems that even a surgery tool may mimic or increase oxidative stress leading eventually to lung tissue damage.

OXIDATIVE STRESS MECHANISMS IN LUNG DISEASES

Many studies show that endogenous oxidative-antioxidative mechanisms may be critical in LD (as reviewed by - 5). As the reactive species of oxygen and nitrogen are ubiquitous in nature, they must be produced by both endogenous and exogenous sources. Endogenous sources may be the cellular organelles responsible for respiratory chain and hydrogen peroxide metabolism (6, 7). In other words, when the unpaired electrons from the mitochondrial transport chain occur during oxidative phosphorylation, they are reduced by the molecular oxygen which leads to superoxide production which easily changes into hydrogen peroxide. This is the time when the peroxisomes are activated and their enzymes contribute to the catalysis of these reactive oxygen species (ROS), but being an enormous source of oxidative stress (8-10).

Studies show that there are many important cellular enzymes that are implicated in lung diseases pathologies (11-14). NADPH oxigenase, cytochrome P_{450}, lipooxygenases may be just some of these enzymes that are produced by various cells in the lung tissue alongside ROS and other molecules.

In this way, neutrophils use to produce a significant ROS concentration due to their antibacterial activity. Therefore, it is in their membrane where the NADPH oxidase generates superoxide which further

activates the reaction cascade in phagocytosis. Alongside the NAPDH oxidase, the myeloperoxidase also is involved in the phagocytosis oxidative reactions mainly producing an extremely reactive Chloride species, the hypochlorous acid (15, 16). However, it has been known that the neutrophils are very few in lung tissues having an extreme mobility during inflammation from the circulation into the lung tissue. Also attracted by the chemical signal of inflammation, the peripheral monocytes may produce significant damage in lung tissue due to their impressive capacity of producing large amounts of superoxide leading to acute injuries (17). In the same way as neutrophils, the alveolar macrophages and eosinophils act like potent ROS producers through the NADPH oxidase activity (18), but the first being active also in physiologic conditions (19) and the latter mostly in allergies or infections (20). Eosinophils also possess peroxidase potential, surprisingly similar to myeloperoxidase.

Endothelial cells, on the other hand, are known to produce ROS in pathological conditions, also being of a great number in lung tissue. Oxidative stress occurs due to these cells during and after hypoxia (21) and in some cases during inflammation (22, 23). They mainly produce superoxide, sent by membrane channels to extracellular compartments of lung tissues, and hydroxyl radicals (24). Smooth muscle cells can also produce ROS through NADPH-like oxidase complexes and may produce airway hyperactivity (25, 26).

Pneumocytes in the alveolar epithelia and lung fibroblasts also possess intense metabolisms being involved in inflammation and tissue reconstruction. Type II pneumocytes may be precursor for type I therefore in the differentiation process a certain oxidative stress occurs (27, 28). Lung fibroblasts are attracted by inflammation signals (such as cytokines) and produce ROS intracellularly and extracellularly (29).

Therefore it seems that each type of cells present in the lung tissue may actually generate ROS, either in physiologic or pathologic conditions. It is not yet sufficiently clear whether if an imbalance in the cellular system of ROS formation and degradation act as a clean pathological trigger. Extremely important should be the fact that most of the ROS play important roles in signalling processes of several survival pathways such as apoptosis.

ROS production from exogenous sources may also play an important role in LD. Environmental toxins or diet could also promote the onset of LD. Classical examples may be the exposure to different herbicides or pesticides that could lead to lung injuries in human by increasing inflammatory factors expression and mitochondrial dysfunction. Also the ethanol poisoning has been shown to lead to lung diseases (30-32) and even ROS-induced cysteine modification in the lungs (33). Even the well-known anti oxidative factors found in the usual diet can influence the inflammatory processes leading to unwanted side effects (34). Other examples may include oxidative stress induction by environmental toxins (arsenic, asbestos, and tobacco) that disrupt the cytochrome P_{450} metabolism (35). All of these changes affect the pulmonary inflammatory response and can finally contribute to lung cancer onset (36-38).

In addition, ROS have been suggested to have a major influence on the rate of postoperatory complications after thoracic surgeries assisted by two-lung ventilation

or one-lung ventilation. It seems that lipid peroxidation may be involved in the atrial fibrillation, a common complication following pulmonary resections (39). Due to high reactive properties, changes in impaired electrons oxygen molecules may be rather difficult to assess, although it might be more suitable to evaluate the ROS production by evaluating the changes that they may lead to different molecules such as proteins. For example, an increase in carbonyl groups may be the result of the increase of hydroxyl radicals which generate protein dimerization following ammonium radical oxidation. Covalent binding of cysteine or methionine protein residues may be associated with excessive oxidation of sulfhydryl groups.

In OLV, it is highly possible that due to the 100% use of oxygen, several changes in oxidative mechanisms occur. As one of the lungs is inactive, the other one must take over the entire activity so that it must provide the entire body the oxygen it needs. In this way, a higher concentration of oxygen must pass through the lung and reach blood. Therefore, it has been shown that higher myeloperoxidase and lower SOD activities may act together in common lung injury caused by hyperoxia due to protein carbonyls formation and high cytokines signalling (40). In the same way, when switching from two-lung to one-lung ventilation, a shunt fraction increases, and oxygenation is impaired. In this way, hypoxemia during OLV may be predicted measuring lung function and distribution of perfusion (41).

ANTIOXIDANT DEFENCE MECHANISMS

While the lung is directly exposed to the environmental oxygen, antioxidant defence is very important (42). Antioxidant defence exists as biochemical systems that help the cells or tissues to restore the proper oxidative balance in order to maintain cellular homeostasis. Obviously, a great part of this defence consists of enzymatic complexes aiming to catalyse the reduction of ROS into low reactive species such as water or to transfer their active potential to other biological important molecules such as NADP(H). The first enzyme in the defence is the superoxide dismutase (SOD) which transforms superoxide in oxygen and hydrogen peroxide. SOD is active both in intracellular and extracellular space with not clear role in antioxidant mechanisms but efficiently decreasing lipid peroxidation and membrane damage (43). As the hydrogen peroxide is produced, the catalase (CAT) and glutathione systems (GS) have the purpose of reducing it to water and oxygen. In spite of SOD, CAT is only active intracellularly (in peroxisomes) alongside the reduced form of glutathione (GSH) (44, 45). Due to the fact that reduction potential of GS bases on the ratio between the oxidized and reduced (GSH) forms of glutathione, and adding that GSH concentration in the alveolar lavage fluid is 100 fold higher than in plasma (44), it seems that GS is a highly active antioxidant system in lung tissues.

Moreover, there are other enzymatic systems that may play direct or indirect roles in the defence against oxidative stress. Among these lie the peroxiredoxins which can reduce hydrogen peroxides, or the thioredoxins which reduce sulfhydryl bonds and they are all implicated in a whole tableau of mutual cellular effort to counteract the damaging effects of normally produced ROS.

Besides the important enzymes that re-

duce the ROS and lipid peroxides such as thioredoxins and GS which in fact were shown to be involved in LD as important protective factors (46, 47), there are also some non-enzymatic endogenous or exogenous antioxidants that can also protect the lung tissue against the peroxidation radicals (48-50). These non-enzymatic antioxidants are mainly "scavengers" of free radicals, such as vitamin C (51), vitamin E (inhibits oxidation of membrane lipids) (52), uric acid (efficient scavenger of peroxynitrite, present in plasma and airway lining fluid) (53), albumin, bilirubin, glutathione or N-acetylcysteine (NAC).

For example, NAC is a potent drug currently used in LD therapies due its potential of directly reacting with ROS and also serving as GSH precursor (50). In this way, it can prevent oxidative stress produced by neutrophil accumulation (54). Many other antioxidant molecules have been studied in LD therapy (55, 56), but currently most of the attention is channeled through enhancing SOD activity (3, 57). Some of these agents reached the clinical trial level, but unfortunately only at a small scale and none of them have yet become commonly used in practice.

PERSPECTIVES IN ANTIOXIDANT THERAPIES

In contrast to the shown fact that the oxidative stress is an important factor in LD, many studies show that the antioxidant therapeutic approach is a matter of efficiency. It seems that many of the trials failed to succeed due to a great number of reasons.

Many of the reasons generally address to the clinical practice such as dosage, delivery and timing. For example, it is known that in active lung injury burst of oxidative stress in a dynamic activity may occur, therefore it is important to calculate the exact amount of antioxidants which must be delivered in order to surpass the oxidative burst but also to be relatively low in order not to impair the oxidant/antioxidant balance in case of an excess. More than that, it seems that the true challenge is the short half-life of the antioxidants in the tissue delivery context being the case of recombinant SOD enzymes (58, 59) which must quickly reach lung tissue through blood stream, a rather problematic matter. In addition, an efficient delivery should be perfectly timed in order that the antioxidant to reach destination than the irreversible tissue damage occurs.

Another important aspect is that ROS may exhibit more than the damaging role of which therapy is addressed to. There might be several interferences of the treatment with the physiological mechanisms. In this way, the immunological response through phagocytosis may be slowed in the case of an antioxidant treatment of airway inflammation, and therefore lung injury (60, 61). Surprisingly, it seems that several studies in 1996 showed that antioxidant therapy may actually increase lung cancer risk due to cell growth promotion potential (62-64). Moreover, an animal model study in 2014 also claims that antioxidant accelerates lung cancer development (65).

Therefore, a more attentive approach should be considered in antioxidant therapies in terms of genetic variation, and regulation of enzymes. There are many genetic variations including polymorphisms and mutations described for many of the antioxidant defence enzymes. For example, in chronic obstruction pulmonary disease, polymorphisms in antioxidant genes related to GS function and all isoforms of SOD

alter the susceptibility to this disease and also the impact on the disease progression (66, 67). Genetic variations in antioxidant enzymes have also been implicated in susceptibility to asthma (68) and acute lung injury. Their importance is crucial because allows the clinicians to target vulnerability rather than the disease itself meaning that a therapy to prevent would be more efficient than a therapy to cure.

CONCLUSIONS

Oxidative damage may be extremely dangerous in terms of lung functions impairment due to their key role in oxygen supplying for the entire body. Many of the lung diseases access the oxidative pathways causing an excess of ROS both intracellular and extracellular in the lung tissue. One-lung ventilation, a common maneuver during thoracic surgery, has also an oxidant component in which, due to the 100% use of oxygen by one lung, the balance between ROS and antioxidant mechanism is impaired. This maneuver must be carefully handled and controlled because most of the after surgery complications occur based on the mechanisms triggered by the excessively produced ROS. Unfortunately, no direct treatment for after-OLV complications is yet available and no antioxidant treatment or preventive therapy can be developed in the absence of a extreme care in assessing OLV maneuver.

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MONITORING OF RECURRENCE IN PATIENTS RADICALLY OPERATED FOR PANCREATIC CANCER

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MONITORING OF RECURRENCE IN PATIENTS RADICALLY OPERATED FOR PANCREATIC CANCER (Abstract): Pancreatic cancer is a diagnosis that carries a poor prognosis. It is the fourth leading cause of cancer death in Europe and the United States, despite advances in operative technique and postoperative management. Furthermore, there is no consensus on the optimal follow-up schedule of patients after surgery for pancreatic cancer, all recommendations on surveillance being based on low level evidence or no evidence and the leading societies propose different guidelines. As a consequence, follow-up strategies may differ between hospitals depending on preference of physicians. The vast majority of patients develop recurrence within 2 years after surgery, suggesting the necessity of a more intensive follow-up the first 2 years after surgery. It usually occurs after surgery as migratory metastases along major upper abdominal arteries and veins to the liver or peritoneum (70%) and less commonly as loco regional disease as masses closely applied to the surgical margins in the neck or body of the pancreas (30%). Currently, there are no effective means to prevent pancreatic cancer recurrence, despite the fact that it is responsible for the majority of postoperative deaths. **Keywords**: PANCREATIC CANCER, FOLLOW UP, RECURRENCE, SURGERY.

DEFINITION/CLASSIFICATION OF RECURRENCE

Local recurrence has been defined as any failure in the retroperitoneum around the superior mesenteric and celiac arteries, including the tumor bed, remnant pancreas, hepatic hilum, or its regional nodes. It is usually associated with cancer infiltration of the nerves, lymphatic vessels, and connective soft tissues to various extents.

In a study on 27 autopsied patients who underwent potentially curative resection of pancreatic cancer between September 1986 and January 2004, Hishinuma et al. have classified the patterns of recurrence as follow: local recurrence, liver metastasis, peritoneal dissemination, para-aortic lymph node metastasis, distant metastasis (not including hepatic metastasis, peritoneal dissemination, and para-aortic lymph node

metastasis). Anyway, of all of these, the two major patterns of recurrence following the curative resection of pancreatic cancer are local recurrence and liver metastasis.

Most studies have assessed the recurrence patterns of resected pancreatic cancer focusing on diagnostic imaging, but this is not considered sufficient, without performing biopsy and obtaining a pathologically proven diagnostic or based on autopsy findings. In some cases recurrence occurs as an infiltrating tissue, without being a tumor mass, so it is not suitable to be detected by the actual imaging methods (1).

Sites of local recurrence after surgery depend mainly on the original tumor site within the pancreas and the lymphatic drainage routes. Because as a result of adjuvant therapy local inflammatory changes cannot be differentiated from recurrent tumor, close follow-up with serial imaging comparing every study with an initial baseline postoperative examination when available is essential (2).

Niedergethmann et al. have classified the site of recurrence as: local, hepatic, peritoneal, lung or other distant recurrences being assessed by ultrasonography, computed tomography, or biopsy during palliative surgery (3).

One of the most challenging features of pancreatic cancer therapy is the poor outcome and almost invariable tumor relapse even after curatively intended resection. This high rate of relapse is likely due to undetected cancer cells in lymphatic and neuronal tissue. Consequently, microscopic dissemination of cancer cells from the primary tumor is likely either to be present before resection or is the result of the resection itself. Failure of pancreatic resection is often due to loco-regional recurrence or development of distant metastases and peritoneal carcinomatosis by cells that have been seeded before or at the time of surgery. Sperti et al. found that of 78 patients who died after undergoing curative resection, local recurrence developed in 72 %, hepatic metastases developed in 62 %, and 97 % had one or the other. Other studies on patterns of recurrence show that the liver is the most common site of failure after pancreatic resection, but in other studies approximately 75 % of patients who die from pancreatic cancer are found to have a local recurrence in the pancreatic bed. Also, there are reports of 50% local recurrence among long-term survivors, and despite adjuvant treatment (4).

Recurrence can be defined as reappearance of tumor tissue after intended curative resection of pancreatic cancer. We are proposing a classification into local recurrence, regional and distant metastases.

Local recurrence is considered to be situated in the retroperitoneum around the superior mesenteric and celiac arteries, including the tumor bed, pancreatic remnant, hepatic hilum.

Regional recurrences include the regional lymph nodes: hepatic hilum, para aortic lymph nodes or any other group in the region.

Distant metastases can be classified into intra abdominal (liver, peritoneal) and extra-abdominal (lung).

PROGNOSTIC/PREDICTIVE FACTORS OF RECURRENCE

Due to the lack of sensitive and specific methods to diagnose pancreatic cancer or the recurrence after intended curative surgery, many efforts have been made to find predictive factors of recurrence of tumor tissue.

Prognostic factors in patients with pancreatic carcinoma have been analyzed based on survival but not on recurrence in

the majority of previously published large series reports.

Shimada et al. retrospectively examined recurrence patterns within a year and predictive factors associated with early recurrence in 133 patients who underwent macroscopic curative pancreatectomy with extended lymphadenectomy for invasive pancreatic carcinoma. More than 80% of the early recurrences were found to be distant and the liver accounted for 46%. Loco-regional recurrence was recognized in only 9% of patients, a lower rate than in previous reports (5, 6).

These findings suggest that microscopic metastases already existed in the liver, peritoneal, or other distant organs at the time of operation. In these patients local cancer control alone could not contribute to long-term survival because the major cause of failure was distant metastases.

Sampling of para-aortic lymph nodes is recommended as a routine examination at laparotomy. When positive nodes are confirmed by frozen section, early recurrence and poor survival are inevitable, even after radical surgery, extended lymphadenectomy included.

This study highlighted that para aortic lymph node metastasis was the definitive predictor of early recurrence and shorter survival (12 months). Based on these results, radical pancreatectomy with extended lymphadenectomy should not be recommended when para-aortic lymph nodal metastasis was confirmed by intraoperative biopsy. Even if there is no apparent hepatic metastasis and peritoneal dissemination after surgical exploration, routine sampling of para aortic nodes for investigation by frozen section is recommended before radical surgery because palpation or intraoperative ultrasound cannot predict para aortic nodal metastases (7).

IMPORTANCE OF SPECIALIZED SURGEONS / HOSPITALS

Among the multiple factors influencing the outcome of patient operated for pancreatic cancer, the grade of surgeons' specialization and hospital volume were taken into the account and therefore analyzed in a few studies.

One complex study regarding these issues has been performed by Sosa et al. under the supervision of Prof. John L. Cameron, at The Johns Hopkins University School of Medicine and published in 1998. They analyzed the hospital discharge data from all non-federal acute care hospitals in Maryland (n = 48), identifying all the patients with pancreatic cancer who underwent a pancreatic resection, palliative procedure, or stent procedure between 1990 and 1995. The hospitals were categorized as high-, medium-, and low-volume providers according to their average annual volume of these procedures. Multivariate regression was used to examine the association between hospital volume and in-hospital mortality rate, length of stay, and hospital charges, after adjusting for differences in case mix and surgeon volume. The result show that increased hospital volume is associated with markedly decreased in-hospital mortality rates and a decreased or similar length of stay for all types of procedures. After adjustment for case mix differences, the relative risk (RR) of in-hospital death after pancreatic resection was 19.3 and 8 at the low- and medium-volume hospitals, respectively, versus the high-volume hospital; after bypasses, the RR of death was 2.7 and 1.9, respectively; and after stents, the RR was 4.3 and 4.8, respectively. So patients with pancreatic cancer who are to be treated with curative or palliative procedures appear to benefit from being operated in high-volume hospi-

tals. These results support previous studies about the better outcomes with greater case volume and also extend the findings of studies that have shown an association between increased hospital volume and better outcomes for pancreatico duodenectomy (8-11).

Improved performance is not associated with patient volume but also with the volume of patients with pancreatic cancer of similar types.

The explanations could reside in the fact that high-volume centers may be able to develop a systematic approach and organizational routines for handling such patients by developing critical pathways and involving case managers which, in turn, may raise the performance of all participants in the health care team.

The data from this study suggest that the combined effect of the whole team of pancreatic cancer care providers at a hospital may be more important than the number of surgeries performed by a particular surgeon. Hospital volume had the strongest and most consistent association with outcomes. A large experienced surgical team, specialized nurses, intensive care specialists, anesthesiologists, radiologists, and hospital monitoring procedures are of big importance for the complex procedures performed in patients with pancreatic cancer (12). Overall, according also to other literature data, patients with pancreatic cancer requiring procedures for their disease may benefit from referral to a high-volume provider, regardless of whether they require curative resection or a palliative bypass or stent and help to decrease the substantial morbidity and mortality rates associated with current patterns of surgical care for patients with pancreatic cancer (13).

A survey of 2.5 million complex surgical procedures in the USA showed a strong inverse relationship between hospital volume and case mortality rates for pancreatic resection. Also, long-term survival after pancreatic cancer resection is superior in high-volume centers (14-16).

DIAGNOSIS OF RECURRENCE

CT is a valuable imaging method used to assess the surgically treated pancreatic cancer patient with or without adjuvant therapy (chemo- or/and radiation therapy). CT can detect postoperative complications, local recurrences or distant metastases. The site of recurrence after surgery is dependent on the site of the primary tumor in the pancreas and lymphatic drainage routes. But local changes following therapy are difficult to be differentiated from recurrent tumor tissue that is why very important is the first postoperative examination which will be used as a baseline for subsequent comparison of findings.

For metastatic disease the authors advocate imaging during the portal venous phase which can better detect liver metastases and the use of thin sections imaging to improve the detection of locally recurrent disease (2).

The possible role of PET in detecting recurrent local or metastatic disease is still a matter of study. It is rather useful in clarifying the CT examinations which are equivocal for recurrent disease. This was suggested by the results of a small study of 20 patients operated for pancreatic cancer using a Whipple procedure (17).

Many reports showed that FDG-PET (fluorine-18-2-fluoro-deoxy-D-glucose positron emission tomography) is useful for the early detection of recurrence in cancers of different organs. But actually there are only a few reporting its use for recurrent pancreatic cancer.

Tanizawa et al. have evaluated the use of FDG-PET for the diagnosis of recurrence of pancreatic cancer. When the recurrence could not be confirmed by computed tomography (CT) or magnetic resonance imaging (MRI), the final diagnosis can be made by FDG-PET. The use of FDG-PET also allowed for early detection of recurrent lesions after surgical resection for pancreatic cancer (18).

Abdominal ultrasound is a reliable method for monitoring the patients with pancreatic resections. It is non-invasive, cheap and it could be used as the first intention imaging technique.

In the management of pancreatic cancer, due to early cancer recurrence and death, the development of surgical techniques was focused on the prevention of early postoperative complications. But the long-term survival following pancreatoduodenectomy is increasing slightly in the last 2 decades and, consequently, the preservation of both endocrine and exocrine function of the remnant pancreas is becoming more important.

Experimental Wirsung duct ligation lead to destruction and progressive fibrosis is replacing the normal pancreatic tissue, although the islets are relatively well preserved for a period of time (19-21). Different degrees of fibrosis of the remnant pancreas following pancreaticoduoden-ectomy have been identified at autopsy, but the assessment of fibrosis in the pancreatic remnant is difficult during life.

Dynamic contrast-enhanced MRI is a reliable and non-invasive method for evaluating fibrotic changes in the remnant pancreas after pancreaticoduodenectomy. A duct-to-mucosa pancreatojejunal anastomosis was associated with a lower risk of residual pancreatic fibrosis than a pancreatojejunoserosal technique (22).

EUS-FNA has a reported sensitivity of 85% and specificity of 98% for the initial diagnosis of pancreatic cancer. Concerning the suspicion of pancreatic cancer recurrence EUS-FNA has a sensitivity ranging from 81% to 93%, which is similar to the sensitivity of 83% reported for fluorodeoxyglucose-positron emission tomography/contrast-enhanced CT.

The main advantage of EUS for this diagnosis is the ability to provide a tissue diagnosis in an otherwise often inaccessible region in which to perform percutaneous biopsy.

Being able to place a needle in these recurrent tumors, we surmise that EUS-FNA may also offer an opportunity to treat or aid in the treatment of these recurrences by fiducially placement, implantation of radioactive seeds, or fine-needle injection of ablative or chemotherapeutic agents (4).

Follow up marker. CA19-9 was first described by Koprowski et al. and is a tumor-associated carbohydrate antigen found on the cell surface of many gastrointestinal tumors. Koprowski immunized mice colorectal carcinoma cell lines and then isolated CA19 9 monoclonal antibody from mice splenocytes. Further research has shown that the antigen is normally present in salivary mucus and exocrine pancreatic juice, so, even if it was originated from colorectal carcinoma cell lines, it is very useful in the diagnosis, assessment and follow-up of the patients with pancreatic cancer (23-25).

Some investigators have tried to demonstrate the usefulness and effectiveness of sequential detection of CA19-9 serum levels in postoperative follow-up period.

Probably the earliest study was performed in 1986 at the National Cancer Institute and the authors found out that a

decrease to normal level after surgery was associated with longer survival compared with the patients whose CA19 9 level remained high. Also they demonstrated that the increase of CA19 9 level >95U/mL or a fourfold increase from the lowest postoperative level predict disease progression (26).

Montgomery et al. found that postoperative CA 19-9 level measurement is the best predictor of disease-free and also of overall survival. They found that patients whose CA 19-9 levels returned to normal by 3 to 6 months had a longer disease-free survival and overall survival than those with a steady level. The survival of patients with postoperative CA 19-9 level of less than 180 U/mL at 1 to 3 months was similar to that of patients whose levels normalized by 3 to 6 months (27).

They concluded that patients who present with undetectable preoperative CA19-9 levels and potentially resectable pancreatic cancer, regardless of advanced stage, should be considered candidates for aggressive therapy.

Adjusted baseline CA 19-9 may be a better clinical application for estimating survival or recurrence risk than serial values. Different treatment strategies can be individualized based on baseline preoperative levels. Chemo radiation or postoperative adjuvant therapy could be planned based on the risk of recurrence estimated by the preoperative adjusted CA 19-9 (28).

Treatment. In most cases re-intervention for local recurrence carry a bad prognosis because many patients have a R1 resection in secondary intervention. Repeat pancreatectomy might bring about a favorable effect on the prognosis in the patients with isolated local recurrence in the remnant pancreas if R0 resection is expected to obtain a repeat pancreatectomy.

Diagnosis of a distant metastasis is an important criterion in selecting candidates for repeat pancreatectomy. During the follow-up after initial pancreatectomy the serum level of tumor marker (CA19-9) should be determined and CT, MRI and PET scans for differentiating the three different patterns of local recurrence (lymph nodes, soft tissue, and remnant pancreas) should be performed.

Repeat pancreatectomy in these patients might bring about favorable effects on the prognosis without an increase in operative morbidity and mortality; the patient should be carefully assessed in order to determine whether he/she might be a good candidate for repeat pancreatectomy (29).

Sunogozaka et al. reported the case of a patient diagnosed with recurrent pancreatic cancer and treated with UFT (uracil-tegafur) combined gemcitabine (GEM) combined chemotherapy. After one cycle of therapy, the anti-tumor effect was progressive disease with obstructive jaundice and enlargement of lymph node metastasis and liver metastasis. Chemotherapy was changed to TS-1 (tegafur + gimestat + otastat potassium) combined GEM chemotherapy and resulted in partial response. The only adverse effect was a grade 3 leukopenia.

The authors support the idea that some cases may be effectively treated by TS-1 combined chemotherapy after treatment failure of UFT combined GEM chemotherapy. The therapy is simple and possible to continue safely on an ambulatory basis while maintaining quality of life (30).

Chemoradiotherapy for local recurrence. In patients with no further surgical option in local relapse, the palliative treatment (chemoradiotherapy) is preferred, radiotherapy having the greatest impact on

locoregional disease control, and is affected by hypoxia-related tumor radioresistance (i.e. pancreatic cancer seems to be extremely hypoxic according to several studies). Several trials using simultaneous chemoradiotherapy (CRT) with gemcitabine, 5-FU or cisplatin have shown that high efficacy can be achieved locally. Based on trials, combined CRT is suggested to be superior to chemotherapy or radiotherapy alone in patients presenting with de novo locally advanced pancreatic cancer.

Stereotactic body radiation therapy-cyberknife. Radiotherapy in the patient with unresectable locally advanced disease shows unsatisfactory local control because therapeutic radiation dose to the pancreatic tumor is limited by the sensitivities of surrounding tissues. The cyberknife system is being used since 2001 to deliver radiation to any human radiosensitive tumor; cyberknife offers a stereotactic boost of radiation therapy alone or in combination with conventional radiation therapy.

There are few studies that demonstrate that cyberknife therapy for unresectable pancreatic carcinoma can be delivered in three fractions with minimal morbidity and a local tumor control rate of 91.7%.

In unresectable pancreatic cancer the most commonly used treatment options are chemotherapy with 5-fluorouracil and/or gemcitabine, palliative surgical bypass procedures, palliative radiation procedures or other palliative measures. For recurrent pancreatic cancer, there are no established therapeutic strategies although the pathophysiological pattern of recurrence is well known (31).

CONCLUSIONS

Recurrence of pancreatic cancer is attributed to either occult systemic disease at the time of diagnosis or the presence of macroscopically (R2) or microscopically (R1) positive surgical resection margins. It usually occurs within 2 years after surgery as migratory metastases along major upper abdominal arteries and veins to the liver or peritoneum (70%) and less commonly as loco regional disease as masses closely applied to the surgical margins in the neck or body of the pancreas (30%).

The treatment of local recurrence may include surgical resection alone or combined chemo radiotherapy.

The vast majority of patients develop recurrence within 2 years postoperatively, suggesting the necessity of a consensus on the optimal and more intensive follow-up during the first 2 years after surgery.

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NEWS

SHEAR BOND STRENGTH OF CALCIUM ENRICHED MIXTURE CEMENT AND MINERAL TRIOXIDE AGGREGATE TO COMPOSITE RESIN WITH TWO DIFFERENT ADHESIVE SYSTEMS

The aim of a study realized by a group of iranian researchers was to evaluate the shear bond strength of recently used pulp capping materials: white mineral trioxide aggregate, and calcium enriched mixture cement to composite resin with the use of etch-and-rinse and self-etch adhesive systems and compare them with the bond strength of commonly used resin modified glass ionomer cement. Forty specimens from each test material were fabricated, measuring 4 mm in diameter and 2 mm in depth. The specimens of each material were divided into 2 groups of 20 specimens according to the adhesive system (Single Bond vs. Clearfil SE Bond) used for bonding of resin composite. The shear bond strength values were measured at a crosshead speed of 1.0 mm/min and fractured surfaces were examined. Data were analyzed using two-way ANOVA and a post hoc Tukey's test ($P<0.05$). The analysis of data showed a significantly higher bond strength for resin modified glass ionomer cement compared to white mineral trioxide aggregate and calcium enriched mixture cement ($P<0.001$); however, no significant differences were observed in the bond strength values of white mineral trioxide aggregate and calcium enriched mixture cement ($P=0.9$). Furthermore, there were no significant differences in relation to the type of the adhesive system irrespective of the type of the material used ($P=0.95$) All the failures were of cohesive type in resin modified glass ionomer cement, white mineral trioxide aggregate and calcium enriched mixture cement. In conclusion, bond strength of resin modified glass ionomer cement to composite resin was higher than that of white mineral trioxide aggregate or calcium enriched mixture cement irrespective of the type of the adhesive system (Savadi Oskoee S, Bahari M, Kimyai S, Motahhari P, Eghbal MJ, Asgary S. Shear bond strength of calcium enriched mixture cement and mineral trioxide aggregate to composite resin with two different adhesive systems. *J Dent (Tehran)*, 2014; 11 (6): 665-671).

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ST SEGMENT DEPRESSION IN ASYMPTOMATIC MALE PATIENT WITH NORMAL CORONARY ARTERIES

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ST SEGMENT DEPRESSION IN ASYMPTOMATIC MALE PATIENT WITH NORMAL CORONARY ARTERIES (Abstract): ST segment depression and T waves inversion are electrocardiographic (ECG) repolarization abnormalities often encountered in clinical medical practice that have been proved to predict future cardiovascular events. We present the case of a 62-year-old male patient, asymptomatic, with ST segment depression and inverted T waves discovered incidentally on resting ECG. Echocardiographic and laboratory examinations ruled out multiple causes of ECG abnormalities. Suspecting a silent myocardial ischemia, an ECG exercise stress test was performed; it revealed pseudo normalization of T waves during exercise and early recovery phase. Being inconclusive, a coronary CT was the final election test; it showed normal coronary arteries with no stenosis, the patient being scheduled for regular follow-up. The possible causes of ST segment depression are reviewed since it is important that early cardiovascular signs especially in asymptomatic patients to be prevented and detected. **Keywords**: ST SEGMENT DEPRESSION, INVERTED T WAVE, ASYMPTOMATIC, NORMAL CORONARY ARTERIES.

ST segment depression and T wave inversion have been identified as markers of future cardiovascular events in patients with or without coronary artery disease (CAD). Most of the ST segment and T wave changes are represented by primary repolarization abnormalities while secondary changes belong to abnormalities of depolarization (1). Theoretically, ST segment displacement should be measured at 40 or 80 ms after the J point in three consecutive beats with a non-modified baseline (2). Our aim is to present a comprehensive evaluation of different causes of ST segment depression in an asymptomatic male patient.

CASE REPORT

We present the case of a 62-year-old male patient, occasional smoker, totally asymptomatic, who was admitted to our department for evaluation of electrocardiographic (ECG) changes discovered incidentally three months earlier when the patient underwent surgery for a lumbar herniated disc.

Patient's case history was short: surgery treatment for herniated disc L4-L5, L5-S1 and recent diagnosis of benign prostatic hyperplasia. The highest known systolic blood pressure (BP) was 140 mm Hg with no other cardiovascular diseases. The patient did not follow any chronic medical

treatment.

Clinical evaluation revealed an overweight patient (body mass index 27 kg/m²), with normal pulmonary and cardiovascular examination findings, resting BP 120/80 mmHg and heart rate (HR) 70 bpm.

Laboratory results showed normal full blood count with no inflammatory syndrome, normal glucose, hepatic and renal parameters. The patient presented borderline values of total cholesterol and LDL-cholesterol (209 mg/dl and 124 mg/dl, respectively) while HDL-cholesterol and triglycerides levels were normal. Potassium and uric acid levels were unaltered.

ECG at rest revealed sinus rhythm, 70 bpm, intermediate axis, ST segment depression (maximum 2 mm) with inverted T waves in precordial leads V3-V6 (fig. 1). Even though R waves in V5 and V6 leads were of moderate amplitude, the patient did not meet any criteria for a diagnosis of left ventricular hypertrophy (LVH) (3).

Taking into consideration the ECG modifications, we performed a 2D Doppler trans thoracic echocardiography that revealed normal left ventricular wall sizes with normal kinetic movement and preserved LV ejection fraction. Thus, the diagnosis of LVH (LV mass index = 96 g/m²) and obstructive hypertrophic cardiomyopathy were ruled out as possible causes of ST segment depression.

As the patient approved to participate in a study assessing subclinical atherosclerosis in patients with no prior diagnosis of cardiovascular or metabolic diseases, we performed the carotid ultrasound and found a moderately increased intima-media thickness (1 mm) with a small calcified plaque on the right carotid bulb. Arterial stiffness was also within the normal range, (aortic pulse wave velocity - 6.9 m/s). Ankle-brachial index was within the normal range.

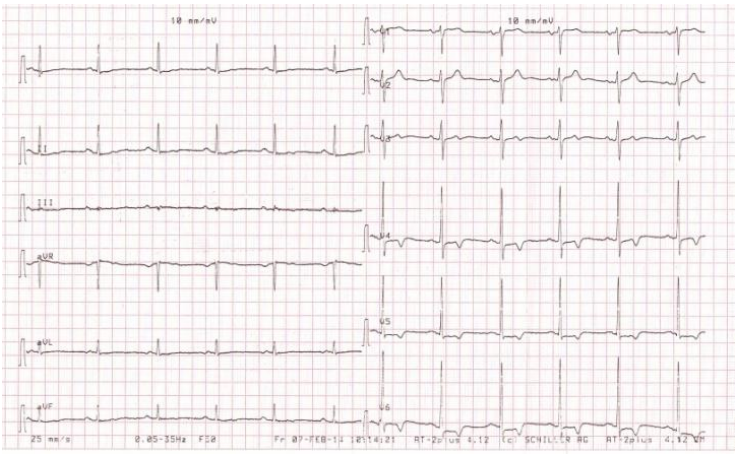

Fig. 1. ECG at rest: sinus rhythm, 70 bpm, ST segment depression and inverted T waves in leads V3-V6

At that moment, we suspected a silent myocardial ischemia. Analyzing the latest guidelines for stable coronary artery disease, the patient presented a 44% intermediate pre-test probability of CAD (4). For intermediate pre-test probability, exercise ECG is the most feasible initial diagnostic test. Thus, an exercise stress testing on

cycloergometer was performed: the patient reached maximum HR (161 bpm, 101% of age-predicted HR), with a work-load of 125 W and 6.5 METs. The patient did not complain of any chest pain or other clinical symptoms. Compared to the resting ECG, T waves were positive in leads V4-V6 and remained positive 6-7 minutes during the recovery phase (fig. 2, 3).

Exercise ECG being inconclusive for making a diagnosis of CAD, we resorted to a more accurate method, the coronary computed tomography angiography (coronary CT) for several reasons. It has a sensitivity of almost 95-99%, being the test with the highest negative predictive value; it is considered an alternative to other stress tests for ruling out CAD in intermediate pre-test probability patients; it is considered in intermediate pre-test probability patients after an inconclusive exercise ECG (4). Our patient meeting all the above mentioned criteria, a coronary CT was performed; it revealed permeable coronary arteries with no stenosis and with Agatston calcium score equal to 0.

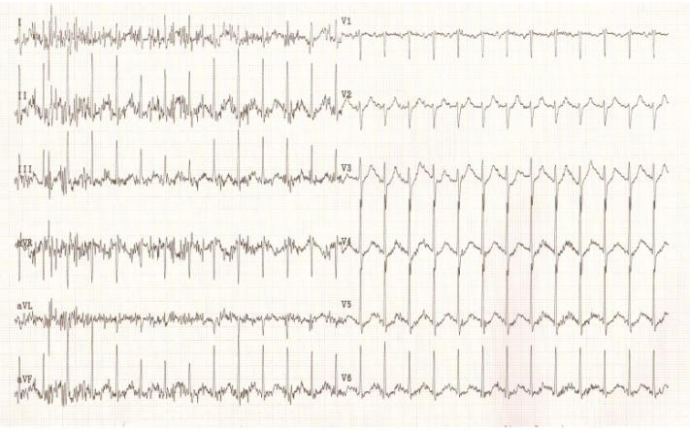

Fig. 2. Exercise ECG – at maximum HR: pseudo normalization of T waves

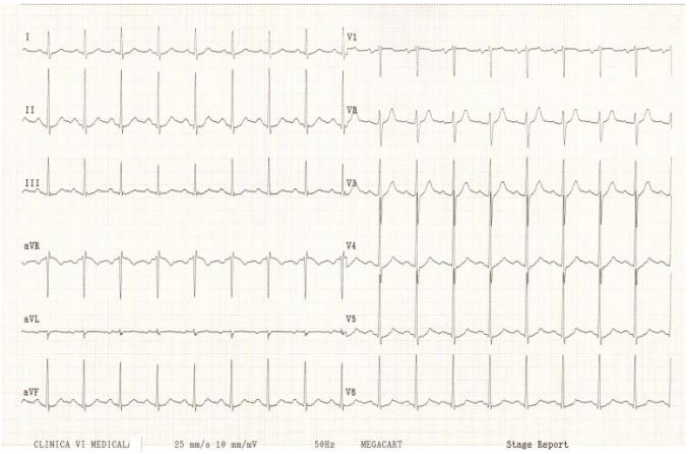

Fig. 3. Recovery phase (5'): still positive T waves with mild ST segment depression

Thus, we excluded all possible causes of ECG ST segment depression. The patient is currently under no chronic treatment and is scheduled for regular follow-up visits in our clinic.

DISCUSSION

ST segment depression and negative T waves are common electrocardiographic changes often seen in medical practice. They need an accurate approach in order to differentiate the possible causes both in acute and in chronic situations.

We will make a summary and comprehensive review of the multiple causes of ST segment depression and T wave inversion based on the above presented clinical case.

When encountering such resting ECG incidentally and not in acute situation, the first diagnostic hypotheses are represented by LVH and myocardial ischemia. Our patient did not meet any of the *left ventricular hypertrophy* ECG criteria (3). However, we performed an echocardiography taking into consideration the poor correlation between ECG and echocardiographic diagnosis of LVH and the result was negative as well. The normotensive status of the patient was another argument in favor of the absence of LVH. On the ECG, the patient did not present *left bundle branch block* and the cardiac ultrasound ruled out the *obstructive hypertrophic cardiomyopathy* as other possible causes of ECG abnormalities.

Another possible cause of ST segment depression could be *Wellens syndrome* which is characterized by two patterns. 75% of cases consist in symmetrically and deeply inversed T waves in leads V2-V4. In 25% of cases, T waves have positive-negative biphasic morphology in leads V2-V4 and the patient usually presents a 90% proximal left anterior descending stenosis (1). However, our patient has no coronary lesions and his ECG findings were different.

There were no *acute causes* that could lead to ST segment depression such as myocardial infarction or pulmonary embolism and by using laboratory results we excluded *hypokalemia*. The patient's medical history ruled out the *digitalis toxicity* as other possible cause.

After interpreting all the results, our main concern was represented by *silent myocardial ischemia*. Asymptomatic myocardial ischemia is present in up to 24% of apparently healthy individuals while in diabetic population the prevalence varies between 12% and 33%. In subjects without prior diagnosis of coronary disease, ST segment depression can predict adverse cardiovascular events and its detection by ambulatory electrocardiogram is a more powerful predictor of mortality than age, exercise duration, hypertension or diabetes (5).

As our patient had an intermediate pretest probability of having CAD, we performed an exercise ECG stress test that showed pseudo normalization of T waves during exercise and within the first minutes of the recovery phase. Nowadays, transient conversion of T waves is considered a nonspecific finding in individuals without prior myocardial infarction and the diagnosis of certainty should be made by using other tests (2).

Finally, the patient having a normal CT coronary angiogram, there were two major hypotheses to explain the ECG abnormalities: an incipient LVH or an atypical *micro vascular angina*.

Coronary micro vascular dysfunction is usually associated with advanced age, hypertension, dyslipidemia and diabetes, but

also with chronic inflammation (high levels of C-reactive protein). Our patient had some of these risk factors, but at lower levels (62 years, arterial hypertension grade I, mild hypercholesterolemia). On the other hand, this specific type of angina is more prevalent in women than in men. However, our patient presented no clinical symptoms at rest or during stress-induced angina. Currently, the gold standard for a certain diagnosis is represented by invasive coronary vasomotor testing using intracoronary injection of acetylcholine (6).

Even if the patient did not meet electrocardiographic or echocardiographic criteria for LVH, this possible diagnosis should be maintained and a regular follow-up should be scheduled. It has been proved that LVH is associated with markers of subclinical atherosclerosis e.g. carotid-intima thickness and carotid plaques as it is the case in our patient (7, 8). However, arterial stiffness was not pronounced and the ankle-brachial index was normal.

It is worth mentioning the research of Kansal et al. who performed a study in which they correlated the presence of resting ST segment depression with coronary angiography in 37 patients free of recent coronary events and with no other cause of ST segment abnormality. Of 37 subjects, 9 had normal coronary angiograms, but 8 of them were females and had a relatively low mean age (43.8 years). The same study reported a double risk for occlusive CAD in men compared to women (9). Another study conducted only on male population pointed out those patients with resting ST segment depression and no prior myocardial infarction had a higher prevalence of severe CAD. After a 4.4-year follow-up, individuals with ST segment depression at rest (with or without prior myocardial infarction) had lower rates of survival compared to general population (10).

CONCLUSIONS

This case report presents a comprehensive management of an asymptomatic male patient, with a moderate cardiovascular risk, that presents with a resting ECG pattern of ST segment depression and inverted T waves in leads V3-V6. By multiple echographic, laboratory and stress tests we ruled out the possible causes of the ECG abnormalities. Using coronary CT, the end result (normal coronary arteries) was surprising so that the exact cause could not be determined and a certain diagnosis made. Thus, it is very important to closely follow-up such patients in order to prevent and promptly diagnose future cardiovascular changes and events.

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NEWS

ANTIOXIDANT ACTIVITY OF PHENOLICS IN RED WINE

It is known that health benefits of red wine have been linked to the presence of phenolic antioxidants. Thus, a group of researchers developed a high performance thin layer chromatographic (HPTLC) method combined with high resolution digital plate images. They visually compared multiple wine samples simultaneously on a single chromatographic plate and quantified levels of four representative classes of phenolics found in wines: gallic acid, caffeic acid, resveratrol and rutin. They also investigated the contribution of these phenolic compounds to the total polyphenolic content (TPC) and total antioxidant capacity (TAC) of the wine samples. The average concentrations of caffeic acid, gallic acid, resveratrol, and rutin in the red wines were 2.15, 30.17, 0.59 and 2.47 mg/L respectively with their concentration below limit of quantification in the white wine samples. The highest concentration of resveratrol and rutin is found in the Cabernet and Shiraz wine samples. The amounts of gallic acid are correlated with TPC ($r = 0.58$). Italian wines have the highest correlation between TPC and TAC ($r = 0.99$) although they do not contain detectable amounts of resveratrol, they contain significant amount of rutin. Researchers concluded that antioxidant properties might be associated with the presence of flavanols in wines. The antioxidant activity of phenols is also related to their chemical structure, especially with the number and arrangement of free hydroxyl groups of polyphenol skeleton (Agatonovic-Kustrin S, Hettiarachchi CG, Morton DW et al. Analysis of phenolics in wine by high performance thin-layer chromatography with gradient elution and high resolution plate imaging. *J Pharmaceut Biomed* 2015; 102: 93–99).

Ioana Mirela Vasicu

MANAGEMENT OF RENAL COLIC DURING PREGNANCY

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MANAGEMENT OF RENAL COLIC DURING PREGNANCY (Abstract**):** The diagnosis of urological diseases in pregnancy involves particularities requiring both the knowledge of the pathophysiological mechanisms of the respective diseases and a experience of the urologist and gynecologist in assessing the physiological conditions of pregnancy. **Aim**: To identify the specific features of renal colic in pregnant women, in the context in which the urological assessment of them is not mandatory. **Material and methods:** The study included a group of 86 pregnant women diagnosed with urological diseases and renal colic as their main symptom. **Results**: There are two major difficulties faced by urologists in these cases: firstly, the impossibility of conducting X-ray imaging investigations to determine the location and type of the obstruction and, on secondly, the ureteral catheterization and insertion of the ureteral catheter in the absence of adequate fluoroscopic control. **Conclusions**: The urologist's experience is decisive in establishing intraoperatively if the introduction of the ureteric probe is possible, the risk of a ureteral perforation with severe consequences requiring a percutaneous nephrostomy to be performed under ultrasound guidance. **Keywords:** PREGNANCY, RENAL COLIC, URINARY DISEASES.

During normal pregnancy there are profound changes in the genitourinary tract affecting the pregnant woman. Management of urological emergencies during pregnancy remains a challenge because clinical signs are subtle diagnostic tools and treatment options are limited.

Mild symptoms of urinary tract infection, urolithiasis or hematuria can progress to true medical emergencies with devastating results for the mother and the fetus if they are not diagnosed and treated promptly (1).

The clinical picture of urological conditions during pregnancy is like the manifestations of these diseases in other categories of patients, but differences may occur in their progression and complications through effects for both mother and fetus.

Urological disorders that may occur during pregnancy are acute hydronephrosis of pregnancy, infections, urinary lithiasis, kidney failure, and clinically these manifestations are dominated by pain, septic status or symptoms associated with impaired renal function.

The aim of this study was to evaluate the issue of the diagnosis of certainty and

the optimal therapeutic choice, related to a symptom that affects relatively frequently (11-20%) pregnant patients - renal colic. Because the number of patients presenting to urology clinics is increasing, it is necessary to standardize the methods for diagnosis and treatment of these diseases, with the implementation of a patient management protocol.

MATERIAL AND METHODS

This retrospective study was performed in a group of 86 patients of different gestational age that experienced this symptom. The reasons for the presentation, age of the patients, gestational age, and the differential diagnosis were considered.

In this research, the STATISTICA program, dedicated to medical research, was used for statistical data processing. The following tests were used: ANOVA, Scheffé, Pearson, Mantel-Haenszel, Fisher test, and specific correlation tests for quantitative and for qualitative variables.

Following these tests, the main parameters of interest were discussed and, according to their values, the conclusions were drawn.

RESULTS

The analysis of the age of patients with urological disorders during pregnancy revealed a high frequency of cases between the age of 25 and 30 years (35.76%). Comparable frequencies were recorded for the age groups 15-20 years - 15.15%, 30-35 years - 16.97%, and 20-25 years - 25.45%. A lower incidence of urological disease in pregnancy was found in patients over 35 years of age.

There was a high frequency of cases in the second and third trimester of pregnancy, with a very low number of pregnant women in the first trimester of pregnancy - 9.09% (fig.1).

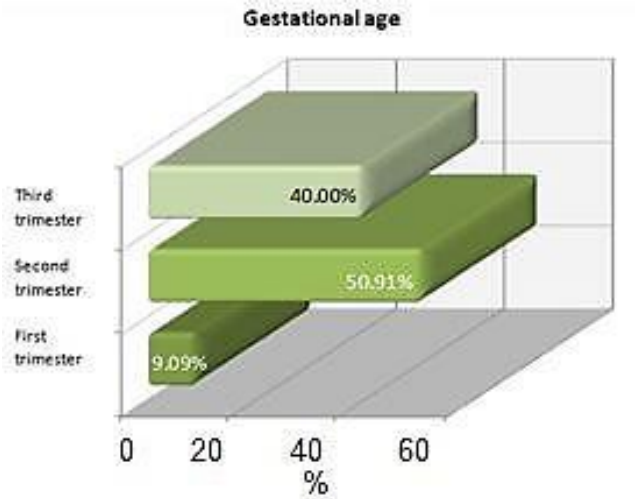

Fig. 1. Distribution of cases by age of pregnancy

The number of pregnancies has a homogeneous distribution, the highest frequency being found for cases with 1 or 2 pregnancies, representing 69.09%. Pregnant women at the 3-rd pregnancy accounted for 30.91% of the study group (tab. I).

TABLE I

Statistical indicators of the number of pregnancies in the study group

Average value of the number of pregnancies	Average -95%	Average +95%	Std. Dev.	Std. Er	Min	Max	Q25	Median	Q75
2.15	1.97	2.33	1.18	0.09	1	6	1	2	3

The main reason for admission was the presence of collateral lumbar pain, which was identified in 82 cases. Absence of lumbar pain was very rare, in just 4 cases.

The diagnosis on admission was ureterohydronephrosis in 52.12% of cases, infected ureterohydronephrosis in 26.67%, and acute pyelonephritis in 15.15% of pregnant women.

A diagnosis of lumbar ureteral calculi was made in 3.3% of cases and of pelvic ureteral calculi in 3.03% (fig. 2).

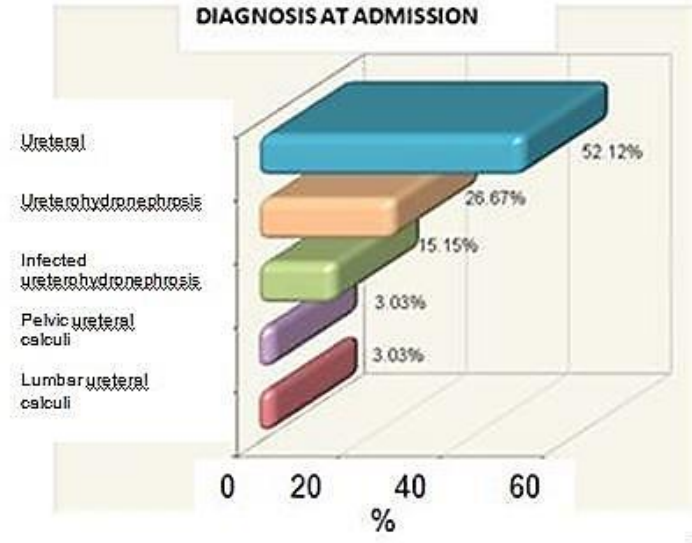

Fig. 2. Distribution of cases based on patient diagnosis

The urological history of pregnant women in the studied group was most frequently represented by the spontaneous elimination of calculi (8.48%) and histological documented lithiasis episode (2.42%).

In our study most, pregnant women did not have a history of urinary disease (89.09%), which shows that pregnancy makes it that the pathological urological aspects that were clinically silent before pregnancy to become clinically manifest.

In this study, the results of the multiple correlations were considered considering

the changes in the admission profile of pregnant women with urological disorders. The insignificant value of the significance level (p) calculated for the intercept variable indicates that besides the factors under study there are no other predictive factors in determining the pursued profile (tab. II, fig. 3).

TABLE II

Multiple correlation of pregnancy age *vs.* clinical aspects on admission

Partial correlation	Coefficient of correlation (Beta)	Std. Err. (Beta)	B	Std. Err. B	t	p *95% interval of confidentiality*
Intercept			2.95090	4.789845	0.61607	0.540818
trimester pregnancy	0.741629	0.106018	3.40026	0.816269	4.16561	0.000132
Renal colic	0.799004	0.131509	3.80784	1.003532	3.79444	0.000423
Microscopic pyuria	0.122716	0.177449	0.13140	0.190001	0.69156	0.492618
Fever, shiver	0.661120	0.109611	2.87819	0.873620	3.29455	0.001879
Bladder	0.075457	0.12871	0.0487	0.83137	0.0586	0.953506

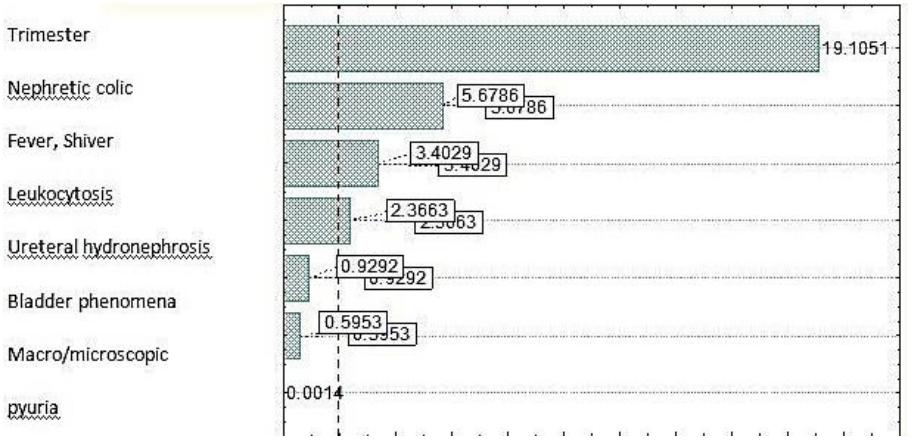

Fig. 3. Statistical t-values in the multiple correlation of the age of pregnancy *vs.* clinical findings on admission

Lumbar pain was located on the right side in 43.64% of pregnant women in this study, while in 18.79% it was bilateral. Left-side location was reported in 35.15%

of cases (fig. 4).

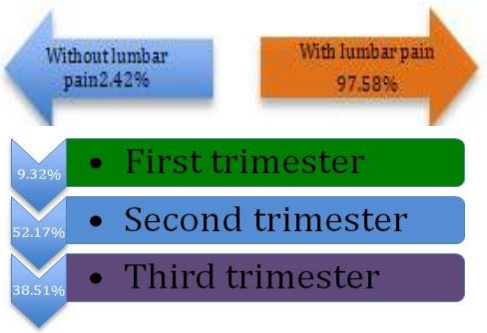

Fig. 4. Clinical aspects on admission - lumbar pain *vs.* trimester of pregnancy

DISCUSSION

Renal colic on admission was particularly noted in patients in the second trimester of pregnancy (52.17%), the results demonstrating a significant correlation between the age of pregnancy and the incidence of lumbar pain ($\chi^2 = 6.14$, $p = 0.036$, 95% CI). The results of the study are consistent with literature data (2).

The statistical data show that the incidence of urological diseases during pregnancy is significantly higher in primiparas women (35.15%) than in the other pregnant categories studied.

The clinical findings on admission have shown a significant correlation with the age of pregnancy, a conclusion demonstrated by the results of the multiple correlation test (3).

In other words, the age of pregnancy significantly influences the clinical aspect of pregnancy-related urological conditions. To highlight the most relevant clinical and laboratory findings depending on gestation age, multivariate analysis was used.

The results of multivariate analysis indicate the predictive factors of the profile of pregnant women with urological disorder and gestational age.

Correlation analysis using nonparametric tests demonstrated the association between the presence of lumbar pain and the trimester of pregnancy by the high value of the non-parametric correlation coefficient and low significance level of the test. In statistical estimations a 95% confidence interval was used.

Ureteral calculi during pregnancy represent not only a diagnostic challenge, but also a management dilemma. Some studies report an incidence of symptomatic urolithiasis during pregnancy of 0.35%, occurring especially in the third trimester of pregnancy (55.5%) (4).

The reported incidence of symptomatic nephrolithiasis during pregnancy varies greatly between 1 in 244 pregnancies to 1 in 1,240 pregnancies (5). The occurrence of urolithiasis during pregnancy is a risk not only for the mother but also for the fetus and may be a factor which contributes to premature birth. The diagnosis during pregnancy is difficult, primarily because of limitations of X-ray use and physiological hydronephrosis during pregnancy (6). In cases requiring invasive treatment, double-J stent or percutaneous nephrostomy may be used, which are the less invasive techniques for urinary drainage. The insertion of a double-J catheter can be performed under control until the end of pregnancy. Percutaneous nephrostomy can also be performed with local anesthesia under ultrasound guidance, realizing an external drainage without irritation of the bladder.

In rare situations where obstruction is bilateral, the colic is accompanied by oliguria or even anuria. These urinary phenomena may be accompanied by reflexive digestive manifestations, namely nausea, vomit-

ing, or ileus which may sometimes be severe. These symptoms may be explained by the direct compression in pregnancy on the celiac plexus and the phenomena of angiogenesis and vascular hypertrophy.

Renal colic is the most common manifestation of acute hydronephrosis in pregnancy and occurs in most cases on the right side. In contrast to colic of lithiasis where there is typically no antalgic position for the patient, in the colic of hydronephrosis the pain improves or even disappears if the pregnant woman is lying on the opposite side to the painful one (7).

During pregnancy, symptomatic urolithiasis associated with ureteral obstruction and upper urinary tract infection is a major urological emergency that may cause perineal urosepsis, or even patient death. Also, the presence of urinary calculi can cause premature rupture of membranes and premature birth in a percentage ranging from 2.9% to 7%, presenting a high degree of risk for the fetus.

The presence of an associated pathology in pregnant women, e.g. hypothyroidism, further complicates the situation and outcomes of treatment, raising doubts about the continuation of pregnancy (8).

These reasons lead to the conclusion that although urolithiasis is a relatively rare condition during pregnancy, its correct approach requires a very good collaboration between obstetricians, urologists, radiologists and anesthetists.

Renal colic during pregnancy can be determined by both acute gestational hydronephrosis and intrinsic obstruction caused by a calculus in the excretory pathway. Differential diagnosis can only be made when the ultrasound directly shows the presence of calculi in the pyelo-ureteral junction or in the first portion of the lumbar ureter. Whatever the cause, renal colic pain should be treated as a matter of urgency. Minimal or non-invasive imaging techniques are preferred. The inflammation of the fascia overlying the neighboring organs can be distinguished by MRI. Anatomical imaging evidence is extremely valuable in differential diagnosis (9,10).

Urological complications in pregnancy are caused by pain and can take the form of uterine contractions and fetal distress. If urine is infected, there may be bacteremia, septicemia, or even toxic-septic shock that may result in fetal and even maternal death. A rare, but very severe complication is the spontaneous rupture of the kidney that is often fatal (11, 12, 13, 14).

CONCLUSIONS

In our study the clinical picture of the patients is almost like the manifestations of other urological diseases; differences that can occur in the disease course and complications could affect both the mother and the fetus. Our statistical data show that the incidence of urological diseases during pregnancy is significantly higher in primiparas women. The imaging investigations should be performed due to the possible harmful effects on the product of conception. In the studied group we were able to make an ultrasound diagnosis so that it was not necessary to use X-rays in any case. The renal colic will be initially treated conservatively by controlled ingestion of fluids, antalgic and antispasmodic drugs, and in the case of hyperalgesicor febrile colic the emergency drainage of the upper urinary tract, most commonly performed by ureteral catheterization or percutaneous nephrostomy.

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NEWS

A NEW APPROACH TO SKIN CANCER DIAGNOSIS: THE ELECTRICAL IMPEDANCE SPECTROSCOPY

In a study by L. Rocha et al. the effect of adding electrical impedance spectroscopy (EIS) measurement at baseline to suspicious melanocytic lesions undergoing routine short-term sequential digital dermoscopy imaging (SDDI) was assessed. Patients with suspicious melanocytic lesions eligible for short-term SDDI were evaluated with EIS measurement which was performed at the first visit following SDDI. Melanocytic lesions with an EIS score ≥ 7 were excised because of the high risk of melanoma while lesions with a score < 7 were monitored with standard SDDI over a 3-month period. The results showed that an EIS score ≤ 3 requires no SDDI and values ≥ 7 require immediate excision of melanocytic lesions reducing the need for SDDI. The study offered a new approach to skin cancer diagnosis and showed that new protocol combining EIS at baseline with SDDI reduced the need to proceed to SDDI in just under half of cases. (Rocha L., Menzies S.W., Lo S. et al. Analysis of an electrical impedance spectroscopy system in short-term digital dermoscopy of melanocytic lesions. *BJD* 2017 Oct. 11. doi:10.1111/bjd.15595).

Ioana -Alina Grăjdeanu

CHRONIC VENOUS DISEASE CASES AT THE IASI DERMATOLOGY CLINIC

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CHRONIC VENOUS DISEASE CASES AT THE IASI DERMATOLOGY CLINIC (Abstract): Chronic venous disease (CVD) is defined as a disturbance of blood return to the deep venous system, superficial venous system, and communicating (perforating) veins. Once present, CVD persists throughout life, so like heart failure it is nowadays considered "a condition for life". Severe CVD of the lower limbs is one of the most common medical problems, affecting up to 25% of the general adult population of industrialized countries. **Aim**: to determine the incidence of CVD among the cases admitted to the Dermatology Clinic of the "Sf. Spiridon" Emergency Clinical Hospital Iasi, CVD incidence by sex, age, area of residence and the main pathogens incriminated in super infection of ulcerative lesions. **Material and methods:** We did a retrospective study of patients admitted to the Dermatology Clinic of the Iasi "Sf. Spiridon" Emergency Hospital over a five years period. **Results:** Of the total number of patients admitted to our clinic (9375), 57% were diagnosed with CVD and 26.61% of them were with CEAP 6 class chronic venous insufficiency. There was an approximately equal sex distribution and most patients (44%) were older than 65 years. **Conclusions: W**e did a large epidemiological study of venous disease, based on revised CEAP classification, showing again that it is not only a very important medical problem, with various clinical manifestation and multidisciplinary approach, but also an important issue for health insurance system. **Keywords:** CHRONIC VENOUS DISEASE, EPIDEMIOLOGY, COMPLICATIONS.

Chronic venous insufficiency (CVI) is not a medical problem exclusive for dermatology, general practitioners, phlebologists, internists also treat patients with this disease. Severe chronic venous disease (CVD) of the lower extremities is one of the most widespread diseases, affecting up to 25% of the general adult population of developing and developed countries (1).

CVI is a common health problem, characterized by symptoms and signs occurring as a result of structural and functional alterations of the veins. Characteristic **symptoms** are heavy, tired, swollen legs, predominantly nocturnal leg cramps, pain and burning sensation in the leg, and itching. **Signs** include telangiectasias, reticular veins, varicose veins, edema, and such trophic skin changes as lipodermato-sclerosis, dermatitis, pigmentation, and, in end stages, ulceration (2, 3, 4). On the basis of this clinical symptoms and signs is made the clinical part of the CEAP classification of venous chronic insufficiency. According to CEAP classifi-

cation venous insufficiency is categorized depending on the severity of signs into 7 classes, C0 to C6, starting with C0 - no visible or palpable sign of venous insufficiency, C1 – telangiectasies or reticular veins, C2 – varicose veins; distinguished from reticular veins by diameter of 3 mm or more, C3 – edema, C4 – changes in skin and subcutaneous tissue secondary to CVD, now divided into 2 subclasses to better define the differing severity of venous disease: C4a – pigmentation or eczema, C4b – lipodermatosclerosis or atrophie blanche, C5 – healed venous ulcer and ending with C6 - varices associated with edema, trophic changes, open chronic leg ulcers. Each of this classes can be subdivided in S – symptomatic or A – asymptomatic. First used in 1994, and revised in 2004, CEAP basis classification is the most important clinical instrument for evaluation of patients with venous chronic disorder, and permits to separate patients with venous disease (C0-C2) from patients with venous insufficiency (C3-C6).

Worldwide, 10-33% of adult women and 10-20% of men have CVI. In the European countries the prevalence is even higher, 20 to 50%. In Romania recent epidemiological studies have shown a prevalence of CVI of 32% (5, 6, 7) in general population.

Factors that hinder venous circulation include: ankylosing arthrites of the knee and leg, plaster casts for lower limb fractures, leg bone malformations, accidental sectioning of lower limb motor nerves, neurological diseases with various motor deficits, oral contraception, etc (8, 9,10). Other important predisposing factors are represented by the association of CVD with cardiovascular diseases, caused and aggravated by alcohol and tobacco use, along with diabetes, obesity and occupations requiring prolonged standing such as: barber, cook, dentist, waiter, housekeeper, and last but not least sedentary life by lack of exercise, walking, outdoor physical activities (8,9,11). Aggravating factors are local infection, local allergy, and autoimmune factors (autoantibodies: against connective tissue, against vessel).

Pathophysiologically, the main deficiency leading to ulcerations and accounting for the difficulties encountered in their healing is the inability to adequately oxygenate tissues. It is an anoxia in a leg with adequate arterial flow. In addition, there is capillary thrombosis developed as a result of stasis, with permanent capillary loss. When the process is slow local fibrosis and sudden necrotic ulceration do occur. Sometimes, on the background of impaired oxygenation, infections or allergic processes develop, which can lead to ulceration if are not treated on time (2, 8, 10, 11, 12).

MATERIAL AND METHODS

This is a retrospective study of 5380 patients admitted to the Dermatology Clinic of the Iasi "Sf. Spiridon" University Emergency Clinical Hospital over a period of five years (January 1, 2009 – December 31, 2013) who met the diagnostic criteria for chronic venous disease. The aim of this study was to determine the incidence of CVD among the cases admitted to the Dermatology Clinic Iasi, the percentage of patients seeking medical attention by CVD stage, CVD incidence by sex, age, and area of residence, predisposing factors, and the proportion of patients in the various CVD classes according to revised CEAP basic classification.

In the patients diagnosed with CEAP class 6 CVI the discharge from ulcer surface was tested bacteriological to determine the degree of colonization of ulcerative lesions by pathogenic microorganisms and their sensitivity/resistance to antibiotics.

RESULTS

Of the 9,375 total number of patients admitted to our clinic over the study interval, 57.38% (5,380 patients) were diagnosed with CVD (fig. 1).

The percentage of patients hospitalized for CVD is significantly higher than patients hospitalized for other pathology - t calculated is 20.5 higher than t tabulated for p 0.001. Relative risk (RR) calculated for patients in the study is 1.2219 (95% CI: 1.1969 to 1.2474), with a z statistic of 19.04.

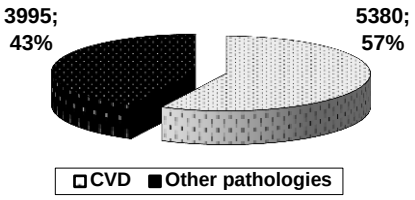

Fig.1. Ratio total admitted patients/CVD patients

From all the patients included in the study, CVD CEAP class 3 was the most common (2,089 patients, 38.83%), followed by CVI classes 6 with 1432 patients (26,61%) CVI class 4 with 1132 cases (21.04%), respectively.

A surprising thing is that throughout the study only 13 patients with CVD class 1 were diagnosed, suggesting that this common disease, with severe consequences, is under diagnosed in the early stages, when preventive measures, hygiene/dietary measures have maximum effect on disease course (fig. 2).

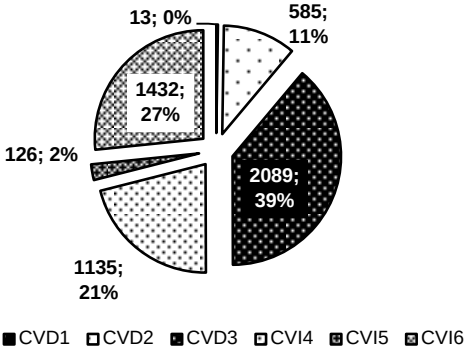

Fig. 2. Distribution of all patients by CEAP stage

The distribution of the patient by CEAP classes is almost the same in the years that we study, the number of cases being around the medium value of all cases introduced in our study (fig. 3).

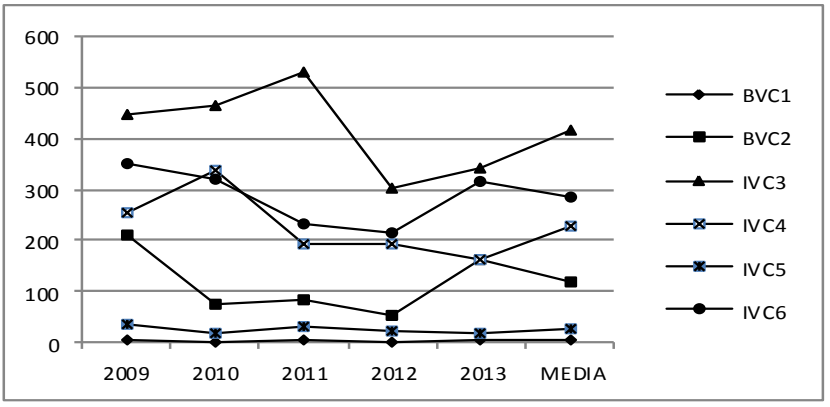

Fig. 3. Distribution of patients by CEAP stage and year

In absolute numbers, of a total of 9,375 patients hospitalized during the study interval, 5,380 had CVD in different stages, of which 1432 CVI CEAP class 6, accounting for 15.37% of all admitted patients in the Dermatology Clinic in the studied period (fig. 4).

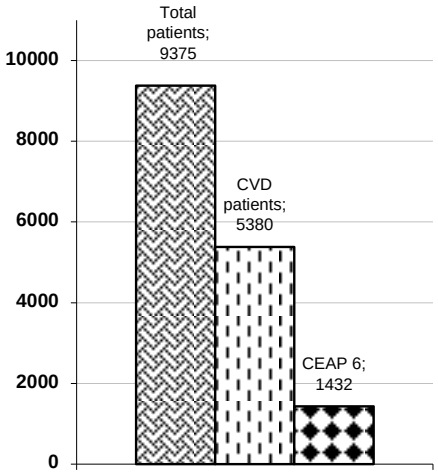

Fig. 4. Ratio total patients/CVD patients/ CVI 6 patients

According to new CEAP classification, from the total number of 5380 patients diagnosed with chronic venous disorder, 598 patients (11.12%) were with chronic venous disease and 4,782 patients (88.88%) with venous chronic insufficiency. As we can see the distribution of patient in venous chronic disease and insufficiency is mainly in the group of venous chronic insufficiency, showing that the prophylactic measures, dedicated to patients in venous disease, for preventing the evolution of pathophysiological changes that lead to irreversible changes in the venous system and the installation of the venous insufficiency are insufficient.

In our study the distribution of patient by sex shows approximately equal rates, although data in the literature show an increased rate of chronic venous disease among female patients (fig.5). Worldwide, 10-33% of adult women and 10-20% of men have CVI. In European countries the prevalence among women is even higher, ranging from 20 to 50% (4, 5, 6, 8).

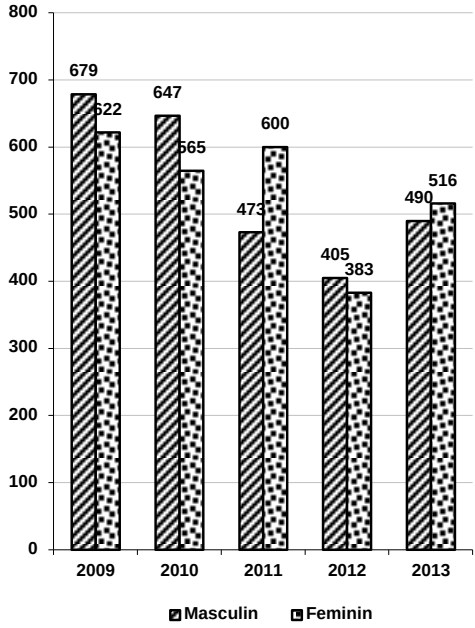

Fig. 5. Number of patients by year and sex

According to the area of residence, most patients (56%) (fig. 6) came from rural areas, probably accounted for by their lifestyle, and also by a delay in seeking medical attention in the early stages of disease, inadequate implementation of primary prevention methods, methods that overlap with general public health education, our data being consistent with the literature data.

As to age group distribution, most patients (44%) were older than 65 years, followed by age group 46-65 years (37%), and

15-45 years (19%), our data being consistent with the literature (fig. 7).

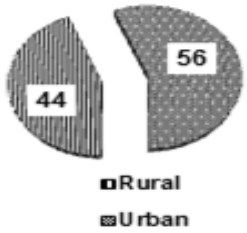

Fig.6. Distribution of patients by area of residence

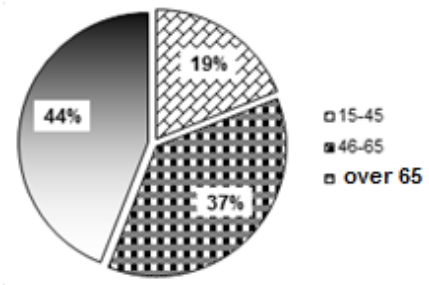

Fig. 7. Age-group distribution of CVD patients

The following predisposing and aggravating factors were identified: genetic predisposition, history of vein thrombosis, obesity, smoking, diabetes, etc.

Given the data resulting from the epidemiological study and their distribution by clinical forms of the disease according to CEAP staging, we focused on patients with CVD CEAP class 6, patients with severe complications, with a major impact on their quality of life, but also on the costs for the healthcare systems, which are being very high.

One of the serious complications of CVD CEAP class 6 is the infection of ulcerative lesions with various pathogens. In descending order of frequency, the main pathogens incriminated in ulcer infections were *Staphylococcus aureus*, *Pseudomonas aeruginoasa*, and methicillin-resistant *Staphylococcus aureus* (MRSA), order maintained throughout the 5 years of study (fig. 8).

Referred to resistance to antibiotics, gentamicin comes first, followed by ciprofloxacin and amoxicillin with clavulanic acid, because of topical and systemic antibiotic abuse, which is a worldwide problem. However, in our study ciprofloxacin proved to be the most sensitive (fig. 9), followed by colistin and teicoplanin (fig. 10).

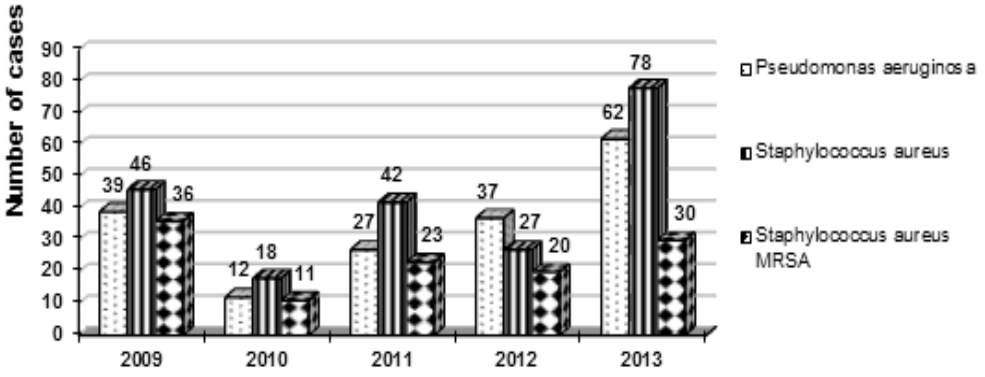

Fig. 8. Most frequent pathogens incriminated in CVI class 6

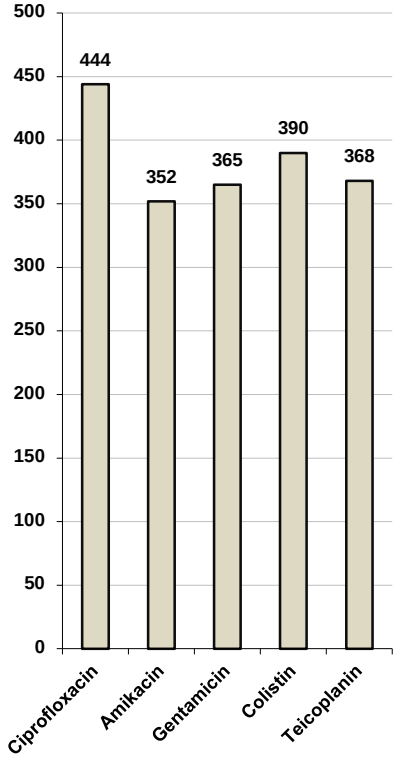

Fig. 9. Antibiotic sensitivity

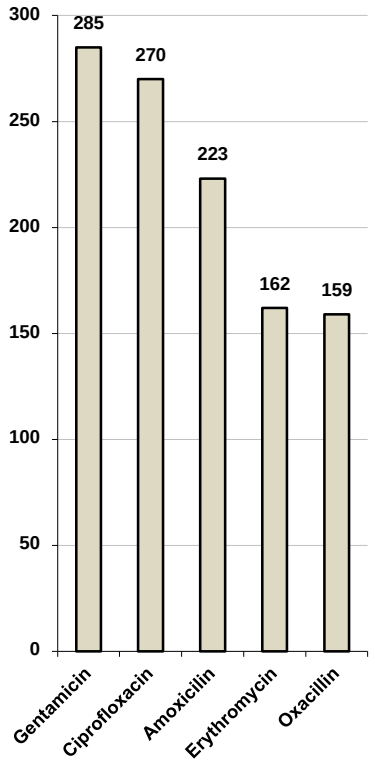

Fig.10. Antibiotic resistance

RESULTS AND DISCUSSION

Data from the present study indicate that chronic venous disorder still raises a serious problem for the healthcare system, even exist a lot of programs dedicated to this disease. Namely, it appears that many patients do not seek medical attention when the disease is in early stages (most patients presenting with classes 3 and 6 according to CEAP classification). The distribution of patients by CEAP classes shows a distribution of the patients mainly in the venous chronic insufficiency (88.88%). Distribution by gender and area of residence was uniform, unlike the worldwide statistical data according to which the disease is more common in females, with a predominance of patients originating from rural environment.

People over the age of 65 were most frequently affected, followed by age groups 46-65 and 15-45 years.

Worrying is the small percentage (11.12%) of patients diagnosed in stage 1 of disease, and also in the group of venous chronic disease (CEAP0-2), these patients being those who can benefit the most from preventive methods, a proper lifestyle, measures that determine a slow progression to advanced disease, and preserve the patient quality of life and ability to work. If prevention programs should be entered correctly if patient education as one of the main goals of primary health care, then we could tip the balance toward those with chronic venous disease.

The main pathogens isolated from ulcerative lesions were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and MRSA, agents that show resistance to common antibiotics due to their improper use.

CONCLUSIONS

The venous chronic disorder is one of the most common health problems meet in clinical practice, not only in dermatology, but also in general practice, family medicine, internal medicine, cardiology, vascular surgery etc. It is an important problem for the healthcare system because of the very high costs associated with long hospital stay. As this study shows, these problems can be reduced by proper preventive measures, by early identification of the CVD, by correct treatment introduced from the very first stages of the disease.

Detection in early stages, regular and thorough check-ups in patients with genetic predisposition, is the first steps to reducing the incidence and prevalence of CVD in the general population. These issues can be positively influenced by effective prevention methods implemented in the early stages of the disease and by patient education and training.

CVD is currently a very important medical problem that requires multidisciplinary approach (family medicine, dermatology, surgery, internal medicine, etc.), in the literature being regarded as the second most common condition after arterial hypertension among cardiovascular diseases. As the study shows, the prophylaxis and correct treatment of patients in early stages can reduce the percent of patients with chronic venous insufficiency, having a positive impact in the hospitalization rate and costs for the healthcare system.

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NEWS

FIBROMUSCULAR DYSPLASIA OF CARDIAC CONDUCTION SYSTEM ARTERIES IN TRAUMATIC AND NON-NATURAL SUDDEN DEATH VICTIMS AGED 0 TO 40 YEARS: A HISTOLOGICAL ANALYSIS OF 100 CASES

Luminal narrowing by fibromuscular dysplasia of the sinus and/or AV-node artery was found in examinations of unexplained deaths and, in many cases, was considered as the cause of death. There are no data on the frequency of fibromuscular proliferations of arterial walls in heart healthy control groups. In numerous case reports, histological evidence of fibromuscular proliferations of arteries of the cardiac conduction system has been suspected as the cause of death when the autopsy and additional investigations could not determine another cause. Cardiac arrhythmia due to ischemia is proposed to be the lethal pathomechanism. While there is an uniform nomenclature for fibromuscular dysplasia as a disease entity of noncardiac arteries, there are several terms for fibromuscular proliferations of cardiac arteries that are only microscopically detectable, e.g., “fibromuscular hyperplasia”, “occlusive lesions of cardiac conducting tissue arteries”, and “small coronary artery disease”. All of the described alterations of the vessel walls appear as a fibromuscular dysplasia. However, systematic histological investigations have rarely been described. For many forensic pathologists and pathologists, it was clearly evident that fibromuscular proliferations of arterial walls are widely spread in a healthy age group of 0 to 40 years. The assessment of the histological findings in cardiac arteries as a cause of death has to be seen critically since functional causes might also be responsible. Furthermore, the diagnosis of “fibromuscular dysplasia of coronary arteries” should not be confirmed without a macroscopic finding. Since fibromuscular proliferations of arteries of the cardiac conduction system and small coronary arteries occur in 84% in an obviously heart-healthy control group (n=100), these findings should be known to every (forensic) pathologist and anatomist. This might reduce the risk of misdiagnosis. The cause of these fibromuscular vessel alterations is still unclear. Possible hypotheses might be either the consequence of the impact of the bloodstream on small vessel branches or a protective mechanism against luminal extension. The term “fibromuscular dysplasia” should not be used for a thickening of coronary arterial walls that can only be seen microscopically. In addition, Zack et al proposes the establishment of a specific nomenclature for such changes (e.g., fibrous and muscular proliferation of arterial wall tissue) (Zack F, Kutter G, Blaas V, Rodewald AK, Büttner A. Fibromuscular dysplasia of cardiac conduction system arteries in traumatic and non-natural sudden death victims aged 0 to 40 years: a histological analysis of 100 cases. *Cardiovascular Pathology* 2014; 23: 12–16.

Doina Butcovan

RARE COMPLICATION OF THE CERVICAL SPINE TRAUMA – TRAUMATIC ESOPHAGEAL FISTULA: CASE REPORT AND REVIEW OF THE LITERATURE

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RARE COMPLICATION OF THE CERVICAL SPINE TRAUMA–TRAUMATIC ESOPHAGEAL FISTULA: CASE REPORT AND REVIEW OF THE LITERATURE. (Abstract): **Background and study aims**: Esophageal fistula due to cervical spine trauma is a rare, potentially fatal complication, which changes significantly the treatment and the prognosis of the trauma patient. There is scarce data regarding this associated pathology of cervical trauma and it consists only of isolated cases, most of them being reported as a complication of the surgical treatment. We present the case of a female patient who suffered a fall from a wagon and who was diagnosed at presentation with cervical fracture and esophageal fistula. Patient: A 65 years old female who suffered a fall from a train wagon presented for intense neck pain and bilateral brachial paresis. The MRI exam showed a C6-C7 fracture with bilateral facet dislocation in the context of a degenerative, spondylotic cervical spine. It also showed an anterior fragment from the C7 body, in contact with esophageal adventitia. The patient underwent surgery. **Results:** a C6 corpectomy was performed, with arthrodesis with bone graft and cervical plate fixation. 24 hours after the procedure, the clinical signs of an esophageal fistula appeared and a CT-scan revealed the typical aspects of that complication. The patient underwent a second surgery in collaboration with the general surgeon with a good outcome and complete neurological recovery. **Conclusion:** in the case of cervical spine trauma the imaging exploration has to look for potential signs of lesions to the neighboring vital structures. Although a rare complication of cervical trauma, esophageal fistula represents a formidable complication and requires immediate surgical sanction, the only factor decisive for a good prognosis. **Keywords:** ESOPHAGEAL FISTULA, CERVICAL SPINE SURGERY COMPLICATIONS

Cervical spine trauma represents a devastating event in the life of the patient especially if associated with lesion of the spinal cord. The presence of an associated lesion of the vital structures neighboring the spine makes an already difficult case even more difficult with direct impact on the outcome. Anterior approaches of the cervical spine are largely used for various pathology of the cervical spine and they are considered as safe procedures useful for both decompression of the neural structures and for the inter-body fusions (1, 2). An 8 to 12% rate of complications has been reported by various studies. Esophageal perforation is one of the rarest of all complica-

tions (0.1%) (3). Being so rare there is virtually no individual experience, especially in the treatment which is very controversial and specific to every particular case. The largest series we found is the one reported by Gaudinez in 2000 (3), which included 44 patients admitted in 25 years in a center dedicated for spinal injuries and a second one published by Newhouse who reviewed the data of the Cervical Spine Research Society and he found 22 cases (4). We present our experience with a 67 years old patient operated for a cervical C6-C7 fracture with facet dislocation complicated with esophageal fistula diagnosed in the early postoperative period.

CASE REPORT

A 67 years old woman who suffered a fall from a train wagon, presented in the emergency department of our hospital, complaining of intense neck pain, bilateral brachial paresis, left cervico-brachial neuralgia. Plain radiographs of the cervical spine were made but these showed no obvious traumatic lesions between C1 and C6. Because of the significant clinical picture it was decided to perform a cervical spine MRI which showed a C7 fracture with bilateral facet dislocation C6-C7 and a narrowed spinal canal in the presence of the retropulsed bone fragment from C7 and spinal cord compression with intramedullary hyperintensity on T2 weighted sequences (fig. 1). The patient underwent surgery: anterior corpectomy C7 with the high-speed drill, followed by an inter body fusion C6 to T1 using an iliac bone graft and anterior plate fixation. There were no incidents during surgery. Given that a good reduction wasn't possible without a high risk, we preferred to perform C7 corpectomy.

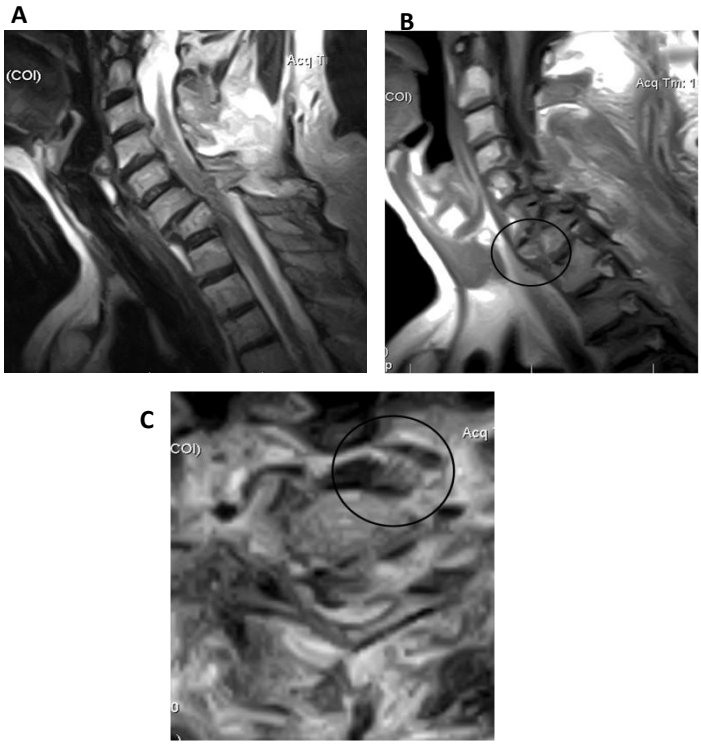

Fig. 1. Preoperative MRI findings showing
A) C7 fracture with bilateral facet dislocation C6-C7,
B) The bone fragment that we found in relation with the site of the perforation,
C) Axial section revealing the bone fragment apparently in relation with esophageal adventitia

Immediately postoperatively the patient performed very well, she was discharged from the intensive care unit, the neck and brachial pain disappeared and she remained with some mild dyparesis. Two days later she developed dysphagia, fever, subcutaneous emphysema of the neck and face, tension at the level of the operatory wound. An emergency CT-scan was performed and it showed the large emphysema and the esophageal defect at the level of the lesion, as well as laryngeal edema (fig. 2).

An emergency reintervention was performed and we found a small bone fragment inserted in the esophagus on his left side (opposite to our operatory view), with a small defect at the adventitia level. Two drainage systems were left in place, a nasogastric tube was inserted and parenteral large spectrum antibiotic therapy (including Imipenem and Metronidazole) was initiated.

The bacteriological results from the operatory wound revealed the presence of gram negative germs sensible at Imipenem. For a week after the second surgery the evolution was good yet, in the eighth day the local inflammatory changes reappeared, with fever and a mild superior digestive hemorrhage and the patient was reoperated. This time an esophageal suture was performed, and a gastrostomy feeding tube was left in place. After the third surgery the evolution was favorable without further complications. The patient remains though, with a permanent gastrostoma.

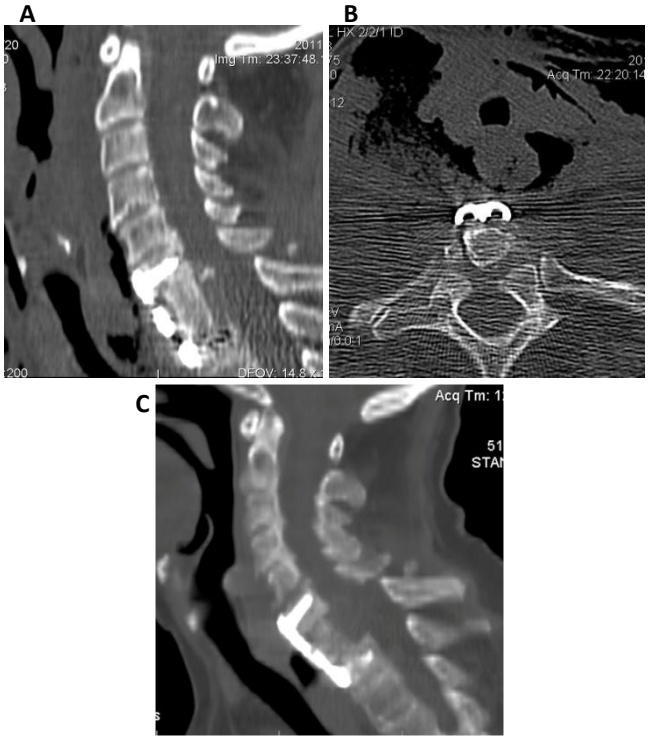

Fig. 2. A and B) CT-scan showing the emphysema in relation with the pre-vertebral plan around the esophagus and in the operatory field with the plans dissected.
C) Control CT-scan, at one month, revealing remnant peri-esophageal emphysema

DISCUSSION

Anterior cervical approach, also known as Smith-Robinson approach, is in our days a very well known procedure which is a part of the surgical arsenal of any neurosurgeon dealing with cervical myelopathy or radiculopathy. The complication rates are variable between a medium of 10% and 19.3% as reported by Fountas (5) in a study on 1015 patients (196 complications).

The authors of this study found the dysphagia as the most common complication (9.5%), followed by postoperative hematoma (5.6%), esophageal fistulas being observed in 0.3% of cases. The list of possible other rare, anecdotal complication may be very large, as they are presented in various publications (5, 6, 7, 8), including carotid artery injury, vertebral artery injury, pneumothorax, glossopharyngeal, facial, hypoglosal paresis, lesions of the cervical plexus, epidural hematomas. Concerning the esophageal fistulas, they appear in different statistics in a range between 0% (6) and 1.62% in a study of Elerkay (9) in patients who underwent corpectomies, like our patient. There are not clearly identified predisposing factors but the patients with cervical spine fracture, esophageal diverticula or reflux disease (10), or who have had a corpectomy (9) might have some additional risk. There are not many protective measures, but what we can do is to visualize well and respect the esophagus during high-speed drilling and at the time of the instrumentation and care must be taken to the placement of the internal retractor blade under the *longus colli* muscle.

We know, as surgeons, that even in the most careful procedure, accidents may happened, but it's important to recognize and to react promptly in face of a very rare situation, any delay or hesitation being associated with a poor outcome. Esophageal perforations occurring after a cervical spine fracture or as a complication of the surgical procedure is one of these very rare conditions, but they are potentially life threatening. The management of this extremely rare condition is not standardized and individual surgeon experience is poor. The diagnostic is mainly on clinical basis, the patient complaints are dysphagia, neck pains, subcutaneous emphysema, hoarseness, fever.

We can discover the complication during the operation or the onset can be acute, in the first 24 hours, sub acute, after 24 hours, or delayed, there is a report of a fistula discovered at 10 years (10).

We did not concluded clearly if, in our particular case, the esophageal perforation was traumatic or iatrogenic, but we consider that the most important is not why that happened but what you have to do when that happened. Most of the authors agreed that the treatment is case adapted. If the lesion is discovered during the surgery it is better to suture primary with protection with large spectrum antibiotics and insertion of a nasal-gastric tube.

The fistulas discovered early after surgery, like our case, have to be explored and the operatory field drained, and we consider now that the suture might be an option, because, even in a small defect, like we experienced, the evolution with drainage, esophageal rest with NG tube, and antibiotics (option indicated by many authors), was not very satisfactory and we were forced, finally, to suture.

We had the impression that the perforation was significantly larger when we reopened. In cases of large defects the suture is indicated and some authors recommended reinforcing the closure with

omental, pectoral or sternocleidomastoid flaps (11, 12).

CONCLUSIONS

Esophageal fistula associated with cervical spine trauma is a very rare complication, but the consequences may be tragic, the evolution can complicate with a mediastinitis or septicemia, that can be fatal.

The predisposing factors are not clearly identified, but the corpectomy, instrumentation, spondylotic changes, esophageal diverticula or gastro-esophageal reflux appear to play a role.

We present a case of a perforation discovered in the early postoperative period and our experience in that case make us to advocate an aggressive attitude, so to suture the defect, even it appear to be small.

The great majority of these complications being iatrogenic, we emphasize once again the role of a meticulous dissection, and anyone of us has to think, during the anterior cervical surgery, at this complication which can devastate a perfectly curable patient.

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POSSIBLE PROGRESSION OF SUB ACUTE LUPUS ERYTHEMATOSUS - CASE REPORT

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POSSIBLE PROGRESSION OF SUBACUTE LUPUS ERYTHEMATOSUS- CASE REPORT (Abstract): Subacute lupus erythematosus (SLE) is a specific form of lupus erythematosus characterized by prevalently cutaneous manifestations usually with a good prognosis. It is more common in patients aged 15 to 70 years, and there is a female predilection. This form accounts for 10% of all lupus erythematosus cases. We present the case of a 57-year-old male patient diagnosed at age 35 with chronic psoriasiform subacute lupus erythematosus, pathologically confirmed at the Iaşi Dermatology Clinic. At the age of 54 years he had multiple ischemic strokes, followed by deterioration of general status, and at 56 years deep vein thrombosis in the right leg. The patient presented the erythematous-squamous lesions specific to psoriasiform SLE localized both on the upper third of the body and knees and associated with submucosal lesions of the lower lip, oral mucosa and appendages. The patient also presented hypo- and hyperpigmentated atrophic scar-like lesions. Laboratory tests performed during the last two admissions showed the presence of anti-ds DNA and antiphospholipid antibodies, inflammatory syndrome, and nitrogen retention syndrome. Treatment consisted of systemic and local dermatocorticoids and associated medication, emollient lotions and creams with SPF 50+, with slowly favorable progression. The peculiarity of the case lies in the chronic progression without significant systemic involvement for 19 years, and then in 2 years the antiphospholipid antibody syndrome and a shift to systemic lupus erythematosus to occur. **Keywords:** SUBACUTE LUPUS ERYTHEMATOSUS, SYSTEMIC LUPUS ERYTHEMATOSUS, ANTIPHOSPHOLIPID ANTIBODY SYNDROME.

Subacute lupus erythematosus (SLE) is a specific form of lupus erythematosus characterized by prevalently cutaneous manifestations usually with a good prognosis. It is more common in patients aged 15 to 70 years, and there is a female predilection (2). This form accounts for 10% of all lupus erythematosus cases (1).

It is characterized by the presence of two clinical forms: annular and psoriasiform.

The etiologic factors involved in SLE

occurrence:

• Genetic predisposition: more commonly affected were found to be the HLA B8 and DR3 individuals.

• Immunological factors: in 80% of cases there is an association with the presence of SSA antibodies, and in 60% of cases with the presence of antinuclear antibodies. Some patients may also present a C2 complement deficiency.

• The environmental factors involved in the occurrence of subacute lupus erythematosus are represented by exposure to UV radiation, some drugs (thiazide diuretics, calcium blockers, statins, angiotensin converting enzyme inhibitors, Terbinafine, Griseofulvin etc), radiation therapy, PUVA treatment for psoriasis (1, 7, 8, 9).

CASE REPORT

We present the case of a 57 year old male patient from a rural area, ex-smoker and former chronic ethanol consumer (until about 2 years ago), known to have SLE since 1992. His past medical history included many diseases aggravated by tobacco consumption (chronic obstructive bronchopneumonia, pulmonary emphysema, chronic bronchitis caused by cigarette smoking, obstructive arteriopathy of the lower limbs) and cardiovascular diseases (essential hypertension, chronic venous insufficiency, strokes, deep vein thrombosis).

The patient was known to have sub acute lupus erythematosus since age 35, pathologically confirmed both in 1994 and in 2011, with chronic progression due to incorrectly applied treatment, drug regimen (Prednisone), and therapeutic non-compliance related to chronic ethanol consumption. After 19 years of sub acute lupus erythematosus evolution, at the age of 54 years the patient had multiple strokes, resulting in general health deterioration. The patient did not come to the Dermatology Clinic for clinical, biological and therapy reassessment visits for 2 years. At age 56 years he developed deep vein thrombosis of the right leg.

Physical examination revealed a vicious position of the lower limbs with genu valgum, atrophy of the left leg, related to left hemiparesis. Pulmonary auscultation revealed sibilant and ronflant rales disseminated on both pulmonary areas.

Dermatological examination revealed erythematous-squamous plaques, well-delimited, with irregular contours, covered by gray scales, multilayered, adherent, and postlesional achromic plaques, well-delimited, with irregular contours, located on the anterior and posterior thorax, upper limbs, knees, face (fig. 1).

Fig. 1. Cutaneous erythematous-squamous and atrophic lesions on the arm

The patient presented both mucosal and submucosal changes consisting of erythematous plaques covered by scales on the lips and erosions surrounded by inflammatory halo on the hard palate, near the molars and changes in skin appendages, nail plate with rough, rugged, irregular, matte surface, with white surface areas, some of them with subungual keratosis deposit, periungual erythema and hyperkeratotic cuticles (fig. 2).

Fig. 2. Ungual and periungual changes

Laboratory tests revealed blood glucose levels within normal ranges until 2013, when a diagnosis of diabetes mellitus type 2 is made and insulin treatment is initiated. Another finding was an alteration of renal function with the occurrence of nitrogen retention and hepatic cholestasis phenomena. Immunologically, the patient had high anti-dsDNA antibody levels upon admission in 2011 and June 2013 which returned to normal with adequate treatment. Although SSA antibodies are specific to subacute lupus erythematosus, the patient showed levels within normal ranges. On two occasions he had high levels (twice the normal value) of antiphospholipid antibodies.

Pathological examination showed a skin fragment with uneven epidermis, with atrophic areas covered by a crust of orthokeratosic keratin associated here and there with plugging of follicular ostia. The granular layer was prominent and papillary dermis contained moderate lymphomonocytic inflammatory infiltrate with isolated appearances of interface dermatitis and rare Civatte bodies (fig. 3). The histopathological appearance corresponds to a lupus lesion.

Direct immunofluorescence was performed on both lesional and healthy skin, and immunnoglobulin G and fibrinogen deposits in the basement membrane were found (fig. 4).

Cardiology consultation detected non-obstructive hypertrophic cardiomyopathy associated with parietal kinetic disorders on echocardiography and left axillary pachypleuritis on chest radiograph.

Given the presence of nitrogen retention a nephrology consultation was requested. The ultrasound showed small kidneys and in association with the absence of proteinuria, the laboratory changes were interpreted in the context of an antiphospholipid antibody syndrome-related ischemic nephropathy.

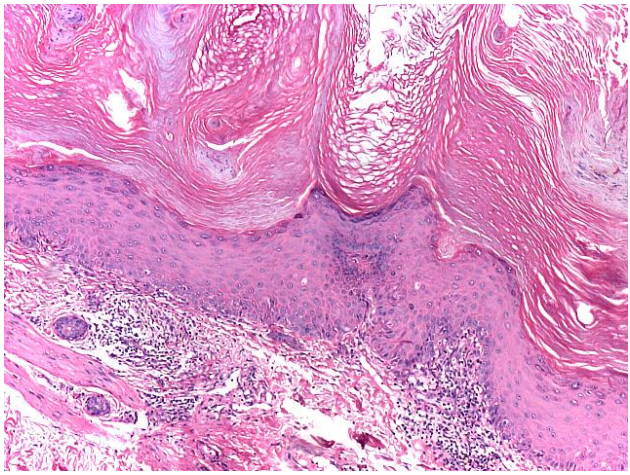

Fig. 3. Histopathologic appearance

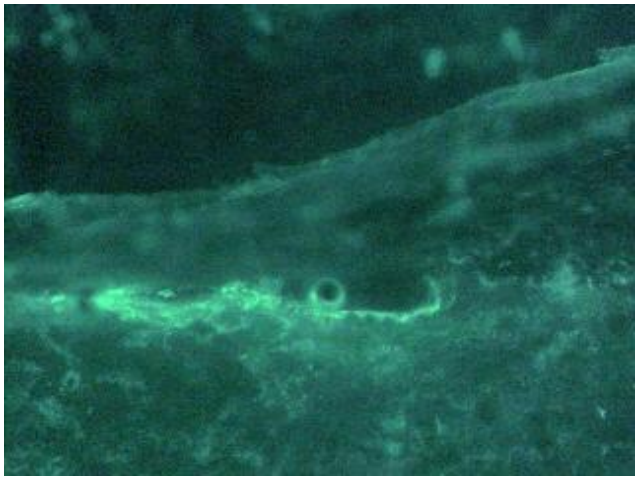

Fig. 4. Presence of IgG in the basement membrane

Based on physical examination and laboratory test results a diagnosis of psoriasiform subacute lupus erythematosus progressed to systemic lupus erythematosus associated with antiphospholipid antibody syndrome was made.

Arguments in favor of subacute lupus erythematosus progression to systemic lupus erythematosus are related to the biological changes, consisting of nitrogen retention, inflammatory syndrome, and presence of anti-dsDNA antibodies, association of antiphospholipid antibody syndrome, multiple strokes, and deep vein thrombosis. Systematization of lupus erythematosus is also demonstrated by kidney and cardiac involvement, positive direct immunofluorescence both on healthy and lesional skin, and therapeutic test, the renal function improving with adequate cortisone therapy.

The therapeutic scheme included topical

dermatocorticoids associated with mechanical and chemical photoprotection, and systemic treatment with Prednisone 15mg/day in combination with Imuran 50mg/day, oral anticoagulant and treatment of associated disorders. Under treatment the evolution was slowly favorable, the patient requiring periodic clinical and biological monitoring.

DISCUSSION

Subacute lupus erythematosus is a form of lupus erythematosus characterized by annular or psoriasiform skin lesions located above the waist (neck, trunk, external aspect of the arms) (7, 8).

Patients with SLE present criteria for systemic lupus erythematosus: photosensitivity, arthralgia, serositis, kidney disease, serologic abnormalities. 50% of SLE patients progress to systemic lupus erythematosus (2).

A study conducted in Philadelphia - USA concluded that of the population of patients with cutaneous lupus erythematosus most do not develop systemic lupus erythematosus, and those who progress to this condition require medium to large doses of dermatocorticoids and immunosuppressive drugs because the systemic manifestations are not significant (10). It was also found that there is an average of 8.03 years between the diagnosis of cutaneous lupus erythematosus and the progression to systemic lupus erythematosus (10).

A study of 44 patients diagnosed with SLE showed the presence of anticardiolipin antibodies in 16% of cases and in 11% the association with antiphospholipid antibody syndrome (3).

Antiphospholipid antibody syndrome is characterized by the production of antibodies against phospholipid binding protein which have a role in coagulation. To make the diagnosis of antiphospholipid antibody clinical criteria were developed (presence of vein thrombosis, pregnancy complications) and laboratory criteria (presence of anticardiolipin antibodies anticoagulant antibodies, elevated anti-β 2 glycoprotein antibodies levels in two or more samples 12 weeks apart) (4).

Depending on the organ affected by thrombus, and the severity of blood obstruction, permanent damage or death can result. The most common clinical manifestations of antiphospholipid antibody syndrome are renal failure and stroke, both due to reduced blood flow to the respective organ. Cardiovascular damage can occur with deep vein thrombosis, deep venous insufficiency and valvular heart disease, and pulmonary damage with pulmonary hypertension and pulmonary embolism. Pregnant women who develop antiphospholipid antibody syndrome may have complications such as miscarriage, preterm birth, preeclampsia (5, 6).

CONCLUSIONS

The presented case is particular due to the fact that psoriasiform skin lesions are located both above the waist and on the knees. Although this form of lupus erythematosus is characterized by the presence of anti-SSA antibodies, the patient had values within the normal ranges at each assessment. The disease had a chronic course without significant systemic involvement for 19 years but because of therapeutic non-compliance, incorrect administration of Prednisone treatment, chronic ethanol consumption, and disregarding photoprotection the patient developed systemic lupus erythematosus associated with antiphospholipid antibody syndrome.

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NEWS

THE PARTICULARITIES OF TREATMENT FOR PATIENTS COINFECTED HIV/TB

In 2012, globally, 13% of people who developed TB were HIV positive. Antiretroviral therapy (ART) reduces morbidity and mortality from TB and reduces by 65% the risk of developing the disease. But, against initiating ART in patients with TB treatment pleads the development of immune reconstitution inflammatory syndrome (IRIS), pharmacological interactions and the large number of pills administered. Influence of the time interval between initiation of antiretroviral therapy (ART) and initiation of treatment for tuberculosis (TB) on patients co-infected HIV/TB has been the goal of many prospective and retrospective studies. CAMELIA study in Cambodia showed that initiating ART within two weeks of starting TB treatment improved survival in severely immunosuppressed patients (CD4 T cells <50/μL). STRIDE global survey and study SAPIT South Africa have shown a decrease in mortality when ART is initiated at the beginning of TB treatment versus initiation at the later stages or after completion of TB therapy. In another retrospective study, Han and collaborators showed that mortality was higher for those diagnosed with TB during ART, but did not reveal differences in prognosis for patients with ART initiation in less than 90 days after starting treatment for TB and those whom ART was initiated late or not started. Based on the results of these studies, guidelines World Health Organization recommends that initiating ART should be made as soon as possible within the first 8 weeks after beginning TB treatment or within 2 weeks for severely immunosuppressed patients.World Health Organization. Global tuberculosis report 2013 (Han Sh, Zhou J, Lee M *et al*. Prognostic significance of the interval between the initiation of antiretroviral therapy and the initiation of anti-tuberculosis treatment in HIV/tuberculosis-coinfected patients: results from the TREAT Asia HIV Observational Database. *HIV Med*. 2014; 15(2):77-85).

Cătălina Luncă

THREE SYNCHRONOUS PRIMARY PELVIC CANCERS – A CASE REPORT

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THREE SYNCHRONOUS PRIMARY PELVIC CANCERS – A CASE REPORT (Abstract). The occurrence of synchronous primary gynaecologic malignancies is a relatively common event. However, the occurrence of three different pelvic cancers is very rare. In this report, we describe the clinical, surgical and pathological findings of a patient with synchronous primary malignancies of the fallopian tube, endometrium and sigmoid colon. To our knowledge, it is the first case described in the literature with such an association of primary synchronous cancers. **Keywords**: SYNCHRONOUS, CANCER, GYNAECOLOGICAL, ONCOLOGY, ENDOMETRIAL, ADNEXAL

Synchronous occurrence of endometrial and adnexal (ovarian or tubal) cancer in female genital tract is a well known event in gynaecological oncology. They may indicate either metastatic or independent neoplasms and the clinical or therapeutic implications and prognosis are very different in each occasion. Compared to metastatic dual cancer, two simultaneous primary cancers are relatively rare and can be easily recognized if the histologic types of each cancer are different. Even more, the occurrence of a pelvic third cancer is an extremely rare event.

The aetiology of synchronous malignancy is uncertain but it has been postulated that embryological similar tissues of the female genital tract may develop synchronous neoplasms when simultaneously subjected to carcinogens(1,2).

Recently, we experienced three primary cancers occurring in both tubes, endometrium and sigmoid colon with three different histologic patterns. This case, along with the diagnosis and the treatment of synchronous female genital malignancy, will be briefly reviewed.

CASE PRESENTATION

In April 2013, a 61 years old white female, gravida 1, para 0, was referred to our clinic with the diagnosis of endometrial cancer and a possible synchronous ovarian cancer. She was menopausal since age of 50 and she described a vaginal bleeding started 3 months before. Her past medical and surgical histories were relatively unremarkable, but her family history revealed a duodenum cancer for her mother, a colon cancer for her father and a stomach cancer for her grand-father. A clinical and ultrasound examination in March 2013 revealed

the uterine origin of bleeding, a normal volume uterus but with a 18 mm thick endometrium with a polyp-like image without apparently myometrial invasion and multiseptated, 111x97x90-mm mass having both solid and cystic components in the right ovary, suggesting malignancy ; the left ovary looked normally. A pelvic computed tomographic scan confirmed the existence of the previously mentioned multiseptated cystic right ovarian mass and minimal ascites in the pelvic cavity. In addition, there were no abnormal findings in the abdominal cavity and thorax, with no extraperitoneal enlarged lymph nodes. She underwent in April 2013 a diagnostic uterine curettage under anesthesia in a private hospital, revealing a grade 3 endometriod adenocarcinoma of the endometrium. The CA-125 was elevated 141.8 U/ml. Routine blood test investigations were normal. The clinical diagnosis was stage IA, grade 3 endometrial carcinoma and a synchronous (primary or metastatic) ovarian cancer.

At laparotomy, a right adnexal tumour involving both tube and ovary, of 11x10x9 cm, but mobile, with smooth surface and both solid and cystic parts was discovered. The frozen section revealed malignant tissue. There was a small amount of ascites in the abdomen. The left ovary and the uterus appeared normal. On the sigmoid colon, a tumour producing bowel stenosis and a retraction of the serosa, very suggestive for malignancy, was discovered. We performed a total abdominal hysterectomy with bilateral adnexectomy, pelvic and paraaortal lymphadenectomy, total omentectomy, appendectomy, recto-sigmoid colon resection (about 20 cm) and peritoneal biopsies. The whole procedure lasted 275 minutes. There were no intraoperative complications. The postoperative recovery was uneventful, and she was discharged home after hospitalisation for 9 days.

The final pathology report described a high-grade (MD Anderson grading system) serous adenocarcinoma of right adnexa, involving both tube and ovary; its origin could not be detected, but contained different microscopic patterns: solid, papillary, cystic, micropapillary and glands (fig. 1). In the left tube, a high-grade serous adenocarcinoma involving the mucosal and muscular layer, but without serosal involvement was found. The lefy ovary was microscopically normal. For these reasons, we consider also the right adnexal tumour of tubal origin. The cytology of the ascites revealed malignant cells. There were no metastases into the appendix, peritoneum, omentum and in the 25 from the right and 35 from the left side pelvic and from the 55 paraaortal lymph nodes. Final pathological staging was a high-grade tubal cancer stage pT1cN0.

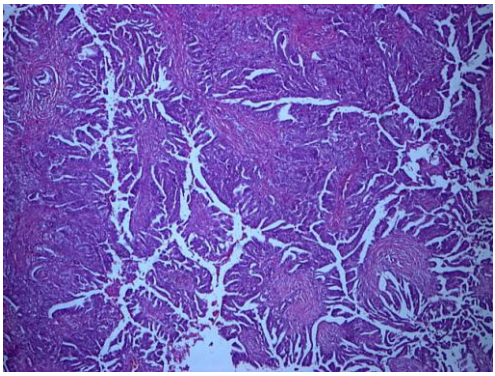

Fig. 1. Solid, papillary, cystic, micropapillary and glands

In the endometrium, an endometrioid type adenocarcinoma grade 3 with mucosal invasion only was found (pT1a grade 3N0) (fig. 2).

The third synchronous cancer was a moderate differentiated adenocarcinoma of

the recto-sigmoid junction with subserosal invasion without metastases in the 17 regional lymph nodes (pT3N0 Dukes-MACB2) (fig. 3).

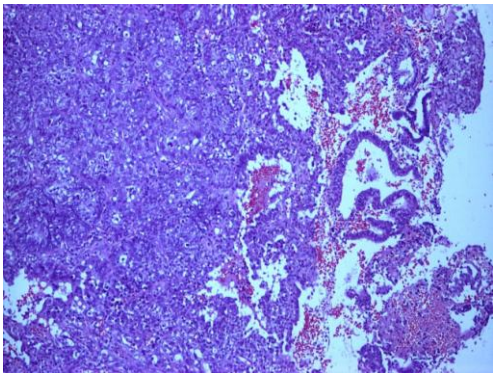

Fig. 2. Endometrioid type adenocarcinoma grade 3, with mucosal invasion

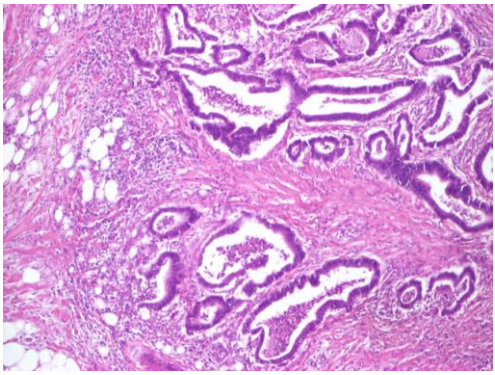

Fig. 3. Moderate differentiated adenocarcinoma of the recto-sigmoid junction with subserosal invasion

The oncology commission in our hospital decided to start the adjuvant treatment focusing on the tubal cancer. The patient started the Carboplatin/Paclitaxel chemotherapy and she is doing well after four courses.

DISCUSSION

The most commonly reported synchronous malignancies were the coexistence of ovarian and endometrial cancers, but genital tract malignancies can arise from more than two anatomical sites, as primary neoplasia. Although the aetiology of synchronous malignancies remains unclear, it has been postulated that the extended mullerian system, comprising ovarian epithelium, fallopian tube, uterus and cervix respond as a single morphological unit to produce primary cancers in different sites. Another theory, which could explain even other sites, suggests that these neoplasms originate in metaplasia occurring in different tissues (4). Till now, limited cases of synchronous primary genital cancers have been reported in the literature, and even less for a third pelvic cancer with an extra-genital origin.

Taking into considerations only 2 cancers out of 3 for our patient (fallopian tube and endometrium), Eisner *et al*(1) described also two synchronous primary cancers of fallopian tube and endometrium, and Atasaver *et al*(8) another 5 sites synchronous cancers involving ovary, both tubes, endometrium and cervix. Our search did not find a similar case in the literature, comprising 3 synchronous malignancies involving 2 genital sites (tube and endometrium) and another pelvic extra-genital one with different embryologic origin (sigmoid colon).

Our pathologic findings fulfilled the conditions described previously for identification of primary synchronous cancers, such as different histologic types (major criterion) or all the following minor criteria: (1) both tumours confined to primary sites; (2) no direct extension between tumours; (3) no lymphvascular tumour emboli; (4) no or only superficial myometrial invasion; and (5) distant metastases (5, 6, 7).

Simultaneous detection of malignancy

in different organs challenges the clinicians and pathologists to make correct diagnosis and arrange proper management (8). Appropriate therapy for synchronous cancers must be planned individually. Different parameters such stage, grade, extension, tumour resection margins, etc should be taken into consideration. As a consequence, our oncology staff decided for this special case that most aggressive tumour necessitating first line adjuvant treatment is the high-grade serous adnexal adenocarcinoma.

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NEWS

THE PROTON PUMP AND INFLUENZA INFECTION

Influenza virus A is responsible for epidemic and pandemic flu favored by a high genetic variability. For obtaining infection control, the scientists developed new strategy that target essential viral needs such as viral dependence on cellular glucose. Initiation of viral replication by releasing the viral genome into the host cell cytoplasm is dependent on the acid pH of the endocytosis vesicle. This pH is maintained by a pump proton cell ATP-dependent. The cell uses the glucose as a source of ATP. Using culture cells, the researchers Kohio and Adamson show that higher glucose concentrations into the host cell, increased viral replication, the cells treatment with glycolytic inhibitors decreased viral infection and addition of extracellular ATP restored infection. In these processes, the vacuolar ATPase proton pump had a central role. These findings suggested that influenza infection could be controlled by altering glucose metabolism (Kohio HP, Adamson AL. Glycolytic control of vacuolar-type ATPase activity: a mechanism to regulate influenza viral infection. *Virology*. 2013; 444(1-2):301-9).

Cătălina Luncă

EFFECTS OF SOME DOPAMINE AGENTS ON MODULATION OF MEMORY PROCESSES PERFORMANCE IN RATS

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EFFECTS OF SOME DOPAMINE AGENTS ON MODULATION OF MEMORY PROCESSES PERFORMANCE IN RATS (Abstract) Apomorphine is a potent dopamine receptor agonist which has been used as a neuro-protective agent in the treatment of Parkinson's disease. SCH-23390 is a synthetic compound that presents selective D1 dopamine receptors antagonist activity and possesses pharmacologic effects similar to standard antipsychotics. **Aim:** Experimental researches on the effects of two substances acting on dopamine receptors on the cognitive processes in rats. **Material and methods:** The experiment was carried out on white male Wistar rats (150-200g) divided into 3 groups of 6 animals each, treated intraperitonealy with the same volume of solution as follows: Group I (Control): saline solution 0.3ml; Group II (coded APO): apomorphine 2mg/kbw; Group III (coded SCH): SCH-23390 0.3mg/kbw. Working memory was assessed using the radial-arm maze. The following measures were recorded: the number of entering an arm containing food, but previously entered (working memory errors); the number of entering an arm that was not baited (reference memory errors); time taken to consume all five baits and the number of arms entered until a repeat entry was made (entries to repeat). The data were presented as mean +/- standard deviation and significance was tested by SPSS Statistics for Windows version 17.0 and ANOVA method. Experimental protocol was implemented according to the recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues. **Results:** In our experimental conditions dopamine agonist apomorphine produced significantly ($p<0.05$) more novel choices in the first eight-arm entries than the saline vehicle. The animals treated with apomorphine entered significantly ($P<0.05$) more arms compared to the control group. D1 receptor antagonist SCH-23390 induced a significant decrease ($p<0.05$) in the number of working memory errors (elements relevant for short-time memory quantification) and average time taken to consume all five baits ($p<0.05$), but did not modify the number of reference memory errors (for long-time memory quantification) compared to the control group, suggesting a short-time memory retention enhancement and an improvement of discriminative spatial learning. SCH-23390 administration resulted in a not quite significant increase in the number of entries to repeat compared to control rats. **Conclusions:** The administration of apomorphine also increased the search efficiency in total arm entries, suggesting that it facilitates the response in the test session of secondary reinforcement, more than rewarding, effect combined with a lack of discrimination. Our research revealed that D1 receptor antagonist SCH-23390 influenced short-time memory, without affecting long-time memory of experimented animals. **Keywords:** APOMORPHINE, SCH-23390, RADIAL ARM MAZE, COGNITIVE

Dopamine is a neurotransmitter with an important role in forming long-lasting memories for some time, especially in episodic memory. Literature data show that dopamine receptor stimulation may be detrimental to the spatial working memory functions in lab animals (1, 2).

Apomorphine chemically designated as 6aβ-Aporphine-10,11-diol hydrochloride hemihydrate, is a non-ergoline potent dopamine receptor agonist which has been used as a neuro-protective agent in the treatment of Parkinson disease (3).

(R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride derivative SCH-23390, a synthetic compound acting as a selective, high-affinity antagonist of D1 receptors, possesses pharmacologic effects similar to standard antipsychotics (4).

MATERIAL AND METHODS

Male white Wistar rats (150–200g) were used in the study. Animals were housed in plastic cages in an animal room maintained at 23 ± 1°C on a 12-hour dark cycle (light period, 07:00 – 19:00).

Animals received water *ad libidum* and were maintained at 85% of their free feeding weight by controlling the amount of their single daily meal 45 minutes after the experimental session. Before the experiment, rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

The animals were divided into 3 groups of 6 subjects each, and treated intraperitoneally as follows:

Group I (Control): saline solution 0.3ml;

Group II (coded APO): apomorphine 2mg/kbw;

Group III (coded SCH): SCH-23390 0.3mg/kbw.

The used drugs, apomorphine, SCH-23390 (Sigma-Aldrich Chemical Company), were dissolved in 0.9% saline, prepared just before use. All drugs were injected intraperitonealy in a constant volume of 1 ml/kg body weight.

The hypothesis that dopamine receptor subtypes are involved in mediation of cognitive pathways is based on a number of experimental reports indicating various activities of different dopamine agents in spatial learning and memory.

To directly prove this hypothesis we investigated the effects of dopamine agonist apomorphine and selective D1 receptor antagonist SCH-23390 on rat memory performance in radial arm maze, a test designed to evaluate lab animals memory processes performances. This standardized test is used as a tool for assessing the neurobehavioral characteristics of memory processes based upon spatial cognition. The substances were administered one hour before the beginning of a behavioral session.

The radial eight-arm maze device consists of central platform (30cm in diameter) elevated from the floor with eight arms (10x80cm) extending radially. The black Plexiglas maze is mounted on an adjustable height tripod (up to 1m). Each arm has lateral walls higher on the proximal side of the arm than on the distal one. On the distal end of each arm, a detachable recessed cup can be installed or replaced by covered up. Food cups for the reinforces were located near the end of each arm.

The design of this device ensures that after checking for food at the end of each arm the animal is forced to return to the central platform before making another choice. The rat always has eight possible options.

After initial acclimation to the laboratory environment there were two daily sessions of 5 min each in which the rat was placed on the maze without bait in the arms. The subject was then trained to run down a single arm to obtain a piece of cheese as the bait. The animals were assigned to learn either the place task or the cue task. Before each session each of the arm was baited with a reinforcer (piece of cheese), placed in a food cup at the end of the arm. The animal was placed on the central platform of the device, allowed to move freely about the maze, and a digital timer was started for a 5 min period of time.

All eight arms of the maze were accessible but four randomly selected were baited.

The arms were not re-baited during the session. Entries into arms previously entered were counted as errors. The session continued until either the animal entered all eight arms or 5 minutes elapsed. The maze was cleaned between trials.

The following measures were recorded: number of novel arms entered during the first eight choices on an un-baited eight-arm maze as well as the total number of arms entered during 5 min, the number of entering an arm containing food, but previously entered (working memory errors); the number of entering an arm that was not baited (reference memory errors); time taken to consume all five baits, and the number of arms entered until a repeat entry was made (entries to repeat). Facilitation of efficient search behavior required an increase in the number of novel arms entered in the first eight choices, rather than simply an increase in total arms entered during the trial.

A camera set above the maze and connected to a monitor in a neighboring room was used to allow us to score behavior without disturbing the animals. All trials were videotaped and subsequently analyzed by a trained observer who was blind to the treatment condition.

Data for each measure were evaluated separately by analysis of variance for repeated determinations. Results were expressed as arithmetic mean ± SD and represented graphically. All values were processed using SPSS for Windows version 17.0 and ANOVA method. P- values less than 0.05 were considered statistically significant compared with those of controls.

Experimental protocol was implemented following the recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues guidelines for handling and use of experimental animals, according to the ethical standards of the European Community.

Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical reasons, all the animals were sacrificed at the end of the experiment.

RESULTS AND DISCUSSION

In our experimental conditions apomorphine administration significantly influenced the data of radial arm maze test compared to control group.

Intraperitoneal injection of dopaminergic agonist apomorphine had an evident effect on increasing search efficiency. It produced significantly ($p<0.05$) more novel choices in the first eight-arm entries than either the saline vehicle.

Statistical analysis showed that animals treated with apomorphine consistently entered significantly ($p<0.05$) more arms compared with the control group.

In the behavioral experimental model

used, D1 receptor antagonist SCH-23390 induced a significant decrease ($p<0.05$) in the number of working memory errors (elements relevant for short-time memory quantification) and average time taken to consume all five baits ($p<0.05$), but did not modify the number of reference memory errors (for long-time memory quantification) compared to control group, suggesting a short-time memory retention enhancement and an improvement of discriminative spatial learning (fig. 1, 2, 3).

SCH-23390 administration resulted in a quite insignificant increase ($p<0.05$) in the number of entries to repeat compared to control rats.

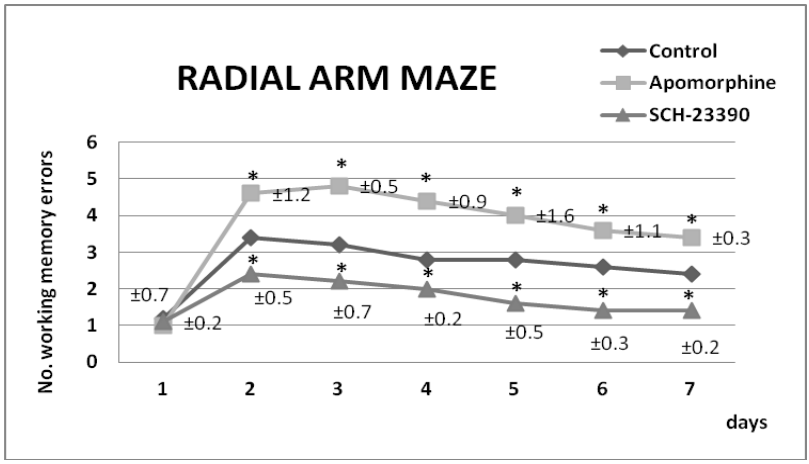

Fig. 1-Effects of apomorphine and SCH-23390 treatment on the number of working memory errors in radial arm maze test. Values were expressed as mean ± S.E.M of the number of working memory errors. *p<0.05, **p<0.01 *vs* control.

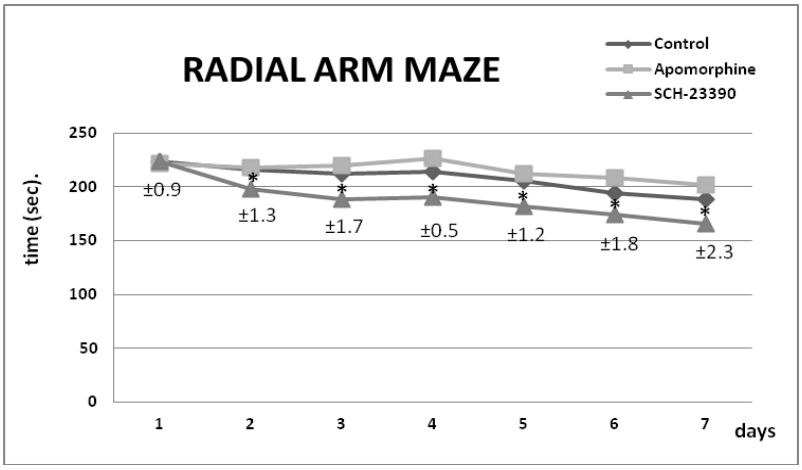

Fig. 2-Effects of apomorphine and SCH-23390 treatment on time (seconds) taken to consume all five baits in radial arm maze test. Values are expressed as mean ± S.E.M of the time taken to consume all five baits. *p<0.05, **p<0.01 *vs* control.

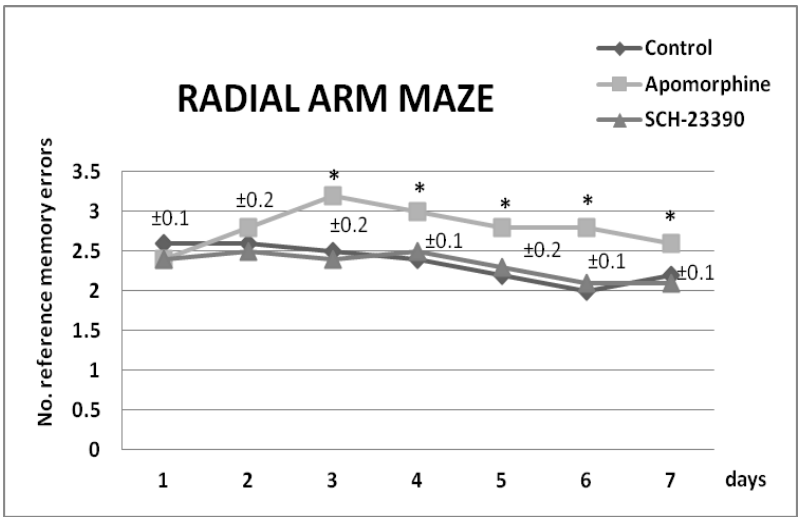

Fig. 3-Effects of apomorphine and SCH-23390 treatment on the number of reference memory errors in radial arm maze test. Values are expressed as mean ± S.E.M of the number of reference memory errors. *p<0.05, **p<0.01 *vs* controls.

Different studies using a variety of approaches suggest that the dopamine pathway may be a key component in modulating activity in the central areas to facilitate behavioral flexibility (1, 10).

The central dopamine receptors, especially D1 receptors in the prefrontal cortex, may be very important for mediating dopamine effects on cognitive functioning (11, 12, 13).

Literature data show that apomorphine stimulates post-synaptic dopamine D_2- type receptors within the caudate putamen in the brain. Different researches revealed that apomorphine improved motor function in an animal model of Parkinson disease and also attenuated the motor deficits induced by experimental lesions in the ascending nigrostriatal dopaminergic pathway in lab animals (14, 15).

Recent cognitive studies proved that apomorphine dose-dependently impaired recognition memory in animals. It is well known the involvement of dopamine receptors in rodent memory and in spontaneous exploration behavior (16).

Experimental studies suggest that SCH 23390 may prevent the spatial working memory disturbances induced by active substances of marijuana, and block the apomorphine-induced stereotypy and methamphetamine-induced lethality in experimental models (17, 18). Also it presented anti-stereotypic, cataleptogenic, and inhibitory effect on amphetamine circling in different laboratory animal species (19).

CONCLUSIONS

The results indicate that facilitation of unconditioned preparatory behavior by dopamine agonist apomorphine applies also to efficient maze search. The administration of apomorphine also increased the search efficiency in total arm entries, suggesting that it facilitates the response in the test session of secondary reinforcement, more than rewarding, effect combined with a lack of discrimination.

Our research revealed that D1 receptor antagonist SCH-23390 influenced short-time memory, without affecting long-time memory of experimented animals. SCH-23390 administration facilitated spatial working memory, as measured by improved choice accuracy in the radial arm maze, when administered to rats.

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PLASTIC SURGERY IN 17TH CENTURY EUROPE. CASE STUDY: NICOLAE MILESCU, THE SNUB-NOSED[1]

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CASE STUDY: NICOLAE MILESCU, THE SNUB-NOSED (Abstract). The rising and the existence of plastic and aesthetic surgery in early modern Europe did not have a specific pattern, but was completely different from one nation to another. Colleges of Physicians could only be found in some places in Europe; different Parliaments of Europe's nations did not always elevate being a surgeon to the dignity of a profession, and being a surgeon did not always come with corporate and municipal privileges, or with attractive stipends. Conversely, corporal punishments for treacherous surgeons were ubiquitous. Rhinoplasty falls into the category of what Ambroise Paré named "facial plastic surgery". The technique is a medical source from which many histories derive, one more fascinating than the other: the history of those whose nose was cut off (because of state betrayal, adultery, abjuration, or duelling with swords), the history of those who invented the surgery of nose reconstruction (*e.g.* Suśruta-saṃhitā or Tagliacozzi?), the history of surgeries kept secret in early modern Europe (*e.g.* Tropea, Calabria, Leiden, Padua, Paris, Berlin), and so on. Where does the history of Nicolae Milescu the Snub-nosed fall in all of this? How much of this history do the Moldavian Chronicles record? Is there any "scholarly gossip" in the aristocratic and diplomatic environments at Constantinople? What exactly do the British ambassadors learn concerning Rhinoplasty when they meet Milescu? How do we "walk" within these histories, and why should we be interested at all? What is their stake for modernity? Such are the interrogations that this article seeks to provoke; its purpose is to question (and eventually, synchronise) *histories*, and not exclusively *history*, both in academic terms but also by reassessing the practical knowledge of the 17th century. **Keywords:** HISTORY OF PLASTIC SUGERY, NICOLAE MILESCU, RHINOPLASTY, NASAL SURGERY, PRACTICAL KNOWLEDGE.

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1. Nose fractures and nasal reconstruction. Why are we interested in the Antiquity in such cases?

We are interested in the Antiquity because history provides historians with reference documents enabling them to make distinctions and construct a narrative. There is historiographic consensus on the fact that the oldest and most consistent written source for the history of surgery is the *Edwin Smith Surgical Papyrus*, dating back to the Djoser period (c.3000-2500 BC) (1). This source provides us, among others, with a first aetiology of the cases of nose swelling or fracture. Consequently, the novelty of the source resides in the fact that it describes pathologies and provides prescriptions. The papyrus, discovered in Thebes and written in Hieratic, left Luxor in 1862 with Edward Smith (1822-1906), its discoverer, who had purchased it from an anonymous Egyptian merchant (1, 2, 29). Smith donated the papyrus to the *New York Historical Society* before his death. For a short period it was held by the *Brooklin Museum*, before being sent to the *New York Academy of Medicine*, where it remained permanently. The text of this precious papyrus was first translated in 1930 by the Orientalist James Henry Breasted, Egyptologist and founder of the *Institute of Oriental Studies* in Chicago (which is, to this date, a sort of Mecca for Orientalists, devoted mainly to the study of Near Eastern civilisations). Breasted was aided by Dr. Arno B. Lukhardt in the translation of the papyrus, especially with regard to the medical details (29). The Egyptologist James P. Allen, Professor at Brown University, Head of the International Association of Egyptologists and curator of the Egyptian Art Section of the *New York Metropolitan Museum*, provided a second critical edition in 2005, an initiative unequalled so far (1). This source reveals the intense medical activity of Imhòtep, a physician and architect at the court of Pharaoh Netjerykhet or Djoser, the first king and founder of the third Egyptian dynasty (c.2760 BC). Imhòtep would be, at least in theory, the first physician to have indicated the fact that a nasal fracture can be examined, diagnosed and cured. To exemplify, we will quote two of the cases of nasal fracture mentioned in the *Edwin Smith Surgical Papyrus*:

Case 1. *A fracture of the nasal cartilage* (5,10-15). *Title*. Practices for a fracture in the pillar of his nose. Examination and Prognosis. If you treat a man for a fracture in the pillar of his nose, and his nose is flattened and his face is flattened out, while the swelling that is on it is high, and he has bled from his nostrils, then you say about him: "One who has a fracture in the pillar of his nose: an ailment I will handle". *Treatment*. You have to wipe it for him with two plugs of cloth. You have to push tow plugs of cloth wet with oil inside his nostrils. You have to put him on his bed in order to reduce his swelling. You have to set for him stiff rolls of cloth so that his nose is restricted from moving, and treat him afterward with oil and honey dressing every day until he gets well. *Explanations.* As for "the pillar of his nose", it is the bridge and his side of his nose, inside his nose in the middle of his nostrils. As for "his nostrils", they are two sides of his nose penetrating to his cheek, starting at the end of his nose and exciting the top of his nose" (1).

Case 2. *A fracture of the nasal bone* (5,16-6,3*). Title.* Practices for a fracture in

the chamber of his nose. *Examination and Prognosis*. If you treat a man for a fracture in the chamber of his nose and you find his nose crooked and his face flattened, while the swelling that is on it is high, then you say about him: "One who has a fracture in the chamber of his nose: an ailment I will handle". *Treatment.* Set it back in its proper place. Wipe for him the inside of his nostrils with two strip of cloth until every eel of blood that is knotted inside his nostrils comes out. Afterward, you have to push two plugs of cloth wet with oil into his nostrils. You have to set for him two stiff rolls of cloth bandaged on it and treat <him> with an oil and honey dressing every day until he gets well. ***Explanations***. As for "a fracture in the chamber of his nose", it is the middle part of his nose down to where it ends between his eyebrows. As for "his nose crooked and his face flattened", it means that his nose is awry and very swollen all over, and his cheeks likewise, so his face is flattened out from it and is not in its right form, because every sinus is distorted with swelling, so his face looks flattened from it. As for "every eel of blood that is knotted inside his nostrils", it is the coagulated blood inside his nostril, similar to the eel that exists in the water" (1).

The structure of Imhòtep's "medical chart" is remarkable in terms of its accuracy, synthesis and solutions: it specifies the name of the disease, defines the pathology, establishes the diagnosis, prescribes the treatment and, finally, offers practical explanations (17, 29, 32). Also remarkable is the distinction between the upper fracture of the nose and the conditions associated with the nasal cavities, more precisely, the deformations at cartilage level, as well as the different treatment approaches. As a common denominator, the concern for the patient constitutes a priority, as evidenced in the medical indications themselves, as well as in the very fact of their systematic recording. For a physician, as well as for the patient, medical prescriptions were of outmost importance.

As far as historians and philologists are concerned, the typology of the text, the author and the event itself are major landmarks. The source also provides an area of interference between two completely different worlds, namely physicians and historians, retracing the ancient approaches to pathology: types of treatment, medical supplies, position towards the patient, and typology of medical chart. For instance, among the instructions referring to the upper nose fracture, the document mentions the use of linen cloths, useful in performing the haemostasis but also during the first steps in stopping the bleeding. Moreover, linen cloths, dressed in a good lubricant such as oil, once introduced in the two nostrils and positioned as high as possible, stop the haemorrhage. In the case of fracture, the deformed nosed can be bandaged with oil and honey. The two relatively stiff rolls inside the nose will be supported with the patient's hand, while the physician handles nasal bleeding or removes the blood coagulated inside the nostrils, respectively in the ears, when necessary. This operation is extremely painful and can impede the patient from speaking(1). Historical exegesis appreciates positively this type of details, since they are clearly distinct from the idea of medicine as assimilated to magical incantations or religious rites. *Edwin Smith Surgical Papyrus* brings to the fore a "rational" medical treatise, as long as the pathology has an etiology, a diagnosis and a treatment. From this perspective, as mentioned above, this papyrus

is the most important document of surgical medicine in the ancient Near Est.

2. *Suśruta-samhitā* and effective nose reconstruction as surgical procedure.

As far as the *tout court* nasal reconstruction techniques are concerned, the first written source on nose reconstruction as a surgical procedure is included in the *Suśruta-samhitā* source in chapter 16.27-31 *Sūtrasthāna*. According to this source, Louis H. Gray explained that the divine physician Dhanvantari (*i.e.*"well-famous"), received the Āyur-Veda from Brahmā trough the successive meditation of Prajāpati and Indra, then taught it to Suśruta and other six colleagues (30). This is a much earlier source than the *Edwin Smith Surgical Papyrus* source, MS. Bower dating back to the Gupta dynasty period (c. IV-V AD). It describes a surgical technique incorporated into one of the first treaties of Āyurvedic medicine (3, 4, 8, 15, 19, 24). The British captain Hamilton Bower discovered this manuscript in 1890 during one of his travels to the Eastern Turkistan, which accounts for the name MS. Bower. The source is considered to date back to the 4th century, approximately a millennium away from a possible original version. Bower offered the voluminous manuscript to the *Asiatic Society of Bengal*. During one of the reunions of the above mentioned Society in Calcutta, the manuscript was exhibited to the public, drawing the attention of Orientalists, especially connoisseurs of the Sanskrit language. A year later, the manuscript was studied by Dr. Augustus Rudolf F. Hoernle (1841-1918), a physician and Indologist of Indo-British nationality and one of the most educated persons in Calcutta Madrasah (31). Calcutta Madrasah is an institution founded primarily for the study of Arab and Persian languages, aiming to a comprehensive understanding of the Koran texts, and which will later introduce the study of exact sciences. Additionally, Calcutta Madrasah is a place where medicine was studied and where medical books and manuscripts were collected (16, 31).

The first studies and translations of the *Suśruta-saṃhitā* treaty were published in the *Journal of the Asiatic Society of Bengal* and, subsequently, in the *Journal of the Royal Asiatic Society of Great Britain and Ireland*. At the same time, Dr. Hoernle started to work on the first translations there. At the beginning of the 20th century, a complete edition translated from Sanskrit into English was prepared by Kunda Lal Bhishagratna. One of the most obvious problems of the translated version, as the reviewers rigorously warn, was related to the fact that the English medical terminology did not always coincide with the homologous Sanskrit vocabulary (29). Thus, it can be noticed that Orientalist studies in Calcutta develop in tune with medical studies at the end of the 19th century and the beginning of the 20th century.

Suśruta and his medical techniques were also adopted in Ancient China and in the Middle Ages his treatise was translated from Sanskrit into Arab (13, 17, 18, 19). During the Renaissance and the Pre-Modern periods, it finally entered Europe (5, 6, 11, 12, 13, 19, 20, 27, 32). The oral tradition was the first channel for the transmission of medical practice, followed by the tradition of widespread manuscripts and the critical editions of *Suśruta-saṃhitā*, which are no less important. As regards the variety of plastic surgery practice, Suśruta provides remedies for conditions associated with the ear lobes and the

lips, in case of excessive swelling. Rhinoplasty remains the most remarkable procedure, considering its secular diffusion and practice. Arion Roşu (1924-2007) an Indologist of Romanian-French nationality explains the connexion between the name of the technique and the technique itself: „Connue sous le nom de 'greffe indienne', pour refaire un nez retranché, cette tehnique comporte une greffe pédiculée à lambeau de joue (*gaṇḍa*), alors que dans la variante moderne de cette opération, révélée à l'Europe à la fin du XVIIIe siècle, le lambeau est frontal" (19).

In brief, the Indian nose reconstruction technique consists primarily in measuring the nose section to be reconstructed; then, a live-tissue section of skin is treated so as to cover the nose, keeping a small pedicle connecting it to the cheek region. The nose section, on which the skin is to be applied, is previously excised (by removing the scar tissue) with a knife. Subsequently, the surgeon carefully sutures the two parts, uplifting the skin and introducing two cylindrical bandages coated in castor oil, directed on the nostrils position, in order to support and shape them. Once the skin is adapted, it has to be powdered with sweet wood or liquorice powder (*Glycyrrhiza glabra*), red sandalwood and barberry powder (*Berberis vulgaris*). Finally, the nose has to be covered in linen and gently, although continuously, padded with sesame oil. When the skin starts to turn uniform, in case the nose is too long or too short, the supporting device (the flat) will be cut in half or modified, as needed (5, 6, 13, 19, 21, 23, 32) (fig. 1).

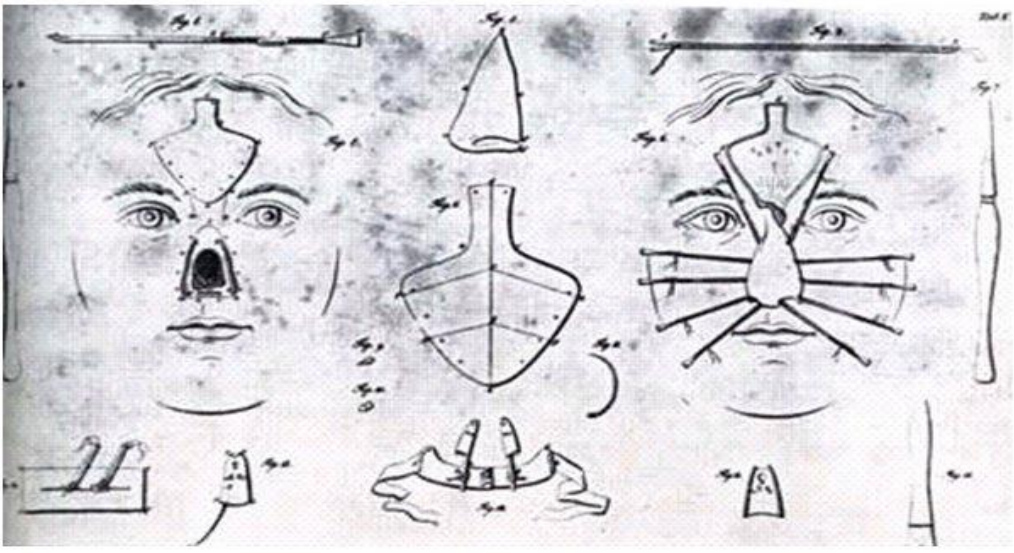

Fig. 1: "Indian flap" for nasal reconstruction apud Sanjay S. (37)

At this point we can be sure that at least the Antiquity provides the distinctions we need in order to differentiate between the virtues of nose treatment in Egypt and those of nasal reconstruction in India. The following conceptual link will refer to the punishments or the various reasons leading to nose mutilation, a link finally leading to the episode featuring Nicolae Milescu, the Snub-Nosed.

3. Punishments and offences.
Why the care for nose integrity?

In Suśruta's India (18), as well as later on, the nose was associated with respect and dignity; this was probably due to the assimilation of body integrity *per se* with the moral norms. Nose mutilation involves dishonour, the stigma of an undesirable sign at the very centre of the face, and automatically means marginalisation and seclusion from the others. Any deviation from the norms of a sexuality controlled by the Brahmin caste and the Vedic/Hindu principles involves a punishment equivalent to the dishonour caused. Similarly, the historian is likely to be familiarised with the punitive measures derived from the Justinian law code, fully functional from late Antiquity to the definitive fall of Constantinople. Crimes related to treason, conspiracy, adultery were not always punished by death, but, just like in India, they could be identified with the image of disgrace. Nose cutting or seclusion in a monastic environment particularly signified political treason or adultery. *Mutatis mutandis*, from pseudo-Aristotle's *Physiognomonikà* or that by Giovanni Battista Della Porta, from Salvadore De Renzi's *Collectio Salernitana* to the screening of the play *Cyrano de Bergerac* by Edmond Rostand or the comedy *Roxanne* (1987) and the famous line: "with that nose you can satisfy two women at the same time", one cannot help distinguishing between *nobilissimus nasorum* and *nasus correspondet praeputio* or virile noses. This is a "detail" that the imagination (scholarly or otherwise) has not ceased to make use of, consolidating the correlation between anatomic characteristics and character or moral features (20). In the 16th century, much more solemnly, the French scientist Laurent Joubert – cited by the historian Luigi Monga (20) – argued that there was not necessarily a correspondence between the proportions of certain anatomical parts: "la portion des membres n'est observée en tous, plusieurs ont une belle trompe de nez qui sont camus du reste et plusieurs camus du nez sont bien appointés du membre principal" (20). We need to remember that, ultimately, most cases of nose mutilation derive from the behaviour of cheated husbands, hence the duel or the nose mutilation punishment. Therefore, a severely punished masculine virility is preferred over a dishonoured femininity killed with stones. But the case of Nicolae Milescu is completely different, though no less exciting.

We will next follow the narrative thread of the chronicler relating the adventure of Nicolae, the Snub-Nosed, stories of Moldavian rulers, then Ottoman chronicles, rumours on unusual subjects from the correspondence of British diplomats at Constantinople, all building, little by little, the story of nose recovery and the luxury of the aesthetic surgery Milescu benefited from in the 17th century at the Prussian court of the Great Elector Frederick William of Brandenburg, most probably under the close supervision of the physician Christian Mentzel.

Power struggle, exile, treason, conspiracy make up, then just like now, the histories of opportunism and of the reigns mediated by the Ottoman Empire, materialising scenarios of political interests. Since historiography does not specify the exact date when Milescu's nose was cut, everything tends to be uncertain. However, after having read exhaustively the polemics between historians, we started looking for the germs of the story in completely different sources than those previously mentioned. For many

scholars in our field, Ion Neculce's chronicle was not a reliable source, which intrigued us even more. Finally, the story of Milescu's nose mutilation could not be sought only in the Romanian Chronicles, which led us to a Turkish chronicle dating back to the 18th century (9, 10). Thus, Naima Targhi briefly gives the following account of: first, the efforts made by Prince Vasile Lupu to redeem his daughter taken hostage by the Ottoman Empire – which revealed his anti-Ottoman views – by promising the damsel's hand to Timush, son of a famous Ukrainian Cossack, Bogdan Zynovii Mykhailovych Khmel'nyts'ki; then, the intention of Eflak *bey*, the Ottoman nickname of Vasile Lupu, to strengthen his army, strong enough reasons to determine the Ottoman Empire to ask for a fabulous sum in return. Finally, Naima Targhi bitterly speaks of a Turkish leader, Bektash *aga*, who facilitated the exchange. In this context, Naima Targhi mentions the initiative of a *devil logothete*, none other than Gheorghe Ştefan (?-1668), one of Vasile Lupu's logothetes, the same who, in the second half of 1653, became involved in the rivalry between the Moldavian Prince and Matei Basarab (1623-1654), which would later undermine his authority.

Why does Naima Targhi consider the logothete (*i.e.* Gheorghe Ştefan) an ungrateful character? Precisely because it was *aga* Matei Basarab who mediated his ascension to the Moldavian throne. George Ştefan is known to be the ruler who employed the copyist Milescu, but the moment of the plot causing the Moldavian scholar to lose his nose was yet to arrive. The 1653 plot against the Moldavian ruler Vasile Lupu fully succeeded, despite the interception of some messages between Matei Basarab and Gheorghe Ştefan or the logothete Costin Ciogolea. Vasile Lupu was aware of conjure, but had to leave the throne. Oddly enough, in 1653, Milescu was in Moldova, supporting Gheorghe Ştefan, despite his links with the Lupu royal family. The whim for power never left Gheorghe Ştefan, not even after he lost, a few years later, the royal seat again; thus, around the years 1659-1660, in similar conditions but with different actors, he tried again to reconfigure the same type of plot, by the same means and for the same purpose: regaining the royal seat of Moldova. This is when Milescu became a part of Gheorghe Ştefan's plan. Neculce wrote about the interception of infamous correspondence, which uncovered the plot, this time with Milescu as a victim, i.e. one of the participants in the plot. As chronicler Neculce mentions, Prince Ştefanită Lupu, a good friend of Milescu's, did not sentence him to death, but handed the executioner his own knife to cut Milescu's nose:

There was a certain boyar, Neculai Milescul, the spatharios (Nicolae the Snub-Nosed Spatharios, by the name of Milescul from Vaslui) from his estate of Vaslui, a learned man and a scholar, who knew many languages: Old Greek, Slavonic, Greek and Turkish. And he was proud and rich, and he would ride in front of the prince, with maces and swords, with harness on the horses. And Prince Ştefăniță treasured and honoured him, placing him at his table and playing cards with him, asking for his advice, for he was a copyist. And then once, for too much good and being too much honoured by Prince Ştefăniță, he set out to write false letters, and sent them in a drilled stick to Prince Constantin "the Old" Băsărabă in Țara Leşească (Poland), urging him to come with the army and remove Prince Ştefăniță from his throne. But Prince

Constantin refused to listen to what was written, and sent the drilled stick and the letters back to Prince Ştefăniță. And Prince Ştefăniță, as soon as he saw the stick and the letters, got angry and asked for Nicolai Milescu to be brought to him, in the small house and had an executioner cut his nose. And Prince Ştefăniță, hastily taking the knife of his belt, he gave it to the executioner to cut his nose. And they did not want to cut his nose with the executioner's knife; they cut it instead with the knife of Prince Ştefăniță himself. Later, Nicolai the Snub-Nosed fled to Țara Nemțească (Germany) and found a doctor, for blood flowed from his cheek and his nose wrinkled, and day after day his blood thickened, and his nose grew back, heeling. And when he returned in the country, under the reign of Prince Iliaș, it could barely be noticed his nose had been cut (11).

Milescu's mutilation presumably took place sometime between 1659 and 1600. This can be deduced from the fact that, in 1659, the chair of Moldavia belonged to Prince Ştefăniță Lupul, and, given the untimely death of the prince, more precisely on the 17th of January 1661, this appears to be the only plausible timeline.

Historical dates, throne succession, intrigues and plots all abound in the history of the Romanian Principalities, but, as regards Gheorghe Ştefan alone, he had the fate of a perpetual wandering prince, asking for support and subsidies from most European countries – a story we will not dwell on here. Milescu left Moldavia in 1660, oscillating between the two Romanian principalities; he was later appointed *kapukahya* at Constantinople (1661-1664), in the service of Prince Grigorie Ghica, the latter being very familiar to the Constantinople environment and a good friend of the princely family Cantacuzino, which also explains the connection between Constantinople and the translation of the *Septuagint* by the Moldavian humanist.

In 1665, Milescu was appointed secretary of the same prince, Grigorie Ghica, and headed in 1666 to Berlin, where he resorted to rhinoplasty. Historical sources do not provide further details on this aspect, but due to the correspondence between two British ambassadors, one in Constantinople, and the other in Tuscany – correspondence published by the British historian Eric D. Tappe and taken over by local historians (34) – it can be inferred that Milescu's snub nose was not overlooked. Why would that be so? The British ambassador, Lord Heneage Winchelsea (1621-1682) wrote a letter dated January 13 - 23, 1667, to his brother in law Sir John Finch, ambassador at the court of the Grand Duke of Tuscany. In this letter, he described in detail Prince Gheorghe Ghica's complaints about an impostor who had betrayed his own master – Grigorie Ghica, none other than his son – and who was using the royal seal in his various escapades in the European courts, taking advantage of the benefits arising from such a recommendation. Finally, as historiography clarifies, Lord Winchelsea mistook the impostor for Constantin Nacul, sent to London in 1666 by Gheorghe Ştefan, a character sharing the same destiny with Milescu: nose mutilation. Despite this confusion, Lord Winchelsea also mentions the mutilation of Milescu's nose and the latter's vast knowledge, recording the episode of the surgery he was subject to in Germany: "He hath a little mark upon the tip of his nose which was cut off by justice for his former knaveries, but in Germany they have so well recovered that deformity that

it is little visible. The name of that person is Nicolai. This you will do well to acquaint the Secretary of State, and cause secret enquiry whether such a person be not at Venice or in Italy"(27).

Sir John Finch (1626-1682) was no common addressee, considering the fact that his main profession was that of physician, Doctor of Medicine, a degree obtained at the University of Padua in 1656. Moreover, Finch was an *extraordinary fellow* of the *College of Physicians of London* and of the *Royal Society*. Undoubtedly, Finch was highly interested in the various types of special surgery, such as, for instance, rhinoplasty. Several sources of medical history indicate the 18th century as the period when the practice of rhinoplasty in Europe becomes known to the British. This may seems rather unrealistic, since the very fact that a British doctor completed his medical studies at a university such as the one in Padua automatically sugests that rhinoplasty was already known in the 17th century and that, in fact, information on nasal reconstruction arrived sometime earlier in England.

In fact, the historian might be tempted to return to the history of the secret medical practices and the European Academies where the study and the practice of surgery was common. In Cambridge, Paris, Leiden, Padua there were the so-called anatomy lecture halls, places designed for carrying out dissections, where the first steps in the study of surgery were made. Together with Italy and England, Germany was part of the barber-surgeon avant-garde; the best-known German surgeon of the time was Wilhelm Fabry von Hilden (1560-1634). However, given the chronological gap, he could not have operated Milescu, nor could have Johannes Schultheiss (1595-1645), the author of a popular treatise on surgery, *Armamentarium Chirurgicum* (1653). Another famous surgeon of Polish origins with comprehensive experience acquired in Germany was Matthaeus Gottfried Purmann (1649-1711), professor of the German doctor Michael Ettmuller (1644-1683). Identifying the surgery practice where Milescu was subject to the nasal reconstruction operation will definitely not be an easy task; we only know that the Medical Academy at the Prussian court in Berlin was represented by Friedrich Hoffmann (1660-1742) and Georg Ernst Stahl (1660-1734). Only Stahl was permanently at the Prussian court in Berlin, elected President of the College of Physicians (*Collegium medicum*), but at the time Milescu resorted to this intervention, Stahl was only 7 years old. Two clues could lead us to believe that the Indian technique of rhinoplasty was known at the court of the Great Elector Frederick William: (i) the fact that he had already been favourable to the idea of organizing trade missions to India, (ii) the presence at the court of the great elector of Andreas Müller (1630?-1694) and his personal doctor Christian Mentzel (1622-1701), both residing in Brandenburg, both familiar with the Asian world and the Chinese language, and both in contact with Jesuit missionaries in the Far East such as, for example, Father Philippe Couplet (1623-1693) (35). Clues of such contacts with the Far East are even traceable in the library of the Great Elector Frederick William, namely in the manuscripts and books briefly described by the Orientalist Julius Klaproth in his *Verzeichniss der Chinesischen und Mandschuischen Bücher und Handschriften der Königlichen Bibliothek zu Berlin*, published in Paris in 1822. The volumes dedicated to Chinese medi-

cine include: *Clavis Medica ad Chinarum doctrinam de pulsibus* and *Medicamenta Simplicia quae à Chinensibus ad usum medicum adhibentur*, both edited with modified titles by Andreas Cleyer and Christian Mentze, but signed by "R. P. Michaelis Boymi Poloni, è Societate Jesu, è Regno Siam, Anno 1658", more precisely, by the Polish Jesuit Michael Boym (1612-1659). (36) The issue of the knowledge of rhinoplasty in mid-seventeenth century Berlin could be summarised as follows: either the Prussian doctor Heinrich von Pfolsprundt spread the method of nasal reconstruction specific to the Branca family, or this information was brought by the Jesuit missioners in China, who provided the Sinologists at the Prussian court with medical information. We tend to believe that the first hypothesis is more accurate, and that the doctor who performed surgery on Milescu in Berlin knew the secrets of nose reconstruction techniques from an autochthonous source. This point of view could give rise to future research, which is likely to map even more precisely the temporal coordinates of the practice of this technique in 17th century Berlin and its influence in other European countries.

4. We do know that Milescu had to choose between the nasal reconstruction of Indian origin and the Tropaean one, practised by the Vianeo brothers in the early 15th century (32). This brings back into discussion the secret surgery practices, more precisely those where nasal reconstruction was assiduously performed. Such practices could be found in central Italy, near Naples and even in some regions of an ethnically composite Sicily, considering the Arab domination and the flow of Sephardic Jews, *genius loci*. We will begin by mentioning the Branca family, since this involves the Prussian doctor Heinrich von Pfolfprundt, the closest to both the secrets of the surgical profession and to Antonio Branca. Pfolfprundt wrote a book in 1460, *Buch der Bündth-Ertznei*, published no sooner than 1868 (5, 32), a volume detailing the technique of nasal reconstruction (fig. 2).

Buch
der
Bündth-Ertznei.
Von
Heinrich von Pfolfprundt,
Bruder des deutschen Ordens.
1460.
Herausgegeben
von
B. Haeser und A. Middeldorpf.
Professoren zu Breslau.
Berlin.
Druck und Verlag von Georg Reimer.
1868.

Fig. 2: Heinrich von Pfolfprundt, Buch der Bündth-Ertznei (1460; 1868)

The Vianeo family also contributed to the creation of a most abundant literature in the history of the practice of nasal reconstruction, not only due to their outstanding skills, but also especially due to a revolution in the technique *per se* (clearly different from the Indian technique), a technique they did not describe themselves, but which was *stolen* and disseminated by Gaspare Tagliacozzi (1545-1599) from Bologna by means of his popular treatise: *De curtorum chirurgia per insitionem* (7, 32).

In the summer of 1947, in Tropaea, a large number of surgery professors from the *Università del Mezzogiorno* celebrated the activity and memory of the Vianeo brothers, within the very precincts of the public hospital *Ignazio Toraldo*, putting Calabria on the map of the history of the nasal reconstruction technique. Ironically or not, this is a somehow misleading history, considering the posterity of their discovery. The secret of the reconstructive technique in the Vianeo manner was dis

closed, as mentioned above, in a luxurious Italian Seicento, in medical publications and in illustrations that reveal, step by step, both the technique and the necessary equipment. We mention to this purpose the study *De curtorum chirurgia per insitionem* by Tagliacozzi, a treatise that is fully and freely available in a Frankfurt edition dating back to 1654 (7). We also need to point out that the interest in rhinoplasty manifested in this period was due particularly to syphilis, an incurable disease, inevitably related to the destruction of nose integrity. Tagliacozzi was a surgeon who dealt with the reconstruction of the nose, the ears and the lips, which ensured his notoriety despite the revolution in the rhinoplasty technique made by the Vianeo brothers, Vincenzo, Paolo and Pietro, as a result of a much slower accumulation. A detailed description of the rhinoplasty technique of Tropaean origin was provided by the French doctor Ambroise Paré, similar to the one made by Tagliacozzi in *De curtorum chirurgia per insitionem*: “Il s’est trouvé en Italie un chirurgien qui par son artifice refaisoit des nez de chair en ceste maniere. C’est qu’il coupoit entierement les bords calleux ou cicatrisez du nez perdu comme l’on fait aux becs de lievre : puis faisoit une incision tant grande et profonde qu’il estoit necessaire au milieu de muscle dit biceps, qui est l’un de ceux qui flechist le bras; puis subit faisoit poser le nez en ladite incision et bandoit si bien la teste avec le bras qu’il ne pouvoit vaciller çà ne là; et certains jours après, qui est ordinairement sur le quarantiesme jour, cognoissant l’agglutination du nez avec la chair duduit muscle, en coupoit tant qu’il en falloit pour la portion du nez qui manquoit: en après la façonnoit de sorte qu’il rendoit le nez en figure, grandeur et grosseur qu’il estoit requis, et traittoit ce pendant la playe du bras comme les autres, lors qu’il a d[é]perdition de substance. Et durant les dits quarante jours faisoit user à son malade des panades, pressis et autres viandes faciles à transgloutir, et quant aux rem[è]des desquels il usoit, estoyet de quelques baumes aglutinatifs” (6).

Then, which technique was applied to Milescu during the nasal reconstruction performed in Berlin? Calling back into discussion the description made by Neculce - *and found a doctor, for blood flowed from his cheek and his nose wrinkled, and day after day his blood thickened, and his nose grew back, heeling* – we can infer that

the Suśruta technique was the technique preferred by the anonymous doctor treating Milescu. From the account made in the chronicle, we can infer that the source of the tissue used in the reconstruction of the nose was the cheek (the genian region), that the transfer of live-tissue indicates a surgical strategy, and that the transfer from the genian region to the nasal region was produced successively. In this case, the resemblance between the source provided by chronicler Neculce and the Indian technique, the one according to the *Suśruta-saṃhitā*, seems more plausible: there is a preliminary period, that of manufacturing the pattern of the defect by means of a leaf, followed by drawing and incising the pedicled flap, which is then transferred into the nasal defect to be reconstructed at various successive stages. This delay technique could be what Neculce describes as: *and found a doctor, for blood flowed from his cheek and his nose wrinkled, and day after day his blood thickened, and his nose grew back, heeling* (6).

However, we should further comment that, based on this description, we cannot distinguish whether the genian flap used involved a random movement – thus explaining the delay – or was an axial (nasogenian) flap. In reconstructing the nasal defect, the hypothesis of a nasogenian flap is difficult to support, since two such flaps would be actually needed, bilaterally rotated. A single ampler genian flap would have produced a noticeable deformation of the cheek and, consequently, a visible deformity of the patient's face. The Indian method consists, *grosso modo*, in harvesting the flap from the genian region, as well as alternatively, designing a pedicled frontal flap. The Sicilian method (Branca, Tagliacozzi) prepares the brachial pedicled flap in the medial part of the arm. The technique of the brachial pedicled flap, as it results from European Medieval and Pre-Modern sources, was diffuse and the ways of accessing the information cannot always be known when publications on the topic are missing.

5. Homage to the Society of Physicians and Naturalists in Iaşi.

We may all be familiar with the novella *Secretul doctorului Honigberger (The Secret of Doctor Honigberger)*, by Mircea Eliade, published in 1940, in *Revista Fundaţiilor Regale*. In the magazine *Cuvîntul* of 30 July 1928, Eliade praised the scientific activity and the contributions made to the history of medicine by Dr. Vasile Gomoiu and Valeriu Bologa, having kept a close correspondence with the latter during his stay in Calcutta (1929-1931) (33). In fact, Eliade uses this eulogy on the occasion of the establishment of the first Academic Society in the Romanian Principalities in order to establish a link between the impressive editorial and scientific activity of George Sarton – founder of the journal *Isis*, the first journal devoted to the history of sciences in the United States – and the assiduous activity of the two physicians, Gomoiu and Bologa, members of the *International Society for the History of Medicine* and founders of the studies in the history of medicine in Cluj during the first decades of the 20th century (33). Returning to Eliade's period in Calcutta, the Indologist and historian of religions Eugen Ciurtin argues that Eliade first found out about Johann Martin Honigberger (1795-1869), the Transylvanian Saxon physician from Braşov, by reading the English edition of his autobiography, published in

1852, *Thirty-Five Years in the East*, which was translated and edited in Romanian in 2004 by Polirom publishing house, with an impressive critical apparatus and an introductory study (9).

Eliade-Honigberger or Honigberger-Eliade? In this case, reality precedes fiction; based on clear and convincing historiographical findings, it has become common knowledge that Johann Martin Honigberger existed indeed, that he had an absolutely impressive biography, of which we will briefly mention no more than two details: first, *Thirty-Five Years in the East*, a volume offering a generous description of rhinoplasty in India (8); second, Honigberger, the anonymous contributor to the politico-literary publication *Albina Românească*, a newspaper founded in 1829 in Iaşi by Gheorghe Asachi, shortly before the establishment of the distinguished Society of Physicians and Naturalists.

This is how Honigberger describes the rhinoplasty procedure in the volume *Thirty-Five Years in the East*: "I introduced to our distinguished guests, Col. Wade and Dr. Murray an Akalee or Nahung, whose nose, ears and hands had been cut off by order of Runjeet Sing (he had even deserved the gallows), and whose nose had been so well restored in the mountains that we were all surprised, and confessed it could not have been better done in Europe. As we know, from history, this operation was even in the remotest antiquity, practised by Hindoos; and they formed the nose out of the cuticle of the forehead, which proceeding is now, and always will be the same. In Europe, where cutting off of noses is only in use, in exceptional cases – as when ulceration or other circumstances make it requisite – this operation is usually performed with the cuticle of the arm, and judiciously too, as, according to our custom, the head mostly is uncovered, and a scar on one's forehead, with a new nose on the face, makes rather an ugly appearance; whilst, in the East, the scar remains hidden beneath the turban" (8).

The contribution of the Transylvanian Saxon physician to the knowledge about Asian medicine, flora, fauna and antiquity studies, together with his political and military contributions were often presented in the pages of the *Albina Românească* newspaper, which determined, most probably, the visit of Honigberger in Iaşi, during one of the reunions of the Society of Phhysicians and Naturalists, an event also signalled by the *Albina Românească*, no. IX, 15 May 1838. Returning from Constantinople – he had no knowledge of Nicolae the Snub-Nosed – Honigberger was received with some ceremony, considering the title (prince of Aracan Aureg-Seib of India) as the *Calendarul pentru poporul românesc*, a magazine from Iaşi, chose to announce him, recalling him (with a slight delay) between 1838 and 1861.

As regards Johann Martin Honigberger's visit at the *Society of Physicians and Naturalists*, besides other highly interesting details, the historian would have more to say.

This is where the circle somehow closes: we argue that the history of Asian studies and the study of Oriental languages contribute to strengthening the epistemological space of the history of medicine, offering adventure and erudition as the ingredients of a huge intellectual horizon, encapsulating the memory of alterities and of the history of practical knowledge.

If the mutilation and reconstruction of Milescu's nose stir such a historiographical interest in the analysis of an extremely unusual excerpt from Neculce's chronicle,

which then leads to a partial explanation of an unclear episode in the adventurous life of the Moldavian scholar, then the effort was worthwhile.

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MARKERS OF INSULIN RESISTANCE IN A CASE OF LAUNOIS-BENSAUDE SYNDROME

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MARKERS OF INSULIN RESISTANCE IN A CASE OF LAUNOIS-BENSAUDE SYNDROME (Abstract): Launois-Bensaude syndrome (benign symmetric lipoma-tosis) is a rare disease characterized by symmetric fat deposits localized in the cervical region, shoulders and proximal parts of upper and lower limbs. We present the case of a 63-year-old male who presented the typical location of fatty masses and a history of chronic alcoholism associated with elements defining the metabolic syndrome. The biological profile indicated high-atherogenic mixed dyslipidemia, high basal insulinemia (30 μU/ml), and multiple markers of insulin resistance (Reaven index, lipid accumulation product, homeostatic model, insulin sensitivity index, and modified glycemic curve following oral glucose load). The particularity of the presented case is the discordance between the severity of metabolic disturbances and their clinical expression, raising the question whether this patient's cardiometabolic risk is increased or rather lowered by the association of benign symmetric lipomatosis. **Kewords**: LAUNOIS-BENSAUDE SYNDROME, INSULIN RESISTANCE.

Launois-Bensaude syndrome (benign symmetric lipomatosis, Madelung's disease) is a rare disease of unknown prevalence, characterized by symmetric fat deposits prevalently localized around the cervical region, in the shoulders and proximal part of the arms and legs. The name of this disease is chronologically disputed by Sir Benjamin Brodie (1846), the first who described this deformity, followed by Otto W. Madelung (1888), a German surgeon, his name being particularly related to the disease eponymous with prevalently cervical location ("Madelung's collar or neck"); in 1898, Raoul Bensaude and Pierre-Emile Launois published the first series of 75 cases and provided a full description of the characteristic features of the disease, adopting the term symmetric lipomatosis (1,2).

Middle-aged (30-60 years) males are most commonly affected, developing the characteristic distribution of body fat after at least 10 years of abusive ethanol consumption. The etiopathogenesis of benign symmetric lipomatosis is not fully known, the hypertrophy of malfunctioning brown fat and the decreased activity of mitochondrial respiratory enzymes that may result in lipolytic pathway depression being incriminated. In all these processes, alcohol is a

possible co-factor. The only effective treatment is surgery, through lipectomy and/or liposuction of fat masses (2, 3).

CASE PRESENTATION

A 63-year-ols male from a rural area presented to the Pulmonary Rehabilitation Clinic of the Iasi Rehabilitation Clinic Hospital complaining of dyspnea, mMRC (*modified Medical Research Council*) breathlessness scale grade 2, intermittent productive cough and mild fatigability.

The patient presented to our clinic for entering a respiratory rehabilitation program having a previous diagnosis of moderate chronic obstructive pulmonary disease (COPD), the symptoms of this chronic lung disease being well controlled with inhaled tiotropium18 μg/day. He also presented grade 2 essential hypertension, blood pressure being optimally controlled with amlodipine 10 mg daily.

What drew our attention during physical examination were the pseudotumoral subcutaneous masses: relatively symmetrical, located mainly in the proximal parts of the upper and lower limbs, with a pseudohypertrophic appearance, and under the chin, behind the ear, and in the nuchal region, resembling a Madelung's collar (fig. 1).

Fig. 1. Relatively symmetric fat deposits in the upper half of the trunk and upper limbs

On palpation, multiple mobile and painless small abdominal lipomas, like the masses in the upper body, were detected (fig. 2). Family history revealed that two patient's brothers present a similar appearance; from personal history we found out that the fat masses with a typical location for Launois-Bensaude syndrome developed progressively over the last 20 years. Surgical removal of one mass located behind the right ear that became painful confirmed its benign nature, lipoma, but resulted in local recurrence.

The patient was a smoker (35 pack-years) and had a history of chronic ethanol consumption (70 international units/week, abstinent for about 6 months); clinically, stigmata of chronic alcoholism persisted, represented by retraction of palmar aponeurosis (Dupuytren's contracture), bilat-

eral parotid gland hypertrophy, telangiectasias on the nose and cheekbone, but laboratory tests confirmed abstinence, serum transaminases, gamma-glutamyltranspeptidase and mean corpuscular volume being normal.

Fig. 2. Abdominal lipomas (left) and under the chin (right)

Patient's anthropometric data showed a body mass index of 30.14 kg/m^2, corresponding to grade I obesity, and a pathological waist circumference of 115 cm, measured in standing position halfway between the costal margin and iliac crest line.

The metabolic profile revealed mixed dyslipidemia at high atherogenic risk (total cholesterol = 6 mmol/l, HDL-cholesterol = 0.9 mmol/l, triglycerides = 1.64 mmol/l, LDL-cholesterol = 4.32 mmol/l), normal glucose levels (4.93 mmol/l, and high basal insulinemia (30 μU/ml), indicator of insulin resistance.

Given the presence of basal hyperinsulinemia, we calculated the markers of insulin resistance derived from previous biological and anthropometric measurements (tab. I).

Also a 3-hour 75-gram oral glucose tolerance test was performed (fig. 3).

TABLE I

Markers of insulin resistance

MARKERS OF INSULIN RESISTANCE	RESULTS	COMMENTS
TG/HDL ratio (Reaven index)	4.17	Reaven index ≥ 3,5 indicates insulin resistance (4)
LAP (lipid accumulation product)	82 cm. mmol/l	Lipid accumulation product correlates well with insulin resistance and metabolic syndrome (5)
HOMA-IR (homeostatic model assessment of insulin resistance)	5.33	Values ≥ 2.6 indicate insulin resistance status; ≥ 4 it is considered prediabetic status (6)
QUICKI(quantitative insulin sensitivity check index)	0.29	Low values (< 0.45) suggest insulin resistance ; < 0.3 in diabetics (7)

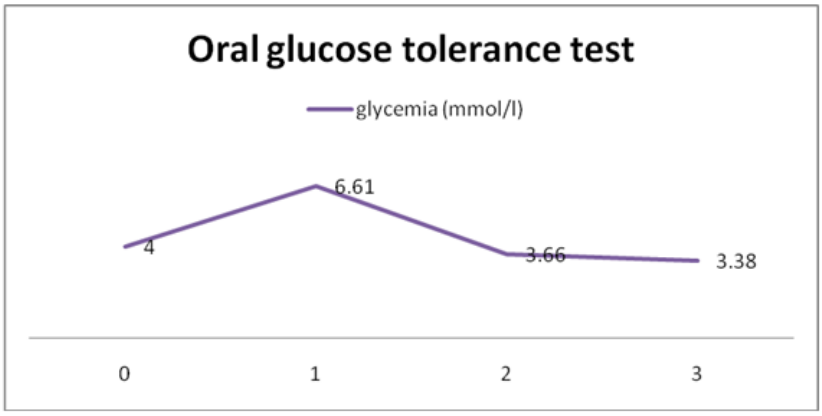

Fig. 3. Evolution of glycemia over the 3-hour 75-g oral glucose load

DISCUSSION

The patient presented a rare disease, benign symmetric lipomatosis, associated with metabolic syndrome, defined in this case by three factors: hypertension, abdominal obesity and low HDL-cholesterol, serum triglycerides being at the limit of diagnostic significance and basal glycemia levels within normal range (8). Simultaneous determination of basal insulinemia and calculation of some markers of insulin resistance (tab. I) revealed frankly pathological values, allowing the assertion of a hyperinsulinemic status associated with insulin resistance.

Remarkable is the discordance between the severity of metabolic disturbances and their clinical expression: the associated pathological conditions (hypertension, COPD) were easily controlled therapeutically and glucose metabolism disturbances were not very obvious. Thus, basal glycemia and the values of oral glucose tolerance test (fig. 3) were normal at fasting or at 2 hours, but prolonged monitoring for three hours revealed a late hypoglycemic dip, result of an abnormal overproduction of insulin in response to glucose load.

The particularity of this case is related to whether the patient's cardiometabolic risk, consequence of the accumulation of risk factors - male, smoking status, hypertension, chronic alcohol consumption, obesity - is increased or rather lowered by the association of Launois-Bensaude syndrome, having in view the mild clinical course despite the severity of underlying metabolic changes?

Review of literature data on Launois-Bensaude syndrome reveals a quite wide phenotypic and clinical variability in this category of patients, ranging from a type of "metabolically innocent" obesity with increased insulin sensitivity by decreased visceral adiposity ascertained by computer tomography (9), to a metabolic picture dominated by insulin resistance, particularly in those patients associating cervical lipomatose deposits and an obstructive sleep apnea syndrome, in its turn generating insulin resistance (10).

The presented case seems to be halfway between these two extremes, but the lack of large studies and validation of any of the models does not allow drawing an indisputable conclusion.

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NEWS

PELTOPHORUM AFRICANUM: ANTIMICROBIAL POTENTIAL AND HEPATOTOXICITY

The antimicrobial activity and hepatotoxicity of *Peltophorum africanum* were evaluated in a study by Okeleye *et al.* Agar well and macrodilution methods were used for antimicrobial activity testing, as well as CellTiter-Blue cell viability assay to assess the toxicity on human liver cells. The results showed both bactericidal and bacteriostatic or fungistatic activity of the extract. The researchers observed good activity against *Plesiomonas shigelloides*. Despite its antimicrobial activity, the extract was toxic to human liver cell lines. Lethal dose at 50 revealed 82.64 ± 1.40 degree of toxicity at 24 hrs, while 95 percentile of cell death dose activity varied between log 3.12 and 4.59. (Okeleye BI, Mkwetshana NT, Ndip RN. Evaluation of the Antibacterial and Antifungal Potential of *Peltophorum africanum*: Toxicological Effect on Human Chang Liver Cell Line. *ScientificWorldJournal* 2013; 2013: 878735. doi: 10.1155/2013/878735).

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DOCTOR-PATIENT DIALOGUE – BASIC ASPECT OF MEDICAL CONSULTATION

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DOCTOR-PATIENT DIALOGUE – BASIC ASPECT OF MEDICAL CONSULTATION (Abstract): Family medicine is the specialty that provides ongoing primary medical care and improves the health status of the individual, of the family and of the community through preventive, educational, therapeutic and rehabilitation measures. The family doctor often makes the interdisciplinary synthesis, in a flexible manner, either alone or in most cases with interdisciplinary consultation. In the latter case, the family doctor initiates the team work and makes the final evaluation by using the longitudinal follow-up of the disease. The doctor-patient encounter represents the "confrontation" with the greatest moral weight, due to the complexity of the values involved, the status of the doctor in a society, and patient's involvement in decision making. The patient is a person who should be treated with respect, honesty, professionalism and loyalty, whatever the clinical status, severity of illness, mental competence or incompetence. A focus, on an international scale, is represented by the characteristics of a good doctor, family physician included, as the latter is the first link in the network of health services. Each model of consultation varies in a more or less subtle way in priorities assignment, and suggests slight differences regarding the role played by doctor and patient in their collaboration. The qualities of a good family physician include not only the strictly professional competences, that also apply to other medical specialties, but also duties, such as, clearly explaining to patients issues concerning their health, informing them about all the possible preventive measures of diseases, making a diagnosis, initiating and supervising a therapy. Medical responsibility lies at the crossroads between medical science and the conscience of the doctor. **Keywords**: DOCTOR-PATIENT DIALOGUE, MEDICAL CONSULTATION, THERAPEUTIC ACT, PREVENTIVE MEASURES, ANAMNESIS, ENQUIRY.

IMPORTANCE OF THE PROBLEM

The disease represents an unfortunate episode in everyone's life, and it has often many negative effects on the entire body. Patient's concern, the fear induced by the disease, but also inappropriately by the family and entourage, sometimes leads to a deep depressive state. In such circumstances, through his conduct, the family doctor has the duty and the professional and moral obligation to create an optimal cooperation climate throughout the period of investigation and care, right from the first contact. This cooperation should be based on inducing a sense of confidence about a good outcome of the therapy and patient's reinte-

gration into family and social life. Respect for the person, as behavior and attitude, represents the fundamental value of the doctor-patient relationship and the essential condition of therapeutic success.

Family medicine brings a global and concise view of the man, represented in a close relationship with his social, existential, and family environment (1).

As the family doctor is the first step into the medical system, he has to be easy to find and approach by patients. The family doctor does not address a specific organ of the body or age group, but the entire person, the interconnections between different systems or organs and the external environment, the general manifestations of diseases, the associated and interdisciplinary pathology (2,3).

Also, family medicine has a nonspecific object of activity shared with other specialties, namely the early diagnosis of diseases, secondary prevention, community- based care in chronic diseases (tertiary prevention), continuous medical care, palliative medical care (4,5).

The family doctor is the only doctor that combines therapy and prevention activities (primary, secondary and tertiary prevention), both on an individual basis and at community level. Through the measures of prevention and health preservation, he is the guarantor of health (5,6).

Primary prevention measures may be effective if they target the individual, the family and the environment through concurrent actions of the family doctor and hygienist (e.g. vaccinations, health education, fighting the internal risk factors by acting on the environment through direct observation). Secondary prevention involves the perception of changes in the individual's health status and early diagnosis during common medical investigations and periodical examination. Tertiary prevention represents the prevention of complications and disease progression (5,6).

The main object of study for the family doctor is the individual, his family, his surrounding community and environment, all these exerting a positive or negative influence on the individual, with which the family doctor must be acquainted.

Family medicine distinguishes itself through the medical care given to healthy persons, primary prevention, and the holistic approach of the patient within the context of his environment, family, and living and working community, respectively (7).

This means that unlike other specialties that have in many cases analytic research methods of certain pathological conditions, that are exhaustively investigated, family medicine is solely based on methods that require synthesis, as the diagnosis is based on the synthesis of data collected through observation, history taking and physical examination of the patient.

Therefore, the research methods of family medicine are influenced by the following aspects: the family medicine is mostly communicative, mostly clinical, and mostly based on synthesis.

The activity of the family doctor mostly pursues clinical and diagnostic objectives, such as providing a diagnostic and therapeutic synthesis, therapeutical and prophylactic measures, as well as maintaining the health status of the healthy individual (7,8). The improvement of clinical methods resulted in the progress of family medicine.

The diagnostic and therapeutic synthesis is essential due to the fact thatthe encountered medical conditions are complex and that the patient should always be monitored (9).

In order to make and confirm a diagnosis, laboratory tests are required. This method of making the diagnosis is specific to all the other specialties, but cannot be ignored by the family doctor (9).

The family doctor should have basic knowledge of laboratory tests, based on which an initial diagnosis is possible. Based on this initial diagnosis the patient is either referred to a specialist or is recommended a first intention treatment. These methods of laboratory examination help the family doctor analyze and understand the diagnostic methods of other specialties, both at admission and upon discharge, in order to move the patient to community based care.

Due to the fact that global clinical laboratory testing uses the results provided by various specialties, where the patient was investigated, we come to the inevitable domain of interdisciplinarity.

DOCTOR-PATIENT DIALOGUE

The doctor-patient dialogue should take place in a relaxed atmosphere. The warm friendly tone, the optimistic attitude of the doctor, the solicitude, the comprehension towards patient's suffering, the kindness, the politeness and the patience with the person in illness, the interest that was proved by the doctor represent not only a real moral support, a source of security, of protection against the aggression affecting the patient. On the other hand, the patient becomes responsive, cooperative, understanding towards the efforts made by the family doctor in order to alleviate his suffering (1,7).

The patient becomes for the doctor a subject of research and of medical practice, but also a rational and social being, with a spiritual life and interest in his own health. Therefore, the doctor must be acquainted with the psychological peculiarities of the patient, irrespective of his disease.

As a good psychologist, the doctor shall make a close interhuman relationship with the patient, for the benefit of the latter. The doctor's emotional openness to the patient represents “the keystone of medical psychology”, as it lessens anxiety and insecurities and it humanizes the medical assistance.

The medical consultation begins with the report of the patient on the signs that were noticed at the onset of his disease. The report of the patient, based on recollection of pathological phenomena is known in the medical terminology as anamnesis or history. In quest of a thorough comprehension of data, the doctor asks questions, in order to set out some aspects. In other words, the doctor performs the medical enquiry (9,10).

The doctor-patient dialogue becomes more and more important, as the doctor helps not only treat the disease, but also solves the problems caused by diseases. In many chronic diseases, the communication is theoretically the only form of treatment. The advice, the support, the information are essential in helping patients adjust to a more limited lifestyle imposed by the disease.

ANAMNESIS COMPLEMENTED WITH INQUIRY

Anamnesis, complemented with inquiry, allows the doctor to gain extensive knowledge of patient’s symptoms. Thus, he has the opportunity to make an evaluation of his/her personality, level of intelligence, and general education, all these providing implicitly more nuanced criteria for evaluating the disturbances of the patient.

The adequate realistic description of symptoms, their succession have a great significance for the medical judgment based on a preliminary diagnostic hypothesis, whose accuracy shall be verified by clinical and laboratory investigations, but also by the results of the applied treatment (10).

The doctor-patient dialogue should be permanent, throughout the whole period of patient care. If the expected results do not occur, the doctor should do history again in order to gain new information that may lead to a reconsideration of the diagnosis (9).

The family doctor often makes the interdisciplinary synthesis in a flexible manner, either alone or in most cases with interdisciplinary consultation. In the latter case, the family doctor initiates the team work and makes the final evaluation by using the longitudinal follow-up. The onset of the disease may be suggestive for the nature and location of the pathological process.

The diseases with sudden onset and loud symptoms stand for acute pathological processes that allow an accurate diagnosis, based on the characteristic signs. An accurate diagnosis can even be made based on history alone. However, diseases such as cancer show progressive and silent clinical manifestations. The various locations of the neoplastic disease may have in early stages uncharacteristic, irrelevant clinical signs, common to other diseases of the affected individual (12). But it is a known fact that in cancer the symptoms are persistent, do not respond to common treatments, and the clinical picture adds further clinical manifestations (13).

In many cases, the description by the patient of the initial clinical symptoms is relevant for certain organs, such as lungs, heart, kidneys, and the presumptive diagnosis is verified through physical examination and laboratory tests. The subjective signs are common to various diseases of the respiratory system but take various forms and aspects, both at disease onset and during its progression, and the inquiry should focus on this problem (9). The infectious and contagious diseases are also important, because they weaken the body resistance and favor diseases of the respiratory system. In heart conditions, the patients complain of suggestive symptoms: pain in the region of the heart (precordial pains) with various characteristics, such as the typical constrictive claw-like pain that irradiates to the left shoulder, left arm and hand, and is common in angina pectoris or in myocardial infarction (7). Another common symptom is the lack of air or the difficulty of breathing that may frequently occur during exercise or even at rest.

The patients with high blood pressure may report such symptoms as seeing dark spots in the eyes or hearing various noises, headaches, and dizziness or sleep disorders. In diseases of the digestive system, the patients describe pains of different location and duration, depending on the affected area (stomach, duodenum, bowel, gallbladder), burning sensation behind the sternum or in the upper abdominal region, bloating. In diseases of the urinary system, the described symptoms include pain and alterations in urine elimination. Some patients report spontaneous urine leakage, that may suggest urinary incontinence, and in other cases urine retention. The qualitative changes in urine are medically significant and always alarming for the patients, when pus or blood is found in urine.

Very important is also that the doctor to

know the collateral genetic antecedents, because many diseases have a genetic basis (9).

During history taking, the patient should give information about age, sex, living and working conditions, conflicting events in the family or at work, if any, climate and geographical features of his neighborhood, unhealthy practices (use and abuse of alcohol and tobacco), treatments administered since disease onset, previous physical examinations and laboratory tests. The patients that received surgery should show the surgical protocol, needed in evaluating the acute disease each of these pieces of information may contribute to a more realistic approach of the investigated disease (14).

An as thorough as possible anamnesis and a correct inquiry represent "the art of interrogating the patients" and "the art of listening the patients". Anamneses that are incomplete and incorrectly recorded may be a source of errors, such as the unnecessary prescription of laboratory procedures, as well as of ineffective treatments. Thus, in some cases of pneumonia, the patients may report abdominal pain, that, as a result of a superficial and inaccurate anamnesis, may lead to a false diagnosis of appendicitis, cholecystitis or ulcer. Atypical clinical forms of severe disorders such as myocardial infarction may result in misdiagnosis and serious therapeutic errors (9). Some forms of myocardial infarctions are associated with digestive disturbances that include epigastric pain, meteorism, regurgitations, eructation that may induce a diagnosis of gastric or duodenal ulcer. In case of myocardial infarction, the treatment requires absolute bed rest, and if the diagnosis is false, this requirement will not be certainly satisfied and the outcome may be fatal (15, 16).

Anamnesis provides 50% of the information needed for a diagnosis. The rest comes from the clinical and laboratory examinations (17). It is common that the family doctor himself treat various diseases, such as, digestive, cardiac, dermatological, ophthalmic disorders, when the patient cannot receive the consultation of a specialist doctor or laboratory examinations, due to objective reasons.

Some medical or surgical specialties may change or event disappear, but in our opinion this is absolutely certain: in all ages, as Hippocrates specified almost two thousand five hundred years ago, the family doctor will be always present with his patients by word and deed and with heart and soul (10).

CONCLUSIONS

The profession of family doctor is a noble one. The family doctor has difficult tasks to undertake, many financial disadvantages, but, on the other hand, he has great satisfactions.

The continuing medical education is essential for the life of a family doctor. The profession of doctor is one of the occupations that necessarily require lifelong education. Moreover, the health education of the population should represent the main aim of the national health policy, along with objectives such as medical preventive measures. The improvement of the level of health education in the general population may be achieved through large educative programs of public health.

Based on the duties of a family doctor, we can conclude that the family medicine represents the most important branch of medicine, because it provides the primary care and differentiates between the truly severe cases, that require hospital admission and the cases that require outpatient

care, under modern and efficient conditions.

What the family doctor does is essential, because he has a holistic view of the patient.

The family doctor should be regarded as "the hub of the world", because he deserves that. But until his status will be acknowledged, the family doctor may be still compared with an armed infantryman, who enters minefields, investigates the enemy, fights successfully or unsuccessfully, while on duty, and watches the sky full of specialists who look at him from aircrafts. However, when brought down to earth and put in the front line, these specialists behave just like untrained infantrymen.

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CHRONIC VENOUS INSUFFICIENCY STAGE V CEAP SECONDARY TO HEREDITARY THROMBOPHILIA AT A YOUNG MAN

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CHRONIC VENOUS INSUFFICIENCY STAGE V CEAP SECONDARY TO HEREDITARY THROMBOPHILIA AT A YOUNG MAN (Abstract): Hereditary thrombophilia is a rare disease of general population, which is responsible for debilitating consequences in severe cases. We present the case of a young male patient whose pathology and symptoms occurred since the age of two, which determined a venous insufficiency stage V with important limitation of the locomotors ability and plenty of complications. The aim of this paper is to draw attention to a disease that sometimes is not diagnosed on a first visit to the doctor.
Keywords: HEREDITARY THROMBOPHILIA, ANTITHROMBIN, FACTOR LEIDEN.

Hereditary thrombophilia is a rare condition that manifests as several abnormalities determined by hereditary thrombophilic factors that can act by reducing the number of inhibitors which can lead to clotting or can increase the number or the function of clotting factors (1, 3, 4, 5, 6).

The factors involved in thrombophilia pathology are: resistance to activated protein C, factor V Leiden, protein C or S deficiency, mutation of the prothrombin gene, anti-thrombin gene, and increase of F VIII a, hyper-homo-cysteinemia.

The antithrombin deficiency of the overall population is around 0.015%-1.00% (1, 2). It is present in 0.5%-7.6% of patients diagnosed with venous thromboembolism. There are three known subtypes of antithrombin deficiency produced by more than 200 different mutations. Most of the mutations are heterozygous; the homozygous type is not life-compatible or determines a severe thrombotic phonotype. From a physiological point of view, antithrombin III has a covalent bond and makes trombone and factors Xa, IXa, XIa and XIIa inactive (1, 3, 5, 6, 7, 9, 10, 11).

Resistance to reactive protein C is given by a mutation at the level of the position 1691 of the factor V Leiden and represents the most frequent thrombophilia cause, being present in more than 40% of the diagnosed cases. Most homozygotes develop at least one thrombotic event throughout life, but have a lower risk than the other factors involved in the pathology of hereditary thrombophilia. In addition, factor V Leiden is met in overall population in a

percentage of up to 15%; on patients with recurrent thromboses it is met in up to 50% (1, 2, 4, 6, 7, 9).

The association of more hereditary thrombotic factors tends to lead to an increase of complications and to the influence of the diagnostic.

Virchow's triad is represented by: injury to the vessel walls, abnormal blood flow and changes in blood structure. Some authors state that about 60% of the patients with hereditary deficiency of antithrombin III develop a thrombotic episode until the age of 60 (1, 2, 3).

CASE PRESENTATION

We are going to present the case of a male patient, aged 28 years, who was admitted at the Cardio-Vascular Clinic of Iasi and accused important pains at the level of the lower right limb, leg ulceration, oedema of inferior legs, asthenia, vertigo, scotomas.

From the hereditary-collateral history it can be identified a familial aggregation of varicose cases located at legs' level (two sisters, one brother, two cousins on the mother line, grandfather on the mother line, mother) and oesophageal varicose (one brother).

The previous pathological history of the young man, ex-smoker of 5 packs a year highlight the fact that he was diagnosed with abdominal varicose and chronic venous deficiency at the age of 2, with varicose at the level of inferior limbs, varicose ulceration at the level of the right calf and deep thrombophlebitis at the age of 6 and in 2007 he received the diagnostic of congenital malformation of the venous system (fig. 1).

The clinical examination on the apparatus and systems reveal the presence of varicose veins at abdominal level and on the inferior legs, as well as the presence of bilateral calf ulceration of 10/5 cm, additional trophic disorders, ochre dermatitis (fig. 2 a, b, c). The examination of the cardio-vascular system reveals a systolic murmur II/III in the mitral valve.

Fig. 1. Varicose veins at abdominal level and on the inferior legs of the patient at puberty

Paraclinical investigations highlight the seriousness of the chronic venous deficiency. Venous Eco Doppler examination does not highlight a venous flow at the level of the superficial femoral veins and popliteal veins. Venous tracts are slightly visible, having a reduced diameter, echogenous on the inside and lacking in flow. Varicose tracts are visible at the calf and thigh level.

Abdominal ultrasounds reveal a spleen with homogenous structures, 152 mm, and small vascular hilum dilatations.

The performed ultrasound revealed a mitral deficiency of degree I-II Doppler,

tricuspid deficiency degree I Doppler, PSAP – 36 mmHg, small calcified structures, especially at the level of the posterior aortic wall.

Fig. 2 a, b, c. Varicose veins at abdominal level and on the inferior legs at the age of 28 years.

Following clinical and paraclinical examination, there is a suspicion of hereditary thrombophilia and a genetic examination is requested. It is recommended to perform diagnostic molecular tests. Following analyses, it was observed an antithrombin III deficiency (51.3% compared to standard values, which are between 75-125%), and a factor V Leiden of 49 (the standard value is 60-140%).

Considering anamnestic, clinical and paraclinical data, it is given the diagnosis of hereditary thrombophilia by antithrombin III deficiency and abnormal factor V Leiden. In addition, there are also given the diagnoses of chronic venous deficiency at legs level, class V CEAP, healed varicose ulcer on the bilateral calves, ochre dermatitis on bilateral calves, slight mitral deficiency, slight tricuspid deficiency, aortic atheromatosis.

DISCUSSION

Hereditary thrombophilia is a rare disorder which affects patient's life quality and implies a guarded prognosis for serious cases. What is so particular about this case are the severe changes observed during clinical and paraclinical examination. The management of this disorder requires the cooperation the patient, family, as well as a medical team made of surgeon, radiologist, internist, geneticist and physical therapist.

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NEWS

EFFECT OF FIBER POST LENGTH AND ADHESIVE STRATEGY ON FRACTURE RESISTANCE OF ENDODONTICALLY TREATED TEETH

When restoring an endodontically treated tooth with excessive loss of tooth structure, a post may be needed to improve the retention of the core build-up material. Post material, design, dimensions, surface roughness and length have been shown to affect fracture resistance and retention. Nowadays, thanks to the optimization of bonding mechanisms of current adhesive composite cements, guidelines regarding post length may be revised. Since fiber posts are bonded within the root canal, their length could perhaps be shortened in light of a less invasive post build-up. The aim of this study was to evaluate the effect of the length of fiber-posts and type of adhesive cement on the fracture resistance of endodontically treated teeth, after fatigue loading. Eighty extracted upper pre-molars were sectioned at the cemento-enamel junction and endodontically treated. After 24 h of water storage at 37 ^{o}C, RelyX Posts (3M-ESPE) were cemented with Panavia F 2.0 (Kuraray) or RelyX Unicem (3M-ESPE). A standardized composite core was built. Specimens were divided into four groups depending on the post–core ratio and submitted to 1,200,000 cycles using a chewing simulator (Willytech). Immediately afterwards, all specimens that survived fatigue loading were fractured using a universal loading device (Micro-tester, Instron). Data were analyzed with ANOVA. Four percent of the specimens failed during fatigue loading. The length of the post into the root affected the fracture resistance, the control group (with the greatest length of the posts) always having the lowest fracture resistance. The type of adhesive cement did not affect the fracture resistance. A prevalence of not-repairable failures was observed in specimens restored with the longest posts, whilst shorter posts led to more repairable failures. The results suggest that shortening the post length and the preservation of more tooth structure, offer the potential for reparability through an in-built fail safe mechanism and may thus reduce the occurrence of catastrophic failures (Zicari F, Van Meerbeek B, Scotti R, Naert I. Effect of fiber post length and adhesive strategy on fracture resistance of endodontically treated teeth after fatigue loading. *Journal of Dentistry* 2012; 40: 312-321).

Gianina Iovan

THE SEMI-QUANTITATIVE CALPROTECTIN RAPID TEST – IS IT USEFUL IN INFLAMMATORY BOWEL DISEASE ?

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THE SEMI-QUANTIATIVE CALPROTECTIN RAPID TEST – IS IT USEFUL IN INFLAMMATORY BOWEL DISEASE? (Abstract): **Aim**: To assess the efficiency of the dosage of the semi-quantitative calprotectin rapid test in evaluating the activity of inflammatory bowel diseases. **Material and methods**: The prospective study included 87 patients with BII (50 with ulcerative colitis, 37 with Crohn disease) hospitalized between January 2011 - May 2012. The diagnosis was confirmed by colonoscopy and histological examination. Fecal calprotectin was measured by means of a semi-quantitative rapid test. **Results**: There were 50 patients with UC and 37 with BC. 96% of the UC patients were evaluated during the active disease. 21 of them had a severe episode of active disease, 16 had a moderate one, while 10 had a mild activity episode. The correlation between the calprotectin values and lesions localization was analyzed, without finding any statistical differences. The data have shown a very strong correlation between the severity of the active disease, assessed through the UCDAI (Mayo) score, and the calprotectin value. Patients with CD manifested a linear correlation between the severity of the active disease and the calprotectin value. **Conclusions**: The rapid test for assessing calprotectin could be a useful non-invasive marker in appreciating the severity of clinical and biological disease activity. **Key words**: CALPROTECTIN, INFLAMMATORY BOWEL DISEASE, ULCERATIVE COLITIS, CROHN'S DISEASE.

Inflammatory bowel disease (IBD) is a group of chronic idiopathic affections represented by ulcerative colitis (UC), Crohn's disease (CD), and undetermined colitis. During the last decades, there has been noted a growth in the occurrence and prevalence of these affections (1). The clinical activity score (CAS) indirectly assesses the activity of the disease and predicts, with low accuracy, the endoscopic inflammatory activity. Endoscopy and histological examinations are precise, but they are expensive and invasive methods. Given the context, there have been some recent rising concerns over the highlighting of the biomarkers that can be used to quantify the activity of the disease and the risk of relapse (2, 3).

Calprotectin is a protein that plays a central part in the immune defense mechanism. Fecal calprotectin can be used as an indirect marker of intestinal inflammation.

It is very stable in feces and its concentration is very well correlated to the activity level of BII (4, 5).

The aim of the study was to assess the efficiency of the dosage of the semi-quantitative calprotectin rapid test in evaluating the activity of inflammatory bowel diseases.

MATERIAL AND METHODS

A descriptive prospective study had been conducted, between January 2011 and May 2012, on 87 BII patients admitted to the Gastroenterology and Hepatology Center of "St. Spiridon" Hospital in Iaşi. The diagnosis was formulated based on clinical, biological, and endoscopic manifestations, and it was confirmed by a histopathological examination. An endoscopic examination established the extension of the lesions. The UCDAI score was used in order to assess the severity of the disease for the UC patients, while the CDAI score was used for the CD patients.

Fecal calprotectin was determined by rapid immunochromatographic semi-quantitative test CalDetect® Sofar. The presence of a first band (T1) indicates a calprotectin concentration lower than 15 µg/g, which is not representative for bowel inflammation. The presence of a second band (T2) demonstrates a fecal calprotectin concentration between 15 µg/g and 60 µg/g. This shows an acute inflammation of the bowel mucosa. The third band (T3) is present at calprotectin concentrations higher than 60 µg/g and it signals a high degree of inflammation of the bowel mucosa.

The Chi-squared test, t-student, and Spearman and Pearson regression tests were used for statistical analysis.

RESULTS

The study included 50 cases of UC and 37 cases of CD. 96% of the UC patients were evaluated during the active disease. 21 of them had a severe episode of active disease, 16 had a moderate one, while 10 had a mild activity episode.

Analysis of individual parameters (age, sex, and location) did not show significant correlations.

The correlation between the calprotectin values and lesions localization was analyzed, without finding any statistical differences (fig. 1).

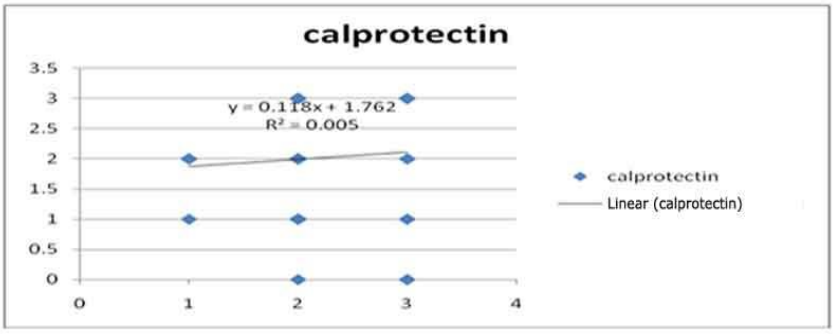

Fig. 1. Correlations between the calprotectin value and the extension of lesions for UC patients

The data have shown a very strong correlation between the severity of the active disease, assessed through the UCDAI (Mayo) score and the calprotectin value (fig. 2), a result confirmed by the Spearman regression (fig. 3).

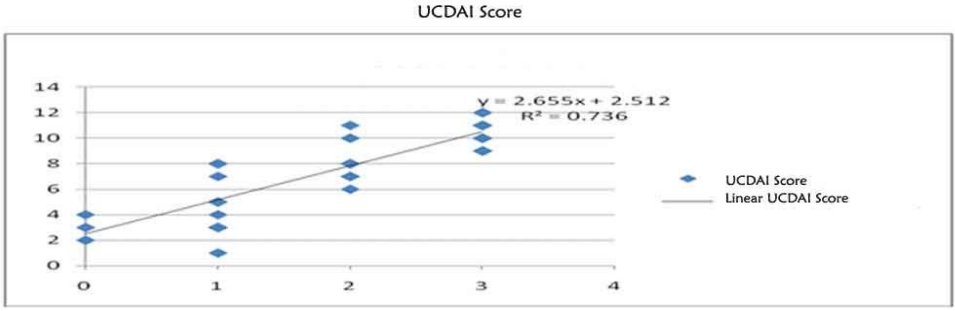

Fig. 2. Correlations between the calprotectin value and the active disease for UC patients

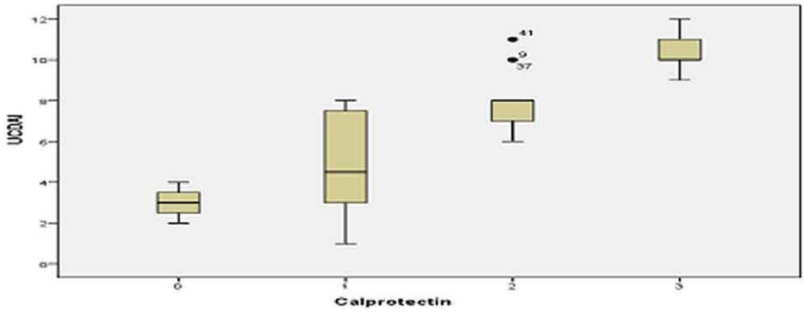

Fig. 3. Correlations between the calprotectin value and the active disease for UC patients

Thirty six patients with UC were studied. Four of them were in remission. As regards the active disease, 11 patients had a moderate active disease, while 11 presented mild active disease. Only 3 patients presented a severe active disease.

Crohn's disease is classified according to the Montreal classification scheme, which uses age, lesions localization, and behavior as individual parameters. The study found no statistically significant correlations between calprotectine and any of these parameters, localization in particular (fig. 4)

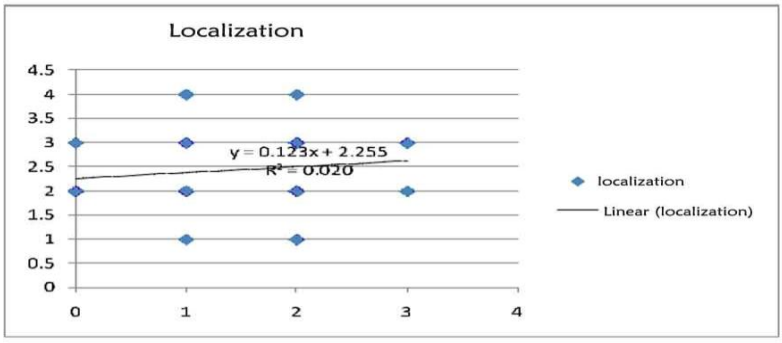

Fig. 4. Correlations between the calprotectin value and the lesions localization in CD

In CD, we noticed a linear correlation between the severity of the active disease, assessed through the CDAI score, and the calprotectin value (fig. 5, 6).

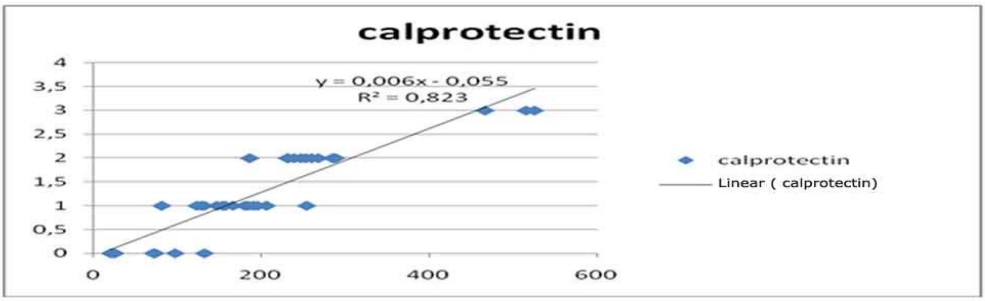

Fig. 5. Correlations between the calprotectin value and the disease activity in CD

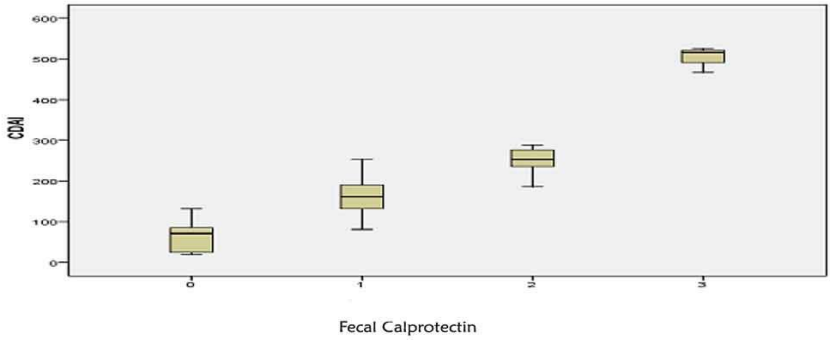

Fig. 6. Correlation between the calprotectin value and the disease activity in CD

DISCUSSION

There is a real concern regarding the discovery of a non-invasive marker with a high power of prediction for inflammatory bowel diseases and severity of active disease. The most promising markers have proven to be the fecal markers: calprotectin and lactoferrin. These proteins were found in large concentrations in intestine inflammation. Numerous recent studies have shown a good correlation between the endoscopic and histological activity of the disease and the fecal calprotectin value (4, 5, 7-10).

Recent studies have highlighted the utility of calprotectin in monitoring the patients in order to predict the relapses (6).

However, few studies use rapid tests in order to assess active disease (9, 10).

This study is prospectively evaluating the importance of the semi-quantitative calprotectin rapid test in assessing the activity.

The univariate analysis of data did not show any correlation between individual parameters (age, sex, location). The data available in literature indicate some correlations between the individual particularities and disease activity, without being statistically significant (3). Surprisingly, the calprotectin value did not correlate significantly with the extension of lesions in UC or the localization in CD. The data available is controversial. Some studies highlight correlations (8), while others

failed in establishing associations with the calprotectin values (6).

The data show a very good correlation between the severity of the active disease and the calprotectin value, not only for UC, but also for Crohn's disease. This suggests that the immunochromatographic semi-quantitative test CalDetect® Sofar is reliable in the evaluation of the disease activity and might be an alternative to the existing tests. The data are similar with those available in literature (9,10).

At the moment, the study does not analyze the correlation with other consecrated inflammation markers (leukocytosis, thrombocytosis, ESR, PCR), nor the endoscopic score, both being cited as having a very good correlation with the calprotectin value.

CONCLUSIONS

The rapid test for assessing calprotectin could be a useful non-invasive marker in appreciating the severity of the clinical and biological disease activity.

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